

SODIUM REGULATION AND ADAPTATION TO FRESH WATER IN THE ISOPOD GENUS *ASELLUS*

By D. W. SUTCLIFFE

*Freshwater Biological Association, The Ferry House,
Far Sawrey, Ambleside, Cumbria LA22 0LP*

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SUMMARY

1. The principal features of the sodium regulatory mechanism are compared in *Asellus communis* Say, *A. aquaticus* (L.) and *A. meridianus* Rac.
2. Water content and total concentrations of sodium and chloride are similar in the three species, but they differ with respect to values for K_{\max} , K_m , the loss rate, and the minimum sodium balance concentration.
3. It is suggested that *A. meridianus*, *A. aquaticus* and *A. communis* represent a natural series of increasing adaptation to fresh water. *A. communis* from North America is completely adapted to fresh water. It has the lowest loss rate, the lowest maximum saturation level (K_{\max}) for sodium influx, and the highest affinity (low K_m value) for sodium ions in the transporting system at the body surface. In many respects *A. meridianus* resembles freshwater populations of *Mesidotea entomon* and *Gammarus duebeni*, and may therefore have had a relatively short history in fresh water.

INTRODUCTION

Physiological adaptation of animals to fresh water involves several mechanisms controlling internal salt and water balance, and these mechanisms have been studied in a wide variety of aquatic animals (Lockwood, 1962; Potts & Parry, 1964). In particular, the process of adaptation to fresh water is now known in considerable detail with respect to sodium regulation in decapod and gammarid crustaceans (Beadle & Cragg, 1940; Shaw, 1961; Lockwood, 1965; Sutcliffe, 1968, 1971 *a, b, d*, 1974). In these two groups of malacostracans the principal features of adaptation are strikingly similar. The maintenance of a high blood concentration relative to the outside medium (hyperosmotic regulation) is achieved by a progressive reduction in the permeability of the body surface to salts, thus reducing salt loss, and by the acquisition of ion transporting systems with a higher affinity for specific ions. For example, the increase in affinity for sodium in the transporting system sited in the gills is reflected in a lower value for K_m , the external concentration at which sodium influx reaches its half-saturation value. Thus a lower K_m value is associated with an increased capacity for active sodium uptake at very low external concentrations. In addition, salt retention in the face of continuous water uptake is commonly assisted by regulating or curtailing water intake via the gut and by renal salt-reabsorption to produce a dilute urine. Further assistance in regulating internal salt and water balance may be obtained by lowering the blood concentration, thereby reducing the gradients

for both active and passive movements of ions and water between the animal and its external medium, and this final step may be accompanied by a reduction in the saturation rate (K_{\max}) of, for example, the sodium transporting system. An animal which has all of these features is regarded as being completely adapted to fresh water, and it may be noted that, singly or in combination, these features help to maintain an optimum internal environment for the tissue cells. Adaptations in regulatory mechanisms at the cellular level are largely acquired when adapting to brackish-water habitats as a prelude to colonization of fresh water, at least in the Malacostraca.

Since the process of adaptation to fresh water in both decapods and gammarids has involved the same sequence of changes in salt and water regulatory mechanisms, it is highly probable that other crustaceans, such as the Isopoda, will have adapted in the same way. Recent studies on *Mesidotea* (Croghan & Lockwood, 1968), *Jaera* (Jones, 1972; Forbes, 1974) and *Sphaeroma* (Harris, 1972) corroborate this view. It was decided therefore, to make a comparative study of sodium regulation in *Asellus* in order to evaluate the relative degree of adaptation to fresh water in the two common species *A. meridianus* Rac. and *A. aquaticus* (L.). The historical status of these two species in fresh water is controversial, but it is thought that *A. meridianus*, widespread in Britain and Ireland, is now being replaced by *A. aquaticus* as the latter extends its range northwest across the British Isles (Williams, 1962, 1963; Hynes & Williams, 1965). The present comparative study also includes *A. communis* Say, a North American species which occurs in one isolated locality in Northumberland (Sutcliffe, 1972; Williams, 1972). Osmotic and ionic regulation has been investigated previously in *A. aquaticus* (Heuts, 1943; Lockwood, 1959*a, b*, 1960).

MATERIALS AND METHODS

A. meridianus was collected from ponds and lakes in the English Lake District and in Northumberland. *A. aquaticus* was collected from Kendal Canal and from ponds at Ferry House. *A. communis* was collected from Bolam Lake in Northumberland.

In the laboratory, the animals were usually acclimated in Windermere lake water containing about 220 μM Na/l; occasionally the sodium concentration was raised by the addition of NaCl. Animals were fed on sycamore and elm leaves, but they were starved for 24 h prior to each experiment. For the influx determinations and some loss rate measurements, animals were acclimated in NaCl solutions made with deionized water. Where seawater media were used, these were made by diluting sea water with deionized water.

Sodium influx was determined by continuously recording the change in radioactivity of NaCl media containing $^{22}\text{NaCl}$ as the tracer (Shaw & Sutcliffe, 1961). Measurements were made on groups of animals over a period of 30–60 min. The techniques and associated experimental procedures used here on *Asellus* were the same as those used on *Gammarus* (Sutcliffe, 1967*a*; 1971*a, b*).

RESULTS

(1) *Haemolymph and total body ion concentrations*

The haemolymph sodium concentration (Na_{in}) was 100 mM/l in a pooled sample obtained from 10 specimens of *A. communis*. In a similar pooled haemolymph sample

Table 1. Water content and total concentrations of sodium and chloride in three species of Asellus

