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REGULATION OF WATER AND SOME IONS IN GAMMARIDS (AMPHIPODA)

I. *GAMMARUS DUEBENI* LILLJEBORG FROM BRACKISH WATER AND FRESH WATER

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Previous work on *Gammarus duebeni* from brackish and fresh waters established that a correlation exists between certain features of sodium regulation and the sodium concentration of the particular habitat occupied by a population. In populations from fresh water sodium losses in the urine are reduced and the transporting system at the body surface has a higher affinity for sodium ions (Sutcliffe, 1971). There is also some evidence to suggest that the sodium concentration in the blood tends to fall more rapidly in animals from brackish-water populations than in animals from freshwater populations when they are exposed to media containing less than 10 mM/l sodium (Sutcliffe, 1967; Sutcliffe & Shaw, 1968). This investigation provides some further information on the regulation of sodium, chloride and potassium in the blood of animals acclimatized to low external concentrations. Chloride regulation at salinities above 2% sea water was studied by Beadle & Cragg (1940*a*), who also found that when *G. duebeni* was placed in distilled water the blood chloride concentration fell to a lower level in animals from a brackish-water locality compared with animals from a freshwater locality (Beadle & Cragg, 1940*b*).

If differences do exist in the osmoregulatory mechanisms of *G. duebeni* from different habitats then it might be supposed that these would be reflected in the total content of water and ions in animals exposed to a range of external salinities. This was found to be the case in *Mesidotea entomon* (Croghan & Lockwood, 1968). Particular attention was given to salinities representing the range found in natural fresh waters up to 2% sea water. Another point of interest concerning *G. duebeni* from freshwater populations is its inability to reproduce in standing fresh water in the laboratory, although it will do so in running fresh water (Hynes, 1954). The effect of stirring the medium on ion regulation was therefore examined, but over a period of a few days only.

MATERIALS AND METHODS

Gammarus duebeni living in brackish water was obtained from a population in salt-marsh pools near Warton, Lancashire. *G. duebeni* living in fresh water was obtained from three populations: Arbory stream at Ballabeg on the southern tip of the Isle of Man (Hynes, 1954) in May 1969; Lough Corrib at Carrick, Co. Galway, Ireland, in September 1970; Windermere experimental population of animals taken from fresh

water on the Kintyre peninsula, Argyll and reared for two years in Windermere water (Sutcliffe, 1970, 1971).

Animals were acclimatized to a temperature of 9 ± 1 °C in a series of media made from Cullercoats sea water diluted with de-ionized water to give a range of salinities down to 2% sea water, or with sodium chloride solutions to give 0·5 and 0·25 mM/l NaCl (NaCl-media). Animals from the brackish-water population were acclimatized to experimental media as follows. In series A, groups of animals were placed directly into the appropriate medium. In series B, animals were step-wise acclimatized to 2% sea water, 0·5 and 0·25 mM/l NaCl. At least 48 h was allowed for acclimatization in sea-water media and 72 h in NaCl-media. Animals were normally starved for several days prior to analysis. All experiments were carried out at 9 °C.

The effect of stirring the medium on salt regulation in brackish-water animals kept in NaCl-media was examined in the following manner. A piece of 'Tygan' screen cloth (mesh size 2 mm) was rolled up to form a tube 100 mm in length and 70 mm in diameter. One end of this tube was cemented to the flat bottom in the centre of a Pyrex crystallizing basin (diameter 190 mm, depth 100 mm). A large magnetic follower was placed inside the tube. This tube was necessary to prevent injury to the animals through collision with the magnetic follower. The animals were placed in the annular space outside the tube. This space was lined with 'Tygan' cloth to provide a surface for the animals to cling to. The crystallizing basin was mounted over a magnetic stirrer and the rate of stirring was adjusted so that the animals were just able to swim against the current.

The body water content was estimated by difference between the wet weight determined after thoroughly blotting with tissue paper, particularly between the limbs, and the dry weight determined after drying in an oven for 24 h at 100 °C. A sample of six animals with similar wet weights was taken from each medium. Weights were determined to the nearest 0·5 mg.

Total body sodium, potassium and chloride were estimated on each animal after determination of the water content. The dried animal was ground into a powder at the bottom of a test tube. 10 ml de-ionized water were added and the contents of the tube were thoroughly shaken at intervals during a period of 2–3 h. Samples digested in nitric acid gave identical results with respect to sodium and potassium, and extraction in water is also suitable for chloride estimations (Cotlove, 1963; Webber & Dehnel, 1968). After centrifuging down particulate material, a 1 or 2 ml aliquot of the extract was diluted to 10 ml with de-ionized water for estimation of chloride (in duplicate) and sodium. Potassium was determined on the original extract.

Blood samples were withdrawn under liquid paraffin. In a few cases the concentration of an ion was estimated on blood taken from one animal, or on a pooled sample obtained from two animals. In most cases the concentrations of sodium, potassium and chloride were estimated on a large sample obtained by pooling blood from 10–17 animals. With these large samples the concentration of each ion was determined in triplicate or quadruplicate on *c.* 6 μl aliquots of blood diluted in 2 ml de-ionized water for sodium and potassium, and diluted in 3 ml nitric-acetic acid solution for chloride.

Sodium and potassium were determined on an EEL flame photometer, sodium accurate to ± 1 %, potassium accurate to about 5%. Chloride was determined on an Aminco Cotlove Chloride Titrator, accurate to ± 1 %. The standard solutions of KCl

used for determination of potassium contained NaCl to give sodium concentrations roughly equivalent to the sodium concentrations in samples of blood or total body extract.

THEORETICAL CONSIDERATIONS

The proportions of the body water in the blood and cells can be calculated from a knowledge of the chloride and potassium concentrations in the blood and in the body water (Croghan & Lockwood, 1968). For this it is assumed that the distribution of chloride and potassium ions in the blood (Cl_o, K_o) and cells (Cl_i, K_i) conforms to the Donnan equilibrium:

$$\frac{Cl_o}{Cl_i} = \frac{K_i}{K_o}. \quad (1)$$

This assumption is reasonably valid for the brackish-water crabs *Carcinus* and *Callinectes* (Shaw, 1955*a*, *b*, 1958*a*, *b*; Hays, Lang & Gainer, 1968).

Now for each ion (X):

$$X_T = X_o V_o + X_i V_i, \quad (2)$$

where X_T is the concentration of the ion in the body water, and V_o and V_i are the proportions of the body water in the blood and cells respectively. Combining (1) and (2) the relative volume of the blood space is defined:

$$V_o = \frac{K_T Cl_T - K_o Cl_o}{K_o Cl_T + K_T Cl_o - 2K_o Cl_o}. \quad (3)$$

As defined above, V_o represents the proportion of body water situated outside the cells. For convenience this will be referred to as the blood space but it should be noted that it includes water and ions held in the extracellular tissue space. The estimated blood space may therefore be larger than the volume of blood actually involved in general circulation.

RESULTS ON *GAMMARUS DUEBENI* FROM A BRACKISH-WATER POPULATION

Water content

The mean water content varied between 76 and 81% of the body wet weight (Table 1). There was a distinct increase in water content at low salinities. Excluding the anomalous high value in 80% sea water, body water represented 76–78% of the wet weight in 100–2% sea water and 79–81% in 0·5–0·25 mM/l NaCl. Bryan (1963) found a similar increase in the water content of the brackish-water isopod *Sphaeroma*.

Sodium and chloride

The pattern of blood sodium regulation is well established for salinities ranging from 150% sea water to fresh water (Shaw & Sutcliffe, 1961; Lockwood, 1964; Sutcliffe, 1967), and Beadle & Cragg (1940*a*) studied blood chloride regulation at salinities between 100% and 2% sea water. Some additional results are given in Table 2, where blood sodium and chloride were estimated on pooled samples from two animals and blood potassium was estimated on pooled samples from five animals in 2% sea water down to 0·25 mM/l NaCl (series B, Table 2). Results given in Table 2 for animals in 100–53% sea water were obtained on pooled samples from ten animals.

Over the salinity range 50–2% sea water both sodium and chloride in the blood are

regulated between about 270 and 300 mM/l. At higher salinities blood sodium is maintained 30–40 mM/l above the sodium concentration of the medium but slightly below blood chloride. The latter is maintained at approximately the same level as the chloride concentration in the medium. At low salinities blood sodium drops sharply when the medium contains less than 1 mM/l sodium. The values for blood sodium in animals from NaCl-media (Table 2) lie between the values obtained previously on *G. duebeni* from brackish-water localities (Shaw & Sutcliffe, 1961; Sutcliffe, 1967), and blood sodium was slightly lower (2–10%) than blood chloride. Stirring the medium apparently had little effect on sodium and chloride levels in the blood of animals acclimatized to these low salinities.

