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SODIUM REGULATION IN THE AMPHIPOD *GAMMARUS DUEBENI* FROM BRACKISH-WATER AND FRESH-WATER LOCALITIES IN BRITAIN

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INTRODUCTION

Osmoregulation in the amphipod *Gammarus duebeni* was studied by Beadle & Cragg (1940*a*, *b*) who showed that the blood concentration is maintained slightly hyperosmotic to normal sea water, but is maintained progressively more hyperosmotic to external concentrations ranging between 50 and 2% sea water. These observations were confirmed by Lockwood (1961, 1964) and by Kinne (1952) who found that *G. duebeni* could also be kept alive in fresh water (Kiel tap water) if the external concentration was gradually reduced. In fact *G. duebeni* has been recorded from a number of coastal fresh-water localities in Britain (Hynes, 1954) and it is common in the fresh waters of Ireland (Reid, 1939; Macan & Lund, 1954). It is therefore of interest to see if the colonization of fresh-water habitats has involved changes in the osmoregulatory mechanisms, particularly since Reid (1939) and Beadle & Cragg (1940*a*) suggested that animals living in fresh-water habitats may constitute a distinct physiological race. With regard to this problem it is worth remembering that the range of sodium concentrations found in natural fresh waters (about 0·1–4 mM/l.) is actually greater than the range of sodium concentrations from brackish to undiluted sea water (about 7–460 mM/l.), and Shaw (1959*a*, 1961) has shown that the African fresh-water crab *Potamon niloticus* is more fully adapted for living in very fresh water than is the European fresh-water crab *Eriocheir sinensis*, which returns to the sea to breed. The present paper investigates sodium regulation in *G. duebeni* obtained from brackish-water and fresh-water localities round the coasts of Britain, and particular attention is given to regulation at very low external sodium concentrations. Sodium regulation in *G. duebeni* from fresh water in Ireland is then examined in a following paper (Sutcliffe & Shaw, 1967*a*).

The general features of the mechanism by which sodium balance was maintained at low external concentrations in *G. duebeni* from a brackish-water locality were described by Shaw & Sutcliffe (1961). It was found that the animals could be acclimatized to live for at least 1 week in 1 mM/l. NaCl and, for shorter periods, at concentrations down to 0·2 mM/l. These were well below the concentrations at which the sodium uptake mechanism was saturated, and acclimatization involved a small in-

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crease in the influx rate and a very large reduction in the sodium loss rate. The experiments were carried out at room temperature, which fluctuated widely during the course of the investigation, and it was realized that the rates of sodium influx and loss in these animals are very sensitive to changes in temperature. So in order to obtain sound quantitative data for comparative purposes, measurements of influx and loss rates have been repeated at constant temperatures. Also, since the original locality at Meggies Burn has been destroyed, the opportunity was taken to investigate sodium balance in other brackish-water populations. This is important as it provides a wider basis for comparison with *G. duebeni* from fresh-water localities.

In the previous paper the reduction in sodium loss rate at low external concentrations could not be explained simply in terms of a lower rate of diffusion across the body surface following a reduction in the blood sodium concentration. Since then Lockwood (1961) has shown that *G. duebeni* can produce urine hypoosmotic to the blood in media less concentrated than 50% sea water. Later, Lockwood (1965) examined the relative losses of sodium in the urine and across the body surface and claimed that, unlike the situation in most other crustaceans which have been studied, only about 20% of the total sodium loss occurs across the body surface when *G. duebeni* is producing urine isosmotic with the blood. This paper re-investigates sodium losses by various routes, and it is shown that a relatively large sodium loss in the urine occurs only in certain special circumstances. However, the contention that the elaboration of a dilute urine is an important part of osmoregulation in hypoosmotic media (Lockwood, 1961, 1965) is shown to be true when *G. duebeni* is exposed to external concentrations lower than about 2% sea water.

MATERIAL

Animals from brackish water were obtained from Budle Bay, Northumberland. They were collected from underneath stones in the tidal zone of a small area of the bay where a small stream discharges on to the beach. Animals were also collected from salt-marsh pools round Morecambe Bay, Lancashire.

Animals from fresh water were collected at three localities in western Britain, viz. the Lizard Peninsula, Cornwall; the Isle of Man; the Kintyre Peninsula, Argyll.

On the Lizard peninsula animals were collected in May 1961 from two small streams at Grochal and Crowgey (see Hynes, 1954). The animals were kept cool in large vacuum flasks on the journey back to Newcastle.

The Isle of Man was visited in September 1964. Collections in several streams on the southern tip of the island, known to contain *G. duebeni* (Hynes, 1954 and personal communication), yielded only a few specimens large enough for experimental work. A few large specimens were then obtained from an old quarry filled with fresh water, situated close to the shore on Scarlett Point, south of Castletown. The actual salt concentration is not known as the water sample was lost in an accident, but since the water tasted fresh it must have had a concentration of less than 8 mM/l. NaCl. The animals were immediately transported to Newcastle by air.

In July 1964 animals were collected from the river in Connie Glen on the Kintyre peninsula. The locality is about 4 miles upstream from the mouth of the river (Sutcliffe, 1967*a*).

METHODS

The experimental media were NaCl solutions varying in concentration from 0·07 to 8 mM/l., and sea water from Cullercoats diluted with tap water at Newcastle and with de-ionized water at Ferry House. The sea-water media provided a range of concentrations down to 10 mM/l. NaCl (about 2% sea water), and are referred to in terms of NaCl solutions with equivalent freezing points.

The animals were acclimatized to experimental media for at least 48 hr. before making any measurements. They were fed on sycamore and elm leaves during intervals between experiments. These were carried out at room temperature with animals from the Lizard peninsula. Most of the other work was done in constant temperature rooms kept at 10 ± 1° C. at Newcastle and 9 ± 1° C. at Ferry House. The animals were acclimatized to these temperatures for about 1 week before starting experimental work.

The experimental techniques were the same as described previously (Shaw & Sutcliffe, 1961; Sutcliffe, 1967*b*).

GAMMARUS DUEBENI FROM BRACKISH-WATER LOCALITIES ACCLIMATIZED TO LOW EXTERNAL CONCENTRATIONS

Survival and sodium balance

Animals from Meggies Burn were able to maintain sodium balance at external concentrations as low as 0·20–0·25 mM/l. sodium at room temperature (Shaw & Sutcliffe, 1961). During acclimatization to these concentrations mortality was very high, and only a few individuals survived at the lowest concentrations. In animals from Budle Bay mortality was also very high when they were acclimatized to 0·25 mM/l. NaCl at room temperature, but mortality was greatly reduced when acclimatized at 10° C. At this temperature the lowest external concentration at which some groups of animals were able to maintain sodium balance was 0·17–0·18 mM/l. sodium. This was found by repeatedly placing a group of animals in a small volume of de-ionized water and allowing the external sodium concentration to reach a steady level. The mean value of the lowest steady-state concentrations in thirteen groups was 0·21 mM/l. sodium, standard deviation 0·025. This is four times higher than the lowest steady-state concentrations in *Gammarus pulex* and *G. lacustris* (Sutcliffe, 1967*b*; Sutcliffe & Shaw, 1967*b*).

It appears that the ability to maintain a steady state at very low external concentrations is very similar in animals from Meggies Burn and Budle Bay. The lower temperature did not substantially alter the ability to maintain a steady state at the lowest external concentrations, but survival was very greatly increased. Kinne (1959) presents evidence which suggests that a temperature of 25° C. is normally near to the lethal point for *G. duebeni*, even at high salinities.

Sodium influx, net uptake and exchange diffusion

Measurements of sodium influx were made with groups of animals acclimatized to 10, 2 and 0·25 mM/l. NaCl. The influxes were measured over a range of external concentrations from 0·25 to 8 mM/l. NaCl. The results are shown in Fig. 1. In animals acclimatized to 10 and 2 mM/l. NaCl the relation between the influx rate and the

external concentration is almost identical, and the results conform closely to the Michaelis equation, 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. This equation has been used previously to describe influx measurements in *Astacus pallipes* (Shaw, 1959*b*) and in *G. duebeni* from Meggies Burn (Shaw & Sutcliffe, 1961). In animals from Meggies Burn acclimatized to 10 mM/l. NaCl at room temperature, sodium influx was described by the equation: influx = 0·95 $[C/(1·5+C)]$. In animals from Budle Bay acclimatized to 10 mM/l. NaCl at 10° C. sodium influx can be described by the equation: influx = 0·8 $[C/(2·25+C)]$, represented by the lower broken curve in Fig. 1. In animals acclimatized to 0·25 mM/l.

