

SODIUM REGULATION IN THE FRESH-WATER AMPHIPOD, *GAMMARUS PULEX* (L.)

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INTRODUCTION

The investigations reported here are an extension of the previous work on *Gammarus pulex* by Shaw & Sutcliffe (1961), where it was shown that an important part of the osmoregulatory mechanism in this fresh-water animal is the ability to take up sodium from extremely low concentrations in the external medium. This is due to the fact that the sodium uptake mechanism has a very high affinity for sodium ions, indicated by the low external concentrations at which the uptake mechanism is saturated and the extremely low concentrations at which the mechanism is half-saturated (K_m value). Shaw (1961) has shown that in several crustaceans the K_m value is progressively lower in species which have increasingly greater powers of osmoregulation in fresh water. Thus the half-saturation concentration for *G. pulex* and *Astacus pallipes* is about ten times lower than in *Gammarus duebeni* and *Eriocheir sinensis*, and about one hundred times lower than in *Carcinus maenas*. The progressive adaptation to fresh water in the Crustacea also involves a reduction in permeability to salts (Shaw, 1961).

These observations, and a comparison of sodium influx and loss in *Gammarus pulex* and *G. duebeni* from brackish water (Shaw & Sutcliffe, 1961), indicate that quantitative measurements of sodium influx and loss rates, made under carefully controlled conditions, may provide a very sensitive method for seeking quite subtle differences in the sodium-regulatory mechanisms of animals adapted to different environments. The gammarid crustaceans are an excellent group for investigations of this kind, as there are a considerable number of species in fresh, brackish and marine environments. This wide range of versatility in a group of closely related species has repeatedly attracted attention in studies of osmoregulatory mechanisms, in particular by Beadle & Cragg (1940*a, b*), Basikalova, Birstein & Taliev (1946*a, b*), Lockwood (1961, 1965) and Werntz (1963). The present paper also forms part of a comparative investigation of sodium regulation in several species of *Gammarus*, and is concerned mainly with sodium regulation at low external concentrations.

For comparison with other species it was considered necessary to be certain that the changes in sodium influx and loss rates at different external concentrations found by Shaw & Sutcliffe (1961) are genuinely characteristic features of *G. pulex*. So the experimental animals were not obtained from the original locality in Co. Durham, but instead they were collected from two widely separated localities in Northumberland,

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and also from a locality in Lancashire. The results show that certain features of the sodium regulatory mechanism can be confidently used for comparative purposes.

An interesting feature of the regulatory mechanism is the ability to reduce the rate of sodium loss when *G. pulex* is kept in the lowest external concentrations at which sodium balance can be maintained. This reduction is greater than could be accounted for simply by a fall in the blood sodium level (Shaw & Sutcliffe, 1961). Since then, Lockwood (1961) has measured the urine concentration in *G. pulex* kept at higher external concentrations and found that it is relatively high, at about 20% of the blood concentration. The possibility that the reduction in loss rate is due to elaboration of a more dilute urine is examined in this paper by an indirect method, employing isosmotic sugar solutions to stop the flow of urine. This approach was possible because Lockwood (1961) and Wernitz (1963) have made independent estimates of the rate of urine flow in several species of *Gammarus*, and Wernitz has demonstrated that the flow rate is proportional to the osmotic gradient between blood and medium.

Although *G. pulex* is normally confined to fresh waters, it has been found living at a salinity of 25‰ in the inland salt waters of Westphalia (Thienemann, 1913), and Sexton (1928) apparently acclimatized this species to undiluted sea water by gradually increasing the external concentration over a period of several weeks. However, rapid acclimatization to concentrations greater than about 50‰ sea water is not usually possible, and the reasons for this are not fully understood. When *G. pulex* is kept in dilute sea water the blood concentration increases steadily, due to an increase in both sodium and chloride (Beadle & Cragg, 1940a; Lockwood, 1961), and Beadle & Cragg, also Dresco-Derouet (1959), showed that the rise in blood chloride concentration is accompanied by a very marked increase in the tissue chloride concentration. Thus the poor survival at high external concentrations may be due to the inability to maintain a low intracellular concentration of ions such as chloride. Also, there appears to be no attempt to offset the rise in blood concentration by producing a more concentrated urine (Lockwood, 1961). Another factor, largely ignored so far, is the imbibition of salt water and the movement of salts and water across the gut wall, as occurs in fresh-water insects immersed in dilute sea water (Wigglesworth, 1933; Sutcliffe, 1962). Observations on some of these aspects of regulation at high external concentrations are also included in this paper.

MATERIAL AND METHODS

Animals were collected at various times of the year from the River Pont at Ponteland, and from a small stream flowing out of Crag Lough. Both localities are in Northumberland. Later, when the author moved to the Windermere Laboratory of the F.B.A., animals were collected from a small stream at Outgate, Lancashire. Large animals, mostly males, were selected and kept in NaCl solutions made with de-ionized water, or in dilute sea water. They were fed with dried leaves of sycamore and elm previously soaked in water for several days, but were starved for about 24 hr. to clear the gut before determining influx and loss rates. All animals were acclimatized to the appropriate external concentrations for at least 48 hr. before making any determinations.

Some early experiments were carried out at a temperature of 12–14° C., but most of

them were carried out in constant temperature rooms kept at $10^{\circ}\text{C.} \pm 1^{\circ}\text{C.}$ at Newcastle, and $9^{\circ}\text{C.} \pm 1^{\circ}\text{C.}$ at Ferry House. Animals brought in from the field were acclimatized to these temperatures for about 1 week before any determinations were made.

The terminology used in this and following papers is that used by Shaw (1959). Thus *sodium influx* refers to the influx of sodium ions measured by the radioactive tracer technique. *Uptake rate* is the true influx of sodium ions, and *net uptake* refers to the difference between the uptake rate and the loss rate, measured by flame photometry. *Loss rate* refers to the total loss of sodium ions by all possible routes under specified conditions. All rate measurements are expressed in terms of μM sodium/animal/hr.

Sodium influx was measured by the technique described previously (Shaw & Sutcliffe, 1961) using ^{22}Na as the tracer. The influx was continuously recorded over a period of 30–60 min. The sodium concentration of the external solution was measured on an EEL flame photometer. Blood sodium concentrations were estimated in the same way after dilution in 2 ml. de-ionized water in small polythene cups, accurate to ± 3 mM/l. sodium.

The freezing-point depressions of blood samples and sea-water media were determined by microcryoscopy, accurate to ± 1.5 mM/l. NaCl (Sutcliffe, 1961). Sea water was obtained from the Dove Marine Laboratory at Cullercoats. This was diluted with tap water at Newcastle, and with de-ionized water at Ferry House, as the tap water here often contained lethal concentrations of metallic ions.

Sodium loss into de-ionized water was determined by placing groups of about ten animals in 50 ml. de-ionized water. The increase in the external sodium concentration was measured by flame photometry at intervals of 10 or 15 min. over a period of 60–80 min. The external sodium concentration towards the end of this period must be kept well below the minimum balance concentration to prevent any marked uptake of sodium. The external concentration was normally not allowed to rise above 0.025 mM/l. sodium. Even so, the sodium loss measured under these circumstances is, strictly speaking, net loss. When loss rates were determined with animals acclimatized to sea-water media, where the loss rates are high, a smaller number of animals and/or larger volumes of de-ionized water were used. To remove sodium chloride from the body surface, animals were washed in de-ionized water for about 5 min. before placing them in the experimental volumes.

