

STUDIES ON SODIUM BALANCE IN *GAMMARUS DUEBENI* LILLJEBORG AND *G. PULEX PULEX* (L.)

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INTRODUCTION

Osmotic regulation in the gammarids, including *Gammarus duebeni* and *G. pulex*, has been previously investigated by Beadle & Cragg (1940*a*) who studied variations in the blood concentration in relation to the concentration of the external medium. *G. duebeni* behaved as a typical brackish-water crustacean in that the blood concentration in normal sea water was only slightly hyperosmotic to the medium, but became progressively more so as the outside concentration was reduced. Below 250 mM/l. the blood concentration remained substantially constant as the external concentration was further reduced, and one feature which distinguished this animal from some other brackish-water forms was the very low external concentration at which a high blood level could be maintained. *G. pulex*, on the other hand, behaved in a manner similar to many fresh-water animals in that the blood concentration rose when the external concentration was increased above the level found in fresh water, and when this exceeded the normal blood concentration the animals were no longer able to survive. Kinne (1952) confirmed these results on *G. duebeni* and, in addition, observed that the animals could be adapted to live in fresh water (Kiel tap water) if the external concentration was gradually reduced. Records of the distribution of *G. duebeni* also indicate the ability of this species to live in fresh water: thus Reid (1939) showed that *G. duebeni* is the dominant fresh-water gammarid in Ireland and Hynes (1954) describes the distribution of the species in fresh-water locations on the West Coast of England and Wales.

Since it has been shown that both in the laboratory and in the field *G. duebeni* is capable of living in fresh water, it is of great interest to see if the osmoregulatory mechanism displayed by the animal when in fresh water is similar to that of the truly fresh-water species, *G. pulex*, or if there are differences which may throw light on the problem of the adaptation of brackish-water animals to fresh water.

Beadle & Cragg (1940*b*) attempted to demonstrate differences by the behaviour of the two species in static distilled water (i.e. in volumes of distilled water changed at intervals). They reported that *G. pulex* survived much longer than *G. duebeni* and that by the end of the experiment the blood concentration of the former was only slightly reduced. They also found that specimens of *G. duebeni* from a fresh-water locality survived in distilled water longer than animals from brackish water, although more extensive experiments of the same kind by Hynes (1954) failed to confirm this.

However, later experiments with *G. pulex* (Beadle & Shaw, unpublished) using continuously running distilled water showed that the animals survived for less than 2 days, and during this time the blood concentration was greatly reduced, being rapidly restored again when the animals were returned to tap water. This pointed to the importance of ion uptake mechanisms in maintaining the normal blood concentration even from the very low concentrations which build up in the static distilled water experiments. This is contrary to the conclusion reached by Beadle & Cragg (1940*b*), who maintained that ion uptake mechanisms were of little importance in the gammarids, although it does not invalidate their suggestion that salt retention may form an important part of the osmoregulatory mechanism. Beadle (1943) suggested that the main factors involved in salt retention were the low permeability of the body surface and the production of a dilute urine, although the active control of salt loss was also envisaged.

The maintenance of the normal blood concentration must result from a balance between the rate of loss of salts, as determined by the factors involved in salt retention, and the rate of active uptake of salts. If these two rates can be measured it is then possible to assess the balance conditions and, hence, the osmoregulatory ability of an animal in low external concentrations can be accurately determined.

Balance conditions, determined in this way, have been reported previously for *Astacus pallipes* (Shaw, 1959*a*) and essentially the same procedure has been adopted to study sodium balance in the two *Gammarus* species. In this paper special attention is paid to a comparison of the properties of the sodium absorbing mechanisms in the two species. Loss rates are measured quantitatively without attempting to evaluate the importance of different factors involved in salt retention.

MATERIALS AND METHODS

Gammarus duebeni was collected from Meggies Burn, a small brackish-water stream in Northumberland, and this is the same locality from which Beadle & Cragg had obtained their specimens. On occasions when the salinity of the stream water was measured it was between 40 and 50% of that of sea water. The animals were maintained in the laboratory in 2% sea water and fed on dead leaves. *G. pulex pulex* was collected from a fresh-water pond at Monkton, Co. Durham (sodium concentration of the water = 0.6 mM/l.) and kept in the laboratory in a 0.3 mM/l. NaCl solution.

In describing the quantitative measurements of rates of uptake and loss of sodium the same terminology has been used as in the previous study of sodium balance in *Astacus pallipes* (Shaw, 1959*a*).

