REGULATION OF WATER AND SOME IONS IN GAMMARIDS (AMPHIPODA)

III. THREE EURYHALINE SPECIES

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INTRODUCTION

Gammarus zaddachi Sexton occupies the low-salinity regions of estuaries, including the fresh-water zone influenced by salt water during periods of high spring tides (Sexton, 1942; Spooner, 1947; Kinne, 1954; Hartog, 1964; Dennert *et al.* 1969). *G. locusta* (L.) and *Marinogammarus finmarchicus* (Dahl) are marine-littoral species which also occur in the more saline regions of estuaries (Spooner, 1947; Segerstråle, 1947; Hartog, 1964; Stock, 1967). In addition, *G. locusta* is found in the Baltic Sea at salinities down to 5·5‰ (Segerstråle, 1950, 1951) and *M. finmarchicus* can tolerate equally low salinities in the laboratory (Werntz, 1963; Sutcliffe, 1968). Both species may therefore be regarded as euryhaline animals with a salinity tolerance range similar to that of the shore crab *Carcinus maenas*, but less euryhaline than *G. zaddachi*. These three gammarids provide an interesting comparison with *G. duebeni*, a highly euryhaline species (Beadle & Cragg, 1940*a*, *b*; Shaw & Sutcliffe, 1961; Lockwood, 1961, 1964; Sutcliffe, 1967, 1971*a*; Sutcliffe & Shaw, 1968).

Several aspects of osmoregulation in *G. zaddachi*, *G. locusta* and *M. finmarchicus* were dealt with by Beadle & Cragg (1940*a*), Derouet (1952), Werntz (1963) and Sutcliffe (1968). This paper is concerned with the body water content, and the blood concentrations and total body concentrations of sodium, potassium and chloride in these gammarids. The proportions of body water in the extracellular blood space and in the intracellular space were calculated from the assumption that the distribution of potassium and chloride ions conforms to a Donnan equilibrium between these two spaces (Croghan & Lockwood, 1968; Sutcliffe, 1971*a*). This assumption is justified by the fact that a Donnan equilibrium exists between the blood and muscle fibres in the euryhaline crabs *Carcinus* (Shaw, 1955*a*, *b*, 1958*a*, *b*) and *Callinectes* (Hays, Lang & Gainer, 1968). It was also shown to be a reasonable assumption in the case of *G. duebeni* and *G. pulex* (Sutcliffe, 1971*a*, *b*).

MATERIALS AND METHODS

G. zaddachi Sexton was obtained during the winter months from underneath stones on the shore where a small stream discharges over the beach near Barrow-in-Furness, Lancashire. *G. locusta* (L.) and *Marinogammarus finmarchicus* (Dahl) were obtained from the tidal zone of the shore at Cullercoats Bay, Northumberland. All of the speci-

mens used in this investigation were individually examined and identified under a binocular microscope (Sutcliffe, 1968).

The experiments were carried out at a temperature of 9 ± 1 °C. Animals were fed until about 48 h before removal for analysis. Experiments on *G. zaddachi* were carried out on three batches of animals (Series A–C). In series A after 3 days acclimatization in approximately 30% sea water the animals were transferred into higher and lower salinities, including 0·25 mM/l NaCl stirred by a magnetic follower (Sutcliffe, 1971a) for a period of 5 days. In Series B the animals were acclimatized to 40% sea water for 24 days, followed by acclimatization to 2% sea water for 2 days and to 0·25 mM/l NaCl for a further 2 days. In Series C the animals were acclimatized to 2% sea water for various periods of up to 18 days before acclimatization to NaCl-free media for 3–5 days.

The proportion of body water in the extracellular space (blood space) was calculated in the manner described in the first paper of this series (Sutcliffe, 1971a). That paper also gives details of the analytical methods employed here.

RESULTS ON *GAMMARUS ZADDACHI*

Water content

The mean water content varied between 74·7 and 79·7% body wet weight over the salinity range 100% sea water down to 0·3 mM/l NaCl (Table 1). These values are practically identical with values for the water content in *G. duebeni* over the same salinity range (Sutcliffe, 1971a) and in *G. zaddachi* there is also a tendency towards increased hydration at low salinities.