Sodium loss into sugar solutions was determined in the same way. Consistent results were obtained with sucrose, but initial experiments with dextrose sometimes gave inconsistent results. 'Analar' grade sucrose and dextrose contain small amounts of sodium chloride, sufficient to give a concentration of about 0.05 mM/l. sodium in a 1 Molar solution. Since the initial sodium concentration in the medium used for determining loss rates must be kept close to zero, the sodium was removed by passing the sugar solutions over Amberlite I.R. 120 in the hydrogen form. It was then found that loss rates into the sugar were sometimes slightly greater than loss rates into de-ionized water. Observations indicated that this was associated with a low pH of about 4.0–3.5, due to H^+ exchanged for Na^+ on the Amberlite resin. Solutions were then passed over a double exchange column consisting of Amberlite I.R. 120 in hydrogen form in the upper half of the column, and Dowex A.G. 1-X8 in hydroxyl form in the lower half. In theory, the Dowex resin exchanges OH^- for Cl^- ions, and H^+ liberated

from the Amberlite resin combines with OH^- to form H_2O . The pH of the effluent was between 6.0 and 7.0, and very consistent results were obtained with sucrose solutions treated in this way.

ANIMALS ACCLIMATIZED TO LOW EXTERNAL CONCENTRATIONS

Sodium influx and net uptake

Measurements of sodium influx were made over a range of external sodium concentrations between 0.06 and 1 mM/l. with animals acclimatized to 0.1 mM/l. NaCl at 10° C. for more than a week. Results with animals from the outflow stream of Crag Lough are shown in Fig. 1; results with animals from the River Pont are shown in Fig. 2. The relation between the influx and the external concentration is very similar

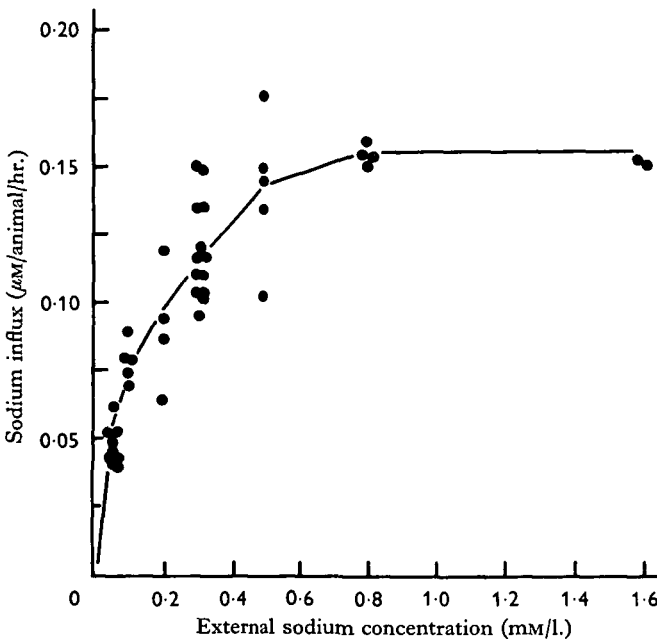


Fig. 1. The relation between the sodium influx and the external concentration in *G. pulex* from Crag Lough outflow stream at 10° C. Each point is an influx measurement on a group of animals acclimatized to 0.1 mM/l. NaCl.

in the two populations. As the external sodium concentration was raised, the influx rate increased rapidly and the sodium transporting system was saturated at an external concentration of about 0.8 mM/l. In the previous study of animals from Monkton Pond, Co. Durham, kept at room temperature, the sodium-transporting system was saturated at an external concentration of about 1 mM/l. NaCl (Shaw & Sutcliffe, 1961). As before, the change in the rate of inward transport of sodium at increasing external sodium concentrations can be described approximately by the Michaelis equation, $\text{influx} = K[C/(K_m + C)]$, where K is the maximum rate of transport, C the external concentration, and K_m the external concentration at which half the maximum influx is reached (Shaw, 1959, 1961; Shaw & Sutcliffe, 1961). For example, in Fig. 2 the

solid curve represents an equation where $\text{influx} = 0.11 [C/(0.10 + C)]$. In both cases the transporting system is half-saturated at an external concentration of 0.10–0.11 mM/l. sodium, and this is almost the same as $K_m = 0.15$ mM/l. found in the Monkton Pond animals kept at room temperature.

At any given external concentration, if the animal is in a steady state with respect to sodium, there will be no net gain or loss of sodium. Thus if the influx of ^{22}Na is a true measure of the uptake of unlabelled sodium ions then, in the steady state, the influx rate should be balanced by an equivalent loss rate. This was found to be so in both *Gammarus pulex* and *G. duebeni* kept at room temperature (Shaw & Sutcliffe, 1961). In animals acclimatized to 0.1 mM/l. NaCl at 10° C. the influx rate was 0.075 $\mu\text{M/hr.}$

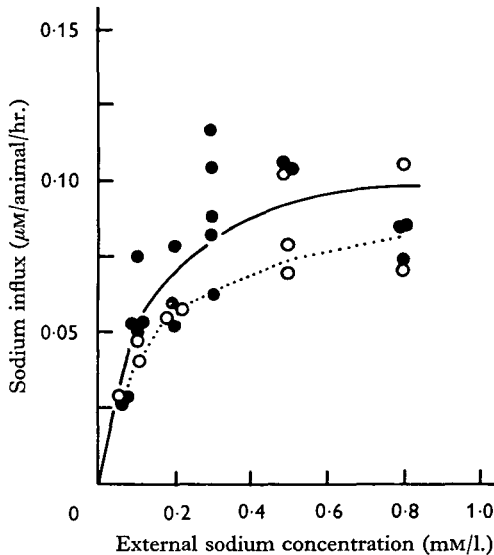


Fig. 2. The relation between sodium influx and the external concentration in *G. pulex* from the River Pont at 10° C. Each point is an influx measurement on a group of animals acclimatized to 0.1 mM/l. NaCl (solid circles) and 1.0 mM/l. NaCl (open circles, broken curve). The solid curve represents the equation, $\text{influx} = 0.11 [C/(0.10 + C)]$.

(Fig. 1) and the loss rate into de-ionized water was 0.06 $\mu\text{M/hr.}$ (Table 1). The fairly close agreement between the two measurements confirms once again the validity of tracer measurements made at low external concentrations. However, in the case of *G. duebeni* acclimatized to 0.25 mM/l. NaCl there was evidence of an exchange diffusion component which represented about 40% of the influx during net uptake of sodium at higher external concentrations. This phenomenon also occurs in *Astacus pallipes* (Shaw, 1959; Bryan, 1960), where the exchange component during net uptake represents about 30% of the influx. Now when animals acclimatized to 0.1 mM/l. NaCl were placed in 0.3 mM/l. NaCl, the initial net uptake of sodium during the first hour at the new external concentration was 0.02 $\mu\text{M/hr.}$ (Table 1). This was measured by placing groups of twenty or twenty-five animals in 20 ml. of 0.3 mM/l. NaCl, and the change in concentration of the external solution was estimated at 30 min. intervals. To this net uptake we may add the loss rate in animals acclimatized to 0.1 mM/l. NaCl, i.e. 0.06 $\mu\text{M/hr.}$, assuming that this loss rate does not change significantly during the

first hour at the new external concentration. To balance this, the uptake rate of sodium must be $0.08 \mu\text{M/hr.}$, but the measured influx rate from 0.3 mM/l. NaCl was $0.12 \mu\text{M/hr.}$ (Table 1). This suggests that about 30% of the influx in *G. pulex* during net uptake of sodium may also be due to some type of exchange diffusion process.