Table 1. *Wet weight, water content and concentrations of total ions in* Gammarus duebeni *from a brackish-water population*

(Mean results from six animals ± 1 standard error.)

Medium	Wet weight (mg)	Water content (% wet wt)	Total ions (mM/kg body H_2O) Na_T	Cl_T	K_T	Ratio Na_T/Cl_T
			Series A			
100% SW	66·0 ± 1·79	76·3 ± 0·86	335·0 ± 8·32	319·3 ± 12·08	66·2 ± 2·74	1·05
80% SW	78·6 ± 1·98	80·5 ± 1·18	293·8 ± 7·54	282·5 ± 12·47	54·3 ± 3·71	1·04
40% SW	73·7 ± 1·26	76·2 ± 0·60	185·7 ± 5·49	156·2 ± 5·29	72·0 ± 1·81	1·19
10% SW	75·3 ± 2·10	76·7 ± 0·92	176·7 ± 7·41	135·2 ± 6·48	67·3 ± 3·43	1·31
2% SW	90·3 ± 1·58	78·1 ± 0·44	178·8 ± 5·56	142·5 ± 4·57	63·8 ± 1·54	1·26
0·25 mM/l NaCl	85·0 ± 1·77	78·6 ± 0·85	116·2 ± 9·79	74·8 ± 10·97	55·3 ± 2·01	1·55
			Series B			
2% SW	73·5 ± 4·90	77·8 ± 0·87	154·8 ± 5·87	124·2 ± 3·52	69·7 ± 1·91	1·25
0·5 mM/l NaCl	84·2 ± 6·64	79·0 ± 1·00	128·2 ± 7·64	97·7 ± 8·04	55·7 ± 1·84	1·31
0·5 mM/l NaCl (stirred)	80·3 ± 2·29	80·3 ± 0·67	133·5 ± 8·18	97·2 ± 3·46	52·5 ± 2·74	1·37
0·25 mM/l NaCl	76·3 ± 3·42	80·8 ± 0·48	126·3 ± 6·59	83·0 ± 6·95	44·7 ± 1·52	1·52
0·25 mM/l NaCl (stirred)	73·7 ± 3·26	80·3 ± 0·56	118·8 ± 5·86	80·5 ± 6·43	49·8 ± 1·54	1·48

Table 2. *Concentrations of sodium, potassium and chloride in pooled blood samples in* Gammarus duebeni *from a brackish-water population*

Medium	Blood ions (mM/l) Na_o	Cl_o	K_o
	Series A		
100% SW (Na = 485, Cl = 550 mM/l)	522	550	12·0 12·2 (4)*
80% SW (Na = 390, Cl = 440 mM/l)	420	430	10·0
53% SW (Na = 254, Cl = 288 mM/l)	290	293	8·0
	Series B		
2% SW	272 (6) ± 4·73	270 (5) ± 8·40	8·5 (2)
0·5 mM/l NaCl	231 (6) ± 6·76	237 (4)	—
0·5 mM/l NaCl (stirred)	201 (6) ± 8·47	207 (6) ± 8·89	6·0 (3)
0·25 mM/l NaCl	180 (7) ± 6·35	199 (4)	—
0·25 mM/l NaCl (stirred)	196 (8) ± 5·69	199 (5) ± 10·05	4·4 (2)

* Each sample represents blood pooled from two animals.

In general, total body sodium and chloride, expressed as mM/kg body water, tend to follow the pattern exhibited by blood sodium and chloride over the salinity range 100% sea water to fresh water (Table 1, Fig. 1), but there is one important difference. Na_T exceeds Cl_T even at the highest salinities where blood chloride is slightly greater than blood sodium (Table 2). In one case only from a total of sixty-six animals Cl_T was slightly greater than Na_T, in an animal from 80% sea water (Cl_T = 326 and Na_T = 319 mM/kg body H_2O). In animals from 100% and 80% sea water the ratio of mean Na_T/Cl_T was close to unity, but this ratio progressively increased at lower salinities

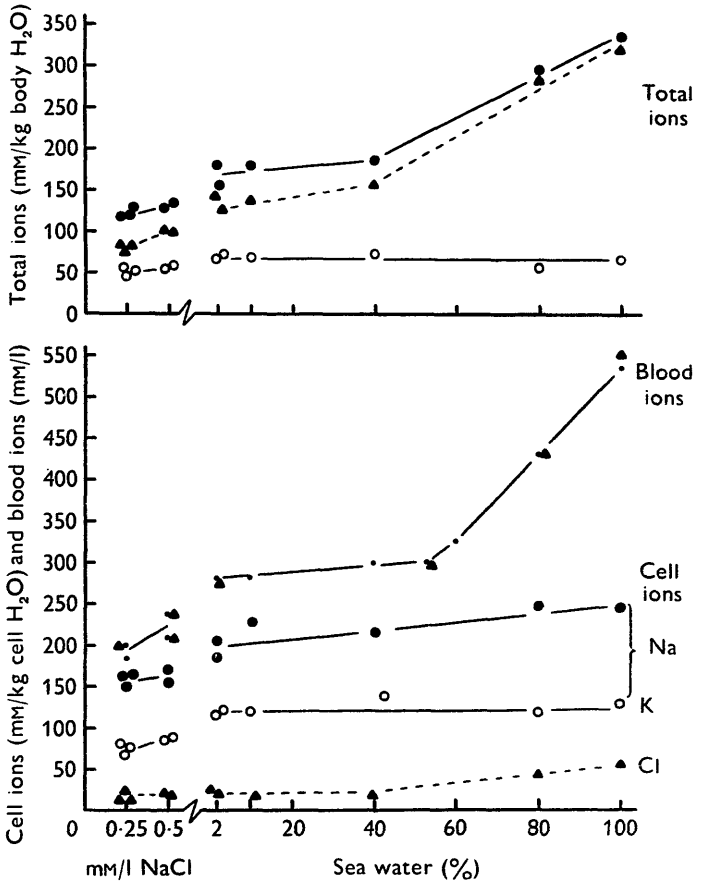

Fig. 1. The concentrations of sodium, potassium and chloride in the body water, blood and cells of animals from a brackish-water population. ▲, Chloride; ○, potassium in body water and cells; ●, sodium in body water and cells; •, sodium + potassium in blood. Note that the cell sodium concentration is added to the cell potassium concentration.

and body sodium was 50% greater than body chloride in animals from 0·25 mM/l NaCl (Table 1). Both the absolute values and Na_T/Cl_T ratios in animals from NaCl-media were not affected by stirring the medium. Since the blood sodium/chloride ratio is close to 1·0 over a wide range of external salinities, the increase in the body sodium/chloride ratio at the lower salinities implies that a greater proportion of the body sodium is held in the tissues.

In 100% sea water the mean body sodium content was 16·8 μM-Na in 66·0 mg wet weight. Lockwood & Andrews (1969) found values of 16·7 μM-Na in freshly moulted

G. duebeni and 14·4 μM-Na in inter-moult animals with a wet weight of 67·7 mg. The animals studied here were in the inter-moult stage and the higher body sodium content is probably due to the higher salinity of '100% sea water' (salinity *c.* 35‰, Table 2) compared with the salinity of 33‰ used by Lockwood & Andrews.

Potassium

Values for blood potassium (Table 2) are similar to the blood potassium concentrations found in other brackish-water crustaceans, *Carcinus* (Shaw, 1955*a*, *b*), *Sphaeroma* (Bryan, 1963) and *Corophium* (McLusky, 1968). In *G. duebeni* acclimatized to NaCl-media blood potassium was less than one-half that in animals from 100% sea water, but the total body potassium showed remarkably little change (Table 1, Fig. 1). The slight fall in K_T of animals in NaCl-media can be attributed largely to an initial net loss of potassium which occurred when the animals were placed in sodium chloride solutions. The initial potassium loss, roughly equivalent to 5% of K_T, raised the external potassium concentration to 0·010–0·015 mM/l and potassium balance was maintained at this concentration for at least 4 days.