Sodium and chloride

A few values for the blood sodium concentration over the range 55% sea water down to 0·25 mM/l NaCl were given by Sutcliffe (1968). Some additional values for blood sodium and chloride are given in Table 2. In animals from 100% sea water the blood sodium and chloride concentrations were identical. The sodium concentration of the blood was about 35 mM/l above that of the medium and the chloride concentration of the blood was about 32 mM/l below that of the medium. Thus the chloride concentration in the blood represented only 94·5% of the chloride concentration in the sea water. Blood chloride was also slightly below the external chloride in 80% sea water. This maintenance of a blood chloride concentration in the blood below that in the external medium distinguishes *G. zaddachi* from *G. duebeni* (Sutcliffe, 1971a) and *Corophium* (McLusky, 1968), and in this respect *G. zaddachi* resembles some marine-estuarine decapods, e.g. *Carcinus* (Shaw, 1955a; Robertson, 1960), *Chionoecetes* and *Pandalus* (Mackay & Prosser, 1970). In *G. zaddachi* at low salinities the blood chloride concentration was also very similar to the blood sodium concentration. Both fell sharply at salinities below 0·5 mM/l NaCl, even when the medium was stirred (Table 2).

The relationship between the external salinity and the total body sodium and chloride concentrations is similar to that shown by the blood sodium and chloride concentrations. Total body sodium and chloride increased at salinities above approximately 40–50% sea water, there was a zone of close regulation at salinities between 40 and 2%

Table 1. *Wet weight, water content and concentrations of total ions in Gammarus zaddachi*

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

Series	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
A	100% SW	62·7±1·84	74·4±0·52	369·0±6·79	343·0±6·02	77·2±2·24	1·08
A	80% SW	63·3±0·95	74·4±0·51	275·5±4·60	233·2±7·66	78·8±1·92	1·18
A	40% SW	72·4±1·96	75·3±0·42	163·5±3·35	128·8±2·55	76·7±1·48	1·27
A	0·25 mM/l NaCl*	65·1±1·45	78·0±0·88	107·2±9·58	58·7±8·56	55·8±4·41	1·83
B	40% SW	70·8±2·38	78·4±0·40	155·7±1·73	122·3±2·11	62·3±1·26	1·27
B	2% SW	61·7±2·11	77·7±0·75	145·2±3·00	113·5±3·37	60·8±3·79	1·28
B	0·25 mM/l NaCl	60·3±2·47	79·7±0·36	102·3±7·53	54·8±10·13	54·8±2·76	1·87
C	2% SW	70·7±1·04	77·8±0·83	157·8±2·44	116·8±3·65	68·2±1·78	1·35
C	0·5 mM/l NaCl*	76·5±1·72	77·0±0·91	148·5±3·33	102·7±3·15	61·8±2·80	1·45
C	0·5 mM/l NaCl	71·3±1·95	75·8±0·56	160·3±4·11	105·0±7·09	65·7±1·67	1·53
C	0·3 mM/l NaCl*	70·4±1·57	78·7±1·06	117·2±5·12	84·7±4·78	56·8±3·43	1·39

* Stirred media.

Table 2. *Concentrations of sodium, potassium and chloride in pooled blood samples from Gammarus zaddachi*

Medium	Blood ions (mM/l) Na_o	Cl_o	K_o
100% SW (Na = 485, Cl = 550 mM/l)	518	518	—
80% SW (Cl = 428 mM/l)	—	420	10·5
2% SW	228	220	7·0
0·5 mM/l NaCl	220	220	—
	207	186	—
0·5 mM/l NaCl*	205	203	—
	195	187	—
0·3 mM/l NaCl*	180	176	5·0
0·25 mM/l NaCl*	—	157	4·0

* Stirred media.

sea water, and a sharp fall in body sodium and chloride occurred at salinities below 0·5 mM/l NaCl (Table 1, Fig. 2). The values for Na_T and Cl_T resemble the values found in *G. duebeni* and the similarity between the two species is further shown by the maintenance of a higher sodium content compared with the chloride content in *G. zaddachi* at low salinities. The ratio Na_T/Cl_T was greater than 1·0 in every individual case, including six animals from 100 % sea water, and the mean ratio increased to about 1·85 in animals acclimatized to 0·25 mM/l NaCl (Table 1). This is higher than the mean ratios of 1·48–1·65 found in *G. duebeni* acclimatized to the same medium (Sutcliffe, 1971 a). In fact the ratio Na_T/Cl_T was higher in *G. zaddachi* when compared with *G. duebeni* at all external salinities up to 100 % sea water.