Table 1. *The initial rates of sodium uptake and loss at an external concentration of 0.3 mM/l. NaCl in Gammarus pulex previously acclimatized to 0.1 mM/l. NaCl*

	No. of groups	$\mu\text{M/animal/hr.}$	
		Mean	Range
Sodium influx	13	0.12	0.10-0.15
Net uptake	8	0.02	0.01-0.03
Loss into de-ionized water	6	0.06	0.05-0.08

Sodium loss and the blood sodium concentration

On the basis of only a few measurements it was previously suggested that a reduction in the rate of sodium loss, coupled with an increase in the influx rate, is an important part of the regulatory mechanism which enables *G. pulex* to survive at very low external sodium concentrations (Shaw & Sutcliffe, 1961). The following experiments examine this reduction in loss rate in greater detail, using animals collected from a small stream at Outgate, in the Lake District. About eighty animals, average weight 38 mg., were acclimatized to 2 mM/l. NaCl at 9° C. for 5 days. The loss rate into de-ionized water was then determined over a period of 80 min. with six groups of ten animals per group. These were returned to 2 mM/l. NaCl and the loss rate was again measured the next day. The animals were then acclimatized to 0.5 mM/l. NaCl and the procedure was repeated, with successive acclimatization to the range of low external sodium concentrations given in chronological order in Table 2. This series of experiments was carried out over a period of 7 weeks, during which the animals were fed at weekends and during acclimatization to each of the external concentrations. With this kind of experiment it is important to note that each measurement of the loss rate will result in a net loss of sodium from the animal. In order to keep the blood sodium concentration at a steady level, particularly in animals acclimatized to the lowest external concentrations, the sodium was immediately replaced by allowing net uptake to occur for about 1 hr. at an external sodium concentration greater than the acclimatization concentration.

From Table 2 it appears that when acclimatized to 0.5 , 0.3 and 0.2 mM/l. NaCl the overall mean sodium loss rate at each of these concentrations remained constant at about $0.07 \mu\text{M/hr.}$, but at 0.1 mM/l. NaCl there was a definite fall in the loss rate to about $0.05 \mu\text{M/hr.}$ or approximately 70% of the loss at 0.2 mM/l. NaCl . Again, as at room temperature (Shaw & Sutcliffe, 1961) this reduction in loss rate is not entirely accounted for by the 15% fall in the blood sodium concentration, shown in Fig. 3. The values for blood sodium at an external concentration of 0.3 and 0.06 mM/l. NaCl at 9° C. are very similar to those found previously at room temperature, and a constant blood sodium level was maintained until the external concentration was reduced to less than 0.18 mM/l. NaCl . A lower level was then maintained over the range 0.10 – 0.06 mM/l. NaCl , and it is clear that the fall in blood sodium level and the loss rate are very closely associated. The possibility that part of the reduced loss rate is due to regulation of the urine concentration is examined later.

Table 2. The rate of sodium loss into de-ionized water in *Gammarus pulex* acclimatized to a succession of low external sodium concentrations at 9° C.

Acclimatization concentration (mM/l. NaCl)	Mean sodium loss rate into de-ionized water ($\mu\text{M}/\text{animal}/\text{hr.}$)	No. of groups	Standard deviation
2.0	0.079	11	0.007
0.5	0.076	6	0.010
0.3	0.061	8	0.007
0.2	0.075	8	0.011
0.3	0.082	6	0.011
0.2	0.067	12	0.011
0.1	0.048	12	0.004
0.3	0.061	6	0.008
0.5	0.062	12	0.009
0.1	0.051	5	0.002
0.06	0.047	5	0.008

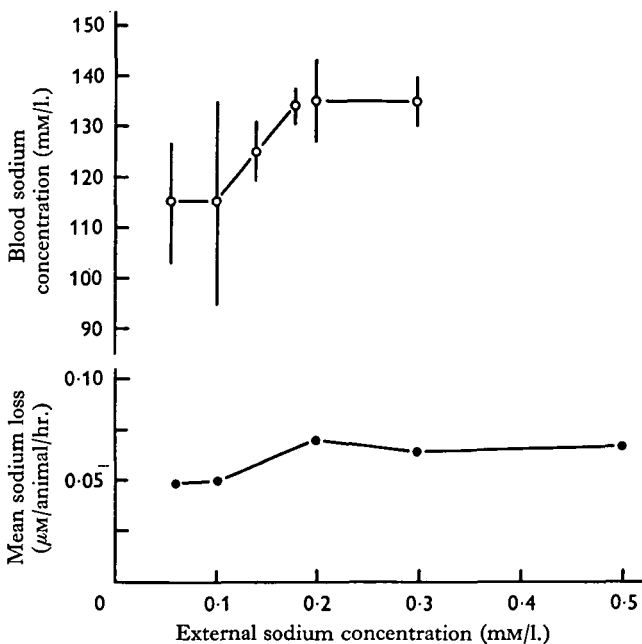


Fig. 3. The relation between the blood sodium concentration and the sodium loss rate into de-ionized water in *G. pulex* acclimatized to low external sodium concentrations at 9° C. Open circles are mean values of blood sodium in five to twelve animals, vertical lines indicate extent of standard deviations from the mean. Closed circles are mean loss rates from data in Table 2.

Effect of temperature on sodium influx and loss

The average weight of animals from the River Pont and Crag Lough was, in both cases, 47 mg. These were of about the same size and weight as the specimens from Monkton Pond used previously. Thus values for influx and loss rates in the three populations can be compared directly. During the previous work the room temperature fluctuated mainly between 18 and 22° C., and the maximum influx rate when the transporting system was saturated was about 0.33 $\mu\text{M}/\text{animal}/\text{hr.}$ in animals acclima-

tized to 0.1 mM/l. NaCl (Shaw & Sutcliffe, 1961), so that in the animals acclimatized to 0.1 mM/l. NaCl at 10° C. (Figs. 1, 2) the maximum influx rate when saturated is only about half that found at the higher temperature. The loss rate is also reduced from about 0.12 μ M/animal/hr. at room temperature (Shaw & Sutcliffe, 1961) to 0.06 μ M/hr. (Table 1), so the effect of lowering the temperature by about 10° C. is to halve both the influx rate and the loss rate of sodium.

This temperature effect on the loss rate was confirmed with animals obtained from the small stream at Outgate. These were acclimatized to an external concentration of 0.5 mM/l. NaCl at $9.0 \pm 1.0^\circ$ C. for 96 hr. before measuring the sodium loss into de-ionized water at 9° C. This was done with six groups of animals over a period of 80 min.; each group contained about twelve animals. They were returned to 0.5 mM/l.