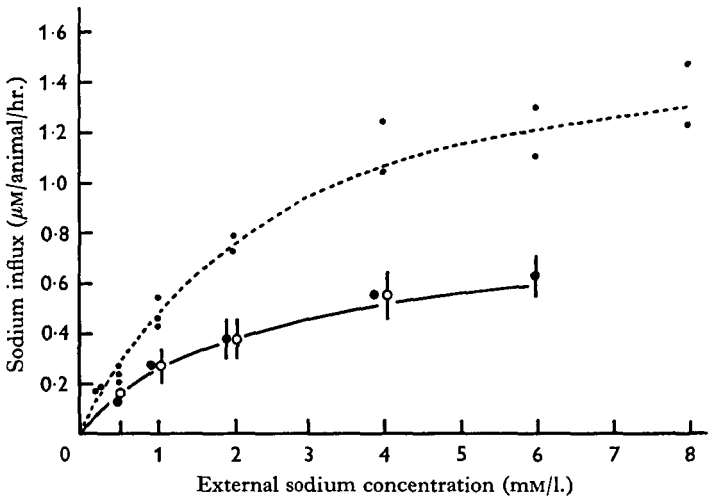

Fig. 1. The relation between the influx and the external sodium concentration in *G. duebeni* from Budle Bay. Groups acclimatized to 10 mM/l. NaCl, ○, and 2 mM/l. NaCl, ●. Vertical lines indicate extent of standard deviations from the means of five to seven groups. The solid curve represents: influx = 0·8 $[C/(2·25+C)]$. Small points represent measurements on individual groups acclimatized to 0·25 mM/l. NaCl. The broken curve represents: influx = 1·7 $[C/(2·4+C)]$.

Table 1. *The rate of sodium influx from steady state concentrations and the loss rate into de-ionized water in* Gammarus duebeni *from Budle Bay at* 10° *C*

Steady-state concentrations (mM/l. NaCl)	Sodium influx (μM/animal/hr.)	No. of groups	S.D.	Sodium loss (μM/animal/hr.)	No. of groups	S.D.
10	0·6–0·7	(from Fig. 1)		0·28	19	0·065
2	0·37	6	0·085	0·24	8	0·040
0·25	0·18	(from (Fig. 1)		0·18	8	0·025

NaCl the influx rates are higher, and this is in agreement with the previous results. Again the results can be described by the equation: influx = 1·7 $[C/(2·4+C)]$.

The value of K_m, which lies between 1·5 and 2·5 mM/l. sodium in these two brackish-water populations, is quite distinct from that found in the fresh-water species *G. pulex* and *G. lacustris*, where the value of K_m lies between 0·10 and 0·15 mM/l. sodium (Sutcliffe, 1967*b*; Sutcliffe & Shaw, 1967*b*). This illustrates the fact that the sodium-

transporting system in the two fresh-water species has a very much higher affinity for sodium ions at low external concentrations than the transporting system in the brackish-water populations of *G. duebeni*.

In animals acclimatized to 10 mM/l. NaCl the initial loss rate into de-ionized water was 0·28 μM/hr. and this is not much greater than the loss rate of animals acclimatized to 2 mM/l. NaCl (Table 1). In fact it is shown later that the loss rate remains constant at external concentrations up to about 270 mM/l. NaCl. However, from Table 1 it appears that the influx rate from the higher external concentrations was considerably greater than the loss rate, despite the fact that the animals were in sodium balance with no net loss or gain of sodium. This suggests that there is a large exchange diffusion component representing up to about 55% of the influx at 10° C. Previously, with animals from Meggies Burn acclimatized to 10 mM/l. at room temperature, the

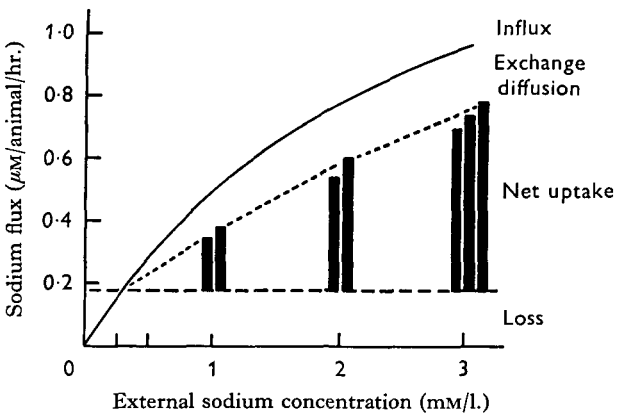

Fig. 2. Net uptake of sodium in *G. duebeni* from Budle Bay acclimatized to 0·25 mM/l. NaCl. Each vertical line represents the net uptake rate of sodium measured in a group of animals over a 30 min. period. The influx curve is taken from Fig. 1. The horizontal broken line represents the mean loss rate into de-ionized water.

loss rate was equal to the influx rate, but in animals acclimatized to 0·25 mM/l. NaCl and then transferred to higher external concentrations there was an exchange component representing about 40% of the influx during net uptake. Room temperature during these experiments fluctuated mainly between 18 and 22° C. These experiments were repeated with animals from Budle Bay acclimatized to 0·25 mM/l. NaCl at 10° C. Groups of twenty or thirty animals were transferred into 20 or 30 ml. of 1, 2 and 3 mM/l. NaCl. The net uptake of sodium was immediately measured during the first hour at the new external concentration. The results are shown in Fig. 2. If the sodium loss rate during the first hour was 0·18 μM/hr. (Table 1), it appears that about 25% of the influx during net uptake was due to some kind of exchange diffusion.

Regulation of sodium influx and loss rates, and the effects of temperature

In animals from Meggies Burn kept at room temperature, survival at the lowest external concentrations was due to a very marked reduction in sodium loss rate, and a slight increase in the influx rate (Shaw & Sutcliffe, 1961). In animals from Budle Bay, acclimatization to low external concentrations at 10° C. also involved an increase in

influx and a decrease in sodium loss rate. When acclimatized to 10 mM/l. NaCl the influx rate from 0·25 mM/l. NaCl was about 0·09 μM/hr. (Fig. 1). This was increased to about 0·18 μM/hr. in animals previously acclimatized to 0·25 mM/l. NaCl, i.e. the influx rate was doubled. At the same time the loss rate was reduced by about 36% (Table 1). However, the influx rate apparently cannot be increased at external concentrations below 0·25 mM/l. NaCl. In three groups brought into sodium balance at an external concentration of 0·20 mM/l. sodium, the influx rates from 0·5 mM/l. NaCl were 0·25, 0·16, and 0·20 μM/animal/hr. with a mean influx rate of 0·21 μM/hr. This is no greater than the mean influx rate of 0·25 μM/hr. from 0·5 mM/l. NaCl in three groups previously acclimatized to 0·25 mM/l. NaCl (Fig. 1).

The relative importance of changes in the influx and loss rates in animals from Budle Bay is the reverse of the situation found in those from Meggies Burn. This difference might be a characteristic feature of the two populations, but more probably it is due simply to the different temperatures at which the experiments were performed. In the next section of this paper it is shown that the loss rate is doubled at a 10° C. rise in temperature. It is shown also in a following paper that in *G. duebeni* from the River Boyne and Lough Melvin, both influx and loss rates were doubled by a 10° C. rise in temperature, and the same effect was found in *G. pulex* (Sutcliffe, 1967*b*). Now the average weight of animals from Budle Bay was 71 mg. and this is almost double the weight of animals from Meggies Burn (about 40 mg.). However, the influx rates in animals acclimatized to 0·25 mM/l. NaCl are almost the same in animals from the two localities at the different temperatures. The size of the influx is probably proportional to a factor which lies between $W^{0·67}$ and $W^{1·0}$ where W is the weight of the animal. In this case the influx rate of the smaller animals from Meggies Burn is almost double that of animals of comparable size from Budle Bay. This difference may be attributed to the temperature difference of approximately 10° C. If this is so, during the experiments with the Meggies Burn animals the influx rate in 10 mM/l. NaCl may already have been near to the maximum possible for a 40 mg. animal, so that it could not be greatly increased on acclimatization to the lower external concentrations.