Sodium influx was measured by use of the sodium isotope, ^{24}Na . The tracer was added to the external solution and the radioactivity of the solution recorded continuously in an apparatus similar to that used for *Astacus* (Shaw, 1960). A diagram of the apparatus is shown in Fig. 1. The Perspex animal chamber contained about 10 ml. of water which was circulated through a flow-type Geiger counter (Twentieth-century Electronics, type FW 10). The isotope was introduced into the water with

sufficient non-active sodium chloride to give the desired sodium concentration, and the radioactivity of the solution was continuously recorded by means of a recording ratemeter. For an influx measurement at an external concentration below 1 mM/l. about ten adult specimens (each weighing about 40 mg.) were introduced into the animal chamber. For higher external concentrations the number of animals was increased up to a maximum of thirty in order to maintain conditions where the total internal sodium greatly exceeded the external sodium. The influx was calculated as before (Shaw, 1959*a*). The sodium concentrations of the external solution and of the blood were measured by means of an EEL flame photometer after appropriate dilution of the sample.

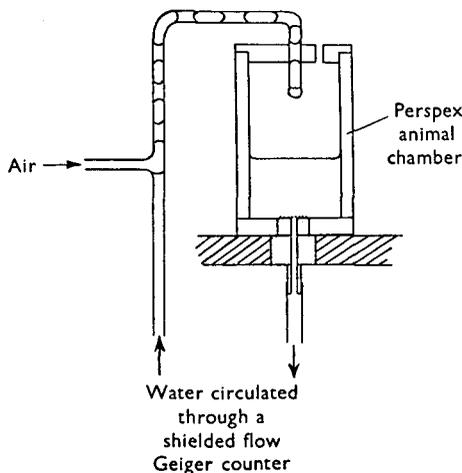


Fig. 1. The apparatus for the measurement of sodium influx.

RESULTS

Gammarus duebeni

(a) Sodium influx

Measurements of sodium influx at different external concentrations for animals from 2% sea water are shown in Fig. 2. The relation between the influx and the external concentration is non-linear and resembles, in certain aspects, that found in *Astacus* (Shaw; 1959*a*, 1960). As the external concentration is increased the influx tends towards a maximum value, indicating that the inward transport of sodium is effected by a rate-limited process. In *Astacus* it was shown that this relationship could be approximated 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. In *Astacus* the value of K_m was between 0.2 and 0.3 mM/l. In *Gammarus duebeni* the same equation can be used: in Fig. 2 the dotted line represents the curve, $\text{influx} = 0.95\{C/(1.5 + C)\}$. An important distinction from *Astacus* may be noted in that the value of K_m (1.5 mM/l.) for *G. duebeni* is about six times greater than that found for the crayfish. This points to the fact that the sodium transporting

system in *Astacus* has a much higher affinity for sodium ions than the corresponding system in *G. duebeni*. It is shown later (p. 10) that *G. pulex* has a transporting system very similar to *Astacus* and that the distinction between the two systems (i.e. between that of *G. duebeni* and *pulex*) is of considerable functional importance.

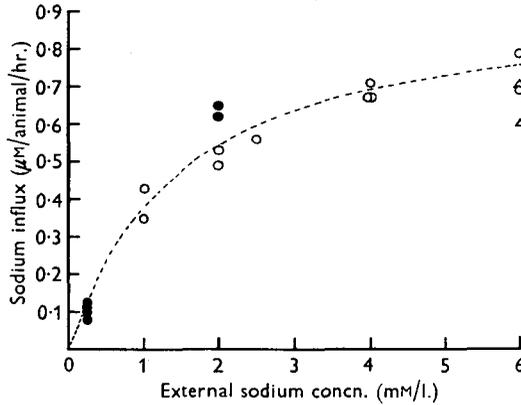


Fig. 2. The relation between sodium influx and the external sodium concentration in *G. duebeni* adapted to 2% sea water. ●, represents measurements made with groups of ten animals in the animal chamber; ○, with groups of twenty and △, with groups of thirty.

The influx measurements for *G. duebeni* were made on animals adapted to 2% sea water with a sodium concentration of about 9 mm/l. It can be seen from Fig. 2 that at this external concentration the influx will be about 0.8 $\mu\text{M/hr.}$ Now if the tracer influx gives a true measure of the rate of uptake of sodium by the animals then, since they were in a steady state at this external concentration, the uptake should be balanced by an equal rate of loss of sodium. Direct measurements of the rate of loss of sodium from animals adapted to 2% sea water confirm the validity of the tracer technique under these conditions.