Potassium

The total body potassium concentrations showed little change at salinities between 100 and 2 % sea water and there was only a slight fall in animals acclimatized to NaCl-media for up to 5 days (Table 1, Fig. 2). Thus the regulation of body potassium, and the blood potassium concentration (Table 2), is practically the same as in *G. duebeni*. However, the detailed features of potassium balance at very low salinities may differ slightly. Some preliminary measurements of the potassium loss rate into 250–1000 ml de-ionized water were made on three groups of *G. zaddachi* kept in 100 % sea water. At 23 °C the initial mean loss rate was 0·25 μM/h/animal. At 9 °C the initial mean loss rate was 0·11 μM/h, identical with the potassium loss rate in *G. duebeni* from 100 % sea water. After a period of 24 h the animals reached a temporary steady state with respect to potassium when the external concentration had increased to 0·027–0·037 mM/l potassium. In this particular experiment the total potassium loss from the animals represented about 20 % of the body potassium. The loss rate was then measured again in 100–400 ml deionized water. The mean potassium loss rates were 0·020–0·023 μM/h at 9 °C, and the 'balance' concentrations after a period of 24 h were 0·027–0·035 mM/l. These are higher than the minimum external potassium concentrations of 0·010–0·015 mM/l required for potassium balance in *G. duebeni* (Sutcliffe, 1971 a), but a more detailed study of potassium regulation in *G. zaddachi* acclimatized to low salinities is necessary for strict comparison between the two species.

Blood space

The mean blood space was calculated from the data given in Table 1 and Table 2. The results are shown in Fig. 1. The blood space is equivalent to about 60 % body H_2O in animals from 100 % sea water and equivalent to 50 % body H_2O in animals acclimatized to 40 % sea water down to 0·5 mM/l NaCl. At lower salinities there was a very marked reduction in the blood space to only 30 % body H_2O in animals from 0·25 mM/l NaCl. In every case the values for the blood space are very close to the maximum calculated on the assumption that all of the body chloride is in the blood space at the concentration found in the blood. This chloride space contains 2·3–4·9 % body H_2O more than the blood space. In contrast the sodium space, calculated on the assumption that all of the body sodium is at the concentration found in the blood, represents approximately 60–70 % body H_2O over a wide range of external salinities (Fig. 1). These values for the blood, chloride and sodium spaces are very similar to the values for the same spaces in *G. duebeni*.

Cell ions

The calculated concentrations of sodium, potassium and chloride in the intracellular water are shown in Fig. 2. The cell sodium concentration was fairly constant, ranging from 65 to 116 mM/kg cell H_2O. In animals from 2% sea water down to 0·25 mM/l
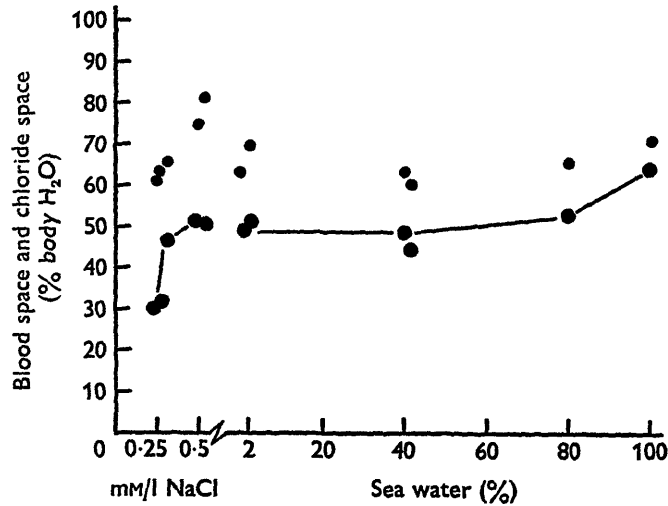

