

## SODIUM AND CHLORIDE BALANCE IN THE PRAWN, *PALAEEMONETES VARIANS*

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### INTRODUCTION

The small euryhaline prawn *Palaemonetes varians* (Leach) is found in estuaries and salt marshes of north-west Europe. It can live in all salinities from 120‰ sea water to 1‰ and some individuals can even survive in 0.5‰ sea water; furthermore, it can tolerate immediate transfer between any salinities in this range. It was observed by Panikkar (1941) that the blood concentration remained remarkably constant within this very wide range of salinities. In this paper we shall try to show how this prawn is able to regulate its blood with such constancy. The prawns in our experiments did not show as close a regulation of the blood as Panikkar's vapour-pressure measurements suggest, but the animals maintain their blood concentration hypo-osmotic to the medium in sea water. In its ability to regulate both hypo-osmotically and hyperosmotically, it resembles euryhaline teleosts (Black, 1957), several genera of crabs (Jones, 1941), *Artemia* (Croghan, 1958), *Sphaeroma* (Riegel, 1959), related palaemonid prawns (Panikkar, 1941), and a number of insects (Nemenz, 1960; Sutcliffe, 1960). The ability to regulate in this fashion would appear to require the reversal of regulatory systems with changing salinity. In order to study this reversible osmoregulation we have investigated the movement of radioactively labelled sodium and bromide (for chloride) ions through *Palaemonetes* in a wide range of salinities.

### METHODS

Most of the work reported here was done at the Plymouth Marine Laboratory using animals caught locally. Some measurements were made in Birmingham using prawns caught in the Plymouth area.

Measurements of the sodium and potassium concentrations were made with an EEL flame photometer and chloride concentrations were measured with a Cotlove chloridimeter. Total body sodium and potassium were estimated after dissolving the prawns in a small quantity of concentrated nitric acid and diluting appropriately. Sodium and chloride concentrations in the blood were measured after diluting weighed quantities of blood with distilled water. Chloride in whole animals was estimated after homogenizing the animals in distilled water and precipitating the protein with Somogyi's zinc sulphate-barium hydroxide reagent. Animals were dried overnight at 105° C. for estimation of water content.

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Flux measurements were made with  $^{24}\text{Na}$ -labelled sodium chloride solution and  $^{82}\text{Br}$ -labelled sodium bromide solution in salinities of 100% sea water (540 mM Cl/kg. water, 460 mM Na/kg. water); 65% sea water, approximately isosmotic with the blood and 2% sea water. The prawns were equilibrated in the appropriate salinity for at least 24 hr. before the experiments were begun. Previous knowledge and the information reported here indicated that this was ample time for equilibration. The ratio of stable ions to radioactively labelled ions (specific activity) was the same in all the experimental media. It was found that the viable salinity limits of the prawns used were close to 1% sea water and consistent results could not be obtained below about 2% sea water. For ease of handling the animals were restrained in short lengths of glass tubing, of slightly larger diameter than the thorax, closed at each end with a small V of stainless steel gauze. The container tubes were emptied and refilled several times when placed in the active medium, to ensure good initial contact. When the animal was placed in the tube with the steel V's in place, the animal's respiratory current provided adequate circulation of the medium through the tube.

Rates of efflux were measured by transferring prawns for  $\frac{1}{2}$  hr. to an appropriate dilution of sea water labelled either with  $^{24}\text{Na}$  or  $^{82}\text{Br}$ . The labelled prawns were washed briefly in four changes of unlabelled medium and then washed by an antero-posterior flow of the unlabelled medium for 2 hr., the eluting fluid running through the tubes at a rate of about 5 ml./min. The radioactivity remaining in the intact animals was measured at  $\frac{1}{2}$  hr. intervals by removing a glass tube with its inhabitant prawn, transferring to 10 ml. of the medium in a test tube, and counting the activity of the whole in a scintillation counter (EKCO, 1 in. well, NaI crystal). The animals do not survive if restrained too closely, but the geometry of the well-type counter allows some movement without significant loss of accuracy. The rate constant  $K$  of the efflux was obtained from the relation  $C_t = C_0 e^{-Kt}$  where  $C_0$  is the initial count rate and  $C_t$  the rate after time  $t$ .