Table 3. *The rate of sodium loss into de-ionized water at different temperatures in six groups of Gammarus pulex acclimatized to 0.5 mM/l. NaCl and 9° C*

Time kept in 0.5 mM/l. NaCl at 9.0° C. before determination of loss rate (hr.)	Loss rate into de-ionized water (μ M/animal/hr.)	Standard deviation	Temperature of de-ionized water (°C.)
96	0.10	0.02	9.0-9.5
24	0.07	0.01	0.3-0.5
2	0.21	0.01	21.0-21.5
48	0.07	0.01	0.3-0.5
2	0.11	0.01	9.0-9.5

NaCl for 24 hr. and the loss rate into de-ionized water was then measured at a temperature of 0.3-0.5° C. over a period of 80 min., and subsequently at a temperature of 21.0-21.5° C. The experimental procedure and results are given in chronological order in Table 3. The average weight of these animals was 50 mg. It is clear that temperature immediately influences the rate of sodium loss, with a Q_{10} of between 1.5 and 2.0.

The reduction in sodium movements into and out of the animals at lower temperatures does not markedly affect the minimum external concentration at which sodium balance can be maintained. Seven groups of ten animals were each placed in about 30 ml. of de-ionized water and the external concentration was allowed to rise to a steady level. The water was then replaced and the procedure repeated until the lowest external concentration was found at which balance could be maintained. The results are shown in Table 4. The mean value of 0.05 mM/l. at 10° C. is very similar to the mean value of 0.06 mM/l. sodium found previously at room temperature.

Sodium loss in the urine

We have seen that there is a very close relationship between the fall in blood sodium concentration at external concentrations below 0.18 mM/l. NaCl and the rate of sodium loss into de-ionized water (Fig. 3). Now Lockwood (1961) measured the freezing point of urine in *G. pulex* kept at external concentrations below 14 mM/l. NaCl and found a mean urine concentration equivalent to that of 27 mM/l. NaCl. If this were, in fact, due entirely to the presence of sodium (chloride) in the urine, then a reduction in urine

sodium concentration at external concentrations below 0.18 mM/l. NaCl would account for part of the observed reduction in sodium loss rate.

Indirect estimates of sodium loss in the urine were made on the assumption that urine flow ceased when animals were transferred into sugar solutions made isosmotic or slightly hyperosmotic to the blood. In support of this, Werntz (1963) has shown that in *G. oceanicus* and *G. fasciatus* the rate of urine flow is proportional to the osmotic gradient between the blood and medium. So, any differences between the sodium loss rates into isosmotic sugar and de-ionized water may be regarded as due to sodium loss in the urine, provided that both sets of determinations are made with the same animals acclimatized to the same external concentration. This also assumes that sodium losses by other routes continue at the same rates into both isosmotic sugar and de-ionized water. The experimental evidence presented in this paper suggests that this is so in the case of animals acclimatized to the range of external concentrations normally found in fresh waters. But the assumption does not hold in the case of animals acclimatized to high external concentrations (see later).

Table 4. *The minimum external sodium concentration for balance at 10° C in Gammarus pulex from the River Pont*

Group no.	External sodium concentration (mM/l.)
1	0.045
2	0.050
3	0.050
4	0.055
5	0.045
6	0.050
7	0.050
Mean	0.05

Experience with *G. duebeni* and *G. zaddachi*, described in following papers, showed that in these two species which have a high sodium concentration in the urine, the loss rate usually slowed down gradually during the first 10–30 min. following transfer to sugar solutions. This was interpreted as due to the gradual cessation of urine flow. The loss rate then remained constant and linear with time, interpreted as due to loss across the body surface. In *G. pulex* this gradual reduction in sodium loss rate was not very marked, and the loss rate was nearly always linear after 5–10 min. in the experimental sugar solution (the animals were washed in sugar solution for about 5 min. before transfer to the experimental solution). Attention is also drawn here to certain difficulties encountered with sugar solutions, dealt with in the section on methods.

Large animals, average weight 60 mg., obtained from Outgate were acclimatized to 2 mM/l. NaCl at 9° C. Loss rates were then measured at room temperature, $20 \pm 1.5^\circ$ C., over a period of about 1 hr. The high temperature was used because it emphasizes differences in loss rates, which are very small in *G. pulex*. Each day loss rates were determined in either de-ionized water or sucrose solution, using five groups of seven or eight animals which were returned to the acclimatization concentration at 9° C. between measurements. The animals were then acclimatized to 0.3 mM/l. NaCl, and

later to 0.06 mM/l. NaCl. The results are shown in Table 5. When acclimatized to 2 mM/l. and 0.3 mM/l. NaCl the loss rate into sucrose was about 84% of the loss rate into de-ionized water, which suggests that about 16% of the total sodium loss was due to sodium in the urine. When acclimatized to 0.06 mM/l. NaCl the loss rates were significantly reduced, but the loss into sucrose was still only about 80% of the loss into de-ionized water. It appears, therefore, that sodium was produced in the urine even at the very low external sodium concentration.

Table 5. *Sodium loss rates into de-ionized water and slightly hyperosmotic sucrose at 20° C. in Gammarus pulex acclimatized to a range of external concentrations at 9° C.*

Acclimatization concentration (mM/l. NaCl)	Loss rate into de-ionized water ($\mu\text{M}/\text{animal}/\text{hr.}$)	Loss rate into sucrose ($\mu\text{M}/\text{animal}/\text{hr.}$)
2.0	0.18 (5) \pm 0.014	0.15 (10) \pm 0.014
0.3	0.19 (5) \pm 0.010	0.16 (5) \pm 0.007
0.06	0.15 (10) \pm 0.024	0.12 (10) \pm 0.022

Estimation of the sodium concentration in the urine

Estimates of urine flow rate in *Gammarus* by Lockwood (1961) and Werntz (1963) using entirely different methods show good general agreement between the fresh-water species *G. pulex* and *G. fasciatus*. In *G. pulex* in fresh water (Cambridge tap water) Lockwood estimated the urine flow rate as about 37% of the body weight per day at 20° C. Applying this value to the animals used for estimating loss rates at 20° C. (Table 5) the urine flow rate would be about 0.93 mg./hr., provided that the urine flow is not increased when animals are transferred into de-ionized water. Now if the difference in loss rates into de-ionized water and sucrose is due to sodium loss in the urine then, from Table 5, urine flow accounted for a loss of 0.03 $\mu\text{M}/\text{hr.}$ at 20° C. in animals acclimatized to 2, 0.3 and 0.06 mM/l. NaCl. Assuming a urine flow of 1 $\mu\text{l}/\text{hr.}$ at 20° C., the sodium loss of 0.03 $\mu\text{M}/\text{hr.}$ would require a urine concentration of 30 mM/l. sodium, and the urine concentration is the same in animals acclimatized to the range 2–0.06 mM/l. sodium.

In another experiment a batch of animals from Outgate (average weight 50 mg.) was acclimatized to 0.5 mM/l. NaCl at 9° C. The mean sodium loss rate in de-ionized water at 9° C. in eleven groups of ten or eleven animals was 0.11 $\mu\text{M}/\text{hr.}$, but the mean loss rate into slightly hyperosmotic sucrose at 9° C. was 0.09 $\mu\text{M}/\text{hr.}$ (see Table 7). The difference is highly significant ($t = 3.4$, $P < 0.001$). In these animals, therefore, urine flow accounted for a loss of 0.02 $\mu\text{M}/\text{hr.}$ or 18% of the total sodium loss. This agrees well with the estimates of 16–20% found at 20° C.