If the loss rates shown in Table 1 are reduced by the ratio 40/71, it appears that the loss rates in the Meggies Burn animals acclimatized to 2 and 0·25 mM/l. NaCl at room temperature (Shaw & Sutcliffe, 1961) are very roughly double those found in the Budle Bay population at 10° C. This again might be attributed to the difference in temperature. But the loss rate in animals from Meggies Burn acclimatized to 10 mM/l. NaCl was about four times greater than it apparently would be in a 40 mg. animal from Budle Bay at 10° C., and the reason for this is unknown.

Sodium loss and the blood sodium concentration

The relation between the reduction in loss rate into de-ionized water and the fall in blood sodium concentration at low external concentrations (Shaw & Sutcliffe, 1961) was investigated in animals obtained from salt-marsh pools at Warton, Lancashire. Large specimens, mostly males, average weight 95 mg., were divided into two batches each containing about fifty animals. The first batch was acclimatized to 10 mM/l. NaCl followed by 1 and 0·5 mM/l. NaCl. The second batch was acclimatized to 2 mM/l. followed by 1 mM/l. NaCl at 9° C. Loss rates into de-ionized water at 9° C. were determined, together with measurements of the blood sodium concentration in six to

eight individuals removed from each batch at the various acclimatization concentrations. The remaining animals in the two batches were then pooled together in 0·25 mM/l. NaCl, where survival was very good, followed by 0·20 mM/l. NaCl in which the animals began to die off during the first 48 hr. A number of blood sodium analyses were made on these animals after 36 hr. in 0·2 mM/l. NaCl. The results are shown in Fig. 3.

The blood sodium concentrations are 15 to 20% higher than those found in animals from Meggies Burn at room temperature (Shaw & Sutcliffe, 1961), but the fall in blood sodium at increasingly lower external concentrations is almost exactly the same. It is of interest to note that the blood concentrations shown in Fig. 3 were obtained from animals which were active and appeared healthy. In a few cases analyses were also made on the blood of animals which experience suggested were likely to die within a few hours. In three animals from 0·5 and 0·25 mM/l. NaCl the blood sodium concentrations were 128, 132 and 123 mM/l. This indicates that the blood sodium level falls by at least 50% in the few hours immediately preceding death. Beadle & Cragg (1940*a*) record a similar fall in the blood chloride concentration.

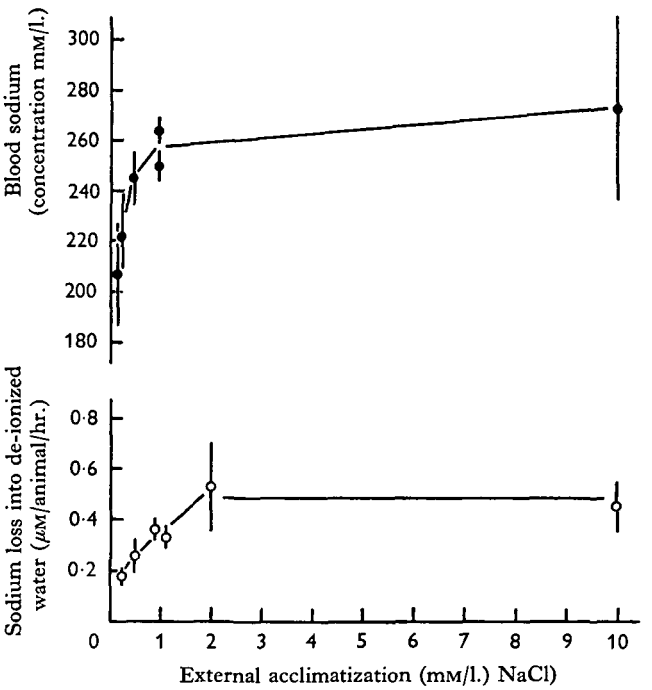

Fig. 3. The blood sodium concentration ●, and sodium loss rates O, in *G. duebeni* from Warton salt-marsh. Blood sodium, each point is the mean of six to eight individuals; loss rates, each point is the mean of six to twelve groups. Vertical lines indicate extent of standard deviations.

In apparently normal, healthy animals the blood sodium concentration at an external concentration of 0·25 mM/l. NaCl was reduced to about 81% of the blood concentration at 10 mM/l. NaCl, whereas the sodium loss rate into de-ionized water was reduced to 40% of the loss at 10 mM/l. NaCl (Fig. 3). It is clear that if sodium loss across the body surface is directly proportional to the blood concentration, part of the reduction in loss rate must be due to some factor other than the fall in blood concentration. One

factor could be a reduction in sodium concentration of the urine, particularly since Lockwood (1961, 1965) has shown that the urine concentration in *G. duebeni* is increasingly hypoosmotic to the blood at external concentrations below about 200 mM/l. NaCl.

Sodium loss in the urine

The amount of sodium lost in the urine was estimated by comparing the loss into de-ionized water with the loss into a sucrose solution made slightly hyperosmotic to the blood. The technique is discussed by Sutcliffe (1967*b*). In some cases, during the first 10–30 min. in sucrose the loss rate was gradually reduced. This reduction was more noticeable in animals acclimatized to high external concentrations. Then followed a period of steady loss, linear with time, during the following 30–60 min. As an example, the results from four out of six groups in one set of determinations are given in Fig. 4, together with results obtained when the animals were then transferred from sucrose into de-ionized water. Results with the other two groups were essentially the same as those shown in Fig. 4. When transferred from sucrose into de-ionized water the loss rate was immediately increased. Note that before transference to the de-ionized water in which the sodium loss was measured, the animals were washed in de-ionized water for several minutes to remove sucrose and sodium chloride. Several hundred determinations of loss rates into de-ionized water have now been made with several species of *Gammarus* and the loss rate is almost always constant from the start of the determination. It seems reasonable, therefore, to interpret the initial reduction in loss rate when in sucrose as due to a reduction in, and final cessation of, a flow of urine containing sodium.

In one experiment large animals from Warton salt-marsh (average weight 95 mg.) were acclimatized to 10 mM/l. NaCl at 9° C. Loss rates into de-ionized water and sucrose at 9° C. were determined on alternate days. The animals were then acclimatized to 0·25 mM/l. NaCl and the loss rates were again determined. In this experiment all of the animals survived the transfer to the low external concentration. The results are given in Table 2. The loss rate into de-ionized water in animals acclimatized to 0·25 mM/l. NaCl was reduced to 40% of the loss in animals acclimatized to 10 mM/l. NaCl. This agrees with the results obtained from a different batch of animals at the same external concentrations (Fig. 3). Similarly, the loss rate into sucrose was reduced to 65% of the loss in animals acclimatized to 10 mM/l. NaCl. If the loss into sucrose represents outward diffusion of sodium across the body surface, a reduction of 35% in the loss rate is greater than the 19% reduction expected from the fall in blood sodium concentration (Fig. 3).

In another experiment slightly smaller animals from Warton (average weight 79 mg.) were acclimatized to 10 mM/l. NaCl at 9° C. The mean sodium loss rate of five groups in de-ionized water at 9° C. was $0{\cdot}31 \pm 0{\cdot}03$ μM/animal/hr., and in sucrose at 9° C. the mean sodium loss was $0{\cdot}14 \pm 0{\cdot}04$ μM/hr. Allowing for the smaller size of the animals, these results agree well with those found in Table 2. Comparative loss rates in these animals were then determined at 20° C. $\pm$ 1·5° C., with the animals kept at 9° C. in between determinations. They were then acclimatized to 0·25 mM/l. NaCl at 9° C., and loss rates were again determined at 20° C. The results are given in Table 3.

Once again, at 20° C. the loss rate into de-ionized water in animals acclimatized to

0·25 mM/l. NaCl was reduced to 45% of the loss in animals acclimatized to 10 mM/l. NaCl. Similarly, the loss into sucrose was reduced to 64% of the former loss rate. This is almost exactly the same as found in the previous experiment at 9° C. (Table 2), and again this reduction is greater than would be expected from a 19% reduction in blood sodium concentration. In passing, we may also note that the loss rates in both de-ionized water and sucrose at 20° C. are approximately double the rates found at 9° C. Furthermore, the effect of a rise in temperature is extremely rapid, since the animals were transferred from 9 to 20° C. within a few minutes; determinations began straight away, and the loss rate did not increase gradually but instead was maintained at a constant high rate in every case right from the start.