Species	No. of Groups	Mean wet weight ± s.e. (mg)	Water content (% wet wt.)	Mean total ion concentration ± s.e. (mm/kg body H ₂ O)			Na/Cl	Starved (days)	Source
				Na	Cl				
<i>A. aquaticus</i>	6	48.9 ± 1.3	75.8	91.2 ± 2.6	50.7 ± 1.8	1.8	0	Windermere	
	6	23.1 ± 1.9	78.3	96.1 ± 3.7	47.9 ± 2.7	2.0	0	Windermere	
	6	50.9 ± 4.3	74.3	70.8 ± 4.8	37.9 ± 3.1	1.9	4	Windermere	
	4	21.0 ± 0.6	80.9	111.2 ± 3.5	55.9 ± 2.9	2.0	9	Windermere	
	6	20.1 ± 1.3	83.3	107.8 ± 2.6	59.5 ± 2.8	1.8	26	Windermere	
	—	—	—	—	109.6	66.2	1.7	—	Calculated from Lockwood, 1959a, b
<i>A. meridianus</i>	5	14.3 ± 0.3	79.0	97.3 ± 5.6	54.2 ± 3.6	1.8	0	Bassenthwaite	
	6	16.7 ± 1.8	80.5	95.2 ± 9.1	65.3 ± 15.0	1.6	0	Brathay	
<i>A. communis</i>	6	31.0 ± 1.2	79.0	109.3 ± 2.5	58.4 ± 2.8	1.9	0	Bolam	
	3	17.7 ± 1.2	79.2	107.2 ± 2.5	51.2 ± 3.4	2.1	9	Bolam	
	4	24.1 ± 1.6	77.7	104.6 ± 1.2	64.0 ± 1.0	1.6	19	Bolam	
	2	—	78-82	168-203	179-180	1.0	—	Croghan & Lockwood, 1968	

Table 2. Ion spaces for body sodium and chloride, expressed as a percentage of body water content in three species of *Asellus*

Species	Remarks	ion, mm/kg body H ₂ O × 100	
		haemolymph ion, mm/l	
		*Na	†Cl
<i>A. communis</i>	Fed	80-109	47-58
<i>A. meridianus</i>	Fed	70-96	48-60
<i>A. aquaticus</i>	Fed	68-94	39-49
<i>A. aquaticus</i>	Starved 26 days	79-108	48-60
<i>A. aquaticus</i>	Lockwood, 1959a	80	53

* Na_{in} = 137-100 mm/l.† Cl_{in} = 125-100 mm/l.

taken from *A. aquaticus*, Na_{in} was 95 mm/l. This value for Na_{in} in *aquaticus* is lower than Lockwood's (1959a) estimate of 137 mm/l for Na_{in} and 125 mm/l for Cl_{in}, but Heuts (1943) gives values for Cl_{in} ranging between 75-109 mm/l. From the investigations of both Heuts and Lockwood it appears that haemolymph concentrations may vary considerably with changes in temperature and season of the year, although the low haemolymph sodium concentrations found in the present study may also be correlated with the low ion concentrations in the lake water (Sutcliffe, 1970).

Water content, and the total ion concentrations for sodium and chloride are given in Table 1. Water content was similar in the three species of *Asellus*, representing 78-83 % of mean wet weights ranging between 14-31 mg, but only 76 and 74 % of mean wet weights 49 and 51 mg respectively. Starvation apparently had little effect on total water content in *aquaticus* and *communis*. The total amounts of both sodium and chloride, expressed in terms of body water content, were very similar in the three species, but in all cases sodium exceeded chloride by 60-100 %. At least some of the excess sodium may be located, as sodium urate, in the cells of Zenker's organ (Lockwood, 1959b), although other freshwater crustaceans also have an excess of extra-haemolymph sodium (Sutcliffe, 1971c). In *aquaticus*, the concentrations of total sodium and chloride were rather variable between different groups, although all of the results shown in Table 1 were obtained at the same time, in November 1973. In general, the concentrations of both sodium and chloride were low in *aquaticus* with a low body water content, and high in *aquaticus* with a high body water content. Table 2 shows the total ion concentrations expressed as the proportion of body water occupied by each ion at the concentration found in the haemolymph. It is clear that a considerable proportion of the total body sodium must be located in the tissues.

(2) Sodium influx

Sodium influx was stimulated to its fastest rate by acclimating animals to very low external sodium concentrations. Prior to, and in between influx measurements, groups of *aquaticus* (average wet weight 20 mg) and *meridianus* (average wet weights 12 and 18 mg) were acclimated to 0.1 mm/l NaCl, and groups of *communis* (average wet weight 40 mg) were acclimated to 0.075 mm/l NaCl. Sodium influx was determined on groups of 10-14 animals at external sodium concentrations (Na_{out}) ranging from 0.1 to 4.0 mm/l at 20 °C. Some of the results are shown in Fig. 1. With *aquaticus* and *meridianus* at Na_{out} > 2 mm/l, measurements of sodium influx were inaccurate

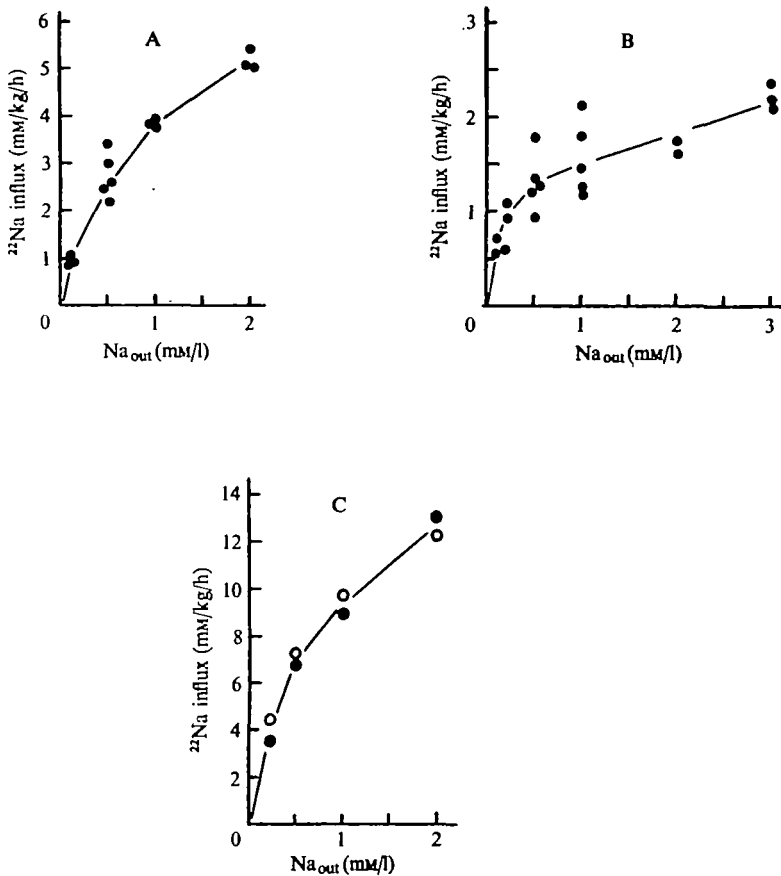


Fig. 1. The relationship between the external sodium concentration and sodium influx in (A) *A. aquaticus*; (B) *A. communis*; (C) *A. meridianus*. In (A) and (B) each point in the figure represents a measurement made on a group containing 20–45 animals. In (C), \circ represents one group of 24 animals (mean wet weight = 18 mg); \bullet represents the mean of three groups (mean wet weight = 12 mg).