Werntz (1963) found that the urine flow rate at a temperature of 25° C. was approximately double the flow rate at 15° C., and it is assumed here that the flow rate in *G. pulex* at 20° C. is double the flow rate at 10° C. Hence the urine flow rate at 9° C. in a 50 mg. animal would be approximately 0.4 $\mu\text{l}/\text{hr.}$, and the sodium loss rate of 0.02 $\mu\text{M}/\text{hr.}$ would then require a urine concentration of about 50 mM/l. sodium.

The above estimates of a urine sodium concentration of 30–50 mM/l. agree very closely with Lockwood's (1961) observations of urine concentrations ranging from 5 to 50 mM/l. NaCl.

Reduction in sodium concentration of the urine

The results given in Table 5 failed to show a reduction in the sodium concentration of the urine in animals acclimatized almost at the minimum balance concentration. The experiment was then repeated using another batch of animals from Outgate, average weight 58 mg., and the entire experiment was carried out at 9° C. The batch of animals was divided into five groups, and the loss rates into de-ionized water and slightly hyperosmotic sucrose were each determined twice on alternate days using animals acclimatized to 0.3 mM/l. NaCl. The batch was then acclimatized to 0.07 mM/l. NaCl and the series of determinations was repeated. The mean loss rates from each set of ten determinations, with standard deviations, are shown in Table 6.

When acclimatized to 0.3 mM/l. NaCl, the difference between the loss rates into de-ionized water and sucrose (0.014 μ M/hr.) is significant ($t = 3.11$, $P < 0.01$). This urine loss rate again represents 18% of the total sodium loss rate. Assuming that the urine flow rate at 9° C. is equivalent to 19% body weight/day, a loss of 0.014 μ M/hr. in the urine would require a concentration of about 30 mM/l. sodium. This is the same as the estimated urine concentration in the previous experiment at 20° C.

Table 6. *Sodium loss rates into de-ionized water and slightly hyperosmotic sucrose at 9° C. in Gammarus pulex acclimatized to 0.3 mM/l. and 0.07 mM/l. NaCl at 9° C.*

Acclimatization concentration (mM/l. NaCl)	Loss rate into de-ionized water (μ M/animal/hr.)	Loss rate into sucrose (μ M/animal/hr.)
0.3	0.078 (10) \pm 0.011	0.064 (10) \pm 0.009
0.07	0.064 (10) \pm 0.007	0.058 (10) \pm 0.008

The difference between the two loss rates into de-ionized water when acclimatized to 0.3 and 0.07 mM/l. NaCl is also significant ($t = 3.42$, $P < 0.001$). But when acclimatized to 0.07 mM/l., the loss into de-ionized water and sucrose is not significantly different ($t = 1.82$, $P > 0.05$). Hence it might be concluded that the urine of animals acclimatized to 0.07 mM/l. did not contain a significant amount of sodium. However, the standard deviations from the means of these measurements are large in comparison with the differences under investigation, since a loss of only 0.014 μ M/hr. represents a urine concentration of about 30 mM/l. sodium. Thus if the urine concentration was 30 mM/l. when acclimatized to both 0.3 and 0.07 mM/l. NaCl, the expected loss rate into sucrose from animals in 0.07 mM/l. would be 0.050 μ M/hr. This rate is, in fact, within exactly one standard deviation from the observed mean loss of 0.058 μ M/hr. (Table 6) which indicates that the determinations may not be sufficiently precise at a temperature of 9° C. to distinguish between urine concentrations of less than 30 mM/l. The same difficulty arises in the interpretation of the loss rate at 20° C. when acclimatized to 0.06 mM/l. NaCl (Table 5) where the standard deviations are again large in comparison with the differences under investigation.

The following conclusions emerge from the determinations of sodium loss rates. When *G. pulex* is acclimatized to the range of external concentrations 2–0.3 mM/l. the urine sodium concentration is approximately 30–50 mM/l. When acclimatized to lower external concentrations the urine concentration is probably lowered to a concentration between 0 and 30 mM/l. sodium; the total urine concentration can certainly be

lowered to only 5 mM/l. NaCl (Lockwood, 1961). This reduction in urine sodium, combined with a fall in the blood sodium level, is sufficient to account for all of the observed reductions in the total sodium loss rate which occur when animals previously acclimatized to external concentrations above 0.2 mM/l. are then acclimatized to concentrations below 0.2 mM/l. NaCl.

It is now possible to make a rough estimate of the rate of sodium re-absorption in the two antennary glands. If it is assumed that the fluid initially entering the glands is isotonic with the blood, i.e. about 135 mM/l. sodium (Fig. 3), and if this fluid flows through the glands at a combined rate of about 0.5 μ l./hr. at 9° C. in a 60 mg. animal, then approximately 0.05 μ M/hr. sodium must be re-absorbed to produce the final urine concentrations of 30–50 mM/l. To reduce the sodium concentration to zero about 0.07 μ M/hr. sodium must be re-absorbed. These rates are very similar to the uptake rate at the body surface required to balance sodium loss by diffusion and in the dilute urine.

ANIMALS ACCLIMATIZED TO HIGH EXTERNAL CONCENTRATIONS

Sodium loss and the blood concentration

Measurements of sodium loss into de-ionized water and sugar solutions were made with animals acclimatized to a series of external concentrations consisting of sea water diluted with tap water or de-ionized water. These concentrations will be referred to in terms of NaCl solutions with equivalent freezing-point depressions. After several days in 115 mM/l. NaCl (about 20% sea water) most of the animals survived transference to 170 mM/l. NaCl, but when they were then transferred to 225 mM/l. NaCl (about 40% sea water) a few days later, a great many died. This occurred on four different occasions at temperatures between 9 and 14° C. when, of those originally acclimatized to 115 mM/l. NaCl, only about 30% survived for more than 24 hr. in 225 mM/l. NaCl. Beadle & Cragg (1940a) record that the majority of *G. pulex* remained normal in 215 mM/l. NaCl, whereas all had practically stopped moving after 24 hr. in 270 mM/l. NaCl. On one other occasion, a few animals survived for 24 hr. in 255 mM/l. NaCl; these were then sacrificed for measurements of the blood freezing-point. The results are shown in Fig. 4, together with freezing-point determinations on the blood of animals kept for at least 48 hr. at the other experimental concentrations. The value of $144(8) \pm 5$ mM/l. NaCl at an external concentration of 0.3 mM/l. NaCl is very similar to that obtained by Lockwood (1961) for animals acclimatized to tap water.

Fig. 4 also shows sodium loss rates into de-ionized water at 12–14° C. in groups of animals obtained from the River Pont in 1963. These were repeated in 1965 at a temperature of 10° C., together with measurements of loss rates into isosmotic dextrose (Fig. 5). On both occasions the average weight of the animals was 60 mg. Because of the uncertainty of determinations with dextrose these results were checked with groups of animals from Outgate in slightly hyperosmotic sucrose at a temperature of 9° C. The results are given in Table 7. The difference between the mean loss rates into de-ionized water and sucrose in animals acclimatized to 0.5 mM/l. NaCl is highly significant ($t = 3.4$, $P < 0.001$). Also, in animals acclimatized to the higher external concentrations, the loss rates into de-ionized water appear to be slightly greater than the loss rates into sucrose, although the differences are barely significant ($P = 0.05$). However, if these differences are regarded as actually due to the excretion of sodium

in the urine of the animals in de-ionized water we may obtain, as in the previous section, a rough estimate of the sodium concentration in the urine of animals acclimatized to 115 and 170 mM/l. NaCl.