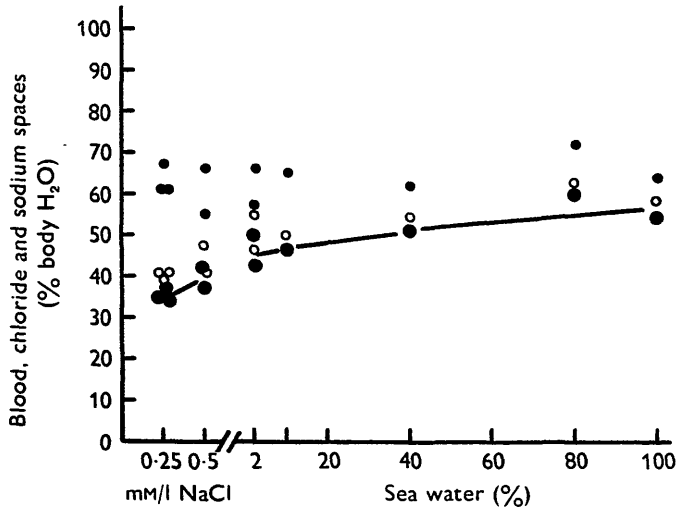

Fig. 2. The mean blood space and the chloride and sodium spaces in animals from a brackish-water population. ●, Mean blood space calculated from equation (3); o, mean chloride space; ●, mean sodium space. Explanation in the text.

Blood space

The mean blood space in groups of six animals was calculated from equation (3) using the data in Table 1 and Table 2. The results are shown in Fig. 2. It appears that the blood space is altered in response to changes in the external salinity. At 100% sea water the blood space contained approximately 55% of the body water, but at 0·25 mM/l NaCl the blood space contained only 35% of the body water despite a slight increase in the body water content (Table 1). The respective blood volumes, approximately 31 and 21 μl in a 75 mg animal, represent about 41 and 28% of the body wet weight.

Direct observation also suggests that there is a marked reduction in the blood volume at salinities below 2% sea water. In animals taken from 2–100% sea water

it was normally possible to obtain at least 3 μl blood by piercing a dorsal intersegmental membrane with a fine needle. Blood was forced through the puncture by hydrostatic pressure in the haemocoel. A similar quantity of blood was further obtained by the application of gentle pressure with forceps along the dorsal region of the body, taking care not to rupture the gut or other tissues. In this manner an average of at least 6 μl blood (roughly 20% of the blood volume) was obtained from animals with a body wet weight of 60–75 mg. A similar amount was also obtained from some animals in salinities below 2% sea water, including 0·25 mM/l NaCl, but in others the amount of blood obtained was rather less (2–5 μl) and in a few cases it was difficult to obtain any blood without exerting considerable external pressure (blood from these animals was discarded). In these individuals the cuticle was shrunken and 'slack' in appearance even before it was ruptured with a needle. This loss of internal hydrostatic pressure was more frequent in animals acclimatized to 0·25 than to 0·5 mM/l NaCl and higher salinities. Beadle & Cragg (1940*a*) noted a loss of hydrostatic pressure in marine gammarids exposed to salinities lower than their normal tolerance range. From these observations it is concluded that the mean blood volume is reduced in *G. duebeni* acclimatized to salinities below 2% sea water, although this reduction may not necessarily occur in all individuals exposed to low salinities.

Fig. 2 also shows the maximum blood space or chloride space calculated on the assumption that all of the body chloride is at a concentration equivalent to that found in the blood. It is interesting to note that in every case this chloride space is only 4–5% greater than the blood space estimated from equation (3), which suggests that the cells probably contain very little chloride. On the other hand the sodium space, calculated in the same way as the chloride space, remained constant over the entire salinity range (100% sea water to 0·25 mM/l NaCl) and is roughly equivalent to 65% of the body water (Fig. 2).

Cell ions

Cell concentrations of sodium, potassium, and chloride were calculated from the estimated cell volume (V_i = total body water $- V_o$) and equation (2) using the data in Table 1 and Table 2. The results are shown in Fig. 1, where cell sodium is added to cell potassium for comparison with the cumulative concentrations of the same ions in the blood.

RESULTS ON *GAMMARUS DUEBENI* FROM FRESHWATER POPULATIONS

Water content

This showed very little variation in animals from three freshwater populations acclimatized to 2% sea water, 0·5 and 0·25 mM/l NaCl (Table 3). The body water represented 79% of the body wet weight in animals from Lough Corrib, but only 74–75% of the wet weight in animals from the Isle of Man. With the exception of one low value (75·4%) in 0·5 mM/l NaCl, the body water also represented 78–79% of the wet weight in animals from the Windermere population acclimatized to a wider variety of media, including animals weighed and dried immediately after removal from the experimental pond (Table 3). Apart from the results on Manx animals, which are uniformly low, the body water content in these freshwater animals is not significantly different from that of brackish-water animals acclimatized to the same media.

Sodium and chloride

Results on large pooled blood samples are shown in Fig. 3. Both sodium and chloride in the blood were regulated very closely over the range 2% sea water to 0·25 mM/l NaCl. In most instances blood chloride was slightly higher than blood sodium. In the extreme case of Manx animals in 0·5 mM/l NaCl the blood chloride level was about 10% higher than blood sodium. The relatively low blood concentrations (< 200 mM/l) found in Windermere animals acclimatized to 0·25 mM/l NaCl were not found in blood samples taken from animals immediately after removal from the pond (Table 4), despite the lower sodium concentration (0·20–0·22 mM/l) of the pond water (Sutcliffe, 1970). The blood values in these animals were also unusual in that sodium was 5–10% higher than chloride.

Table 3. *Wet weight, water content and concentrations of total ions in* Gammarus duebeni *from three freshwater populations*

(Mean results from six animals ± 1 standard error.)

Medium	Popn.*	Wet weight (mg)	Water content (% wet wt)	Total ions (mM/kg body H_2O) Na_T	Cl_T	K_T	Ratio Na_T/Cl_T
2% SW	C	56·5 ± 8·38	78·9 ± 1·47	146·2 ± 7·17	116·7 ± 7·13	68·0 ± 3·44	1·25
	W	88·5 ± 1·97	77·8 ± 1·12	145·3 ± 2·94	112·8 ± 4·23	57·5 ± 2·28	1·29
	M	67·4 ± 1·69	74·2 ± 0·69	156·0 ± 3·25	110·8 ± 4·17	70·5 ± 1·52	1·41
0·5 mM/l NaCl	C	52·7 ± 1·54	79·2 ± 0·78	136·8 ± 4·11	103·8 ± 3·83	59·2 ± 1·60	1·32
	W	88·4 ± 0·84	75·4 ± 0·68	139·7 ± 3·43	95·5 ± 3·34	60·0 ± 0·68	1·46
	M	57·6 ± 1·04	75·0 ± 1·26	136·5 ± 3·67	86·0 ± 4·20	64·0 ± 2·83	1·59
0·25 mM/l NaCl	C	49·8 ± 4·50	78·9 ± 1·70	123·5 ± 4·10	91·2 ± 6·71	59·8 ± 3·32	1·35
	W	85·1 ± 1·54	77·7 ± 0·56	127·2 ± 3·90	84·5 ± 2·95	50·3 ± 1·43	1·51
	M	64·8 ± 0·70	74·5 ± 0·62	128·5 ± 7·68	77·8 ± 8·50	60·3 ± 1·96	1·65
0·25 mM/l NaCl + 0·02 mM/l KCl	C	52·6 ± 3·48	79·2 ± 1·16	124·8 ± 3·75	91·8 ± 3·03	61·0 ± 3·83	1·36
Direct from pond (1969)	W†	80·8 ± 3·81	77·6 ± 0·93	144·8 ± 2·89	104·2 ± 4·48	59·5 ± 2·31	1·39
Pond water (1970)	W†	62·0 ± 1·14	78·5 ± 0·72	150·6 ± 2·56	105·0 ± 1·48	58·2 ± 2·27	1·43
0·25 mM/l NaCl + 0·01 mM/l KCl (1970)	W†	62·9 ± 1·66	79·1 ± 0·61	151·7 ± 2·06	110·7 ± 3·99	56·0 ± 2·22	1·37

* C = Lough Corrib, M = Isle of Man, W = Windermere experimental population.
† Animals provided with food until removed for analysis.