because net uptake of sodium by large groups of animals progressively lowered Na_{out} ; determination of the influx by the method employed here requires that Na_{out} should remain relatively constant during the period of measurement (Shaw, 1959). Influx measurements at the higher concentrations were therefore discarded. Nevertheless, they did indicate that when $\text{Na}_{\text{out}} > 2$ mm/l the influx continued to increase above the levels shown for *aquaticus* and *meridianus* in Fig. 1 A and 1 C, whereas in *communis* the influx was approaching saturation level at a Na_{out} of 3 mm/l (Fig. 1 B). Since the influx apparently displays saturation kinetics, as in other crustaceans, it was assumed that the relationship between sodium influx and the external sodium concentration can be described by a curve derived from the Michaelis-Menten equation; $\text{influx} = K_{\text{max}} C / K_m + C$, where K_{max} = maximum influx K_m = external sodium concentration at which the influx is half-saturated, $C = \text{Na}_{\text{out}}$ (Shaw, 1959; Shaw & Sutcliffe, 1961; Sutcliffe, 1968; Stobbart, 1974). Values for K_{max} and K_m were obtained from a Lineweaver-Burk plot of the reciprocal of sodium influx against the reciprocal of Na_{out} (Fig. 2), where it is seen that the reciprocals of influx measurements for each

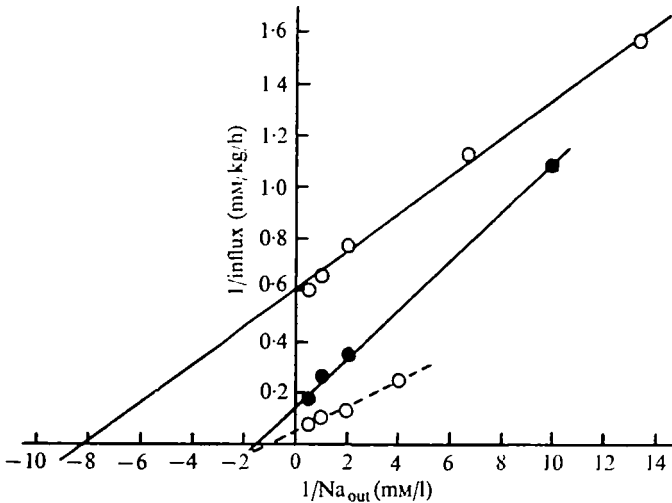


Fig. 2. Lineweaver-Burk plot of sodium influx in *A. communis*, ○—○; *A. aquaticus* ●—●; *A. meridianus* ○--○. The intercept on the ordinate = $1/K_{\max}$, and on the abscissa = $1/K_m$, in the Michaelis-Menten equation, $\text{influx} = K_{\max} C / K_m + C$ (see text). Values for K_{\max} are given in Table 3.

Table 3. Values for K_m and K_{\max} in three species of *Asellus*

Species	K_m (mmol/l)	K_{\max} (mmol/kg/h)
<i>A. meridianus</i>	0.91	18.2
<i>A. aquaticus</i>	0.62	6.5
<i>A. communis</i>	0.12	1.7

species fall on reasonably straight lines. The appropriate values derived from Fig. 2 are given in Table 3. Two points emerge clearly from Table 3. First, the values for K_m in *meridianus* and *aquaticus* are much higher than the value for K_m in *communis*. Second, the saturation rate for sodium influx in *aquaticus* is about four times greater than in *communis*, and the rate in *meridianus* is about ten times the saturation rate in *communis*.

(3) Sodium loss

(a) Sodium losses in the urine

An attempt to estimate sodium loss in the urine was made by comparing sodium losses in deionized water and in an isosmotic solution of sucrose in deionized water. For this, five groups each containing 30 *aquaticus*, average wet weight 24 mg, were acclimated to 2% sea water at 9 °C. Sodium losses over a period of 1 h were determined for each group in deionized water and, after a 2 h recovery period in 2% sea water, sodium losses were then determined in isosmotic sucrose. The same procedure was used on these animals following acclimation in 10% sea water at 20 °C, and acclimation in 2% sea water at 20 °C. The results are given in Table 4. In a *t*-test there were no significant differences between sodium loss rates in isosmotic sucrose compared with deionized water; values for *P* are given in Table 4. Using the same method, Croghan & Lockwood (1968) reported negligible sodium loss in the urine of *Mesidotea entomon* (L.). However, in the case of *aquaticus* acclimated to 10% sea water at 20 °C, if the difference of 0.58 mmol/kg/h (Table 4) was in fact due entirely to sodium loss in urine,

Table 4. Mean sodium loss rates from five groups of *A. aquaticus* in deionized water and isosmotic sucrose at 9 °C and 20 °C

Acclimation medium	Temp. for loss rate (°C)	Mean sodium loss \pm S.E. (mm/kg/h)		Difference (urine)	P
		Deionized water	Sucrose		
2% sea water	9	1.60 \pm 0.07	1.48 \pm 0.07	0.12	> 0.2
10% sea water	20	2.75 \pm 0.22	2.17 \pm 0.05	0.58	> 0.05
2% sea water	20	1.95 \pm 0.10	1.91 \pm 0.15	0.04	> 0.2

Table 5. Mean sodium loss rates in *Asellus* acclimated to a range of sodium concentrations at 20 °C