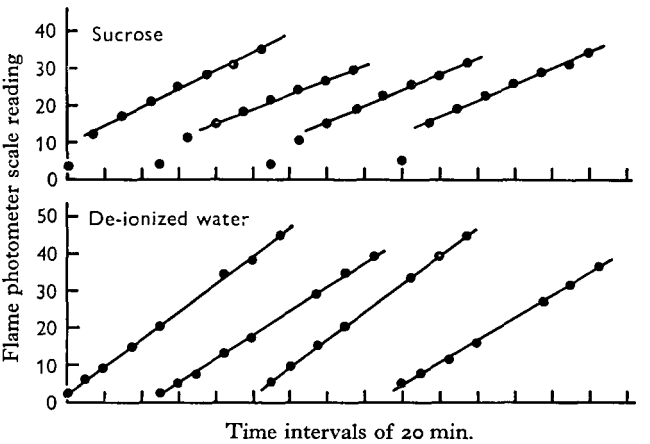

Fig. 4. Sodium loss into 50 ml. sucrose followed immediately by sodium loss into 50 ml. de-ionized water at 9° C. in four groups of *G. duebeni* from Warton salt-marsh acclimatized to 10 mM/l. NaCl. Each group contained seven animals. The groups are placed in the same order in both series. Fifty divisions on the flame photometer scale were equivalent to a concentration of 0·10 mM/l. sodium.

Table 2. *Sodium loss into de-ionized water and slightly hyperosmotic sucrose at* 9° *C. in* Gammarus duebeni *from Warton salt-marsh acclimatized to* 10 *mM/l. NaCl and* 0·25 *mM/l.* NaCl.

Acclimatization concentration (mM/l. NaCl)	Loss rate in de-ionized water (μM/animal/hr.)	No. of groups	S.D	Loss rate in sucrose (μM/animal/hr.)	No. of groups	S.D.
10	0·38	12	0·10	0·17	12	0·03
0·25	0·15	12	0·01	0·11	12	0·01

The results presented in this section can now be briefly summarized, comparing the situation in animals acclimatized to 0·25 mM/l. NaCl with the situation found when previously acclimatized to 10 mM/l. NaCl. The blood sodium concentration is reduced by about 20%, but the loss rate of sodium across the body surface into sucrose is reduced by about 35%. The total sodium loss into de-ionized water is greatly reduced, by 35–60%, and sodium loss in the urine now represents only 25% of the total. When

acclimatized to 10 mM/l. NaCl, sodium loss in the urine represents 50% of the total loss into de-ionized water. It is clear, therefore, that there is a very considerable reduction in sodium loss via the urine.

Table 3. *Sodium loss into de-ionized water and slightly hyperosmotic sucrose at* 20° C. *in* Gammarus duebeni *from Warton salt-marsh acclimatized to various external concentrations at* 9° C.

Acclimatization concentration (mM/l. NaCl)	Loss rate in de-ionized water (μM/animal/hr.)	No. of groups	S.D.	Loss rate in sucrose (μM/animal/hr.)	No. of groups	S.D.
10	0·62	10	0·11	0·33	5	0·05
0·25	0·28	5	0·02	0·21	5	0·01

Sodium concentration in the urine

Lockwood (1961) estimated that the rate of urine flow in *G. duebeni* kept in Cambridge tap water was approximately equivalent to 56% body weight/day at 20° C. This represents a urine flow rate of 1·8 μl./hr. in 79 mg. animals at 20° C. (Table 3), where the mean loss attributed to sodium in the urine was 0·29 μM/hr. in animals acclimatized to 10 mM/l. NaCl. With a urine flow of 1·8 μl./hr. the sodium concentration in the urine would be about 160 mM/l. On the other hand, when acclimatized to 0·25 mM/l. NaCl the sodium loss attributed to the urine was 0·07 μM/hr. and this would require a urine concentration of only 39 mM/l.

In the case of urine loss rates measured at a temperature of 9–10° C. it is assumed that the urine flow rate is reduced to half the estimated rate at 20° C. This assumption is based on the observation that in *Gammarus oceanicus* and *G. fasciatus* the urine flow rate at 25° C. was approximately double the rate found at 15° C. (Werntz, 1963). Hence a 95 mg. animal in de-ionized water at 9° C. will produce about 1·1 μl. urine/hr. From Table 2, in animals acclimatized to 10 mM/l. NaCl the mean difference between loss rates into de-ionized water and sucrose was 0·21 μM/hr. at 9° C. If this was due to sodium loss in 1·1 μl of urine then the mean sodium concentration of that urine must have been about 190 mM/l. Similarly, when acclimatized to 0·25 mM/l. NaCl the mean difference of 0·04 μM/hr. represents a urine sodium concentration of about 63 mM/l.

From the above calculations it is concluded that in animals acclimatized to 10 mM/l. NaCl the urine sodium concentration was probably about 160–190 mM/l. When the animals were then acclimatized to 0·25 mM/l. NaCl the urine sodium concentration was reduced to about 36–39 mM/l. These estimated concentrations compare very well with the range 48–142 mM/l. NaCl for the total urine concentration found in *G. duebeni* kept in media less concentrated than 14 mM/l. NaCl (Lockwood, 1961). This close agreement leaves little doubt that the method of analysis employed here is a realistic one.

GAMMARUS DUEBENI FROM FRESH-WATER LOCALITIES ACCLIMATIZED TO LOW EXTERNAL CONCENTRATIONS

Gammarus duebeni *from the Lizard Peninsula, Cornwall*

Experiments on these animals were carried out at room temperature in the summer of 1961. Most of the experiments were carried out with animals from the stream at

Grochal, where the sodium concentration on the day of collection was 2·5 mM/l. All the streams containing *G. duebeni* on the Lizard peninsula have a sodium concentration greater than about 2 mM/l. (Hynes, 1954).

These animals were readily acclimatized to 0·25 mM/l. NaCl, but they began to die rather quickly after 24 hr. in 0·15 mM/l. NaCl. Animals collected from the stream at Growgey behaved in the same way.

Sodium influx in animals acclimatized to 0·5 and 0·25 mM/l. NaCl was measured to see if the influx rate is increased at the lower external concentration, as it is in animals from brackish water. The results are shown in Fig. 5, where it appears that the influx rate was doubled in animals acclimatized to 0·25 mM/l. NaCl. At the same time the loss rate into de-ionized water was reduced by 40% compared with the loss rate in

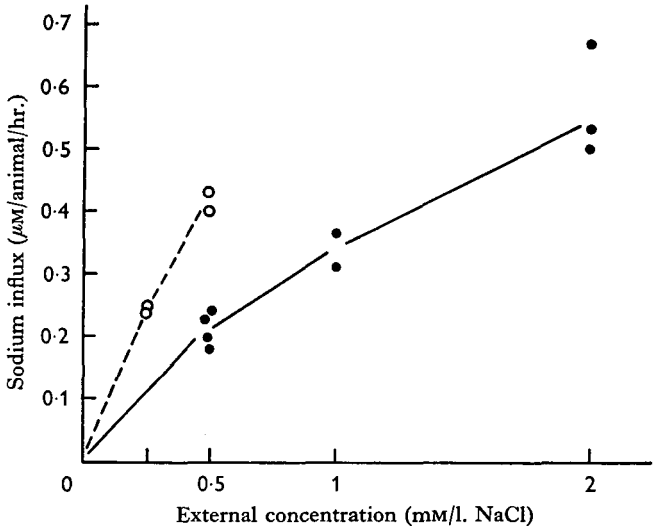

Fig. 5. The relation between the influx and the external sodium concentration in *G. duebeni* from the Lizard peninsula. ●, groups acclimatized to 0·5 mM/l. NaCl; O, groups acclimatized to 0·25 mM/l. NaCl.