The concentrations of total body sodium and chloride were virtually identical in animals from three freshwater populations (Table 3, Fig. 3) and are not significantly different from the concentrations found in the brackish-water population. Again there is an increase in the ratio Na_T/Cl_T in progressively more dilute media (Table 3). This ratio is similar in animals from both freshwater and brackish-water populations acclimatized to the same media.

The relatively large changes in Na_T/Cl_T at low salinities might be due simply to differences in the rates of uptake and loss of sodium and chloride, particularly in 0·5 and 0·25 mM/l NaCl, where the maintenance of a steady state with respect to sodium is critically dependent on a balance between the rates of uptake and loss (Shaw &

Sutcliffe, 1961; Sutcliffe, 1967; Sutcliffe & Shaw, 1968). This was tested on animals from the Windermere population. Chloride loss into 150 ml de-ionized water was measured on two groups of 50 animals previously acclimatized to 0·5 mM/l NaCl for a period of 2 weeks (Fig. 4) and one group of 30 animals taken directly from the population exposed to Windermere water with a chloride concentration of *c.* 0·24 mM/l (Fig. 5). During the first 3–6 h in de-ionized water chloride and sodium losses were similar, and further observations after a period of 24 h showed that a steady state was reached at the same concentration (0·10–0·12 mM/l) for both chloride and sodium. These results suggest that changes in the Na_T/Cl_T ratio are not due to an inability to maintain chloride balance at low external salinities, but reflect differences in the internal regulation of sodium and chloride at the cellular level.

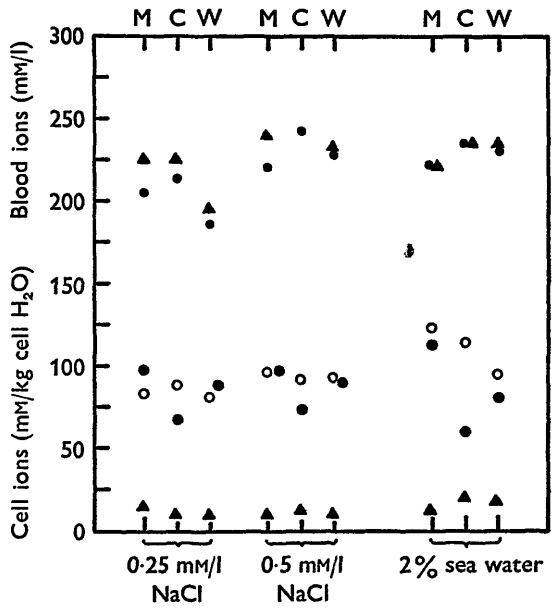

Fig. 3. The concentrations of sodium and chloride in the blood, and sodium, potassium and chloride in the cells of animals from three freshwater populations. M = Isle of Man, C = Lough Corrib, W = Windermere population. ●, Sodium; ○, potassium; ▲, chloride. Blood concentrations were estimated on pooled samples taken from 13–17 animals.

One further point of interest is that, in comparison with animals from all three freshwater populations acclimatized to NaCl-media, the values for body sodium and chloride were markedly higher in animals exposed to Windermere water obtained from the experimental pond, and in animals taken directly from the pond for analysis (Table 3). The maintenance of high body sodium and chloride values similar to those found in animals acclimatized to 2% sea water may be connected with the provision of food right up to the time of analysis, rather than to the presence of natural media compared with simple NaCl solutions. Values for Na_T and Cl_T were also high in Windermere animals acclimatized to 0·25 mM/l NaCl with KCl added to give a potassium concentration of 0·01 mM/l (Table 3). These animals were also provided with the same food source – leaf material taken from the experimental pond. In contrast, all of the other groups of animals were starved for several days before estimates of total ions or blood ions were made.

Potassium

Determinations of blood potassium were made on the blood samples pooled for determination of sodium and chloride. The results for blood potassium are given in Tables 4 and 5. Total body potassium is given in Table 3. In Manx and Windermere animals the blood potassium level was very similar to that found in brackish-water

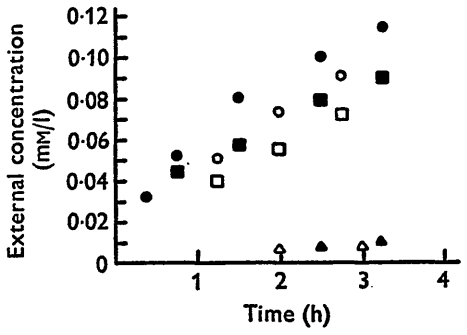

Fig. 4

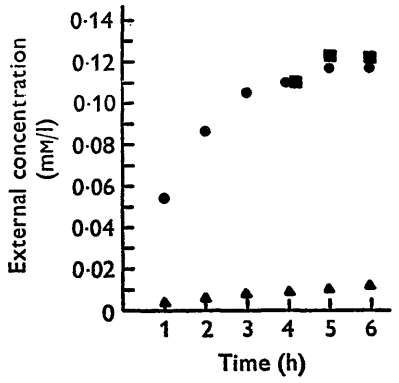

Fig. 5

Fig. 4. Sodium, potassium and chloride loss into 150 ml de-ionized water in two groups of Windermere animals acclimatized to 0·5 mM/l NaCl. ○, ●, Sodium; □, ■, chloride; △, ▲, potassium.

Fig. 5. Sodium, potassium and chloride loss into 150 ml de-ionized water in Windermere animals taken directly from the experimental pond. Symbols as in Fig. 4.

Table 4. *Concentrations of sodium, chloride and potassium in blood samples taken directly after removal of* Gammarus duebeni *from the Windermere experimental population*

Date	Na_o (mM/l)	Cl_o (mM/l)	K_o (mM/l)
September 1969	249	227	6·0
	248	225	—
May 1970	227	215	5·0

Table 5. *Concentrations of potassium in the blood of* Gammarus duebeni *from freshwater populations*

	Population		
Medium	L. Corrib (K_o, mM/l)	Isle of Man (K_o, mM/l)	Windermere (K_o, mM/l)
2% sea water	10·0	7·0	7·0
0·5 mM/l NaCl	8·5	5·0	6·0
0·25 mM/l NaCl	8·0	6·0	5·0
0·25 mM/l NaCl +0·02 mM/l KCl	9·5	—	—

animals over the same salinity range. In animals from Lough Corrib blood potassium was slightly higher, particularly in 0·25 mM/l NaCl, where the addition of KCl to give a potassium concentration in the medium of 0·02 mM/l apparently raised the blood potassium concentration from 8·0 to 9·5 mM/l potassium, but did not significantly

alter the body potassium content (Table 3). In the freshwater populations K_T was slightly higher than K_T in brackish-water animals acclimatized to NaCl-media, but no particular conclusions can be drawn from these results concerning differences in the body potassium concentrations between populations or in different media. Prior exposure to sodium chloride solutions will have affected body potassium to varying extents dependent on the amount of potassium loss required to raise the external potassium to the minimum equilibrium concentration, and also on the frequency with which animals were moved from one medium to another. However, it may be noted that, unlike body sodium and chloride, K_T was not increased in the three groups of Windermere animals provided with food until the time of analysis (Table 3).

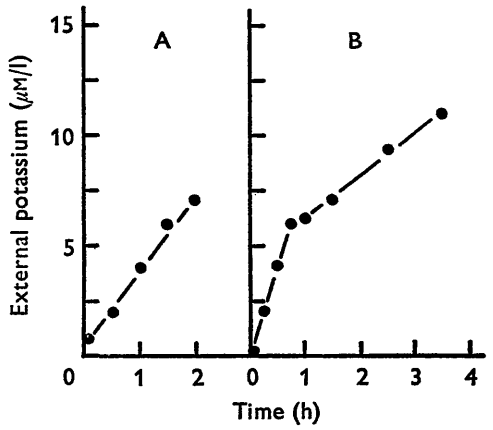

Fig. 6. Potassium loss in Windermere animals. (A) loss into 150 ml de-ionized water in animals taken from the pond, (B) loss into 500 ml de-ionized water in animals acclimatized to sea water.

The initial rate of potassium loss into potassium-free media is surprisingly fast. For example, potassium loss into de-ionized water was about 0·01 μM/h from animals previously acclimatized to low-salinity media (Figs. 4, 5). In another group of 42 animals taken directly from the Windermere population and placed in 150 ml de-ionized water the potassium loss rate during the first 2 h was also 0·01 μM/h (Fig. 6A). This group of animals was then acclimatized to 100% sea water for 2 days, and potassium loss was determined on 35 animals in 500 ml de-ionized water. The initial loss rate was 0·11 μM/h during the first hour, followed by an abrupt change to half this rate for the next 2·5 h (Fig. 6B). This tenfold increase in the initial loss rate is equivalent to a loss of 3% body potassium/h. Bryan (1963) found a very similar loss rate for potassium in *Sphaeroma* transferred to distilled water from 100% sea water.