Fig. 1. The mean blood space (●—●) and the sodium space (●) in *Gammarus zaddachi*. Explanation in the text.
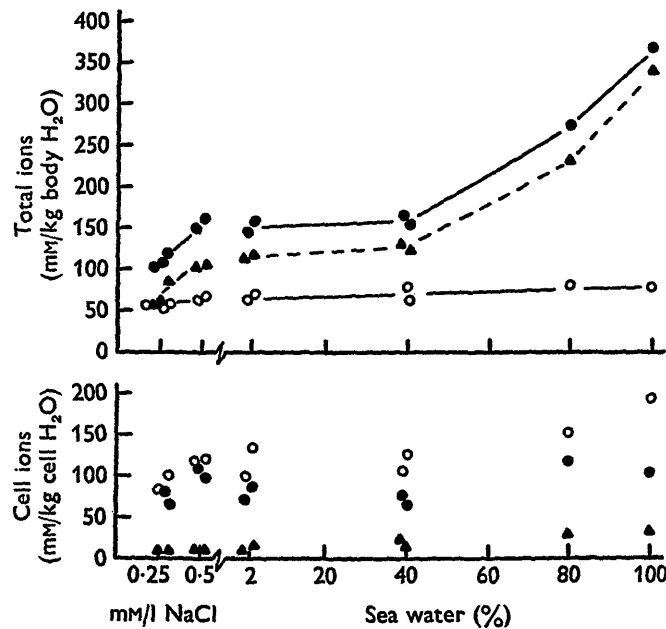

Fig. 2. The concentrations of sodium (●), potassium (○) and chloride (▲) in the body water and in the intracellular space of *Gammarus zaddachi*.

NaCl the concentrations of cell potassium-plus-sodium range from 226 to 163 mM/kg cell H_2O, and these are equal to the concentrations of potassium-plus-sodium in the blood. In animals from 80 to 100% sea water the concentrations of cell potassium-plus-sodium were 267–296 mM/kg cell H_2O, representing about 50–56% of the blood

concentration. The cell potassium concentration in *G. zaddachi* from 80 to 100% sea water is higher than the cell potassium concentration in *G. duebeni*, otherwise the concentrations of the three ions are similar to those calculated for *G. duebeni* and similar to the concentrations found in muscle and some other tissues of marine, brackish and fresh-water decapods.

RESULTS ON *MARINOGAMMARUS FINMARCHICUS* AND *GAMMARUS LOCUSTA*

Water content

In animals from 100% sea water the body water content was equivalent to approximately 72% body wet weight in both species (Table 3, Table 4). At lower salinities the water content in *M. finmarchicus* increased to 76% body wet weight in animals from 20% sea water. The water content of these two species is slightly lower compared with *G. zaddachi* and *G. duebeni*.

Table 3. *Wet weight, water content and concentrations of total ions in* Marinogammarus finmarchicus

(Mean results from six animals ± 1 standard error)

Medium (% SW)	Wet weight (mg)	Water content (% wet wt.)	Total ions (mM/Kg body H_2O)			Ratio Na_T/Cl_T
			Na_T	Cl_T	K_T	
100	67·9±1·93	71·5±0·65	280·2±2·48	226·3±4·88	98·2±2·02	1·24
80	74·0±3·67	73·8±0·77	241·7±5·58	197·8±5·13	89·3±1·43	1·22
40	59·4±2·44	73·9±1·29	190·7±6·93	152·5±8·99	88·3±2·16	1·25
20	62·6±1·33	76·1±0·68	167·2±10·50	128·3±13·17	82·2±2·94	1·31

Table 4. *Wet weight, water content and concentrations of total ions in six* Gammarus locusta *acclimatized to 100% sea water*

Wet weight (mg)	Water content (% wet wt.)	Total ions (mM/kg body H_2O)			Ratio Na_T/Cl_T
		Na_T	Cl_T	K_T	
336·5	72·0	310	273	77	1·14
319·5	74·5	326	295	78	1·10
303·5	70·5	277	235	91	1·18
113·0	72·5	305	254	84	1·20
64·5	72·0	295	248	90	1·19
59·5	72·0	256	200	102	1·28
Means	72·2	295	251	87	1·18