Rates of influx were measured by placing the prawns in their containers in a labelled medium for an accurately determined period of time, usually 5–20 min., washing and drying thoroughly and then sampling the blood (2–20 mg.) from the heart or dorsal abdominal vessel.\* The blood samples were transferred to weighed planchettes, and weighed before and after desiccation to determine their water content, and the radioactivity was measured with a GM-4 end-window counter. Reference samples of the medium were taken at the same time and treated similarly. The rate constant of the influx was determined from the relation:

$$K = 2.3 \log_{10} \frac{C_{\infty}}{C_{\infty} - C_t},$$

where  $C_t$  = count rate/unit weight of blood at time  $t$ ,  $C_{\infty}$  the count rate at equilibrium.  $C_{\infty}$  was calculated from the activity of the medium on the assumption that at equilibrium the specific activities of both body fluid and medium would be the same.

Changes in the rate of loss of sodium from the prawns during short intervals of time were determined by transferring prawns labelled with  $^{24}\text{Na}$  through a succession of test tubes, each containing 10 ml. of fresh medium. The animals were transferred

\* Samples of blood were obtained by inserting fine glass capillaries into the abdominal thoracic vessel and the heart.

from one tube to another at every  $\frac{1}{2}$  min. and the radioactivity remaining in the solutions was measured by scintillation counting.

All radioactive measurements were corrected for physical decay and background. The experiments were made at  $15 \pm 1^\circ$  C. throughout.

Electrical potential difference between the blood and the medium was measured by using KCl-filled glass micro-electrodes, a cathode follower and a pen recorder. The electrode was introduced into the animal through an intersegmental membrane into the dorsal vessel of the abdomen, but small movements of the animal often broke the electrodes or widened the hole to short circuit the system, so that many attempts were abortive. Consistent results could not be obtained in the greatest dilution, and measurements are reported for 100, 75 and 10% sea water. The electrical system was calibrated by the introduction of a known potential across the electrodes. These measurements were made by Mrs M. D. Fox.\* As the individual measurements varied with time Table 2 records the range of potentials found, not their means.

#### INTERPRETATION OF RESULTS

The principal results of this investigation appear in Tables 1 and 2. The variation in the exchange rates of sodium and bromide ions with the change in concentration of the medium, and the changes in the potential difference between the animal and the medium can be interpreted as follows. In isosmotic (65%) sea water the animals are in a passive equilibrium with the medium. In more dilute media the sodium and chloride content of the blood is maintained mainly by an active uptake of chloride ions which produces a potential difference. This in turn helps to maintain an equilibrium of sodium, although at the lowest viable concentration studied (2% sea water) some active sodium uptake also occurs. In full strength sea water, ionic balance is maintained by the active outward transport of sodium ions which produces a potential difference sufficient to maintain the passive equilibrium of chloride ions.

Table 1. *Sodium and chloride content of medium, of blood and of whole animals*

	100% sea water			65% sea water			2% sea water		
	Na	Cl	K	Na	Cl	K	Na	Cl	K
Medium (mM/kg.)	460	540	—	300	350	—	9	11	—
Blood (mM/kg.)	$328 \pm 10$ (7)	$342 \pm 10$ (6)	—	$308 \pm 5$ (9)	$312 \pm 6$ (6)	—	$204 \pm 10$ (8)	$212 \pm 10$ (6)	—
Whole animals (mM/kg. water)	$166 \pm 3$ (12)	$151 \pm 4$ (5)	$103 \pm 2$ (6)	$148 \pm 2$ (12)	$154 \pm 7$ (6)	$101 \pm 4$ (6)	$108 \pm 2$ (10)	$123 \pm 12$ (6)	$90 \pm 3$ (6)
Water content	100% sea water			65% sea water			2% sea water		
Whole animals (%)	$76.9 \pm 0.3$ (6)			$77.6 \pm 0.6$ (6)			$78.4 \pm 0.7$ (6)		

Figures in parentheses are the number of samples analysed.

The evidence on which this view is based is discussed more fully below.