The potassium loss rate of 0·01 μM/h at low salinities may be compared with the sodium and chloride loss rates of about 0·13 μM/h in the same animals (Figs. 4, 5). Since the blood sodium and chloride concentrations are approximately 40 times greater than the blood potassium concentration it appears that the permeability of the body surface to potassium must be three times greater than the permeability to sodium and chloride. Shaw (1959*a*) also found a higher permeability to potassium in *Potamon niloticus*, and this freshwater crab reached potassium equilibrium at a minimum external concentration of 0·07 mM/l, slightly higher than the minimum sodium equilibrium concentration of 0·05 mM/l. In contrast, potassium balance in *G. duebeni*

was established at an external concentration of 0·01–0·015 mM/l, compared with the minimum sodium equilibrium concentration of 0·10 mM/l (Sutcliffe, 1971). Potassium balance in unfed animals was maintained at the minimum equilibrium concentration for periods of at least 3–4 days in bowls containing 0·5–1·0 l of medium. Thus *G. duebeni* also differs from the crayfish *Astacus* and the mosquito larva *Aëdes aegypti*, which are unable to achieve a steady state with respect to potassium at very low concentrations (Stobbart, 1965).

Blood space

The mean blood space in groups of six animals was calculated from equation (3) using the data given in Tables 3–5. The results are given in Fig. 7. It appears that the blood space was reduced from about 45% to 35% of the body water during step-wise acclimatization to the salinity range 2% sea water down to 0·25 mM/l NaCl. This reduction was similar in extent to that found in brackish-water animals acclimatized

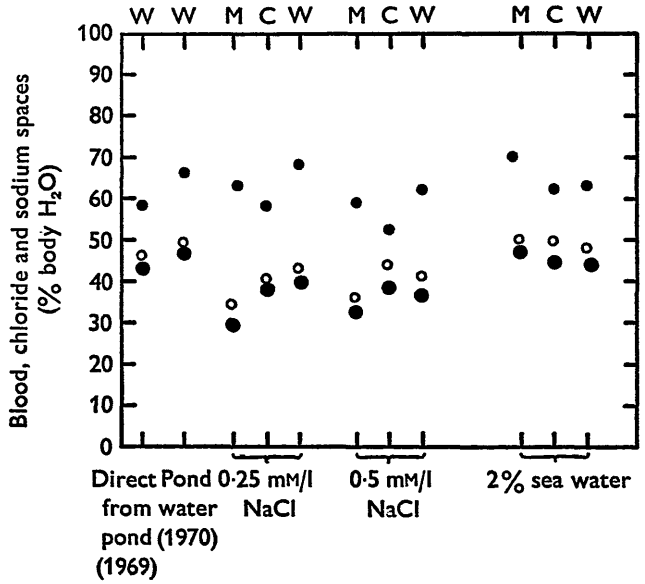

Fig. 7. The mean blood space and the chloride and sodium spaces in animals from three freshwater populations. M = Isle of Man, C = Lough Corrib, W = Windermere population. ●, mean blood space calculated from equation (3); ○, mean chloride space; •, mean sodium space. Explanation in the text.

to the same media. However, unlike the observations made on brackish-water animals there was no clear evidence of a reduced blood volume when blood samples were taken, apart from a few individuals which showed symptoms of a reduced hydrostatic pressure in the haemocoel. These were discarded. In the remainder the average volume of blood extracted from each animal was calculated from estimates made on the total volume of large pooled blood samples (Table 6). In all cases the average volume was the same in animals from 2% sea water and from NaCl-media.

Nevertheless, the blood space calculated from equation (3) is very close to the maximum space calculated on the assumption that the blood contains all of the body chloride (Fig. 7). Consideration of these values for the chloride space indicates that the blood space must be reduced to less than 40% of the body water in freshwater

animals acclimatized to 0·5 and 0·25 mM/l NaCl, including animals from the Windermere population. It is therefore interesting to note that in Windermere animals kept in water obtained from the experimental pond, and in animals taken directly from the pond for analysis, the blood spaces calculated from equation (3) and the chloride spaces are higher than the same estimates made on animals acclimatized to NaCl-media. Instead the estimates of the blood spaces closely resemble those made on animals acclimatized to 2% sea water (Fig. 7). Apart from their exposure to natural Windermere water these animals were also fed right up to the time of removal for analysis. The sodium space was calculated on the assumption that all of the body sodium is at the concentration found in the blood. The results (Fig. 7) show that, unlike the chloride space, the sodium space remained constant at about 60% of the body water.

Table 6. *The average volume of blood extracted from freshwater* Gammarus duebeni *acclimatized to low salinities*

		Medium		
Population	Body weight (mg)	2% sea water (μl blood/ animal)	0·5 mM/l NaCl (μl blood/ animal)	0·25 mM/l NaCl (μl blood/ animal)
L. Corrib	40–50	2·8	2·6	2·9
Isle of Man	50–65	3·8	3·8	3·6
Windermere	80–95	5·3	5·2	5·2

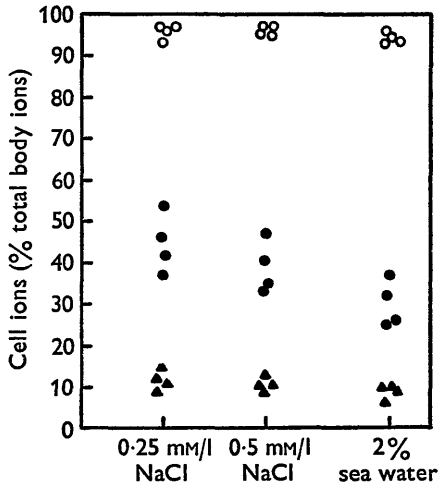

Fig. 8. The proportions of total body ions situated in the cells of animals from one brackish-water population and from three freshwater populations acclimatized to low salinities. ○, Potassium; ●, sodium; ▲, chloride.

Cell ions

The concentrations of sodium, potassium and chloride in the cells were calculated from the cell volume and equation (2) using data given in Tables 3–5, and the blood sodium and chloride concentrations given in Fig. 3. The results are shown in Fig. 3. In general, the concentrations of cell sodium and potassium are practically identical, and there are no major differences between these values and the estimated cell concentrations of sodium, potassium and chloride in brackish-water animals (Fig. 1).

Fig. 8 shows the proportions of the total body ions situated inside the cells of freshwater and brackish-water animals acclimatized to 2% sea water and NaCl-media. As expected, 93–97% of the body potassium and only about 10% of the body chloride is held in the cells. But the proportion of body sodium in the cells rises from about 30% in animals from 2% sea water to 40–50% in animals from 0·25 mM/l NaCl. In animals from 80–100% sea water the cell sodium represents approximately 17–18% of the body sodium.

DISCUSSION

The principal aim of this investigation was to see whether there were any differences between the water content and total ion contents of *Gammarus duebeni* from populations living in habitats ranging from coastal brackish waters to Windermere water with a very low salt content. Since the standard errors for body water and total ion concentrations are similar in the samples from one brackish-water population and three freshwater populations, and since the differences between the mean values in the four populations are generally less than twice the standard errors, it is concluded that no major differences exist in the values for body water or total ions and blood ions with respect to sodium, potassium and chloride in animals acclimatized to 0·5 and 0·25 mM/l NaCl. This conclusion needs to be qualified since it applies strictly to the experimental results obtained from biased samples of the various populations. So far as it was possible to judge from external appearances, only vigorous individuals were chosen for analysis, so that the samples are biased in favour of animals able to osmoregulate well at low salinities. A true estimate of variability within populations would require random sampling on a large scale to include individuals which were relatively inactive and assumed to be poor osmoregulators. In some of these individuals there was a marked loss of hydrostatic pressure in the haemocoel; in addition the blood sodium level had fallen to around 50% of the normal concentration (Sutcliffe, 1967). These animals were not included in the samples. Nevertheless the impression was gained that brackish-water animals are more variable than freshwater animals. For example, at low salinities there is a tendency towards slightly greater hydration and lower blood concentrations in some brackish-water animals, and mortality is certainly higher. This was particularly noticeable in previous work at temperatures above 9–10 °C (Shaw & Sutcliffe, 1961; Sutcliffe, 1967; Sutcliffe & Shaw, 1968). A high water content (and low blood concentration) presumably represents the initial stages of the eventual breakdown in water economy leading to the loss of hydrostatic pressure in the haemocoel. With this in mind it is also concluded that a greater proportion of animals from brackish water is less able to maintain a high blood concentration than of animals from fresh water when exposed to very low salinities. In fact a comparison of blood sodium analyses presented here and in the previous work shows that over the salinity range 2% sea water down to 0·25 mM/l NaCl the mean blood sodium level fell by 23–33% in brackish-water animals but fell by only 15% or less in animals from freshwater populations.