Sodium and chloride

Values for the blood sodium concentration in *M. finmarchicus* at salinities from 100 to 10% sea water were given by Sutcliffe (1968). Some additional analyses of blood sodium and chloride were made on pooled samples (Table 5). These were obtained from 15 to 16 animals with some exceptions noted in the table. Table 5 also includes samples taken from animals exposed to sea water which was then allowed to become slightly concentrated by evaporation. At salinities above 80% sea water the sodium concentration in the blood was maintained at or slightly above the sodium concentration in the medium, but the chloride concentration in the blood was distinctly

lower than that in the medium. In this respect *M. finmarchicus* resembles *G. zaddachi*, and a low blood chloride concentration is also characteristic of *G. locusta*. Two large specimens of *G. locusta* were acclimatized to slightly concentrated sea water with a chloride concentration of 630 mM/l. The blood chloride concentrations in the two animals were 580 and 550 mM/l respectively. Beadle & Cragg (1940a) found a blood chloride concentration of about 420 mM/l in *G. locusta* from sea water containing *c.* 495 mM/l chloride, and the blood chloride level was slightly below that of the external chloride in 75% sea water. Beadle & Cragg also found a lower chloride concentration in the blood of *Marinogammarus obtusatus* compared with the chloride concentration in undiluted sea water.

Table 5. *Concentrations of sodium, potassium and chloride in pooled blood samples from* Marinogammarus finmarchicus

(All concentrations in mM/l)

Medium (% SW)	Medium		Blood		
	Cl	Na	Cl_o	Na_o	K_o
115	630	—	578† 582‡	—	—
113	618	512	535*	512*	—
100	548	487	472	495	12·0
80	432	—	432	—	—
40	210	195	372	382	—
20	110	—	300*	—	10·5*

† Blood from one animal.
‡ Blood from four animals.
* Blood from eight animals.

In animals from 100% sea water the values for the total body sodium and chloride concentrations in *M. finmarchicus* (Table 3, Fig. 4) and *G. locusta* (Table 4) are lower than the values found in *G. zaddachi* and *G. duebeni* despite the lower water content of the two marine-littoral species. Another distinguishing feature is the higher value ot the ratio Na_T/Cl_T. This ratio was approximately 1·2 in the marine-littoral gammarids (Table 3, Table 4) reflecting the lower blood chloride levels maintained in 100% sea water, although a lower chloride concentration in the cells would also increase the ratio. Furthermore, the ratio Na_T/Cl_T remained constant in *M. finmarchicus* at salinities down to 40% sea water, and showed only a small increase in 20% sea water (Table 3).

Potassium

The blood potassium concentration showed little change in *M. finmarchicus* from 100 to 20% sea water (Table 5). In one large specimen of *G. locusta* exposed to slightly concentrated sea water the blood potassium concentration was 13·5 mM/l (blood chloride = 550 mM/l). In both species the concentrations of total body potassium at 100% sea water were slightly higher than the concentrations found in *G. zaddachi* and *G. duebeni*, but the blood potassium concentrations in all of these gammarids were maintained at the same level. This suggests that the intracellular potassium concentration might be maintained at a higher level in the less euryhaline marine-littoral gammarids compared with the more euryhaline species *G. zaddachi* and *G. duebeni*.

Blood space

The calculated blood space (= extracellular water) in *M. finmarchicus* at salinities between 100 and 20% sea water is shown in Fig. 3. A blood chloride concentration of 372 mM/l (Table 5) was used to calculate the lower value for the blood space (= 36% body H_2O) at 40% sea water. However, the blood chloride concentration may have been lower than this in the animals used to estimate the concentrations of total body ions. If the blood chloride concentration was 350 mM/l in these animals from 40% sea water, the blood space would then be equivalent to 39.5% body H_2O.
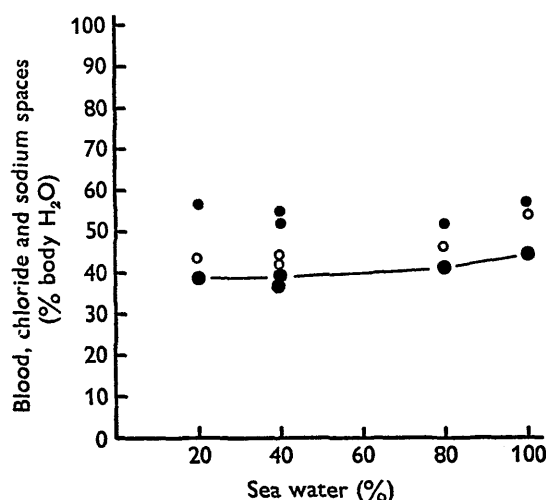

Fig. 3. The mean blood space (●—●), chloride space (o) and sodium space (●) in
Marinogammarus finmarchicus.