Species	Acclimation concentration (mM Na/l)	Sodium loss into deionized water (mm/kg/h)
<i>A. communis</i>	2.0	0.65 (3)
	0.6	0.60 (5) \pm 0.04
	0.075	0.26 (3)
<i>A. aquaticus</i>	25.0	2.40 (2)
	10.0	2.53 (5) \pm 0.14
	2.0	2.10 (4) \pm 0.05
	0.6	0.90 (5) \pm 0.14
	0.25	1.27 (5) \pm 0.11
<i>A. meridianus</i>	10.0	3.30 (1)
	0.7	2.67 (3)
	0.2	1.70 (1)
	0.1	1.73 (2)
	0.1	1.92 (1)

then the concentration of that urine would be 56–70 mM/l, assuming a daily urine flow rate equivalent to 20–25 % body weight (Lockwood, 1962; Potts & Parry, 1964). This estimated urine sodium concentration in *aquaticus* is similar to the urine concentrations found in *Gammarus pulex* acclimated to dilute sea water (Lockwood, 1961). From Table 4 it also appears likely that sodium loss in the urine probably represents less than 10 % of the total sodium loss in animals acclimated to media containing less than 10 mM/l sodium. Hence sodium loss from *Asellus* in deionized water may be regarded as almost entirely due to extrarenal net sodium movement across the body surface.

(b) Sodium loss rates at 20 °C

On various occasions sodium loss rates were determined in deionized water at 20 ± 1.5 °C, using animals acclimated at 20 °C to media with Na_{out} ranging from 0.075–25 mM/l. The average wet weights of these animals were: *communis* 40 mg, *aquaticus* 20 mg, *meridianus* 18 mg. The results are compared in Table 5. In each species, sodium loss rates tended to increase when animals were acclimated to the higher sodium concentrations. But there was also a specific difference that is particularly noticeable in animals acclimated to $\text{Na}_{\text{out}} < 1$ mM Na/l, representing the range of sodium concentrations normally encountered in fresh water. Here, *A. communis* had the lowest sodium loss rates (0.3–0.6 mm/kg/h). By comparison, in *aquaticus* the loss

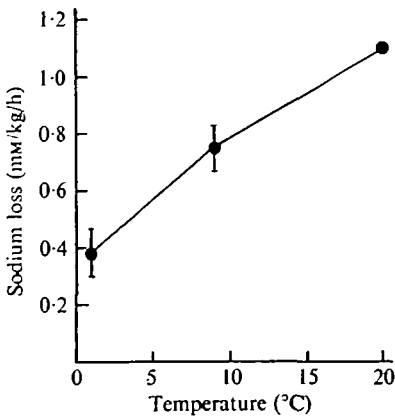


Fig. 3

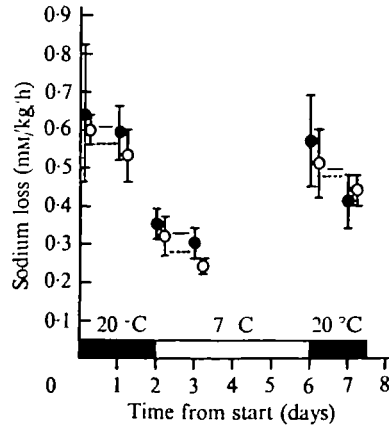


Fig. 4

Fig. 3. Net sodium loss in deionized water at three temperatures in *A. aquaticus* acclimated to Windermere lakewater at 9 °C. Each point is the mean of 4 groups; each group contained 20 animals. The vertical lines indicate the extent of the standard errors of the means.

Fig. 4. Net sodium loss in deionized water at 20 °C and 7 °C in *A. aquaticus*, ●, and *A. communis*, ○. Each point represents the mean of 5 groups \pm standard errors (vertical lines). The horizontal lines represent the combined mean values for sodium loss at each temperature, — *A. aquaticus*, ····, *A. communis*. Bars on the abscissa show the temperature regime experienced by the animals during a 7-day experiment.

rates were approximately twice as fast (0.9–1.3 mm/kg/h, and in *meridianus* they were roughly doubled again (1.7–2.7 mm/kg/h). These specific differences were also noted at lower temperatures (e.g. Table 6).

Table 6. *The effect of low temperature on sodium loss rates in Asellus acclimated to 0.7 mM/l sodium at 12 °C*

Species	<i>A. communis</i>	<i>A. aquaticus</i>	<i>A. meridianus</i>
Sodium loss at 12 °C (mm/kg/h)	0.40 (2)	0.86 (1)	1.51 (2)
Initial sodium loss at 1 °C (% of loss rate at 12 °C)	26	99	52
Sodium loss after 24 h at 1 °C (% of loss rate at 12 °C)	26	53	43

(c) *Effect of an abrupt change in temperature on sodium loss rates*

In *aquaticus* acclimated to 2 ‰ sea water (10 mM/Na/l) at 9 °C the sodium loss rates were 22–29 ‰ faster at a temperature of 20 °C compared with the rates at 9 °C (Table 4). The mean wet weight of these animals was 24 mg. An even greater increase in the loss rate at 20 °C was found in *aquaticus* with a mean wet weight of 42 mg after acclimation to lake water containing 0.25 mM Na/l. On the first day, sodium loss rates were determined in deionized water at 9 °C, followed on successive days by measurements at 1 °C and at 20 °C respectively. For this, the animals were first washed in deionized water at 9 °C for a period of *c.* 5 min. They were then transferred to deionized water at either 1 °C or 20 °C, washed for *c.* 5 min, and the net loss of sodium was then determined during the next 60 min. The results from four groups of animals are shown in Fig. 3. Compared with the loss rate at 9 °C, there was a 51 ‰ reduction

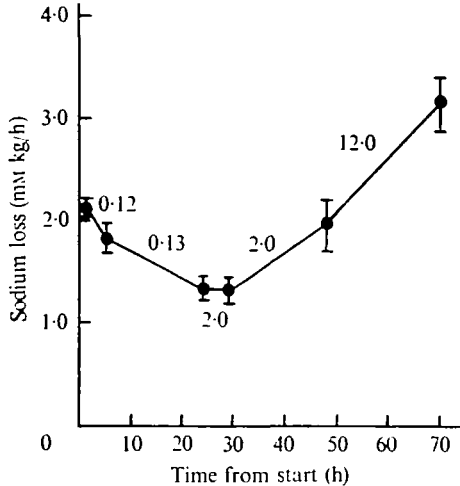


Fig. 5. Net sodium loss in deionized water at 20 °C in *A. aquaticus*. Each point is the mean of four groups \pm standard errors (vertical lines). Values given in the figure refer to the external sodium concentration at which the animals were held in between each loss determination. Further explanation in the text.