The results of blood analyses on animals from Lough Corrib confirm the previous suggestion that in *G. duebeni* from Ireland the blood sodium level does not drop sharply until the external concentration falls below at least 0·25 mM/l sodium. However, this feature is not characteristic of Irish animals alone, but is now seen to be a feature shared by other populations living in fresh water in Britain. In fact the present

study has provided no further evidence to support the possibility that Irish animals living in fresh water may constitute a distinct physiological race (Sutcliffe & Shaw, 1968; Sutcliffe, 1971). Since the values for body water content, total ions and blood ions in *selected* samples of brackish-water animals kept in NaCl-media are very similar to the values obtained on freshwater animals, it is further concluded that the results are consistent with the previously adopted view that freshwater populations represent a naturally selected proportion of the coastal populations living in brackish water, without any major change in the osmoregulatory mechanism of the selected individuals. This natural selection favoured animals with a high-affinity sodium uptake system at the body surface and in the antennary glands. The result is a notable change in the mean affinity for sodium within the population, directly correlated with the sodium concentration in the habitat occupied by the population (Sutcliffe, 1971). Stock & Pinkster (1970) found morphological differences between populations of *G. duebeni* and these authors propose that some populations living in fresh water, including those in Ireland, constitute a subspecies, *G. duebeni celticus*.

The selective advantage to *G. duebeni* with a higher affinity for sodium ions is thought to be the faster uptake rate of sodium permitted by a high affinity at the very low sodium concentrations found in fresh water. Thus sodium loss across the body wall and the minimal losses via the urine can be more effectively countered by sodium uptake from the medium, resulting in the more stable blood and body sodium concentrations found in freshwater animals. Selection is likely to be particularly severe at the time of moulting, when the body permeability is suddenly increased and there is a very high rate of sodium uptake (Lockwood & Andrews, 1969). The higher total sodium content in fed Windermere animals might be due to higher transport rates as found in *Aëdes*, where the exchange and net uptake rates of sodium are faster in fed larvae than in starved larvae (Stobbart, 1959, 1960). The fast transport rates in *Aëdes* were attributed to the synthesis of more carriers in the transporting system and to the supply of more energy for removal of sodium from the carrier (Stobbart, 1967). An increased sodium transport rate in fed *G. duebeni* might also explain the consistently higher values for sodium influx obtained by Lockwood & Andrews (1969) on both intermoult and moulting animals. Their animals were fed during the experiments. Like *Aëdes*, *G. duebeni* is a small animal with a high rate of turnover of ions. For example, sodium uptake required to balance sodium losses in fresh water is equivalent to about 2% body Na/h compared with only 0·2–0·4% body Na/h in *Astacus* (Shaw, 1959*b*; Bryan, 1960*a*, *b*). It seems reasonable to assume that this high turnover rate of sodium ions in *G. duebeni* will require more energy and perhaps more frequent replacement of carriers than in an animal like *Astacus* with a low turnover rate of sodium. *Astacus* can withstand starvation for long periods (Huf, 1933; Scholles, 1933) whereas *Gammarus* begins to die off after 2–3 weeks without food. In a number of small crustaceans the blood concentration is lowered by starvation, and it has been suggested that some ions are partly obtained from the food, e.g. *Daphnia*, *Branchipus* (Krogh, 1939), *Triops* (Parry, 1961) and *Corophium* (McLusky, 1970).

Direct measurement of water and ions in the tissues is difficult because the small muscles in *G. duebeni* are not easily removed without extensive damage to the fibres. In their study of chloride regulation in the tissues of *Gammarus*, Beadle & Cragg (1940*a*) cut each animal into two and drained off the blood by pressing the two portions

between pieces of filter paper. Derouet (1952) apparently used the same technique, which does not remove all of the blood from the haemocoel. Personal observations were made on animals cut into several pieces and pressed firmly between tissue paper to remove as much blood as possible. It was observed that some blood remained in the fine appendages (antennae, legs and gills) and this may represent a considerable proportion of the total blood volume. The 'tissue' (whole animal minus blood) studied by Beadle & Cragg therefore probably contained some blood with a high sodium chloride content, and this would raise the apparent chloride content of the tissues. Their values for tissue chloride in *G. duebeni* from 2% and 100% sea water were respectively about 70 and 150 mM-Cl/kg wet tissue, compared with the present estimates of 20 and 50 mM-Cl/kg cell H_2O (Figs. 1, 3). The apparently large differences between the two sets of values for tissue and cell chloride can be accounted for in the following manner. It is assumed that 80% of the tissue wet weight was due to water and that 20% of the tissue water is held in the extracellular tissue space with a chloride concentration equal to that of the blood (Robertson, 1961, 1970; van der Kloot, 1966; Hays *et al.* 1968; Lang & Gainer, 1969; Mackay & Prosser, 1970). In animals from undiluted sea water with a blood chloride concentration of 500 mM/l (Beadle & Cragg, 1940*a*) it was calculated that the extracellular tissue space would contain 88 mM-Cl and the intracellular space would contain 150 − 88 = 62 mM-Cl at a concentration of 97 mM-Cl/kg cell H_2O. In the present study the estimated intracellular concentration was 50 mM-Cl/kg cell H_2O. Hence 97 − 50 = 47 mM-Cl/kg cell H_2O remains outstanding. This amount of chloride represents approximately 9% of the chloride in the blood, excluding the extracellular tissue space, when the blood space is equivalent to 55% body H_2O. Agreement between the two sets of results is therefore obtained by assuming that the 'tissue' studied by Beadle & Cragg contained a residual amount of blood equivalent to about 7·5% of the total blood space. A similar calculation for the tissues of *G. duebeni* in 2% sea water (Beadle & Cragg, 1940*a*) gives 43 mM-Cl in the extracellular tissue space (blood Cl = 270 mM/l) and an intracellular concentration of 42 mM-Cl/kg cell H_2O. Hence 42 − 20 = 22 mM-Cl/kg cell H_2O remains outstanding. This amount of chloride represents approximately 10% of the total blood space when the latter is equivalent to 45% body H_2O.

From the above considerations it is inferred that the mean chloride concentration in the intracellular water probably lies between 20 and 40 mM/kg H_2O in animals from 2% sea water, and between 50 and 100 mM/kg H_2O in animals from sea water. These values are very similar to intracellular chloride concentrations found in the muscles of the brackish-water and freshwater decapods *Eriocheir* (Krogh, 1939, p. 85), *Carcinus* (Shaw, 1955*a*, *b*), *Potamon* (Shaw, 1959*a*), *Orconectes* (van der Kloot, 1966), *Pacifastacus* (Kerley & Pritchard, 1967) and the marine arthropods *Nephrops* (Robertson, 1961) and *Limulus* (Robertson, 1970). The estimated intracellular potassium concentrations of 100–125 mM/kg H_2O in *G. duebeni* from 2–100% sea water (Figs. 1, 3) also agree with muscle potassium concentrations found in the decapod crustaceans and in the isopod *Mesidotea*. On the other hand the intracellular sodium concentrations of 75–80 mM/kg H_2O in *G. duebeni* from 2% sea water and about 120 mM-Na/kg H_2O in sea-water animals are a little on the high side compared with the muscle sodium concentrations found in marine and brackish-water decapods, including *Carcinus*, but agree more closely with values obtained on the freshwater crayfish *Orconectes* and

the freshwater race of *Mesidotea* from L. Mälaren (Croghan & Lockwood, 1968). These values are also similar to the elevated muscle sodium concentrations found when the blood sodium was raised to a high level in *Potamon* (Shaw, 1959*a*) and in *Astacus* (Bryan, 1960*b*). Hence the estimated intracellular concentrations of both sodium and chloride in *G. duebeni* support the assumption made here that a Donnan equilibrium exists between potassium and chloride in the blood and cells, since any radical departure from this equilibrium would lead to estimated mean sodium and chloride concentrations in the cells much higher than the concentrations found in the muscles of decapods and other aquatic animals (Robertson, 1961, 1965; Potts & Parry, 1964).