When an aquatic animal is in salt balance the influx and efflux of an ion must be equal. Ions may be gained from the medium by active uptake and by passive inward diffusion, while ions may be lost by passive outward diffusion, by active excretion

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across the body wall, and by excretory loss in the urine. At equilibrium it follows that:

$$\text{inward diffusion} + \text{active uptake} = \text{outward diffusion} + \text{loss in urine} + \text{extrarenal excretion.}$$

Table 2. Sodium and 'chloride' fluxes in different salinities

	100 % sea water		65 % sea water		2 % sea water	
	Na	Br	Na	Br	Na	Br
Rate constant of influx (hr. <sup>-1</sup> )	1.25 ± 0.17 (8)	0.27 ± 0.036 (7)	0.56 ± 0.023 (12)	0.121 ± 0.017 (7)	0.192 ± 0.011 (8)	0.172 ± 0.017 (8)
Rate constant of efflux (hr. <sup>-1</sup> )	1.09 ± 0.17 (7)	0.36 ± 0.03 (8)	0.712 ± 0.15 (8)	0.16 ± 0.03 (7)	0.213 ± 0.059 (8)	0.28 ± 0.046 (8)
Mean	1.17	0.33	0.64	0.14	0.20	0.23
Sodium flux (mm/kg. animal/hr.)	150		73.5		18	
Chloride flux (mm/kg. animal/hr.)	50		20.7		28.2	
Medium	100 % sea water		75 % sea water	50 % sea water	25 % sea water	10 % sea water
Potential range (mV)/(blood negative)	13-40		7-17	7-10	7-33	11-32

Figures in parentheses are the number of samples analysed.

When there is no potential difference across the barrier between the blood and the medium the rate of inward diffusion will be proportional to the concentration of the ion in the external medium, and the rate of outward diffusion will be proportional to the concentration in the blood. Thus:

$$\frac{\text{inward diffusion}}{\text{outward diffusion}} = \frac{\text{concentration of ion in medium}}{\text{concentration of ion in blood}}.$$

However, a potential difference between the blood and the medium will enhance or retard the diffusion of an ion, and the relationship will then become:

$$\frac{\text{inward diffusion}}{\text{outward diffusion}} = \frac{\text{concentration in medium}}{\text{concentration in blood}} e^{\pm zEF/RT}, *$$

where  $z$  is valency,  $R$  is the gas constant,  $T$  is absolute temperature,  $E$  is potential difference and  $F$  is the Faraday. Even a small potential difference will produce a large change in the rates of diffusion. As is well known, a potential difference of 58 mV across a membrane will balance the rates of diffusion of monovalent cations between two solutions which differ in concentration by a factor of ten, if the membrane is negative toward the more concentrated solution. However, a potential difference of this polarity will enhance the rate of diffusion of anions from the more concentrated to the less concentrated solution and retard the diffusion of anions the other way.

If the simplifying assumption is made that the electrical field across the membrane

\* This relationship is derived from the Nernst equation:

$$E = \pm \frac{RT}{zF} \log \frac{\text{concentration in medium}}{\text{concentration in blood}}.$$

is uniform, the passive efflux  $\mathcal{J}_E$  across a membrane from a solution of constant concentration  $C$  varies with the potential  $E$  according to the relationship (House, 1963):

$$\frac{\mathcal{J}_{E_2}}{\mathcal{J}_{E_1}} = \frac{[(FE_2/RT)C \exp(FE_2/RT)]/[1 - \exp(FE_2/RT)]}{[(FE_1/RT)C \exp(FE_1/RT)]/[1 - \exp(FE_1/RT)]}$$

If  $E_1$  is set at zero the denominator then equals  $-1$  and  $\mathcal{J}_{E_2}/\mathcal{J}_{E_1}$  can then be evaluated (Fig. 1).