in the loss rate at 1 °C and a 47% increase in the loss rate at 20 °C. In another experiment, five groups of *aquaticus* (mean weight = 15 mg) and five groups of *communis* (mean weight = 16 mg) were acclimated to lake water containing 0.7 mM Na/l at 20 °C. Loss rates in deionized water were then determined at temperatures of 20 °C and 7 °C as indicated in Fig. 4. The results show that at 7 °C the combined mean sodium loss rates of both *aquaticus* and *communis* were reduced to approximately one half of the combined mean rate found initially at 20 °C. Following a 4-day period of acclimation at 7 °C, the combined mean sodium loss rates of both species at 20 °C were 80–84% of the initial loss rates at that temperature (Fig. 4). In a similar experiment, two groups of *communis*, two groups of *meridianus*, and one group of *aquaticus* were acclimated to lake water containing 0.7 mM Na/l at 12 ± 2 °C. Sodium loss rates were measured at 12 °C, and on the following day at 1.0 ± 0.5 °C. After holding the animals for 24 h at 1 °C, in lake water, sodium loss rates were measured, for the third time. The results, expressed as a percentage of the loss rates at 12 °C, are shown in Table 6. In this particular experiment *aquaticus* did not respond immediately to the abrupt changes in temperature, although the loss rate was reduced by 47% after 24 h exposure to 1 °C. However, both *communis* and *meridianus* did respond immediately; in *communis* there was a very marked reduction to only 26% of the rate at 12 °C (Table 6).

(d) Effect of the external sodium concentration on sodium loss rate

From both Table 4 and Table 5 it appears that the rate of sodium loss from *Asellus* usually increases when animals are acclimated to an increased level of sodium in the external medium. The following experiment, carried out at 20 °C, illustrates this point more clearly (Fig. 5). Four groups of 30 *aquaticus* (mean weight = 19 mg) were acclimated to a 2 mM/l solution of NaCl. Each group was then placed in 30 ml deionized water and the loss rates were determined during the first 60 min. After 90 min

the external sodium and chloride concentrations were determined; the mean value for Na_{out} was $55.5 (4) \pm 2.9 \mu\text{M/l}$ (S.E.) and the mean value for Cl_{out} was $54.8 (4) \pm 4.1 \mu\text{M/l}$ (S.E.). Apparently sodium loss from *Asellus* is accompanied by an equal amount of chloride. The animals were left in this dilute sodium chloride solution for a total period of 5 h, when Na_{out} had risen to a mean value of $119.8 (4) \pm 7.4 \mu\text{M/l}$ (S.E.). Each group was then transferred into 50 ml deionized water and the sodium loss rate was measured again. The loss rate had been reduced to 86 % of the initial rate (Fig. 5). The animals continued to lose sodium until Na_{out} was raised to a mean value of $130.0 (4) \pm 10.3 \mu\text{M/l}$ (S.E.). 20–30 % of the animals died during this second period of sodium loss. The remainder were again transferred to 50 ml deionized water; the sodium loss rate was now only 63 % of the initial rate (Fig. 5). After 90 min in deionized water, each group of *aquaticus* was placed in a 2 mM/l NaCl solution for a period of 3 h, followed by another determination of the sodium loss rate in deionized water. The loss rate had not altered, but after a further period of 19 h in 2 mM/l NaCl it rose to 93 % of the initial rate. These animals were subsequently placed in 12 mM/l NaCl for 22 h, and the loss rate then increased to 150 % of the initial rate found when acclimated to 2 mM/l NaCl (Fig. 5).

The most likely explanation for the changes in sodium loss rate observed in the above experiment is that the loss rate is altered in response to internal changes following a net loss or net uptake of sodium (and chloride). This has been observed previously in a number of crustaceans (Bryan, 1960; Lockwood, 1964, 1965; Sutcliffe, 1967*a, b*). The following section shows how the sodium loss rate in *Asellus* changes when the total body sodium content is reduced.

(e) Relationship between sodium loss and body sodium content

Experiments described in this section were carried out at 20 ± 1.5 °C. Five groups of *aquaticus* (mean weight = 24 mg) acclimated to 2 ‰ sea water were each placed in 50 ml deionized water for three successive 90 min periods. Sodium loss rates were measured during the first 60 min in each period. The mean loss rates found in the second and third periods were then expressed as a percentage of the initial mean loss rate found during the first period. At the end of each 90 min period the amount of sodium lost from each group was measured, and this amount was subtracted from the total body sodium content (Na_T) of the animals in each group. The reduced value for Na_T was then expressed as a percentage of the initial value for Na_T ; the latter was derived from estimates based on data given in Table 1. In this way it was found that when Na_T was reduced to 94 % of the initial amount, the sodium loss rate fell to 80 % of the initial rate. Similarly, after a further reduction in Na_T to 89 % of the initial amount, the loss rate fell to 70 % of the initial rate. Following a subsequent, prolonged period of net sodium loss in a large volume of deionized water, Na_T was reduced to 58 % of the initial amount and the sodium loss rate fell to only 25 % of the initial rate found in the same animals at the start of the experiment. The results of this experiment are shown in Fig. 6A, together with results from two similar experiments on *aquaticus* acclimated to 5 ‰ sea water and to lake water containing 0.25 mM Na/l. Fig. 6B shows a further series of more detailed measurements made on three groups of *aquaticus*.