(b) Sodium loss rate

Sodium loss rate was measured by the rate of increase in sodium concentration of a volume of deionized water into which the animals were placed. For each experiment ten animals were placed in 50 ml. of water and the sodium concentration was measured at intervals over a period of 1–2 hr. During this period the rate of increase of sodium concentration was linear, so that the rate of loss of sodium from the animals could be easily calculated. The results of these measurements are shown in Table 1. The mean rate of loss was 0.76 $\mu\text{M/hr./animal}$ and this was in reasonable agreement with the expected value (0.8 μM) from the influx measurements.

The validity of the tracer measurements is further demonstrated by measurement of the net loss of sodium into solutions of sodium chloride with a concentration lower than 9 mm/l. and from which the sodium influx was already known by previous measurements (see Fig. 2). The results are shown in Fig. 3 where at three different external concentrations (1, 2 and 3 mm/l.) the sum of the net sodium loss

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rate and the influx is approximately equal to the total loss rate in the absence of sodium influx (i.e. the loss rate into deionized water).

In addition, these experiments show that animals adapted to 2% sea water are not in balance at lower concentrations, since there is always a net sodium loss.

Table 1. *The rate of sodium loss in Gammarus duebeni from 2% sea water*

Group no.	Sodium loss rate ($\mu\text{M/hr./animal}$)
1	0.75
2	0.81
2	0.67
4	0.74
5	0.84
Mean	0.76
S.D.	± 0.07

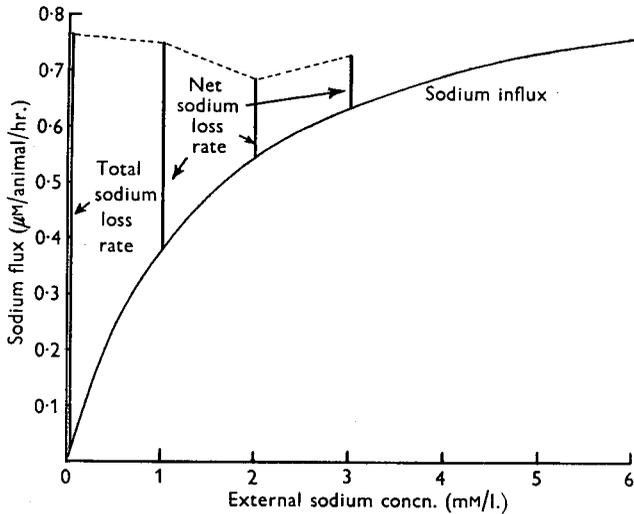


Fig. 3. The net rate of sodium loss at different external concentrations in *G. duebeni* adapted to 2% sea water. The net loss is indicated by the vertical lines. The influx is represented by the smoothed curve from Fig. 2. The dotted line represents the calculated total loss rate.

(c) *Adaptations to low concentration*

Despite the fact that in short-term experiments with animals from 2% sea water, sodium balance cannot be maintained at lower external concentrations, the animals may be gradually adapted to these concentrations. Thus in the laboratory, animals were successfully adapted to very dilute sea water and to sodium-chloride solutions with a sodium concentration of 2 mM/l. and often with a concentration as low as 1 mM/l. A progressively smaller percentage of the animals survived in lower concentrations of sodium chloride and the limit appeared to lie between 0.2 and 0.3 mM/l.

Clearly adaptation to these low concentrations must involve a change in the balance conditions, and must be effected either by an increase in the influx or a decrease in the loss rate, or by a combination of both. These rates were therefore measured in a group of animals which had been successfully adapted to an external sodium concentration of 0.25 mM/l. Measurements of sodium influx and the net sodium movement were made within a range of external concentrations from 0 to 2 mM/l. The results are shown in Fig. 4. Balance was maintained at the concentration to which the animals were adapted (0.25 mM/l.). At higher concentrations (at 0.75 and 1.5 mM/l.) a net uptake of sodium took place, whereas in animals adapted to 2% sea water a net loss occurred at these concentrations. The loss rate (calculated from the difference between the influx and the net sodium movement—represented in Fig. 4 by the dotted line) appeared to increase with increasing external concentration. This was also observed in *Astacus* (Shaw, 1959*a*) and is probably due to an exchange diffusion component in the influx at high net uptake rates.