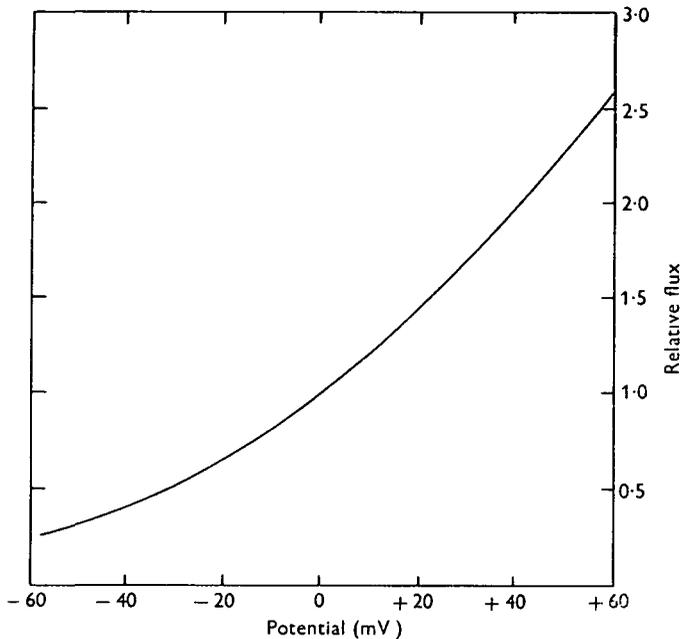


Fig. 1. The effect of an electrical potential on the diffusion of a monovalent ion. Convention: positive potential facilitating diffusion, negative potential retarding diffusion. Relative flux means unidirectional flux from a given concentration relative to the flux at the same concentration and zero potential.

It can be seen from Fig. 1 that a potential of 30 mV, blood negative, will increase the efflux of negative ions by a factor of 1.7 and decrease the efflux of positive ions by a factor of 0.52. Note that the factors are not reciprocal. Similarly a potential of 58 mV will increase a flux by a factor of 2.58 or decrease it by a factor of 0.258. This accords with the familiar Nernst relationship that a tenfold difference between ionic concentration across an ion-permeable membrane can be balanced by a potential difference of 58 mV of the appropriate polarity.

In considering the ion balance of *Palaemonetes varians* in relation to the flux and potential measurements reported here, it is necessary to review the loss of ions in the urine within this range of external salinity. It has been established previously (Parry, 1955) that the urine is approximately isosmotic with the blood in all media studied, but that the rate of urine flow varies with the concentration of the medium in a curious fashion. In isosmotic conditions the rate of urine flow was found to be minimal, approximately equivalent to 0.25% body weight/hr. In more dilute media the rate increased in proportion to the concentration difference between the blood and the medium and

reached 2.0% body weight/hr. in the most dilute solutions. This increase is to be expected from the concentration gradient, but a similar increase in the rate of urine flow in more concentrated media is more surprising. In 100% sea water the urine flow reached 1.5% body weight/hr., but no further increase in flow was found in still greater salinities. This anomalous increase in hyperosmotic solutions may be associated with the maintenance of a low concentration of divalent ions in the blood. Little is known of the ionic composition of the urine of *P. varians*. In the related species *Palaemon serratus* the urine appears to be an important route for the removal of divalent ions (Parry, 1954); in all media sodium and chloride were the major ions found in the urine of *P. serratus*, and this is probably also true for *P. varians*. It is not clear from the urine-flow measurements how water balance is maintained in the hyperosmotic media. However, if the permeability to water remains constant, and measurements using heavy water suggest that this is so (Parry, 1955), the water loss by exosmosis in 100% sea water will amount to 1% of the body weight/hr. In 2% sea water the rate of urine production amounts to 2% of the body weight/hr. and the osmotic gradient is about twice that in 100% sea water. If we assume that the animals swallow and absorb 2½% of their body weight/hr. and remove the associated salt extrarenally, as the teleosts do, they would remain in water balance, producing 1½% of their body weight/hr. of urine. The increase in sodium and chloride fluxes in 100% sea water suggest that this is so.

#### *Fluxes in 65% sea water*

In isosmotic solutions the low potential difference measured (Table 2) suggests that no active transport is taking place and that the animals are in passive equilibrium with the medium. If an appreciable active transport of either cations or anions were taking place a potential difference should be measurable. On the other hand electrical neutrality could be maintained if both cations and anions were being transported in the same direction, but a concentration difference would then be established between the blood and the medium. In the isosmotic medium the rate constant of sodium flux is 0.64/hr. and the sodium content 148 mM/kg. wet weight, so that the rate of sodium flux is 73.5 mM/kg./hr. (Table 2). The sodium loss in the urine (assuming sodium concentrations in urine and in blood to be similar) is less than 1 mM Na/kg./hr., so that almost all the sodium loss must be passive loss by diffusion.