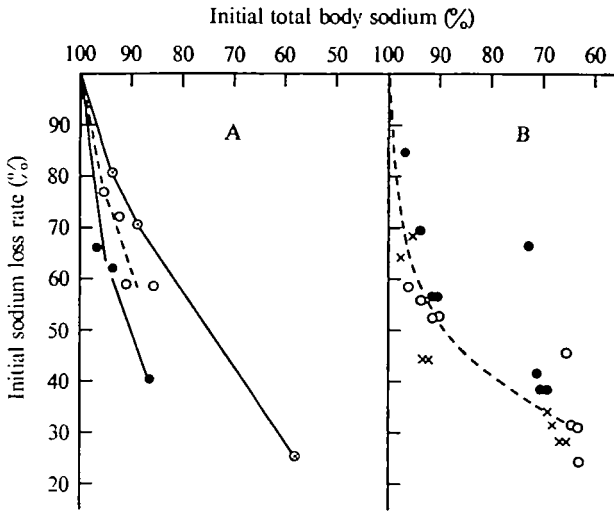


Fig. 6. Net sodium loss in deionized water at 20 °C in *A. aquaticus* acclimated to: (A), 5% sea water, means of two groups, O--O; 2% sea water, means of five groups, ⊙-⊙; lakewater (0.25 mM Na/l), means of four groups ●-●; (B), lakewater (0.25 mM Na/l), three separate groups, curve fitted by eye. Further explanation in the text.

(4) *The minimum sodium balance concentration*

It was shown above that when *Asellus* is exposed to a continuing net loss of sodium, the rate of sodium loss is progressively diminished. However, a continuing net loss of sodium is an experimental artifact induced either by the removal of sodium from the external medium or by keeping the animal in a relatively large volume of medium so that Na_{out} remains below a certain minimum value. This minimum value represents the external concentration at which the sodium uptake mechanism, when stimulated to work at its fastest rate, can just balance sodium losses, so that there is no net movement of sodium between the animal and the external medium. The minimum balance or steady state concentration is a very useful and easily determined measure of the relative capacity of the ion regulating mechanism to operate at low external concentrations of particular ions. It also provides an estimate of the lowest concentration that can be tolerated by an animal; a natural population would not be expected to occur where the ion concentration is below the minimum balance concentration. To determine the minimum sodium balance concentration it is first necessary to remove some 5–10% of the internal body sodium by repeated salt depletion in a limited volume (50–100 ml) of deionized water. On each occasion Na_{out} is allowed to rise to a steady state concentration, normally attained in about 24 h. The salt-depletion process is repeated until a more or less constant value for Na_{out} is obtained at the steady state (Shaw, 1959; Shaw & Sutcliffe, 1961; Sutcliffe, 1968). This was done with *communis*, *aquaticus* and *meridianus*, using three groups of each species acclimated at 12 °C. Mean values, with standard errors, for the minimum sodium balance concentrations in the external medium, were $6.5 \pm 1.0 \mu\text{M/l}$ (*communis*), $12.8 \pm 2.9 \mu\text{M/l}$ (*aquaticus*), $15.8 \pm 1.2 \mu\text{M/l}$ (*meridianus*). These values for Na_{out} increased slightly during a further 24 h at 12 °C (Table 7, column 1). The temperature of the external medium was then lowered, over a 30 min period, to 1.5 ± 0.5 °C, and Na_{out} monitored at

Table 7. *Minimum sodium balance concentrations ($\mu\text{M/l}$) for Asellus at temperatures of 12 °C and 1.5 °C. Mean values \pm S.E. for three groups*

Duration of exposure to temperature (h)	24	48	72
Temperature (°C)	12	1.5	12
<i>A. communis</i>	7.0 \pm 1.53	7.7 \pm 1.86	9.0 \pm 3.47
<i>A. aquaticus</i>	13.7 \pm 2.97	17.7 \pm 4.10	20.3 \pm 3.18
<i>A. meridianus</i>	17.0 \pm 1.00	22.3 \pm 0.88	26.0 \pm 2.00

Table 8. *Minimum sodium balance concentrations ($\mu\text{M/l}$) for Asellus at temperatures of 7 °C and 20 °C. Mean values \pm S.E.*

Duration of exposure to temperature (h)	60	24	24
Temperature (°C)	7	20	7
<i>A. communis</i>	13.3 (6) \pm 0.56	13.8 (6) \pm 1.01	13.3 (6) \pm 1.05
<i>A. aquaticus</i>	22.8 (5) \pm 3.81	30.6 (5) \pm 5.14	29.6 (5) \pm 4.97
<i>A. meridianus</i>	23.0 (5) \pm 5.50	35.6 (5) \pm 5.80	30.2 (5) \pm 4.25

intervals during the next 48 h. In two groups of *meridianus* there was a small increase in the balance concentrations during the first 24 h exposure to the low temperature, and all groups of *aquaticus* and *meridianus* had increased the minimum balance concentrations by an average of 30% at the end of the 48 h period at 1.5 °C (Table 7, column 2). The temperature of the external medium was then raised back to 12 °C (over a 30 min period) and after 72 h reacclimation to this temperature the balance concentrations were again determined. In one group of *communis* there was a slight decrease in Na_{out} . In one group from each species there was no change in Na_{out} , and in the remainder there was a slight increase, so that the mean values for Na_{out} had again increased by about 17% (Table 7, column 3). A *t*-test for significance was made on the differences between the mean values for Na_{out} within each species. In *meridianus* the difference between Na_{out} at 12 °C compared with 1.5 °C was significant ($P < 0.05$). Other values for Na_{out} within each species were not significantly different, but it seems reasonable to conclude that Na_{out} showed a general tendency to rise during the 114 h period of the measurements. Possibly the values for Na_{out} given in column 3 represent the minimum balance concentrations for a true steady state at 12 °C rather than the values given in column 1 (Table 7). In another experiment, using new batches of animals divided into five or six groups, very similar values for the minimum sodium balance concentrations were found at 7 °C (Table 8, column 1). The temperature was then raised to 20 °C, and in 24 h Na_{out} had increased for both *aquaticus* and *meridianus* (Table 8, column 2) although the intraspecific differences were not significant ($P > 0.05$). When the temperature was lowered back to 7 °C there was a small decrease in Na_{out} for *meridianus* (Table 8, column 3).