Further speculation on regulation at the cellular level is not warranted until an independent estimate of the blood space in *G. duebeni* is available (e.g. by inulin injection), but three points are worth noting. First, intracellular sodium may make a major contribution to the osmotically active components in some cells, e.g. muscle cells, and this might explain the marked retention of sodium at low salinities where the blood concentration is lowered (Fig. 8). Secondly, cell volume regulation (Lange, 1968) may be inadequate in some individuals in the freshwater populations at times of stress, e.g. moulting, injury or food shortage. Thirdly, the ionic composition of the cells appears to be similar in *G. duebeni* from freshwater and from brackish-water populations, and there are no apparent differences in the relative volume of the blood space. Croghan & Lockwood (1968) found a higher water content and larger blood volume in *Mesidotea* from fresh water compared with animals from the Baltic Sea.

Gammarus duebeni illustrates the effects of natural selection on the osmoregulatory mechanism during the process of adaptation to fresh water. In this species the basic osmoregulatory features essential for survival in fresh water (Beadle & Cragg, 1940*a*; Shaw, 1961) exist in some individuals in the brackish-water populations, i.e. lowered permeability to salts, a high-affinity ion uptake system, reduced salt loss in hypotonic urine (Sutcliffe, 1968). Colonization of fresh water has been achieved by selection in favour of these individuals. The very low concentrations in the blood and tissues which are characteristic of many freshwater animals are not an essential part of adaptation in all gammarids. Several genera and species with a long history in fresh water have blood concentrations as high as *G. duebeni*; for example, *Eulimnogammarus* in Lake Baikal (Basikalova, Birstein & Taliev, 1946) and *Dikerogammarus haemobaphes* which extends from the Caspian Sea to the upper reaches of the Ural and Volga rivers (Belayev & Birstein, 1944; Romanova, 1959). The estuarine species *G. zaddachi* might repay further study since it does not penetrate fresh water above the limits of high spring tides, despite the fact that its osmoregulatory mechanism closely resembles that of *G. duebeni*. Perhaps *G. zaddachi* cannot breed in fresh water, although some limited success has been achieved in an experimental pond (Sutcliffe, 1970). Adult specimens of *Eriocheir* live in fresh water but return to the sea to breed. In this crab the eggs and zoeae are sensitive to low salinity (de Leersnyder, 1967). In contrast, the zoeae of *Hymenosoma orbiculare* can live in fresh water, allowing this estuarine–marine crab to breed in a freshwater lake with a chloride content of about 4 mM/l (Forbes & Hill, 1969). The importance of reproductive adaptations for complete penetration into fresh water is also shown by the work of Smith (1950) on the viviparous *Nereis limnicola*, which protects the sensitive larval stage in coelomic fluid with a high salt content. This adaptation may have been sufficient to permit *N. limnicola* to colonize

fresh water, although there is some evidence that the permeability to salts in this species is lower than in *N. diversicolor* (Smith, 1963; Oglesby, 1965). Like *G. duebeni*, adult *N. diversicolor* tolerate salinities down to fresh water without any changes in the osmoregulatory mechanism in populations from different areas, but the sensitivity of the larvae to low salinities may prevent this species from colonising fresh water (Smith, 1955, 1964; Bogucki, 1963).

SUMMARY

1. *Gammarus duebeni* from brackish water was acclimatized to salinities ranging from 100% sea water down to 0·25 mM/l NaCl at 9 °C.

2. The body water content increased from 76 to 81% body wet weight. The ratio of total body sodium/chloride increased from 1·04 to 1·52. The sodium space remained constant, equivalent to about 65% body H_2O. The chloride space decreased from about 60% body H_2O down to 35% body H_2O.

3. Total body potassium remained almost constant and showed only a small decrease in dilute NaCl-media. Potassium balance was maintained for several days at an external potassium concentration of 0·010–0·015 mM/l.

4. The proportion of body water in the extracellular blood space was calculated from the assumption that potassium and chloride ions were distributed in a Donnan equilibrium between the blood and intracellular spaces. The blood space was slightly smaller than the chloride space.

5. The mean intracellular concentrations of sodium, potassium and chloride were calculated. Sodium fell from 120 to 75 mM/kg cell H_2O, potassium fell from 125 to 75 mM/kg cell H_2O and chloride fell from 55 to 12 mM/kg cell H_2O. These concentrations are similar to the concentrations found in the muscles of decapods and in the tissues of other animals.

6. About 10% of the body chloride and 93–97% of the body potassium is situated in the cells. The proportion of intracellular sodium increased from 17–18% body sodium at 100% sea water to 40–50% body sodium at 0·25 mM/l NaCl.

7. *G. duebeni* from three freshwater populations were acclimatized to 2% sea water, 0·5 and 0·25 mM/l NaCl. The body surface is three times more permeable to potassium than it is to sodium and chloride. Potassium balance in starved animals was achieved at 0·010–0·015 mM/l K. Fed animals had a higher body sodium and chloride content than starved animals.

8. The regulation of body water and ions in animals from the freshwater populations was essentially the same as in animals from brackish-water populations. The significance of the results is discussed in relation to the process of adaptation to fresh water.

REFERENCES

BASIKLOVA, A. J., BIRSTEIN, J. A. & TALIEV, D. N. (1946). Osmotic pressure of the body fluids in the amphipods of Lake Baikal. *Dokl. Akad. Nauk CCCP* **53**, 377–9.

BEADLE, L. C. & CRAGG, J. B. (1940*a*). Studies on adaptation to salinity in *Gammarus* spp. I. Regulation of blood and tissue and the problem of adaptation to fresh water. *J. exp. Biol.* **17**, 153–63.

BEADLE, L. C. & CRAGG, J. B. (1940*b*). Osmotic regulation in fresh-water animals. *Nature, Lond.* **146**, 588.

BELAYEV, G. M. & BIRSTEIN, J. A. (1944). A comparison between the osmoregulatory ability in Volga River and Caspian amphipods. *Dokl. Akad. Nauk CCCP* **45**, 304–6.

BOGUCKI, M. (1963). The influence of salinity on the maturation of gametes of *Nereis diversicolor* O. F. Müller. *Polsk. Arch. Hydrobiol.* **11**, 343–47.

BRYAN, G. W. (1960*a*). Sodium regulation in the crayfish *Astacus fluviatilis*. I. The normal animal. *J. exp. Biol.* **37**, 83–99.

BRYAN, G. W. (1960*b*). Sodium regulation in the crayfish *Astacus fluviatilis*. III. Experiments with NaCl-loaded animals. *J. exp. Biol.* **37**, 113–28.

BRYAN, G. W. (1963). The accumulation of ^{137}Cs by brackish water invertebrates and its relation to the regulation of potassium and sodium. *J. mar. biol. Ass. U.K.* **43**, 541–65.

COTLOVE, E. (1963). Determination of the true chloride content of biological fluids and tissues. II. Analysis by simple, non-isotopic methods. *Analyt. Chem.* **35**, 101–5.

CROGHAN, P. C. & LOCKWOOD, A. P. M. (1968). Ionic regulation of the Baltic and freshwater races of the isopod *Mesidotea* (*Saduria*) *entomon* (L.). *J. exp. Biol.* **48**, 141–58.

DEROUET, L. (1952). Influence des variations de salinité du milieu extérieur sur des Crustacés cavernicoles et épigés. II. Étude des teneurs en chlore du milieu intérieur et des tissus. *C. r. hebd. Séanc. Acad. Sci., Paris* **234**, 888–90.

FORBES, A. T. & HILL, B. J. (1969). The physiological ability of a marine crab *Hymenosoma orbiculare* Desm. to live in a subtropical freshwater lake. *Trans. R. Soc. S. Afr.* **38**, 271–83.

HAYS, E. A., LANG, M. A. & GAINER, H. (1968). A re-examination of the Donnan distribution as a mechanism for membrane potentials and potassium and choride ion distributions in crab muscle fibers. *Comp. Biochem. Physiol.* **26**, 761–92.