#### *Fluxes in 2% sea water*

In 2% sea water, the rate of sodium flux (Table 2) is only 18 mM Na/kg./hr., and of the efflux 4 mM will be loss in the urine, so that the passive loss is only 14 mM/kg./hr. This decline in the passive diffusion loss, from 73.5 mM in isosmotic media to 14 mM/kg./hr. in 2% sea water, is only to a small extent due to the change in blood sodium which declines by a third (Table 1). The remaining part of the decline in the efflux, by a factor of 0.29, must be the result either of a fall in the permeability of the body wall, or the development of a potential difference across the skin of about 50 mV (blood negative). The measured potential difference in 10% sea water (Table 2) shows that the latter explanation is the more likely. The increase in rate constant of the bromide flux in 2% sea water, compared with that in 65% sea water, is also in accord

with the potential measurements and conflicts with the alternative hypothesis of a change in permeability. The permeability of *P. varians* to heavy water (Parry, 1955) showed no change with change in external salinity. A negative potential of 50 mV in 2% sea water will have a considerable effect on the influxes of sodium and chloride. In the absence of a potential difference the fall in the external concentration, compared with that in 65% sea water, will reduce the passive sodium influx from 73.5 mM/kg./hr. to 2.2 mM but the potential will increase the passive flux by a factor of 2.6 (Fig. 1) to 5.5 mM. This will not be sufficient to balance the efflux of 18 mM and the remaining 12.5 mM must be attributed to an active uptake of sodium. However, in brackish water of concentration equivalent to 10% sea water or higher the prawn can probably maintain sodium balance without any active uptake of sodium.

The negative potential in 2% sea water combined with the low external concentration will reduce the passive inward diffusion of chloride ions to only 0.12 mM/kg./hr., the rest of the observed influx, 20.6 mM, must be attributed to the active uptake of chloride ions. This active uptake of chloride ions will produce the observed negative potential.

The increase in the rate constant for the bromide efflux from 0.14/hr. to 0.23/hr. on transference from 65% sea water to 2% sea water may also be attributed to the development of a negative potential between the animal and the medium. The increase in the efflux ( $\times 1.65$ ) is equivalent to a potential difference of about 25 mV compared with a potential of about 50 mV calculated from the sodium fluxes. The cause of this discrepancy is not clear. If it is significant it implies that the permeability to ions declines slightly in 2% sea water but it may not be significant in view of the considerable scatter of the results. The animals show large individual variations in rate constants, and the sodium and bromide fluxes were measured at different times of the year.

#### *Fluxes in 100% sea water*

In full strength sea water the prawns are electrically negative with respect to the medium (Table 2) although the rate of sodium efflux is increased from 74.5 to 150 mM/kg./hr., compared with isosmotic sea water. This increase must result from the active excretion of sodium and produces the observed potential difference. The negative potential will also cause an increase in the rate of sodium influx (also 150 mM/kg./hr.) which is proportionally greater than the increase in the concentration of the external medium, but some of the increase, about 10 mM may be due to the swallowing, and absorption of sea water to maintain water balance, as in the teleosts. If we allow for this the potential required to increase the sodium influx in the observed manner, from 73.5 to 150 mM/kg./hr., is 10 mV, assuming no change in the permeability of the prawn, but allowing for the increase in external sodium concentration, from 300 to 460 mM/kg.

The sodium efflux will consist in part of urine loss, in part of passive diffusion and in part of active extrusion. The urine loss can be estimated from published data (Parry, 1955) as 4 mM/kg./hr. The passive efflux, estimated from the passive efflux in 65% sea water, allowing for the increased blood concentration and a negative potential of 10 mV, will be  $73.5 \times 328/308 \times 0.8$ , or 62.5 mM/kg./hr. The remaining 87.5 mM/kg./hr. must be attributed to the active extrusion of sodium ions, causing the negative potential.