Two main conclusions emerge from these experiments. First, the minimum sodium balance concentration for both *aquaticus* and *meridianus* is approximately 20–30 $\mu\text{M/l}$, whereas *communis* can achieve a steady state at about 9–13 $\mu\text{M/l}$ or one half the concentration required by the other two species. Second, the minimum balance concentration for *communis* is not affected by temperature changes in the range 1–20 °C, and these had only a small effect, if any, on *aquaticus* and *meridianus*.

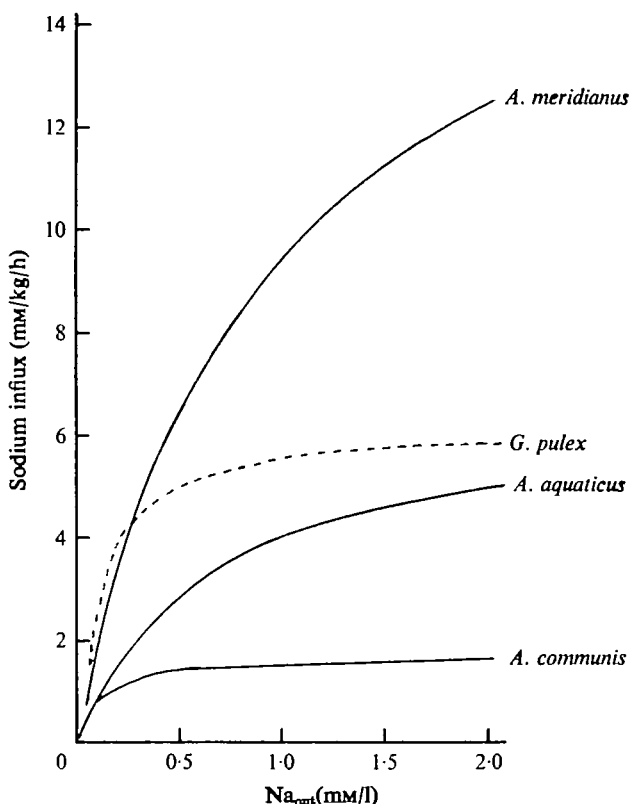


Fig. 7. A comparison of sodium influx at 20 °C in three species of *Asellus* and in *Gammarus pulex*. The curves are derived from the Michaelis-Menten equation, $\text{influx} = K_{\text{max}} C / K_m + C$, where $C = \text{Na}_{\text{out}}$ (see text). Values for K_{max} and K_m in *Asellus* were taken from Table 3. For *G. pulex*, $K_{\text{max}} = 6.2 \text{ mm/Kg/h}$, $K_m = 0.12$ (Sutcliffe, 1968). The curves represent the fastest or maximum influx in each species.

DISCUSSION

Some of the results on *Asellus* are summarized and compared with results on other isopods in Table 9 and Fig. 7. Compared with the two common British isopods *A. meridianus* and *A. aquaticus*, it is clear that the North American *A. communis* has a sodium regulatory mechanism which is better adapted to deal with very low sodium concentrations in fresh water. This is evident from the fact that *A. communis* has lower values for no less than four principal features of the regulatory mechanism, viz. K_m , K_{max} , the sodium loss rate, and the minimum sodium balance concentration. In *A. communis* the K_m value for sodium influx (0.12 mm/l) is identical with the K_m value in the British freshwater amphipods *Gammarus pulex* and *G. lacustris* and lower than the K_m value of 0.23 mm/l found in *Crangonyx pseudogracilis*, a common freshwater amphipod in North America (Sutcliffe, 1974). In contrast the K_m values for sodium influx in both *A. aquaticus* and *A. meridianus* are relatively high for freshwater crustaceans; K_m in *A. aquaticus* is similar to the half-saturation values found in freshwater populations of *Gammarus duebeni* thought to be still in the process of adapting to fresh water, and K_m in *A. meridianus* is only slightly below K_m in other

Table 9. *A comparison of some principal features of sodium influx and loss in fresh-water and brackish-water isopods*

Species	Sodium in acclimation media (mM/l)	Temp. (°C)	K_m (mM Na/l)	K_{max} (mM Na/Kg/h)	Loss rate (mM Na/Kg/h)	Source
<i>Aeolus communis</i>	0.075-2	20	0.12	1.7	0.3-0.7	This paper
<i>A. aquaticus</i>	0.1-2	20	0.62	6.5	0.9-2.1	
<i>A. meridiannus</i>	0.1-0.7	20	0.91	18.2	1.7-2.7	
<i>Mesidotea entomon</i> (L. Mälaren)	0.7	5-10	2.6	c. 15	1.6	Croghan & Lockwood, 1968
<i>M. entomon</i> (Baltic)	10	5-10	9	20	3.2	
<i>M. entomon</i> (Baltic)	7	8.5	—	—	1.5	Vinogradov, 1973
<i>Sphaeroma rugicauda</i>	10	16-20	2.3	c. 15	4.1	Harris, 1972
<i>S. serratum</i>	85	16-20	15	> 30	16.5	
<i>Jaera albifrons</i>	40	10-15	—	c. 30	c. 30	Forbes, 1974

Euryhaline gammarids (Sutcliffe, 1968, 1971*a*). The low K_m value in *A. communis* indicates a high affinity for sodium ions in the transporting system at the body surface, and this is reflected in the animal's ability to achieve a steady state with respect to sodium when the external medium contains only 9–13 μM Na/l. This ability is enhanced by the very low rate of sodium loss, reduced to a minimum of 0.26 mm/kg/h at 20 °C (Table 5). The relative permeability is similar to that of the decapods *Potamon niloticus* and *Austropotamobius pallipes*, which are the least permeable of aquatic crustaceans (Shaw, 1961; Sutcliffe, 1974). On the other hand, values for the minimum sodium loss rate in *A. aquaticus* (0.9 mm/kg/h) and *A. meridianus* (1.7 mm/kg/h) are similar to the minimum loss rates found in freshwater gammarids, and the value of K_{max} in the influx of *A. aquaticus* is similar to K_{max} in *G. pulex* and *G. lacustris*. Comparing *A. meridianus* and the freshwater population of *Mesidotea* from L. Mälaren, when both isopods are acclimated to media containing 0.7 mm Na/l (Table 9), values for K_{max} and the sodium loss rate in *A. meridianus* are very roughly one half the values in *Mesidotea* (allowances were made for the effects of the different temperatures at which the rates were measured).