From Fig. 3 it appears that there was remarkably little change in the blood space despite a considerable fall in the blood concentration at salinities between 100% and 20% sea water (Table 5; Sutcliffe (1968), Fig. 6). The values for the blood space in *M. finmarchicus* are lower than the values for the blood space in *G. zaddachi* and *G. duebeni*. This also applies to the values for the chloride and sodium spaces (Fig. 3). These spaces were calculated in the manner described for *G. zaddachi*. Very similar values were obtained for *G. locusta* in 100% sea water, assuming blood concentrations of 472 mM/l chloride and 12 mM/l potassium. In this case the blood space is 49.4% body H_2O, the chloride space is 53.5 and the sodium space is 59.6.

Cell ions

The calculated concentrations of sodium, potassium and chloride in the intracellular water of *M. finmarchicus* are shown in Fig. 4. All three ions appear to be maintained at fairly constant levels. For each ion its intracellular concentration in *M. finmarchicus* closely resembles its intracellular concentrations in *G. zaddachi* and *G. duebeni*. In *G. locusta* from 100% sea water the calculated intracellular concentrations of sodium, potassium and chloride were practically identical with the concentrations in *M. finmarchicus* at 100% sea water (Table 6). Thus the suggestion made earlier that the higher total body potassium concentration in the marine-littoral gammarids might

reflect a higher intracellular potassium concentration is not supported by these results. This is because the undoubtedly higher potassium content in the cells of these gammarids from 100% sea water is offset by the higher water content of the cells,

Table 6. *Calculated mean intracellular concentrations of sodium, potassium chloride in* Gammarus locusta *from 100% sea water*

Cell ions (mM/Kg cell H_2O)
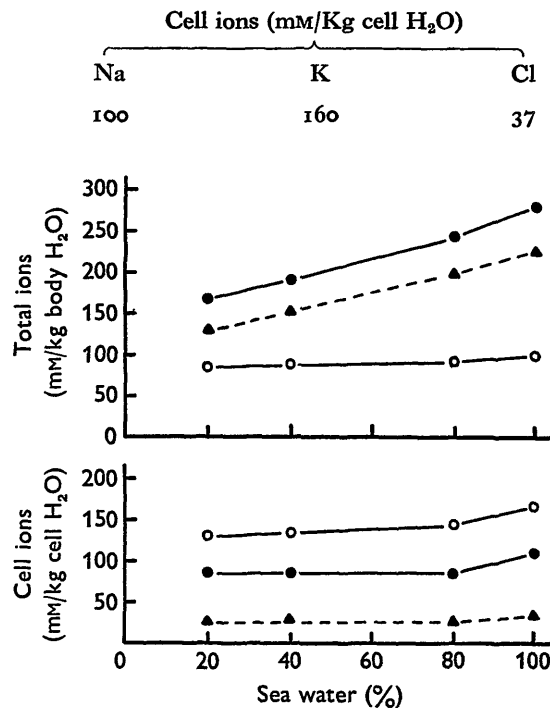
Na	K	Cl
100	160	37

Fig. 4. The concentrations of sodium (●), potassium (○) and chloride (▲) in the body water and in the intracellular space of *Marinogammarus finmarchicus*.

reckoned to be equivalent to 50-60% body H_2O. In comparison the water content of the cells in *G. zaddachi* and *G. duebeni* from 100% sea water was reckoned to be equivalent to 40-45% body H_2O.