HUF, E. (1933). Über die Aufrechterhaltung des Salzgehaltes bei Süsswasserkrebsen (*Potamobius*). *Pflüg. Arch. ges. Physiol.* **232**, 559–73.

HYNES, H. B. N. (1954). The ecology of *Gammarus duebeni* Lilljeborg and its occurrence in freshwater in Western Britain. *J. Anim. Ecol.* **23**, 38–84.

KERLEY, D. E. & PRITCHARD, A. W. (1967). Osmotic regulation in the crayfish, *Pacifastacus leniusculus*, stepwise acclimated to dilutions of sea water. *Comp. Biochem. Physiol.* **20**, 101–13.

VAN DER KLOOT, W. G. (1966). The exchange of radioactive cations by somatic and cardiac muscles of the crayfish. *Comp. Biochem. Physiol.* **17**, 1019–43.

KROGH, A. (1939). *Osmotic Regulation in Aquatic Animals.* Cambridge University Press.

LANG, M. A. & GAINER, H. (1969). Isosmotic intracellular regulation as a mechanism of volume control in crab muscle fibers. *Comp. Biochem. Physiol.* **30**, 445–56.

LANGE, R. (1968). Isosmotic intracellular regulation. *Nytt. Mag. Zool.* **16**, 1–13.

DE LEERSNYDER, M. (1967). Influence de la salinité et de l'ablation des pédoncules oculaires sur la mue et sur le développement ovarien d'*Eriocheir sinensis* H. Milne-Edwards. *Cah. Biol. mar.* **8**, 421–35.

LOCKWOOD, A. P. M. (1964). Activation of the sodium uptake system at high blood concentrations in the amphipod, *Gammarus duebeni*. *J. exp. Biol.* **42**, 59–69.

LOCKWOOD, A. P. M. & ANDREWS, W. R. H. (1969). Active transport and sodium fluxes at moult in the amphipod, *Gammarus duebeni*. *J. exp. Biol.* **51**, 591–605.

MACKAY, W. R. & PROSSER, C. L. (1970). Ionic and osmotic regulation in the king crab and two other North Pacific crustaceans. *Comp. Biochem. Physiol.* **34**, 273–80.

MCLUSKY, D. S. (1968). Aspects of osmotic and ionic regulation in *Corophium volutator* (Pallas). *J. mar. biol. Ass. U.K.* **48**, 769–81.

MCLUSKY, D. S. (1970). Osmoregulation in *Corophium volutator* – the effect of starvation. *Comp. Biochem. Physiol.* **35**, 303–6.

OGLESBY, L. C. (1965). Water and chloride fluxes in estuarine nereid polychaetes. *Comp. Biochem. Physiol.* **16**, 437–55.

PARRY, G. (1961). Chloride regulation in *Triops*. *Nature, Lond.* **192**, 468–9.

POTTS, W. T. W. & PARRY, G. (1964). *Osmotic and Ionic Regulation in Animals.* Pergamon Press.

ROBERTSON, J. D. (1961). Studies on the chemical composition of muscle tissue. II. The abdominal flexor muscles of the lobster *Nephrops norvegicus* (L.). *J. exp. Biol.* **38**, 707–28.

ROBERTSON, J. D. (1965). Studies on the chemical composition of muscle tissue. III. The mantle muscle of cephalopod molluscs. *J. exp. Biol.* **42**, 153–75.

ROBERTSON, J. D. (1970). Osmotic and ionic regulation in the horseshoe crab *Limulus polyphemus* (Linnaeus). *Biol. Bull. mar. biol. Lab., Woods Hole* **138**, 157–83.

ROMANOVA, N. N. (1959). The distribution and ecological character of the North Caspian Amphipoda and Cumacea. *Dokl. (Proc.) Acad. Sci. U.S.S.R. (Biol. Sci.)* **121**, 637–40.

SCHOLLES, W. (1953). Über die Mineralregulation wasserlebender Evertebraten. *Z. vergl. Physiol.* **19**, 522–54.

SHAW, J. (1955*a*). Ionic regulation in the muscle fibres of *Carcinus maenas*. I. The electrolyte composition of single fibres. *J. exp. Biol.* **32**, 383–96.

SHAW, J. (1955*b*). Ionic regulation in the muscle fibres of *Carcinus maenas*. II. The effect of reduced blood concentration. *J. exp. Biol.* **32**, 664–80.

SHAW, J. (1958*a*). Further studies on ionic regulation in the muscle fibres of *Carcinus maenas*. *J. exp. Biol.* **35**, 902–19.

SHAW, J. (1958*b*). Osmoregulation in the muscle fibres of *Carcinus maenas*. *J. exp. Biol.* **35**, 920–9.
SHAW, J. (1959*a*). Solute and water balance in the muscle fibres of the East African freshwater crab, *Potamon niloticus* (M. Edw.). *J. exp. Biol.* **36**, 145–56.
SHAW, J. (1959*b*). The absorption of sodium ions by the crayfish, *Astacus pallipes* (Lereboullet). I. The effect of external and internal sodium concentrations. *J. exp. Biol.* **36**, 126–44.
SHAW, J. (1961). Sodium balance in *Eriocheir sinensis* (M. Edw.). The adaptation of the Crustacea to fresh water. *J. exp. Biol.* **38**, 153–62.
SHAW, J. & SUTCLIFFE, D. W. (1961). Studies on sodium balance in *Gammarus duebeni* Lilljeborg and *G. pulex* (L.). *J. exp. Biol.* **38**, 1–15.
SMITH, R. I. (1950). Embryonic development in the viviparous nereid polychaete, *Neanthes lighti* Hartman. *J. Morph.* **87**, 417–65.
SMITH, R. I. (1955). Comparison of the level of chloride regulation by *Nereis diversicolor* in different parts of its geographic range. *Biol. Bull. mar. biol. Lab., Woods Hole* **109**, 453–74.
SMITH, R. I. (1963). A comparison of salt loss rate in three species of brackish-water nereid polychaetes. *Biol. Bull. mar. biol. Lab., Woods Hole* **125**, 332–43.
SMITH, R. I. (1964). On the early development of *Nereis diversicolor* in different salinities. *J. Morph.* **114**, 437–64.
STOBBART, R. H. (1959). Studies on the exchange and regulation of sodium in the larva of *Aëdes aegypti* (L.). I. The steady-state exchange. *J. exp. Biol.* **36**, 641–53.
STOBBART, R. H. (1960). Studies on the exchange and regulation of sodium in the larva of *Aëdes aegypti* (L.). II. The net transport and the fluxes associated with it. *J. exp. Biol.* **37**, 594–608.
STOBBART, R. H. (1965). The effect of some anions and cations upon the fluxes and net uptake of sodium in the larva of *Aedes aegypti* (L.). *J. exp. Biol.* **42**, 29–43.
STOBBART, R. H. (1967). The effect of some anions and cations upon the fluxes and net uptake of chloride in the larva of *Aëdes aegypti* (L.), and the nature of the uptake mechanisms for sodium and chloride. *J. exp. Biol.* **47**, 35–57.
STOCK, J. H. & PINKSTER, S. (1970). Irish and French fresh water populations of *Gammarus duebeni* subspecifically different from brackish water populations. *Nature, Lond.* **228**, 874–5.
SUTCLIFFE, D. W. (1967). Sodium regulation in the amphipod *Gammarus duebeni* from brackish-water and fresh-water localities in Britain. *J. exp. Biol.* **46**, 529–50.
SUTCLIFFE, D. W. (1968). Sodium regulation and adaptation to fresh water in gammarid crustaceans. *J. exp. Biol.* **48**, 359–80.
SUTCLIFFE, D. W. (1970). Experimental populations of *Gammarus duebeni* in fresh water with a low sodium content. *Nature, Lond.* **228**, 875–6.
SUTCLIFFE, D. W. (1971). Sodium influx and loss in fresh-water and brackish-water populations of the amphipod *Gammarus duebeni* Lilljeborg. *J. exp. Biol.* **54**, 255–68.
SUTCLIFFE, D. W. & SHAW, J. (1968). Sodium regulation in the amphipod *Gammarus duebeni* Lilljeborg from freshwater localities in Ireland. *J. exp. Biol.* **48**, 339–58.
WEBBER, H. H. & DEHNEL, P. A. (1968). Ion balance in the prosobranch gastropod, *Acmaea scutum*. *Comp. Biochem. Physiol.* **25**, 49–64.