Table 4. *Sodium loss rate into de-ionized water in* Gammarus duebeni *from the Grochal stream, Lizard peninsula, when acclimatized to a series of decreasing external concentrations at room temperature*

Acclimatization concentration (mM/l. NaCl)	Sodium loss rate (μM/animal/hr.)	No. of groups	S.D.
2·0	0·42	6	0·06
0·25	0·25	6	0·03
0·15	0·17	2	—

animals acclimatized to 2 mM/l. NaCl, and there was a further reduction of 32% when acclimatized to 0·15 mM/l. NaCl for 24 hr. (Table 4). A single measurement of the influx rate in a group of animals acclimatized to 0·15 mM/l. NaCl gave a value of 0·16 μM/animal/hr.

In a single group of animals from the Crowgey stream acclimatized to 2 mM/l. NaCl

the sodium loss rate into de-ionized water was 0·38 μM/animal/hr. This was reduced by 40% when the group was acclimatized to 0·5 mM/l. NaCl, and reduced by a further 17% when acclimatized to 0·25 mM/l. NaCl.

The sodium concentrations in the blood of animals acclimatized to 2 and 0·25 mM/l. NaCl are given in Table 5. Each sample used for determining the sodium concentration was obtained by pooling the blood from two to four individuals. The blood sodium concentrations are very similar to those found in animals from brackish-water localities. In animals from the Grochal stream the blood sodium level fell by about 15%

Table 5. *The blood sodium concentration in* Gammarus duebeni *from the Lizard peninsula*

Stream	Acclimatization concentration (mM/l. NaCl)	Mean blood sodium concentration (mM/l.)	No. of samples	Standard deviation
Grochal	2·0	246	6	24
	0·25	210	4	—
Crowgey	0·25	188	5	14

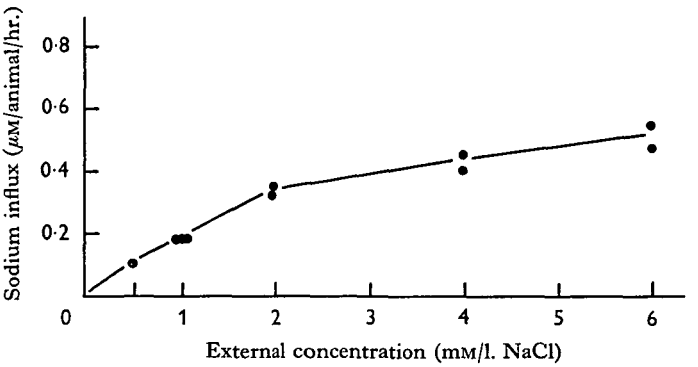

Fig. 6. The relation between the influx and the external sodium concentration in *G. duebeni* from the Isle of Man acclimatized to 0·25 mM/l. NaCl.

when acclimatized to 0·25 mM/l. NaCl, and it may have fallen by a greater amount in the case of animals from the Crowgey stream. These rather large reductions in the blood sodium concentration, coupled with the successive reductions in sodium loss rate and increases in the influx rate at low external concentrations, closely resemble the situation found in *G. duebeni* from brackish water.

Gammarus duebeni *from the Isle of Man*

These animals, average weight 43 mg., were also easily acclimatized to 0·25 mM/l. NaCl at 10° C. After repeated sodium loss into de-ionized water, two groups maintained sodium balance at an external concentration of 0·1 mM/l. sodium over a 48 hr. period. After further sodium loss in de-ionized water several animals died in both groups, and the remainder balanced at 0·07 and 0·1 mM/l. respectively over a 38 hr. period. These temporary balance concentrations are lower than in the brackish-water animals and, in fact, are not much greater than the balance concentrations achieved by the fresh-water species *G. pulex* and *G. lacustris*.

A few sodium influx measurements made with animals acclimatized to 0·25 mM/l. NaCl are shown in Fig. 6. The influx rate continued to increase at external concentrations up to at least 6 mM/l. NaCl, and the sodium-transporting system is apparently half-saturated at an external concentration greater than 1 mM/l. In this respect the transporting system is very similar to the system found in animals from brackish water (Fig. 1).

Gammarus duebeni *from the Connie River, Kintyre*

In July 1963 the sodium concentration of the river was only 0·55 mM/l. and in July 1964 the sodium concentration was 0·60 mM/l. These concentrations are comparable with those found in the fresh waters of Ireland, and are lower than the sodium (and chloride) concentrations found in all of the other fresh-water localities containing *G. duebeni* outside Ireland (Sutcliffe, 1967*a*).

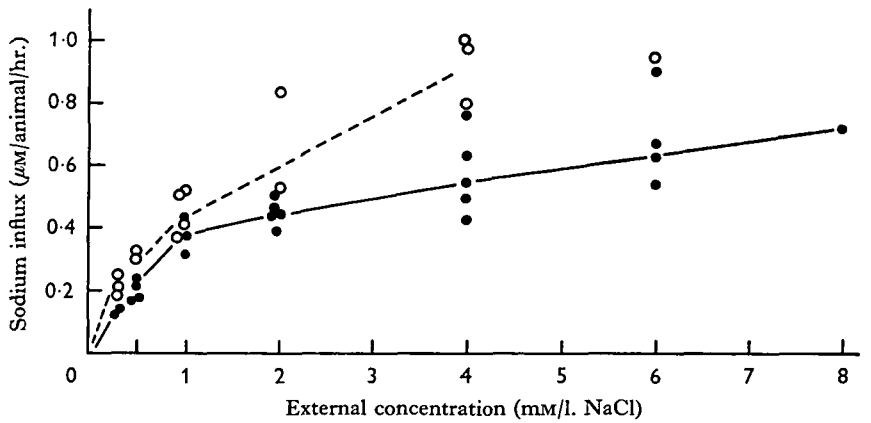

Fig. 7. The relation between the influx and the external sodium concentration in *G. duebeni* from the Connie River, Kintyre. ●, groups acclimatized to 0·25 mM/l. NaCl; ○, groups acclimatized to 0·15 mM/l. NaCl.

In the laboratory the animals were easily acclimatized to 0·15 mM/l. NaCl at 10° C, with only a few deaths. In fact sodium balance was maintained for short periods at even lower external concentrations. Three groups of animals were placed in 30–40 ml. de-ionized water at 10° C. and the external sodium concentration was allowed to increase to a steady level. Some of the medium was then replaced with fresh de-ionized water, and the process was repeated. On the third occasion, the three groups maintained a steady external sodium concentration of 0·07, 0·10 and 0·13 mM/l. respectively over a 24 hr. period. Again these balance concentrations are distinctly lower than the balance concentrations maintained by *G. duebeni* from brackish water. The relation between sodium influx and the external concentration in animals acclimatized to 0·25 and 0·15 mM/l. NaCl is shown in Fig. 7. Although a greater number of measurements is required to confirm the point, it looks as if the influx rate continued to increase at external concentrations up to about 10 mM/l. NaCl, and the sodium-transporting system certainly appears to be only half-saturated at an external concentration slightly greater than 1 mM/l. NaCl.

The mean sodium loss rate into de-ionized water from six groups of animals acclimatized to 0·25 mM/l. NaCl was 0·15 μM/animal/hr., standard deviation 0·05. This agrees

closely with the influx measurements at this concentration. In four groups acclimatized to 0·15 mM/l. NaCl the mean sodium loss into de-ionized water was 0·16 μM/hr., and again this agrees with the influx curve in Fig. 7 for the same animals. The average weight of the animals was 50 mg.

Summary of observations on Gammarus duebeni *from fresh-water localities*

The general picture which emerges from these observations on *G. duebeni* from coastal fresh-water localities in Britain is that the sodium-transporting system has the same characteristics as the system found in *G. duebeni* from brackish-water localities. Thus the system is unsaturated at external concentrations below about 10 mM/l. and the system is only half-saturated at about 1–2 mM/l. NaCl. To achieve sodium balance at concentrations below 10 mM/l. the influx rate is increased and the loss rate is greatly reduced.

The chief difference from the brackish-water animals seems to be the far better survival at very low external concentrations, and this is reflected in the ability to achieve temporary sodium balance at an external sodium concentration of about 0·1 mM/l. at 10° C. instead of about 0·2 mM/l. as found in *G. duebeni* from brackish-water localities.