For this, it is assumed that the urine flow rate is about 0.5 μ l./hr. at 9° C. in animals acclimatized to fresh water. An allowance is also made for the increased blood concentration in animals acclimatized to 115 and 170 mM/l. NaCl (Fig. 4), so that urine flow is proportional to the increased osmotic gradients when transferred to de-ionized

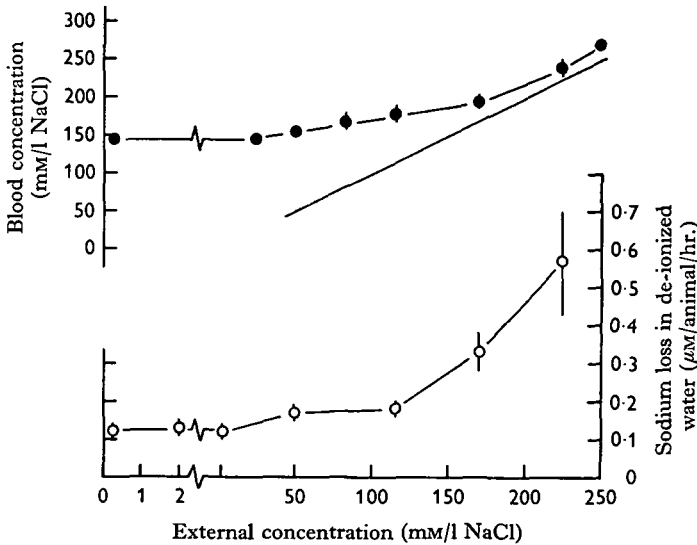


Fig. 4. The total blood concentration (closed circles) and the rate of sodium loss into de-ionized water (open circles) in *G. pulex* acclimatized to a range of external concentrations at 12–14° C. Blood concentrations obtained from freezing-point determinations on individuals; each point is the mean of eight individuals, vertical lines indicate extent of standard deviation (± 5 –12 mM/l.). Loss rates are means of six to fourteen groups, with standard deviations (± 0.02 –0.14 μ M/hr.).

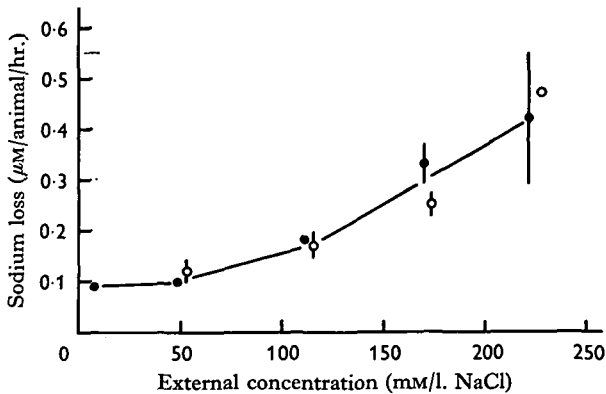


Fig. 5. Sodium loss into de-ionized water (closed circles) and into isosmotic dextrose (open circles) in *G. pulex* acclimatized to a series of increasing concentrations of diluted sea water at 12–14° C. Each point is the mean of six groups, except for the open circle at 225 mM/l. NaCl (mean of four groups only). Vertical lines indicate extent of standard deviation where this is greater than ± 0.02 μ M/hr.

water. The urine flow rates are then about 0.6 and 0.7 $\mu\text{l./hr.}$ respectively. Thus the apparent differences of 0.04 and 0.06 $\mu\text{M/hr.}$ in Table 7 would require urine sodium concentrations of roughly 70 and 90 mM/l. respectively. These values are, in fact, not much greater than values obtained by Lockwood (1961) on urine collected from *G. pulex* acclimatized to sea-water media ranging up to about 140 mM/l. NaCl.

Table 7. Sodium loss rates into de-ionized water and slightly hyperosmotic sucrose in *Gammarus pulex* acclimatized to 0.5 mM/l. NaCl and diluted sea water at 9° C.

Acclimatization concentration (mM/l. NaCl)	Loss rate into de-ionized water ($\mu\text{M/animal/hr.}$)	Loss rate into sucrose ($\mu\text{M/animal/hr.}$)
0.5	0.11 (11) \pm 0.018	0.09 (10) \pm 0.008
115	0.21 (10) \pm 0.05	0.17 (5) \pm 0.02
170	0.33 (5) \pm 0.05	0.27 (5) \pm 0.03

The above analysis serves to emphasize the point that the large increases in loss rates observed in Fig. 5 and in Table 7 cannot be due mainly to excretion of sodium in the urine. Also, from the measurements of total blood concentration, it appears that the blood sodium concentration will have increased by no more than 20% at an external concentration of 115 mM/l. NaCl, and by no more than 55% at 225 mM/l. NaCl. But the loss rates in animals acclimatized to 225 mM/l. NaCl are four times greater than in animals acclimatized to 50 mM/l. NaCl (Fig. 5), so this large increase cannot be due simply to an increased rate of diffusion down the concentration gradient from blood to external medium unless it is postulated that the permeability of the body surface in the direction in-to-out is increased in animals acclimatized to high external concentrations. However, it is first necessary to examine the possibility that the increased sodium loss is due to leakage from the gut through the mouth and anus.

Intake and expulsion of fluid from the gut

G. pulex takes water into the gut, even when in fresh water. This was demonstrated by adding a small quantity of the red dye Amaranth (Azo-Rubin S). The solution was filtered to remove small particles of dye and detritus. Animals were starved for several days to empty the gut, and were then gently transferred to dyed media of the same concentration as the acclimatization media at 9° C. After 48 hr. dye was faintly visible in the gut of animals kept in 0.5 mM/l. NaCl. In dilute sea water equivalent to 10 mM/l. NaCl the gut was full of dye in every case, and this was readily visible through the body wall. In 115 mM/l. NaCl and more concentrated media the entire gut appeared bright red within 12 hr. This suggests that a considerable amount of the medium was imbibed, presumably through the mouth, although both oral and anal intake of water are common in crustaceans (Fox, 1952). Fox did not observe anal intake of water in *Gammarus*. When animals treated in this way were then moved to clean sea-water media of the same concentration the dye was visible in the medium 12–24 hr. later, which indicates that fluid is also expelled from the gut.

As in the locust (Treherne, 1957), Amaranth is apparently not absorbed from the gut of *G. pulex*. Normally the dye was visible only in the gut. When an animal died in sea-water media containing Amaranth the entire animal turned bright red. Under

a binocular the dye could be seen throughout the body cavity, including the antennae, other limbs and the gills. This was also observed in living animals which appeared to be in very poor condition and which died a short time afterwards. It also occurred in animals which, after removal to clean media, died before the dye was completely removed from the gut (this takes several days). These observations indicate that the gut wall becomes abnormally permeable at this time, as it does in mosquito larvae (Wigglesworth, 1933), *Chaoborus* (Schaller, 1949) and fresh-water caddis larvae (Sutcliffe, 1962) when kept in sea-water media isosmotic with the blood.