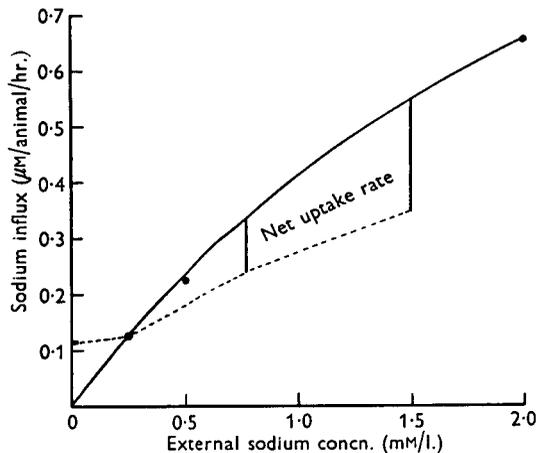


Fig. 4. The relation between the influx and the external sodium concentration, together with measurements of the net sodium uptake in a group of *G. duebeni* adapted to 0.25 mM/l. NaCl.

The balance conditions were clearly different from those found in the animals from 2% sea water and it was important to determine whether this was due to changes in the influx or in the loss rate. The effect of adaptation to a low external concentration on the influx was first considered. In one series of experiments the influx at an external concentration of 0.25 mM/l. was measured for a group of animals adapted to 2% sea water. The group was then adapted to 0.25 mM/l. sodium chloride and the measurement repeated. This was successful in a few cases: in most, however, some of the animals died during the course of adaptation. In the other series of experiments a large group of animals were adapted to the low external concentration and then smaller groups taken from those which had survived. This method had the advantage that many animals were available for the experiment, but introduced an element of selection into the comparison. The results of both series

of experiments are shown in Table 2. A comparison of the mean influx in animals from 2% sea water and from 0.25 mM/l. NaCl shows that the adaptation has involved an increase in the influx of only about 25%. In the case of the adapted animals selected from a large group the increase in the influx was greater (about 65%). However, it cannot be argued that any individual animal had increased its influx by this amount, since the technique may simply have selected out those animals which had a high influx initially. In any event it is clear that the influx level found in the animals adapted to 0.25 mM/l. NaCl is quite insufficient to balance the loss rate, if this was maintained at the same level as it was in the animals from 2% sea water. Thus an increase in the influx is not a major part of the mechanism of adaptation to the lower concentrations.

Table 2. *The effect of adaptation to low external concentrations on the sodium influx in Gammarus duebeni*

Groups adapted to 2% sea water	Sodium influx ($\mu\text{M/hr./animal}$)	Active animals selected from a large group adapted to 0.25 mM/l. NaCl	Sodium influx ($\mu\text{M/hr./animal}$)
1	0.116	1	0.163
2	0.096	2	0.146
3	0.110	3	0.146
4	0.08	4	0.146
6	0.118	5	0.231
Mean	0.102	Mean	0.166
S.D.	± 0.015	S.D.	± 0.037
Groups adapted to 0.25 mM/l. NaCl	Sodium influx ($\mu\text{M/hr./animal}$)		
1	0.126		
2	0.118		
3	0.118		
4	0.143		
Mean	0.126		

The effect of adaptation to a low external concentration on the rate of loss can now be considered. The rate of loss of sodium from animals adapted to 2 mM/l. NaCl and to 0.25 mM/l. NaCl was measured in the same manner as for animals from 2% sea water. The results are shown in Table 3. The reduction in the rate of loss is striking. In animals adapted to 2 mM/l. NaCl the loss rate is one-half of that of animals from 2% sea water and in the animals from 0.25 mM/l. NaCl it is halved again. This is clearly the most important factor in the adaptation of the animals to the low external concentrations.

The mean rate of loss for animals adapted to 0.25 mM/l. NaCl was 0.17 $\mu\text{M/hr.}$, and this was somewhat higher than the measured influx at this concentration found in animals adapted to 2% sea water (Table 2). Thus it would appear that only those animals with a relatively high influx in 0.25 mM/l. NaCl will be able to achieve balance at this concentration and this would seem an adequate explanation of why only a relatively small proportion of the animals survive the adaptation.