GAMMARUS DUEBENI FROM BRACKISH-WATER LOCALITIES ACCLIMATIZED TO HIGH EXTERNAL CONCENTRATIONS

Sodium loss and the blood sodium concentration

Sodium loss in animals acclimatized to sea-water media was determined in a batch of *G. duebeni* from Budle Bay (average weight 71 mg.) initially used to determine loss rates when acclimatized to 10, 2 and 0·25 mM/l. NaCl (Table 1). One-half of the batch was acclimatized to a sea-water medium equivalent to 56 mM/l. NaCl at 10° C. and the loss rate was determined at 10° C. The animals were then acclimatized to a series of increasing external concentrations, viz. 270, 425 and 530 mM/l. NaCl. This last concentration was undiluted sea water. The remaining one-half was successively acclimatized to 115, 225, 370 and 480 mM/l. NaCl. The loss rates into de-ionized water at 10° C. in animals acclimatized to these increasing concentrations are shown in Fig. 8. The total loss rate remained constant in animals acclimatized to concentrations up to 270 mM/l. NaCl, but then it increased sharply in animals acclimatized to increasingly higher concentrations.

In order to assess the relative contribution of sodium loss in the urine and across the body surface the loss rates into slightly hyperosmotic sucrose, and some into de-ionized water, were determined with animals from Warton salt-marsh acclimatized to a series of increasing external concentrations at 9° C. This was the same batch of animals previously used to determine loss rates at low external concentrations (Table 2). The results are shown in Fig. 9, together with a value for the blood sodium concentration at an external concentration of 10 mM/l. NaCl, drawn from Fig. 3. The other two points represent mean values on blood from six and seven individuals. The broken line corresponds to blood sodium concentrations at other external concentrations given by Lockwood (1964).

First, from Figs. 8 and 9 we may note that the loss rates into de-ionized water are very similar in animals from the two localities, particularly in view of the difference in

their average weights. Secondly, from Fig. 9 it appears that although the blood sodium concentration is increased by about 83% over the external range of concentrations 10–530 mM/l. NaCl, the loss rate into slightly hyperosmotic sucrose increased by only 47% over the same range of external concentrations. This was checked by transferring the animals acclimatized to 530 mM/l. NaCl back into 115 mM/l. NaCl. After 5 days

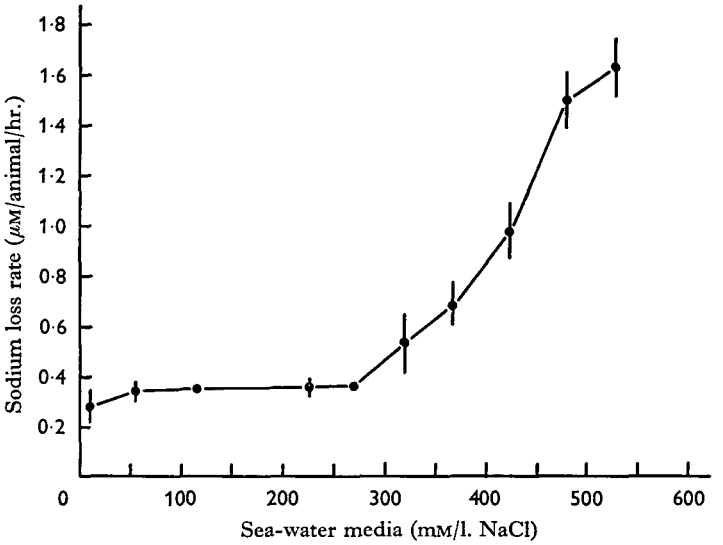

Fig. 8. Sodium loss into de-ionized water in *G. duebeni* from Budle Bay acclimatized to a series of increasing external concentrations. Each point represents the mean loss in five to nine groups, vertical lines indicate the extent of standard deviations (±0·03—±0·12).

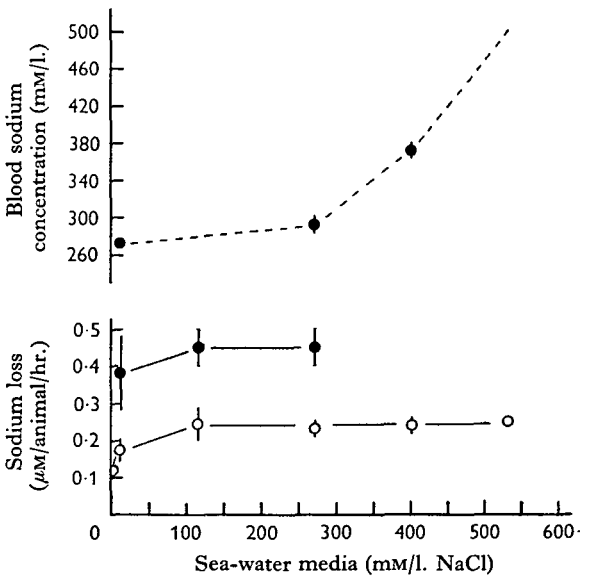

Fig. 9. Sodium loss and the blood sodium concentration in *G. duebeni* from Warton salt-marsh acclimatized to a series of increasing external concentrations. O——O, loss into sucrose, ●——●, loss into de-ionized water; each point represents the mean loss in five or six groups. ●---●, blood sodium concentration. Vertical lines indicate the extent of standard deviations.

acclimatization at the lower concentration the mean sodium loss rate of four groups in sucrose was 0·26 μM/hr., compared with 0·24 μM/hr. found when previously acclimatized to 115 mM/l. NaCl (Fig. 9). The animals were then again acclimatized to 530 mM/l. NaCl for 6 days and the mean loss rate of four groups in sucrose was 0·24 μM/hr., compared with 0·25 μM/hr. found when previously acclimatized to this external concentration. Also, the blood sodium concentrations given in Fig. 9 were obtained from animals removed from the above series of loss-rate determinations when the batch of animals was acclimatized to the appropriate external concentrations. Thus there seems to be no doubt that, in this experiment, the loss rate of sodium across the body surface into sucrose remained constant in animals acclimatised to the range of external concentrations 115–530 mM/l. NaCl, despite the fact that the blood sodium concentration must have increased by about 80% over this same range. It appears therefore that, as was found at low external concentrations, the loss rate across the body surface is not directly proportional to the blood sodium concentration.

Sodium concentration in the urine

From a comparison of Figs. 8 and 9 it appears that in animals acclimatized to external concentrations up to 270 mM/l. NaCl about 53% of the total sodium loss into de-ionized water was due to loss across the body surface. Now in animals from Warton salt-marsh acclimatized to 115 and 270 mM/l. NaCl the sodium loss attributed to the urine was about 0·21 μM/hr. (Fig. 9). As in an earlier section it is assumed that the urine flow rate at 9–10° C. is equivalent to about 28% body weight/day for an osmotic gradient of 300 mM/l. NaCl between the blood and external medium. The urine flow rate into de-ionized water is then about 1·1 μl./hr., and a sodium loss rate of 0·21 μM/hr. would require a urine concentration of approximately 190 mM/l. sodium. This is the same concentration as that estimated previously for urine produced in the same animals when acclimatized to 10 mM/l. NaCl (Table 2).

A similar estimate may be made for 71 mg. animals from Budle Bay (Fig. 8) if it is also assumed that the sodium loss rate across the body surface into de-ionized water at 10° C. was similar to the observed loss in 95 mg. animals from Warton (Fig. 9), i.e. about 0·2 μM sodium/hr. Sodium loss in the urine of Budle Bay animals acclimatized to the range of concentrations from 55 to 270 mM/l. NaCl is then about 0·15 μM/hr., and if the urine flow rate was 0·83 μl./hr. at 10° C. the urine sodium concentration must have been approximately 180 mM/l.

The above estimates, combined with those made in an earlier section, indicate that when *G. duebeni* was fully acclimatized to a series of concentrations increasing from 10 to 270 mM/l. NaCl (i.e. from about 2 to 50% sea water) the urine sodium concentration remained surprisingly constant at about 160–190 mM/l. or 55–70% of the total blood concentration. These concentrations are very similar to most of the values for the total concentration of the urine given by Lockwood (1961) for *G. duebeni* acclimatized to the same range of sea-water media. At external concentrations above 270 mM/l. NaCl the greatly increased loss rate into de-ionized water (Fig. 8) appears to be closely associated with the increase in blood sodium concentration (Fig. 9), and was presumably due to the production of urine isotonic with the raised blood concentration since the urine is isosmotic with the blood at these high external concentrations (Lockwood, 1961).