Now the movement of external medium into and out of the gut may continue to occur in animals suddenly transferred from high external concentrations into the de-ionized water and sucrose solutions used to determine sodium loss rates. Thus animals acclimatized to a sea-water medium equivalent to 170 mM/l., with a sodium concentration of about 140 mM/l., might be expected to expel from the gut a fluid also containing about 140 mM/l. sodium. In this case, if each animal expelled only 1 μ l./hr. of fluid, the sodium loss rate would apparently increase by about 0.14 μ M/hr., and this would be sufficient to account for the observed increase in loss rate into sucrose (Fig. 5).

The expulsion of fluid from the gut was examined in the following way. A batch of animals from Outgate was acclimatized to a series of increasing external concentrations over a period of 7 days at 9° C., and was finally acclimatized to a sea-water medium containing 160 mM/l. sodium. A small quantity of Amaranth, about 0.5–1 g./l., was then dissolved in the medium; 12 hr. later the most active animals were selected to determine loss rates, and were first washed for 1 hr. in a medium of the same concentration containing no dye. This removed all traces of dye from the body surface. In two groups of eight animals the loss rates into 50 ml. de-ionized water were 0.29 and 0.33 μ M/animal/hr., and in two other groups the loss rates into 50 ml. slightly hyperosmotic sucrose were 0.31 and 0.33 μ M/hr. at 9° C. These loss rates are very similar to those found previously in animals acclimatized to a medium containing about 140 mM/l. sodium, i.e. at 170 mM/l. NaCl (Fig. 5). The loss rates were determined over a period of 1 hr., and it was noted that both lots of de-ionized water contained very slight traces of Amaranth. Both lots of sucrose contained stronger traces of the dye. These and some further observations indicated that a very small amount of gut fluid containing dye was usually expelled into de-ionized water, and that a rather large amount was expelled into sucrose solutions. An experiment was then designed to obtain a rough estimate of the quantity of this fluid.

Estimation of the quantity of fluid expelled from the gut

The batch of animals used above was replaced in the sea-water medium containing Amaranth for 20 hr. Five groups of eight animals were then washed for 1 hr. in medium containing no dye, and were then transferred to 50 ml. de-ionized water (one group) and 50 ml. sucrose (four groups). The groups were also washed in the appropriate solutions for 5 min. before placing them in the experimental solutions. After 1 hr. at 9° C. the solutions were decanted and the concentration of Amaranth present in the solutions was estimated on a Unicam Spectrophotometer at a wavelength of 520 m μ . with reference to de-ionized water and a series of increasing concentrations of the dye. These consisted of 5 μ l. aliquots of the sea-water medium containing Amaranth, added to 50 ml. de-ionized water.

The results are given in Table 8, which shows the estimated volumes of fluid expelled from the animals during 1 hr. These were estimated on the assumption that all of the Amaranth present in the solutions is derived from the gut, and that the concentration of dye in the gut is not altered by movements of water across the gut wall into or from the blood. At external concentrations of less than 170 mM/l. NaCl there might well be a considerable osmotic uptake of water from the gut, but this must be reduced to a very small amount when the blood becomes only slightly hyperosmotic at concentrations above 170 mM/l. NaCl (Fig. 4). Furthermore, there is presumably a great deal of mixing between the gut fluid and external medium, as the animals at very high external concentrations are continuously drinking and expelling fluid from the gut. Hence it may be concluded that the concentration of dye in the gut is the same as the concentration of dye in the experimental sea-water medium.

Table 8. *Estimated volumes of gut fluid containing Amaranth expelled into de-ionized water and sucrose solutions at 9° C. by Gammarus pulex acclimatized to an external sodium concentration of 160 mM/l.*

Experimental medium	Volume expelled by groups of eight animals (μ l./hr.)	Volume expelled by one animal (μ l./hr.)
Sucrose	9.0	1.1
	14.0	1.75
	13.0	1.6
	7.5	0.94
		Mean 1.35
De-ionized water	4.5	0.56

From Table 8 it appears that the animals in sucrose expelled two to three times more fluid containing dye than animals in de-ionized water, with a mean volume of 1.4 μ l./animal/hr., and if the fluid contained 160 mM/l. sodium this would represent a loss rate of about 0.22 μ M/hr. In fact, the fluid in the gut may be isotonic with the blood, due to diffusion across the gut wall, and it would then have a sodium concentration approaching 200 mM/l. With a sodium loss rate across the external body surface of 0.1 μ M/hr. (Fig. 5) or slightly more, about 0.13 μ M/hr. due to the increased blood sodium concentration in animals kept in the sea-water medium (from Fig. 4), the sodium loss rate of about 0.32 μ M/hr. in sucrose is entirely accounted for.

On the other hand, the volume of fluid expelled into de-ionized water was only about 0.6 μ l./animal/hr. If this also contained from 160 to 200 mM/l. sodium, the sodium loss by this route would be 0.10–0.12 μ M/hr. and, allowing also for sodium loss across the external body surface, approximately 0.08 μ M/hr. remains to be accounted for. With a urine flow rate of about 0.7 μ l./hr. into de-ionized water at 9° C. (see earlier section) the urine concentration required to produce this amount would be approximately 115 mM/l. sodium. This is not much greater than the urine concentration of 90 mM/l. estimated previously for animals acclimatized to 170 mM/l. NaCl, and Lockwood (1961) records one value of about 90 mM/l. NaCl for the urine of *G. pulex* kept at 140 mM/l. NaCl.

It seems reasonable to conclude that the large increases in sodium loss rate into

de-ionized water and sucrose solutions can be explained without invoking changes in the permeability of the body surface.

DISCUSSION

The experiments with *G. pulex* at low external concentrations establish two points which are valuable for making comparisons with other species of *Gammarus*. The first point is that the relationship between the external sodium concentration and the rate of sodium influx is very consistent in *G. pulex* from three localities. Thus there is no doubt that the very high affinity for sodium ions in the sodium-transporting system can be characterized by the value of 0.10–0.15 mM/l. for the sodium concentration at which the system is half-saturated. This value for the external concentration is slightly lower than the corresponding value for *Astacus pallipes* and about ten times lower than in *Eriocheir sinensis* (Shaw, 1961).

The second point is that when the animals are in a steady state with respect to sodium the total sodium loss rate remains approximately constant over the range of external concentrations from 2 to about 0.2 mM/l. sodium. Hence the maintenance of the blood sodium concentration at a steady level over this range can be achieved without any large changes in the rate of sodium uptake. This is illustrated in Fig. 6 where the influx curves are based on Figs. 1 and 2, and the loss-rate curve is drawn from Fig. 3. In Fig. 6 the minimum influx (curve 1) is sufficient to maintain sodium balance at external concentrations above about 0.3 mM/l. An increase of only 25–30% in the influx (uptake) rate is enough to maintain balance at concentrations between 0.3 and 0.1 mM/l. This relatively small increase in the influx is sufficient because the loss rate is simultaneously reduced by a similar amount. In the final attempt to balance at the minimum external sodium concentration, achieved by a large increase in the uptake rate, the influx (curve 3) is approximately double the influx in curve 1 and, in fact, the influx rate was increased by a factor of 1.5 when *G. pulex* was moved from 0.3 to 0.06 mM/l. NaCl (Shaw & Sutcliffe, 1961).