Table 3. *The effect of adaptation to low external concentrations on the rate of sodium loss in Gammarus duebeni*

Groups adapted to 2% sea water	Sodium loss rate ($\mu\text{M/hr./animal}$)	Groups adapted to 0.25 mM/l. NaCl	Sodium loss rate ($\mu\text{M/hr./animal}$)
Mean value from Table 1	0.76 \pm 0.07	1	0.14
		2	0.13
Groups adapted to 2 mM/l. NaCl		3	0.13
1	0.23	4	0.18
2	0.58	5	0.14
3	0.42	6	0.20
4	0.37	7	0.21
5	0.46	8	0.20
6	0.24	9	0.16
Mean	0.38	10	0.22
S.D.	\pm 0.13	Mean	0.17
		S.D.	\pm 0.03

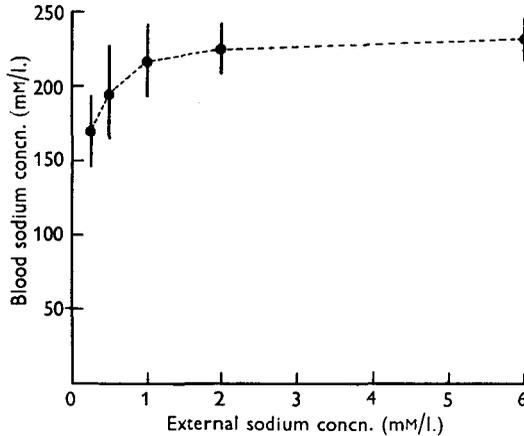


Fig. 5. The relation between the sodium concentration of the blood and the external solution in *G. duebeni*

(d) Blood sodium concentration

It is reasonable to suppose that the rate of loss of sodium is directly proportional to the blood concentration. The level of sodium in the blood is thus a factor concerned in determining the loss rate, and the reduction in the loss rate when the animals are adapted to low external concentrations could follow from large reductions in the blood concentration. Measurements of blood sodium were therefore made on animals which had survived adaptation to various low external concentrations. The results are shown in Fig. 5. A decrease in the external concentration from 6 to 1 mM/l. brought about only a slight change in the blood concentration. At external concentrations below 1 mM/l. there was a steep fall in blood concentration, although at the lowest concentration in which the animals would survive the blood concentration was still nearly 75% of its initial value. Hence a reduction in the blood concentration is not directly responsible for the decrease in loss rate. It

follows, therefore, that the reduction in loss rate must result from either a decrease in the permeability of the body surface or a reduction in the loss of salts through the urine, or a combination of both. It is difficult to decide between these possibilities. It is probable that *G. duebeni* can produce a dilute urine, since the antennal gland is morphologically similar to that of *G. pulex* and in both it differs from that of a marine species such as *G. locusta* (Schwabe, 1933; Hynes, 1954). But it does not follow that this will allow an effective regulation over the loss rate. Even if the urine could be varied between a very dilute and an iso-osmotic solution it would require a very rapid rate of production to produce large changes in the loss rate. Thus if animals from 2% sea water produced an iso-osmotic urine and animals from 0.25 mM/l. NaCl produced a very dilute urine, and if the difference in loss rate between the two (i.e. $0.59 \mu\text{M/hr./animal}$ —from Table 3) is brought about solely by the difference in urine concentration, then the urine must be produced at a rate equivalent to about 7.4% of the body weight per hour. This is a fast rate but not an impossible one. If measurements of urine production rate ultimately show that this rate is not achieved, then one must postulate a mechanism by which the animal can regulate its surface permeability.

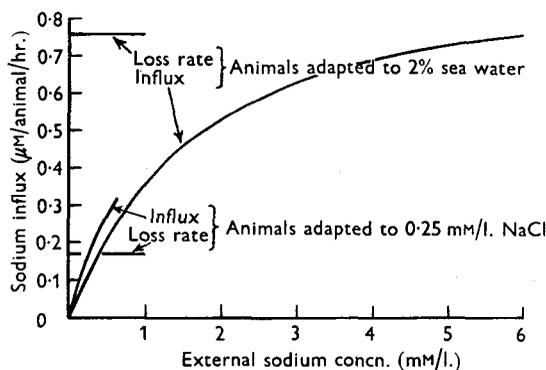


Fig. 6. A summary of the balance conditions in *G. duebeni*. The curves represent the influx for animals adapted to 2% sea water and to 0.25 mM/l. NaCl and the horizontal lines represent the loss rates under these conditions.