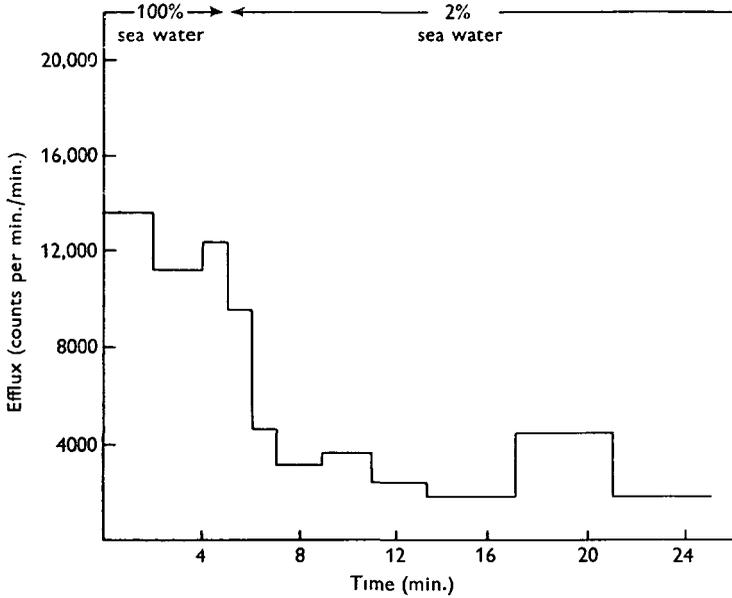


Fig. 2. The efflux of sodium ( $^{24}\text{Na}$ ) from *Palaemonetes varians* before and after transfer from 100 to 2% sea water. The temporary increase in efflux between 17 and 21 min. is probably due to the release of urine. See Fig. 4.

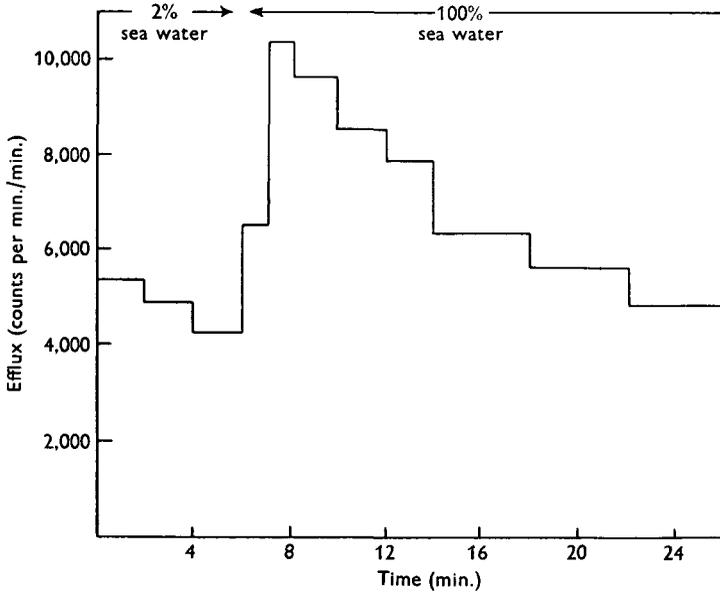


Fig. 3. The efflux of sodium ( $^{24}\text{Na}$ ) from *Palaemonetes varians* before and after transfer from 2 to 100% sea water. The apparent decline in efflux in 100% sea water is due to the decline in total  $^{24}\text{Na}$  load.

*Chloride fluxes in 100% sea water*

The increase in the external chloride from 350 to 540 mM/kg. would increase the influx from 20.7 to 32 mM/kg./hr., but a negative potential would decrease this again to 26 mM/kg./hr. The difference between this and the observed 50 mM/kg./hr. may be evidence of water swallowing and associated salt elimination as in the teleosts. The increase in the blood concentration from 312 to 342 mM/kg. will increase the passive efflux of chloride to 22.7 mM/kg./hr. and the negative potential will increase it again to 27.3 mM/kg./hr. The rate of urine production also increases in 100% sea water, to 1% of the body weight/hr., and this will remove a further 3 mM/kg./hr. of chloride. If the prawns swallow sea water and excrete the monovalent ions extrarenally a further 11 mM of chloride must be absorbed and removed, making a total of 41 mM/kg./hr., in fair agreement with the observed 50 mM/kg./hr.