The normal sodium concentration in the urine is relatively high compared with *Astacus* (Bryan, 1960) and a number of other fresh-water animals (Potts & Parry, 1964), but even so the urinary sodium losses constitute only 18% of the total sodium loss rate. This can be further reduced by elaborating a more dilute urine at very low external concentrations, and at the same time the 15% fall in the blood sodium concentration results in a similar fall in the rate of outward diffusion of sodium across the body surface. Thus the overall reduction in loss rate at concentrations below 0.2 mM/l. is shared more or less equally between the active reduction in urine concentration and the passive reduction in diffusion losses, and the apparent changes in permeability found in *Gammarus duebeni* (Shaw & Sutcliffe, 1961; Sutcliffe, 1967) may not occur in *G. pulex*.

The rather sharp drop in blood sodium concentration at external concentrations between 0.2 and 0.1 mM/l. is due to the manner in which the sodium uptake and loss rates are regulated. Fig. 6 shows that at external concentrations approaching 0.2 mM/l. the loss rate exceeds the minimum influx rate (curve 1) and hence the blood sodium concentration will be lowered by a net sodium loss. As this occurs the passive element in the loss rate is reduced, but at the same time the loss rate is reduced even further

by elaboration of a more dilute urine. The lower blood sodium concentration can now be maintained at about 0.1 mM/l. because the influx rate is increased to curve 2. A further drop in blood sodium concentration at the lowest external concentrations tolerated by the animals is prevented by increasing the influx to its maximum (curve 3).

Now, in theory, the fall in blood sodium concentration and in the rate of loss could be prevented simply by increasing the influx rate to its maximum at external concentrations below 0.3 mM/l. But this does not happen, and the very close association which exists between changes in all three of these variables lends strong support to the suggestion that in *G. pulex* the rate of sodium uptake is increased following a relatively small drop in the blood sodium concentration (Shaw & Sutcliffe, 1961). The same

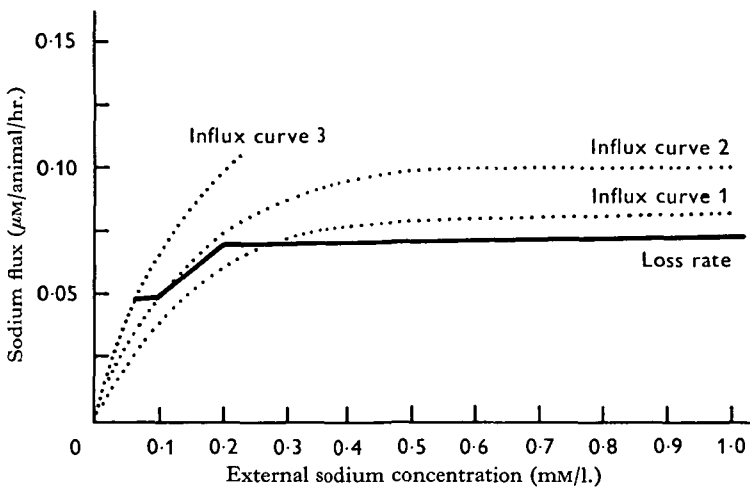


Fig. 6. Illustrating the relationship between the rates of sodium influx, sodium loss and the external sodium concentration. Explanation in the text.

effect has been demonstrated in *Astacus* by Shaw (1959) and Bryan (1960*a, b, c*), and Shaw (1964) proposes that the ion-transporting systems are activated (regulated) by a primary controller, possibly hormonal in nature, which responds to relative changes in the concentrations of perhaps several of the blood ions, and not necessarily to the change in concentration of blood sodium alone. Since it is likely that the elaboration of a more dilute urine in *G. pulex* will require an increase in the rate of sodium uptake in the antennary glands, it seems reasonable to suppose that both the increased rates of uptake at the body surface and in the antennary glands are due to simultaneous activation of the sodium-transporting systems by a single regulator as the blood sodium concentration falls. A controlling mechanism acting in this way in *Astacus* has been described by Bryan (1960*c*) and the simultaneous activation of the sodium-transporting systems also occurs in *Gammarus duebeni* (Lockwood, 1961, 1964; Sutcliffe, 1967) and *G. lacustris* (Sutcliffe & Shaw, 1967).

The normal production of hypotonic urine containing about 30–50 mM/l. sodium has reduced urinary losses to less than 20% of the total sodium loss rate. The main advantage gained by producing this dilute urine seems to be the considerable reduction in the uptake rate required at the body surface to balance sodium losses. It can be

calculated that if *G. pulex* produced urine isotonic with the blood when in fresh water urinary losses would then contribute about 50% of the total sodium loss, and this would be similar to the urinary losses in *G. duebeni* when the animals are producing isotonic urine (Sutcliffe, 1967). In this case the uptake rate at the body surface would need to be twice as great as the actual rate required to balance sodium losses when hypotonic urine is produced. Thus, by elaborating a hypotonic urine, the total sodium uptake is shared equally between the transporting systems at the body surface and in the antennary glands. As well as being more efficient from an energetic point of view, the reduction in uptake rate at the body surface may have permitted the transporting system to develop a greater affinity for sodium ions at very low external concentrations.

The drinking behaviour of *G. pulex* in sea-water media closely resembles the behaviour in a number of fresh-water insects when these are also immersed in dilute sea water (Wigglesworth, 1933; Schaller, 1949; Sutcliffe, 1962). At concentrations approaching that of the blood, mosquito larvae, *Chaoborus* and caddis larvae drink and expel large quantities of salt water, and the cells lining the gut wall eventually disintegrate. Drinking is presumably a response to water shortage, and it is interesting to note that in a sea-water medium slightly hyperosmotic to the normal blood concentration the amount of salt water expelled, and therefore imbibed, by *G. pulex* is equivalent to the normal urine flow rate when in fresh water. This suggests that drinking is controlled, as it is in salt-water insects (Sutcliffe, 1962), but a net gain of salt-free water can only be obtained by producing urine hyperosmotic to the external medium, and *G. pulex* appears to be unable to do this.

SUMMARY

1. Sodium influx and loss rates in *Gammarus pulex* were measured at constant temperatures. The sodium loss rate was immediately influenced by a change in temperature, with a Q_{10} of 1.5 to 2.0 at temperatures between 0.3 and 21.5° C. The sodium influx rate is apparently influenced in the same way.

2. The sodium uptake mechanism in *G. pulex* from three localities was half-saturated at an external concentration of 0.10–0.15 mM/l. sodium.

3. The total sodium loss rate remained approximately constant in animals acclimatized to the range of external concentrations from 2 to about 0.2 mM/l. sodium. 18% of the sodium was lost in urine with a sodium concentration estimated at 30–50 mM/l. The remainder of the sodium loss was due to diffusion across the body surface.

4. In animals acclimatized to concentrations below about 0.2 mM/l. sodium the sodium loss rate was reduced, due to (a) a lower diffusion rate following a fall in the blood sodium concentration, and (b) the elaboration of a more dilute urine.

5. There was a very close association between changes in the blood sodium concentration, the elaboration of a very dilute urine, and the rate of sodium uptake at the body surface. The results indicate that a fall in the blood sodium concentration leads to simultaneous activation of the sodium uptake mechanisms at the body surface and in the antennary glands.

6. It is estimated that, by producing a dilute urine, total sodium uptake in *G. pulex* is shared equally between the renal uptake mechanism and the mechanism situated at the body surface.

7. In sea-water media *G. pulex* drinks and expels fluid from the gut. In a medium slightly hyperosmotic to the normal blood concentration the amount imbibed was equal to the normal rate of urine flow when in fresh water.

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