(e) Summary of balance conditions

The results of the influx and loss rate measurements on *G. duebeni* are summarized in Fig. 6. This shows that in 0.25 mM/l. NaCl only those animals with an influx above average are able to maintain balance. On the other hand, in 0.5 mM/l. NaCl the majority of animals will maintain balance, although some individuals with a low influx or high loss rate may not. At an external sodium concentration of 1 mM/l. balance can always be achieved, and there is some margin of safety. These conclusions are borne out by the survival of the animals during adaptation and by the measurements of the blood concentration. At external concentrations down to 1 mM/l. survival is good and the blood concentration is maintained. Below this the survival rate becomes progressively poorer and the blood concentration falls rapidly.

Gammarus pulex pulex

(a) Sodium influx

Measurements of sodium influx were made over a range of external concentrations from 0.05 to 2 mM/l. NaCl in animals which had been previously adapted to 0.1 mM/l. NaCl for several days. The specimens were approximately the same weight and size as the specimens of *G. duebeni* and therefore the influx values could be directly compared. The results are shown in Fig. 7. The relation between the

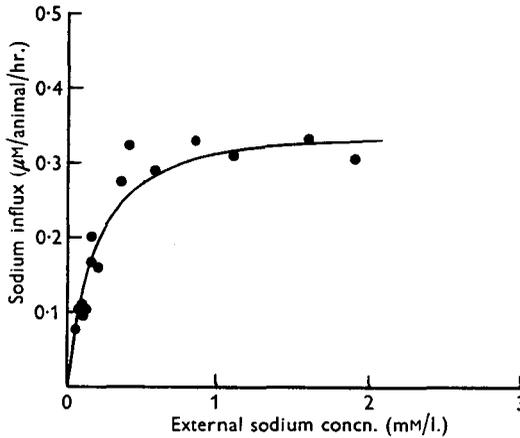


Fig. 7. The relation between the influx and the external sodium concentration in *G. pulex* adapted to 0.1 mM/l. NaCl.

influx and the external concentration has the same general form as that found in *Astacus* (Shaw, 1959a) and in *G. duebeni*. However, there are important differences between the relationship as shown in the two *Gammarus* species which may be listed as follows: (a) the maximum influx for *G. pulex* is less than half that for *G. duebeni*, (b) the maximum influx for *G. pulex* is reached at a much lower external concentration than for *G. duebeni*, and (c) the value of K_m for *G. pulex* (0.15 mM/l.) is much lower than the corresponding value (1.5 mM/l.) for *G. duebeni*. This points to the fact that although the maximum rate of sodium transport is lower in *G. pulex* than in *G. duebeni*, the transporting system in *G. pulex* has a much higher affinity for sodium ions. It follows, therefore, that at the lower external concentrations the uptake rate for *G. pulex* may exceed that for *G. duebeni*. The importance of this fact for the maintenance of sodium balance at low external concentrations is discussed below. The relation between the influx and the external concentration as found for *G. pulex* bears a much closer resemblance to that of *Astacus*, and it is suggested that the high affinity of the transporting system for sodium ions is a special feature of truly fresh-water forms.

(b) Survival in low external concentrations

Gammarus pulex surpasses *G. duebeni* in its ability to survive in low external concentrations, as might be expected from the normal fresh-water habit of the species. Thus *G. pulex* can be readily adapted to sodium chloride solutions with a concentration as low as 0.1 mM/l. and the percentage survival is high. It has been shown that *Astacus pallipes* (Shaw, 1959*a*) and *Potamon niloticus* (Shaw, 1959*b*) could be characterized by the minimum external sodium concentration at which sodium balance could be maintained. A similar situation was found for *G. pulex*. Groups of ten animals were placed in a limited volume (50 ml.) of deionized water and the external concentration allowed to rise until a steady state was reached. The water was then replaced and the procedure repeated until the lowest external concentration at which balance could be maintained was reached. The results of these experiments are shown in Table 4. The mean value was 0.06 mM/l. It may be noted that this value is very similar to that found for *Astacus* (0.04 mM/l.; Shaw, 1959*a*) and for *Potamon* (0.05 mM/l.; Shaw, 1959*b*). This external concentration is sufficiently low to allow the animals to survive in all normal fresh waters found in this country. It is probable that a value around 0.05 mM/l. is characteristic of the true fresh-water Crustacea.