Estimation of sodium losses across the body surface and in urine of animals when in dilute sea water

Using the data obtained with *G. duebeni* from Warton salt-marsh, the rates of sodium loss in the urine and across the body surface can be calculated for animals fully acclimatized to external concentrations below 270 mM/l. NaCl, where the blood concentration is maintained strongly hyperosmotic to the medium (Fig. 10). For this it was assumed that the urine flow rate is proportional to the osmotic gradient between the blood and external medium (Werntz, 1963; Lockwood, 1965), with a flow rate equivalent to 28% body weight/day at 9° C. for an osmotic gradient of 300 mM/l. NaCl. It was also assumed that sodium loss across the body surface is proportional to the concentration gradient between the blood and external medium, with a loss rate

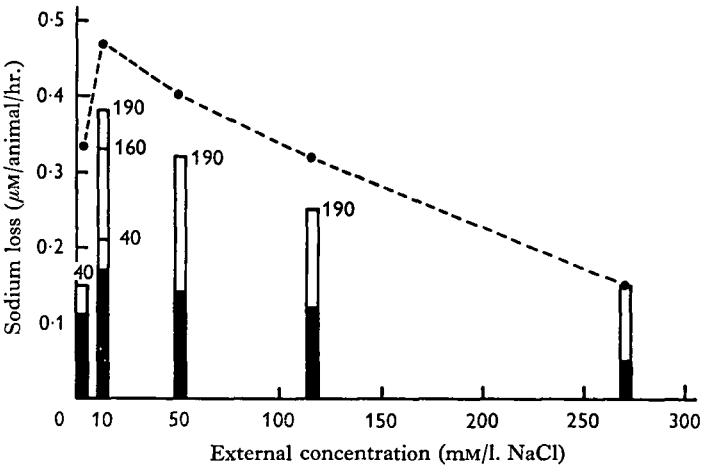

Fig. 10. Estimated and observed sodium loss rates in *G. duebeni* from Warton salt-marsh at external concentrations below 270 mM/l. NaCl. Solid vertical bars represent sodium loss across the body surface. Open vertical bars represent sodium loss in urine with sodium concentrations (mM/l.) given alongside. The dotted line indicates the total sodium loss rate when the urine is made isotonic with the blood sodium concentration.

of 0·17 μM/hr. at 9° C. for a gradient of 273 mM/l. sodium. This assumption does not appear to be strictly true in all cases, particularly at the lowest external concentrations, and the observed loss rate in animals acclimatized to 0·25 mM/l. NaCl is given in Fig. 10.

It is seen that even when the urine is made isotonic with the blood at concentrations down to 10 mM/l. NaCl, sodium losses across the body surface account for at least 35% of the total sodium loss rates. This minimum value is nearly double the estimate of 20% made by Lockwood (1965), which is true only in the special case of a very large osmotic gradient, and hence a large urine flow rate, obtained when animals acclimatized to 150% sea water are placed in de-ionized water. In this case, using the data employed to construct Fig. 10, it is calculated that sodium loss in urine isotonic with the very high blood concentration would account for 81% of the total sodium loss rate during the first hour in de-ionized water. This is an excellent agreement with Lockwood's estimate. Nevertheless, the results substantiate the general point that

urinary losses in *G. duebeni* are rather larger than in some other crustaceans which produce urine isosmotic with the blood (Lockwood, 1965), including *Palaemonetes varians* (Potts & Parry, 1964).

From Fig. 10 it is also clear that if *G. duebeni* in 10 mM/l. NaCl produced urine isotonic with the blood, the total sodium loss rate would be about three times greater than the total loss rate when in 270 mM/l. NaCl. On the other hand, if the sodium concentration in the urine of animals in 10 mM/l. NaCl was reduced to only 40 mM/l., the total loss rate would be more than halved, and would then be nearly the same as in 270 mM/l. NaCl. This confirms the suggestion that the production of a hypotonic urine could have the effect of keeping the total sodium losses almost constant (Lockwood, 1965). However, the actual determinations (Lockwood, 1961) and estimates presented here show that, although the urine is hypotonic to the blood, in most instances the urine concentration is not reduced to a very low level until the animals are acclimatized to media which are fresh rather than brackish, i.e. concentrations below 10 mM/l. NaCl. In fact the urine concentration is only reduced to 60–70% of the blood concentration at external concentrations ranging from less than 270 mM/l. down to about 10 mM/l. NaCl. As a result, when the external concentration is gradually reduced over this range the total sodium loss rate tends to increase until it reaches a maximum at about 10 mM/l. NaCl, and this maximum rate is roughly double the rate of loss in 270 mM/l. NaCl (Fig. 10). It follows that the sodium uptake rate must also tend to increase to double the uptake rate at 10 mM/l. compared with the rate required to balance sodium losses at 270 mM/l. NaCl, and Lockwood (1965) in fact found that the sodium influx from 2% sea water was roughly double the influx rate from higher external concentrations.

DISCUSSION

The evidence presented by Lockwood (1961, 1965) and in this paper strongly suggests that in *G. duebeni* an increase in the sodium uptake rate at the body surface is linked with renal sodium uptake, resulting in a hypotonic urine. This possibility has already been noted (Lockwood, 1964), and it accounts for the rather curious fact that a strongly hypotonic urine containing about 40 mM/l. sodium is not usually elaborated until the animals are acclimatized to very low external concentrations, below 10 mM/l. NaCl. Here the sodium-transporting system at the body surface is less than fully saturated, and the system is fully activated to achieve the maximum rates of uptake; at the same time the urine concentration is strongly reduced. The same effect is seen in *G. pulex* (Sutcliffe, 1967*b*).

In view of the fact that activation of the sodium-transporting systems at the body surface and in the antennary glands is brought about by changes in the blood sodium concentration in *G. pulex* (Sutcliffe, 1967*b*) and in *Astacus* (Bryan, 1960; Shaw, 1959, 1964) it seems likely that the same regulatory mechanism occurs in *G. duebeni*. When the external concentration is reduced from 270 to 10 mM/l. NaCl the blood sodium concentration falls by approximately 10% (Fig. 6, and Lockwood, 1964) and a reduction by this amount is more than sufficient to activate the sodium-uptake system in both *G. pulex* and *Astacus*. This accounts for the increased influx rate and production of hypotonic urine at concentrations between 270 and 10 mM/l. NaCl, and the

further reduction in blood sodium at concentrations below 10 mM/l. would fully activate both the renal and body surface uptake systems. This also seems to provide an adequate explanation for the results obtained by Lockwood (1964) with *G. duebeni* moved from very high salt concentrations to lower concentrations, where the influx rate was greatly increased and hypotonic urine was formed despite the fact that the final blood sodium concentration was as high as that in control animals where the uptake systems were not activated. But the experimental treatment produced a very large and sudden fall in the blood concentration, and Shaw (1964) has indicated that the internal regulator responsible for activating the transporting systems may be responding to relative changes in perhaps several of the blood ions, and not necessarily to the absolute concentration of sodium in the blood. Under these circumstances an internal regulator in *G. duebeni* could temporarily activate the uptake systems and then be withdrawn as the correct ion balance in the blood was restored.

Since the blood concentration is only regulated strongly at external concentrations below about 270 mM/l. NaCl it may be presumed that an internal regulator, generally assumed to be a hormone, will not be released when the blood concentration has risen steeply in animals acclimatized to increasing concentrations above about 270 mM/l. NaCl. In this case net uptake of sodium would be reduced to a minimum, both at the body surface and in the antennary glands, resulting in an isosmotic urine. Thus the basic sodium regulating mechanism in *G. duebeni* appears to operate in the same way as that proposed for *Astacus* by Bryan (1960).