Changes in the principal features of the sodium regulatory mechanism associated with adaptation to fresh water in decapod and gammarid crustaceans are summarized in the Introduction. A strikingly similar sequence of changes in the same features is now evident within several genera of isopods, including species with differing tolerances to low salt concentrations (Table 9). It therefore seems reasonable to suggest that the sequence *A. meridianus*:*A. aquaticus*:*A. communis*, seen in Table 9 and in Fig. 7, represents a natural series of increasing adaptation to fresh water. The inference here is that *A. aquaticus* is better fitted than *A. meridianus* for survival at low sodium concentrations, although both species reached the same minimum sodium balance concentration during short-term experiments lasting for up to six days. However, these experiments were done on animals in the intermoult stage, when the permeability of the body surface is at its lowest. If there is a large increase in permeability at the moult in *Asellus*, as in *Idotea* (Lockwood & Inman, 1973) and in *Gammarus* (Lockwood & Andrews, 1969), then the combination of lower permeability and higher affinity for sodium in *A. aquaticus* might result in better survival when moulting at low sodium concentrations. Both *A. aquaticus* and *A. meridianus* occur in the English Lake District at sodium concentrations down to approximately 150 μM /l (Moon, 1957). Although this is well above the minimum sodium balance concentration of 20–30 μM /l, when *A. aquaticus* was exposed to a medium containing less than 100 μM Na/l the blood sodium concentration was maintained below its normal level (Lockwood, 1960). If the regulatory mechanism in the intermoult stage of *A. aquaticus* is unable to maintain the normal internal steady state level for sodium when the external concentration is below 100 μM /l, then it may be supposed that an external concentration of 150 μM /l must be near to the limits of tolerance for successful completion of the life-cycle in both *A. aquaticus* and *A. meridianus*. Consequently it is of interest to note that during the last fifteen years, *A. aquaticus* has become more numerous and widespread in Esthwaite Water and Rydal Water, and *A. meridianus* has colonized or become more numerous in Ullswater (Moon, 1968). During the same period the sodium chloride content of these and other neighbouring lakes has increased, sometimes by a substantial amount. For example, in Rydal Water the sodium concentration currently

stands at about $220 \mu\text{M/l}$ compared with a mean value of $157 \mu\text{M/l}$ in 1954–56. Similarly, sodium in Ullswater has increased from 143 to about $180 \mu\text{M/l}$.

One further point of ecological interest concerns the historical status of *A. meridianus* in fresh water in northern Europe (Williams, 1962, 1963). The subgenus or genus *Proasellus*, in which *A. meridianus* is placed, is thought to have had a long preglacial history in fresh water and to be mediterranean in origin, whereas *A. aquaticus* (*Asellus s.s.*) is considered to have originated in Siberia, during the Ice Age, from a group of eurasiatic species (Birstein, 1951; Henry & Magniez, 1970). The physiological evidence presented here suggests that *A. aquaticus* (or its ancestors) has been in fresh water probably for a longer period of time than has *A. meridianus*. It is suggested that the latter species has had a relatively short history in fresh water, at least in regions such as the Lake District where the ion concentrations are relatively low (in the early postglacial period most fresh waters in formerly glaciated regions probably had higher ion concentrations than at present). In terms of physiological adaptation to dilute fresh water, *A. meridianus* resembles freshwater populations of *Gammarus duebeni* (Sutcliffe, 1971*a, b*) and *Mesidotea entomon* in L. Mälaren and L. Vättern (Croghan & Lockwood, 1968 and personal communication), except that *A. meridianus* has the added advantage of a reduced haemolymph concentration. Both *G. duebeni* and *M. entomon* are thought to be postglacial colonizers of fresh water. In contrast, *A. communis* is completely adapted to dilute fresh water and presumably has had a long history in such waters; this and other species grouped in *Conasellus* are thought to have evolved independently from the palearctic *Asellus s.s.* and *Proasellus* (Henry & Magniez, 1970). Apart from the single anomalous record in England, *A. communis* is confined to North America, where it is particularly common in the northeastern region east of the Great Lakes (Williams, 1970). Few natural waters in that area have sodium concentrations less than $100 \mu\text{M/l}$ (2.3 mg Na/l) but, as elsewhere in North America, many of the rivers and lakes do have chloride concentrations well below $100 \mu\text{M/l}$ (3.5 mg Cl/l), in some instances as little as $6 \mu\text{M/l}$ (0.2 mg Cl/l) (Clarke, 1920; Eriksson, 1955). Although crustaceans (and fishes) in general have separate transporting systems for the uptake of sodium and chloride ions, the control of the internal steady states for these two ions is to some extent linked (Shaw, 1964; Maetz, 1971), and it is therefore possible that the acquisition of a highly adapted sodium regulatory mechanism in *A. communis* is linked with adaptation to extremely low chloride concentrations in the external medium. In oceanic areas of northwestern Europe, natural waters usually contain more chloride than sodium.

Brief mention must be made of the effect of changing temperature on sodium influx and the sodium loss rate. As in other crustaceans, both are reduced when the temperature of the medium is lowered, and both are increased when the temperature is raised. Lockwood (1960) also found that the sodium influx in *A. aquaticus* is reduced by lowering the temperature, but he concluded that the sodium loss rate was relatively independent of temperature. This conclusion, however, was based on measurements of ^{22}Na efflux in animals exposed to a stream of deionized water for periods of at least 24 h. With a loss rate equivalent to about 1% total body sodium/h (Lockwood, 1960), it is clear from Fig. 6 that a 25% reduction in total body sodium over a 24 h period in deionized water would lead to a much reduced rate of loss irrespective of temperature, and under these circumstances it would be more difficult to detect any alteration in

the rate of loss due to a change in temperature. In fact, Lockwood showed that during the first 5 h in deionized water the loss rate in *A. aquaticus* is faster at 16 °C than at 2 °C. The experiments reported here confirm this. A change in temperature has an immediate effect on the loss rate, and the new rate then remains relatively unchanged in circumstances where the animal does not incur a large net loss of sodium.

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