Table 4. *The minimum external sodium concentration for balance in Gammarus pulex*

Group no.	External sodium concentration (mM/l.)
1	0.06
2	0.055
3	0.06
4	0.07
5	0.05
6	0.065
Mean	0.06

G. pulex is thus able to survive in concentrations well below the limit for *G. duebeni*. It is interesting that the limiting concentration for *G. duebeni* is similar to that found for the brackish-water crab, *Eriocheir sinensis* (0.2–0.5 mM/l.; Krogh, 1939; Koch & Evans, 1956). *G. duebeni* resembles this crab in other physiological features too. For example, they both maintain a high blood concentration and are able to survive in full-strength sea water—features which distinguish them from many true fresh-water animals.

(c) Balance conditions at different external concentrations

At any given external concentration balance results from the equality between the rates of sodium uptake and loss, both of which may be variable. In *Astacus*, adaptation to the lower concentrations is brought about largely by an increase in the uptake rate whereas in *G. duebeni*, at concentrations below 2‰ sea water, this is

Table 5. *The effect of adaptation to low external concentrations on the sodium influx and the rate of sodium loss in Gammarus pulex*

Groups adapted to	Sodium influx ($\mu\text{M/hr.}$)		Sodium loss rate ($\mu\text{M/hr.}$)
	From 0.3 mM/l. NaCl	From 0.06 mM/l. NaCl	
0.3 mM/l. NaCl	0.16	—	0.18
0.06 mM/l. NaCl	0.25	—	0.12
0.06 mM/l. NaCl after further sodium loss	—	0.084	0.09

achieved largely by a reduction in the loss rate (p. 7). In *G. pulex* both features were found to be important. Measurements were made of the influx and loss rate in animals adapted to 0.3 mM/l. NaCl and to their minimum equilibrium concentration (0.06 mM/l.). The results are given in Table 5, which shows the influx and loss rate measurements on three groups of animals. The first was adapted to 0.3 mM/l. NaCl, the second to 0.06 mM/l. and the third adapted to 0.06 mM/l. and then subjected to further sodium loss. In the third group the blood concentration was reduced below that of the first two groups, but still required an external concentration of 0.06 mM/l. to maintain balance. It can be seen that where the influx was measured at the balance concentration (groups 1, 3) there was good agreement between the influx and the loss rate. Where the influx was measured at the same external concentration (groups 1, 2) it can be seen that adaptation to the lower concentration had involved an increase in the influx by a factor of 1.5 and a reduction in the loss rate by a similar amount. Additional sodium loss (group 3) led to a further reduction in the loss rate.

The increase in the influx must have been brought about by activation of the transporting system, but the reduction in loss rate might have been due simply to a fall in blood concentration. Therefore measurements of the blood sodium concentration were made on groups of animals which had been similarly treated, and the results are shown in Table 6. The fall in blood concentration was not sufficient to account for the reduction in loss rate so that, as in *G. duebeni*, some other explanation must be sought to account for this phenomenon. Again this might be due to regulation of the urine concentration, but a decision on this must await measurements of urine production rate.

Table 6. *The effect of adaptation to low external concentrations on the blood sodium concentration in Gammarus pulex*

Groups adapted to	Blood sodium concentration (mM/l.)	S.D. \pm mM/l.	n
0.3 mM/l. NaCl	127	16	9
0.06 mM/l. NaCl	113	18.5	8
0.06 mM/l. NaCl after further sodium loss	105	7	8

DISCUSSION

The work of Beadle & Cragg (1940*a*) and Kinne (1952) showed that *G. duebeni* behaves as a typical brackish-water crustacean which can maintain its blood concentration at low external concentrations. It has now been shown that it owes its ability to survive in these low concentrations to the fact that it can reduce the rate of salt loss under these conditions. This ability has allowed the species to extend into at least part of the fresh-water range, although it is still unable to compete with *G. pulex* in its ability to survive in concentrations below 0.5 mM/l. It remains to be discovered whether the mechanism of the reduction of loss rate can be explained solely in terms of the ability to produce a dilute urine, or whether an active mechanism for controlling surface permeability must be looked for.

When *G. duebeni* is adapted to low concentrations the rate of salt loss is not very different from that of *G. pulex*, so that the superiority of the latter species in surviving in low concentrations clearly lies in the special properties of its sodium uptake mechanism. In both species sodium uptake plays a vital role in the maintenance of sodium balance; in the absence of this mechanism sodium is lost from both at a rate which would at least halve the total body sodium in the course of a single day. The sodium uptake mechanisms of *G. pulex* bears many resemblances to that found in *Astacus pallipes*, particularly in that (a) the uptake mechanism has a very high affinity for sodium ions, and the maximum transport rate is reached at an external concentration of about 1 mM/l.; and (b) the rate of uptake is increased by a relatively small drop in the internal sodium content. Apart from the ability of *G. pulex* to reduce the rate of loss in response to a lowered blood concentration (a fact which was not demonstrated in *Astacus*) the mechanisms of sodium balance in the two species have much in common. These features may be characteristic of fresh-water crustaceans in general.