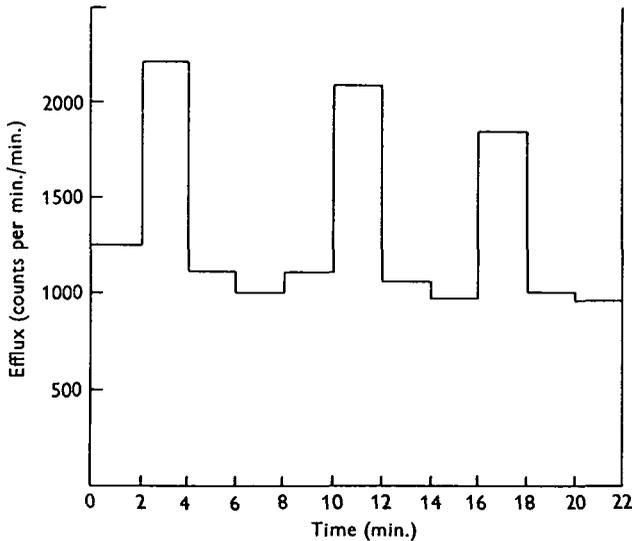


Fig. 4. Efflux of sodium ( $^{24}\text{Na}$ ) from *Palaemonetes varians* in 2% sea water. Periodic release of urine between 2 and 4 min., 10 and 12 min. and 16 and 18 min.

*Rate of adaptation to a change of medium*

Measurements of the rate of sodium efflux show that the prawns can adapt to a change of medium very rapidly. A typical experiment is shown in Fig. 2. On transfer of a prawn from 100 to 2% sea water the rate of efflux has adapted to a new level within about 4 min., most of the adaptation occurring in the first two minutes. Adaptation is equally rapid when the change is reversed, from 2 to 100% sea water. Adaptation is complete before the blood concentration is changed substantially (Fig. 3). For example, the animal in Fig. 2 must be actively taking up sodium and chloride ions while the blood concentration is still higher than in an animal adapted to 65% sea water, where no active uptake occurs. This must surely imply that salinity receptors (or the responding effectors) are not stimulated (or mediated) by blood changes, but by some other means. The transport systems respond to changes in ionic composition,

not to change in osmotic pressure, as there is no increase in the rate of efflux of sodium on transfer from 65% sea water to a solution of 65% sea water containing 1/3 molar sucrose, isosmotic with 100% sea water.

The high urine flow in hyperosmotic conditions, reported previously, is confirmed indirectly by the present experiments, but implies considerable water flow, perhaps to be explained by analogy with the sea-water drinking of teleosts. In general the urinary losses of monovalent ions seems to play a relatively unimportant part and it might be interesting to consider the excretory organs of the prawn as volume regulators in hypo-osmotic conditions and a means of excreting divalent ions in hyperosmotic conditions.

Table 3. *Rate constants, K, of sodium influx in Palaemonetes varians in dilute brackish water*

Salinity	2% sea water	1% sea water	0.5% sea water
K (hr. <sup>-1</sup> )	0.192 ± 0.011 (8)	0.134 ± 0.006 (10)	0.074 ± 0.009 (10)

Figures in parentheses are the number of samples analysed.

In very dilute solutions the influx of ions declines approximately in proportion to the external concentration (cf. *Astacus*; Shaw, 1959). The rate constant for the sodium influx is 0.192/hr. in 2% sea water (Table 2). After transfer to 1% sea water the rate constant for the sodium influx is 0.134/hr.; after transfer to 0.5% sea water it is only 0.074/hr. (Table 3). If the rate constant of the efflux remains constant, around 0.2/hr., the animals will suffer a net loss of salt. Individuals which can survive in salinities as low as 0.5% sea water are probably animals of less than average permeability or more than average rate of uptake.

#### SUMMARY

1. The exchanges of sodium and bromide (for chloride) ions between the brackish-water prawn *Palaemonetes varians* and its environment are described.
2. In an isosmotic medium the exchange of sodium and chloride ions takes place by passive diffusion.
3. In full-strength sea water sodium ions are actively removed extrarenally, the potential difference produced by the active extrusion of sodium ions maintaining the chloride ions in passive equilibrium. There is some evidence of an increased flux of ions in hyperosmotic sea water associated with water-swallowing to obtain water for water balance.
4. In 2% sea water chloride ions are actively absorbed, the potential produced by this active uptake helping to maintain sodium balance; but some active sodium uptake also occurs.
5. In salinities below 2% uptake of ions declines and the animals can no longer maintain equilibrium.

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