Although the influx rate can be doubled over the external concentration range 10–0·25 mM/l. NaCl, the amount of sodium uptake is very small at these low concentrations due to the low affinity for sodium ions in the transporting system at the body surface. Consequently, as found earlier (Shaw & Sutcliffe, 1961), the reduction in total sodium loss rate forms a major part of regulation at low external concentrations, although it must be noted that this regulation does not prevent a progressive lowering of the blood sodium concentration. However, it is also necessary to account for the fact that the reduction in total loss rate is not entirely due to the production of a very dilute urine and the fall in blood sodium concentration. It is difficult to avoid the conclusion that the rate of sodium loss across the body surface can be controlled, particularly since this appears to have also occurred in animals acclimatized to high external concentrations. The reason for exercising control at high external concentrations is hard to see, but there is no doubt about its effectiveness at very low external concentrations.

In brackish water sodium losses across the body surface and in the urine are balanced by sodium uptake at the body surface through a transporting system which is both fully saturated and operating at a low rate, and these presumably represent optimum working conditions for the system. But in fresh water this transporting system is not fully saturated, and although the loss rate is reduced the uptake rate must be increased to balance the loss. This is illustrated in Fig. 11 for external concentrations below 1 mM/l. NaCl, where the minimum rate of influx, A, is the influx rate in animals from brackish-water localities acclimatized to the range 2–10 mM/l. NaCl. In 1 mM/l. NaCl this rate must be increased to balance the loss rate L_3, and in 0·5 mM/l. NaCl the influx rate must be increased further, almost to the maximum, B, to balance the loss rate L_2. Temporary balance is achieved at about 0·2 mM/l. NaCl by

reducing the loss rate to the minimum L_1. Since the maintenance of high influx and low loss rates presumably requires a greater expenditure of energy, and since the blood sodium concentration falls rapidly at external concentrations below 1 mM/l. sodium, it is suggested that the sodium concentration range of 0·1 to 0·5 mM/l. found in most inland fresh waters in Britain and Ireland is unsuitable for animals from brackish-water localities. But these animals could live in fresh water with a higher sodium concentration, particularly when this is greater than 1 mM/l. Following the Venice Symposium (1959) the distinction between fresh and mixohaline brackish water is arbitrarily placed at 0·5‰ salinity, or approximately 7 mM/l. sodium. With the exception of Ireland and the Kintyre peninsula, all other fresh-water localities in north-west Europe containing *G. duebeni* are streams and lochs with sodium concentrations greater than about 1 mM/l. (Sutcliffe, 1967*a*). It is suggested, therefore, that these fresh-water localities have been colonized from populations in the estuaries without any modifications in the sodium-regulatory mechanism characteristic of brackish-water animals. This also appears to be applicable to the population living in fresh water on the Kintyre peninsula.

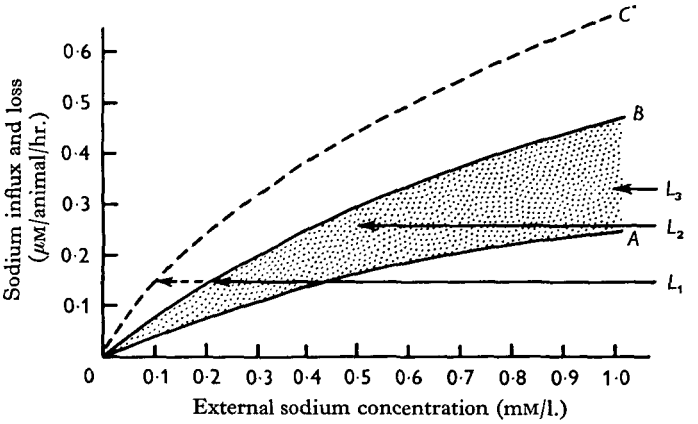

Fig. 11. The relationship between sodium influx and loss rates in *G. duebeni* at external concentrations below 1 mM/l. sodium. *A* and *B* are influx curves for animals from brackish water (Budle Bay) acclimatized to 10 and 2 mM/l. NaCl (A) and acclimatized to 0·25 mM/l. NaCl (B). *C* is the influx curve for animals from fresh-water localities. L_1 represents the observed minimum sodium loss rate, L_2 is the loss rate when acclimatized to 0·5 mM/l. NaCl, L_3 is the loss rate when acclimatized to 1 mM/l. NaCl in animals from brackish-water localities. Explanation in the text.

The suggestion that no adaptive changes have occurred in *G. duebeni* living in fresh water outside Ireland, is based on the fact that the affinity for sodium ions in the transporting system at the body surface of animals from three of these localities is no greater than the affinity for sodium in animals from brackish-water localities. Since the minimum total sodium loss rates are also similar it might be expected that sodium balance in the fresh-water animals should be achieved at the same minimum external concentration as in brackish-water animals. But the fresh-water animals achieve sodium balance at a lower concentration, and this appears to be due to natural selection of individuals in which the maximum influx rate is faster than the average maximum rate (curve *B* in Fig. 11) in a population of animals living in brackish water. This higher influx rate is illustrated by curve *C* in Fig. 11 where *C* represents the

maximum influx in animals from the Lizard peninsula. Curve *C* also represents the maximum influx rate in animals from the Connie River, Kintyre when this is multiplied by a factor of 1·4 to compensate for the smaller size of these animals. From Fig. 11 it is seen that with a minimum loss rate equal to L_1, this is balanced by the influx rate *C* at an external concentration of 0·1 mM/l.

In the case of *G. duebeni* living in the Connie River where the sodium concentration is less than 1 mM/l., selection of individuals capable of maintaining a high rate of sodium uptake would be particularly severe. That selection of individuals with a high uptake rate is possible was demonstrated by Shaw & Sutcliffe (1961). When a large batch of *G. duebeni* from a brackish-water locality was suddenly transferred to 0·25 mM/l. NaCl at room temperature more than 50% died, and the average influx rate in the most active of the survivors was about 65% higher than the average influx rate in animals very gradually acclimatized to 0·25 mM/l. NaCl by successively lowering the external concentration. Moreover, in the latter case some of the animals also died, so that selection was also operating here.

SUMMARY

1. A quantitative study of sodium influx and loss rates was made on *Gammarus duebeni* obtained from brackish-water localities. Both influx and loss rates were immediately doubled by a rise in temperature from 10 to 20° C.

2. It is estimated that when animals are fully acclimatized to a series of media decreasing from 50 to 2% sea water the rate of sodium uptake at the body surface is doubled to balance the rate of sodium loss, which is also doubled. The increased loss rate is due equally to an increase in the rate of diffusion across the body surface and to loss in hypotonic urine containing about 160–190 mM/l. sodium. Diffusion losses normally account for at least 35% of the total losses, even when the urine is isotonic with the blood.

3. The sodium-transporting system at the body surface is fully saturated at an external concentration of about 10 mM/l. NaCl (2% sea water). The system has a low affinity for sodium ions and is only half-saturated at 1·5–2·5 mM/l. sodium. The overall rate of uptake is increased to its maximum rate to balance sodium losses when in fresh water.

4. When acclimatized to fresh water (0·25 mM/l. NaCl) the sodium loss rate is greatly reduced. This was partly due to a lower rate of diffusion across the body surface following a fall in the blood sodium concentration, and mainly due to elaboration of a very dilute urine.

5. It is suggested that increases in sodium uptake in the antennary glands, resulting in a hypotonic urine, are linked with increases in uptake at the body surface. Both uptake systems are possibly activated by a single internal regulator responding to changes in the blood concentration.

6. Sodium regulation at concentrations below 10 mM/l. NaCl was examined in *G. duebeni* obtained from fresh-water streams on the Lizard peninsula, the Kintyre peninsula, and the Isle of Man. The regulation of sodium uptake and loss is very similar to regulation in brackish-water animals, and the sodium-transporting system has the same low affinity for sodium ions at concentrations below about 10 mM/l.

7. It is suggested that fresh-water localities in north-west Europe, excluding Ireland, have been colonized from brackish water without any modifications in the sodium-regulatory mechanism. But the fresh-water animals tolerate very low sodium concentrations better than brackish-water animals. This is apparently due to natural selection of individuals in which the sodium uptake rate is higher than the average uptake rate in brackish-water animals.

This investigation started as a joint study with Prof. J. Shaw, who made the sodium influx measurements on animals from the Lizard peninsula. I wish to thank him for critical comments on the manuscript of this paper. I also wish to thank Mrs R. Sutcliffe for valuable assistance in the field. The work done at Newcastle and travelling expenses were generously supported by a grant from D.S.I.R.

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