The mechanism of sodium uptake in *G. duebeni*, although basically similar to that of *G. pulex*, shows several important differences upon which the ability to maintain balance at low external concentrations can be seen to depend. The mechanism in *G. duebeni* has a relatively low affinity for sodium ions and, although the maximum transport rate is greater than in *G. pulex*, this rate is not reached until the external concentration is about 10 mM/l. Further, a relatively large fall in blood concentration below 200 mM/l. has little or no effect on increasing the uptake rate.

The importance of the distinction between the two types of mechanism displayed by *G. duebeni* and *G. pulex* can be seen by reference to Fig. 8, where the curves relating influx to external concentration in the two species are compared. It can be seen that whereas above 1 mM/l. the influx in *G. duebeni* may greatly exceed that in *G. pulex*, at external concentrations below this the situation is reversed. At 0.1 mM/l., for example, the influx in *G. pulex* is nearly three times greater than in *G. duebeni*. Moreover, on decreasing the external concentration from 2 to 0.1 mM/l., in *G. pulex* the influx only drops to 40% of its value, whereas in *G. duebeni* it falls to as low as 10%. It is therefore suggested that the high affinity for sodium

displayed by the uptake mechanism of *G. pulex* is an important adaptation to survival in low external concentrations.

The problem of the occurrence of populations of *G. duebeni* in apparently true fresh-water habitats remains to be settled. It must be emphasized that the specimens of *G. duebeni* used in this work were derived from a permanently brackish-water locality, and it has been suggested previously (Beadle & Cragg, 1940*b*) that the animals from fresh-water habitats constitute a physiological variety of the species. This view was questioned by Hynes (1954) who failed to find differences in survival time between animals derived for brackish and fresh water when these were exposed to distilled water. However, it is felt that the possibility could well be re-examined now that more sensitive methods are available. It may be that the fresh-water forms have evolved an uptake mechanism which is closer to the *G. pulex* type. An examination of these animals holds promise of some interesting eco-physiological problems.

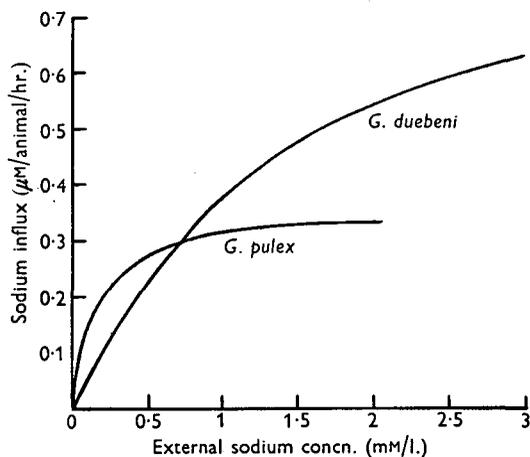


Fig. 8. A comparison between the influx/external concentration curves for *G. duebeni* and *G. pulex*.

SUMMARY

1. The mechanisms of sodium balance in *Gammarus duebeni* and *G. pulex*, adapted to various external concentrations, were compared.

2. *G. duebeni* could be adapted to live in 1 mM/l. NaCl solution and, in some cases, to concentrations down to 0.2 mM/l. *G. pulex* could survive in concentrations as low as 0.06 mM/l.

3. The sodium loss rate in *G. duebeni* adapted to 2% sea water was much higher than in *G. pulex* but was reduced to about the same level when the animals were adapted to low external concentrations.

4. In both species there was a non-linear relationship between sodium influx and the external sodium concentration. In *G. duebeni* the uptake mechanism was saturated at an external concentration of about 10 mM/l., whereas in *G. pulex*

saturation was reached at a much lower concentration. The maximum rate of uptake was greater in *G. duebeni* than in *G. pulex*.

5. In both species adaptation to low concentrations involved a small increase in the sodium influx and a reduction in the loss rate.

6. The most important factor in the superiority of *G. pulex* over *G. duebeni* in surviving at low external concentrations is the high affinity for sodium displayed by the uptake mechanism in *G. pulex*.

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