DISCUSSION

The distribution of water and ions in the blood space and in the intracellular space was calculated from the assumption that there is a Donnan equilibrium between potassium and chloride ions in these two spaces (Sutcliffe, 1971*a*). On this basis the blood spaces in *Marinogammarus finmarchicus* and *Gammarus locusta* were equivalent to 44 and 50% body H_2O respectively in animals from 100% sea water. Similar values were obtained for the blood spaces in the more euryhaline *G. duebeni* and *G. zaddachi* from 2 to 40% sea water, and in *G. pulex* from fresh water. In the case of *G. duebeni* and *G. pulex* it was shown that it is reasonable to assume that a Donnan equilibrium exists between potassium and chloride in the blood and cells (Sutcliffe, 1971*a, b*). It is suggested that this assumption is also reasonable as an approximation in the case of the euryhaline gammarids considered in this study. Otherwise it is clear that the proportion of body water in the blood space must be lower than that calculated here, and

the blood spaces would then contain less than 40% of the body water. In fact, if the extracellular tissue space represents 20% of the tissue water, then the blood situated outside the extracellular tissue space would contain as little as 20% of the body water in animals from low salinities, i.e. approximately 10 mg H_2O in an animal of 65 mg body wet weight. There is no direct evidence in support of blood volumes as low as this. Up to 5 mg blood can be obtained without difficulty from a 65 mg animal through a small puncture made in a dorsal intersegmental membrane, and this technique only removes blood from the part of the haemocoel immediately surrounding the gut.

In comparison with *G. duebeni* and *G. zaddachi* the less euryhaline *G. locusta* and *M. finmarchicus* maintain higher blood concentrations at salinities between 20% and 100% sea water. They also have a lower body water content, a smaller sodium space, and slightly lower concentrations of sodium and chloride in the body water than animals from 80 to 100% sea water. When taken together these observations indicate that it is unlikely that the tissue ion content is maintained at a higher level in the two marine-littoral species. Rather, they lead to the conclusion that the mean intracellular concentrations of sodium, potassium and chloride are practically the same in all of these euryhaline gammarids. This is in agreement with direct measurements made on stenohaline and euryhaline arthropods. In *Nephrops* and *Paralithodes* (Robertson, 1961; MacKay & Prosser, 1970) the concentrations of sodium, potassium and chloride in the muscles are substantially the same as in *Eriocheir*, *Carcinus*, *Potamon*, *Pacifastacus*, *Callinectes* and *Limulus* (Krogh, 1939; Shaw, 1955a, b, 1959; Kerley & Pritchard, 1967; Hays, Lang & Gainer, 1968; Robertson, 1970).

The above conclusion is also in agreement with the work of Beadle & Cragg (1940a) who found that the chloride concentrations in the 'tissues' of both *G. locusta* and *M. obtusatus* from 100% sea water were slightly lower than the corresponding values in *G. duebeni*. In *G. locusta* Beadle & Cragg found approximately 120 mM-Cl/kg wet tissue against a blood chloride concentration of 420 mM/l. Derouet (1952) also found about 128 mM-Cl/kg wet tissue against a blood chloride concentration of 440 mM/l in *G. locusta* from 100% sea water. Now the mean intracellular chloride concentration in this 'tissue' can be calculated in the manner described for *G. duebeni* and *G. pulex* (Sutcliffe, 1971a, b), assuming that the extracellular tissue space contained 20% of the tissue water with a chloride concentration equal to that of the blood. The extracellular tissue space would then contain 67–70 mM-Cl/160 g H_2O, leaving 53–58 mM-Cl/640 g H_2O in the cells. This gives an intracellular chloride concentration of 83–91 mM/kg cell H_2O compared with the estimate of 37 mM/kg cell H_2O given in Table 6. At least part of the difference would be due to a residual amount of blood in the appendages of the 'tissue' preparations (whole animal minus blood), since it is impossible to remove all of the blood (Sutcliffe, 1971a). The entire difference would be accounted for if only 85% of the initial blood volume was removed during the preparation of the 'tissue'.

A comparison of the intracellular concentrations of sodium and chloride is shown in Fig. 5, where the cell concentrations are plotted against the blood concentrations in a series of gammarids. In *M. finmarchicus*, *G. zaddachi* and *G. duebeni* the intracellular chloride concentration is approximately proportional to the blood concentration, and this type of regulation is also found in the muscle fibres of *Carcinus* (Shaw, 1955b). In contrast the freshwater species *G. pulex* lacks the ability to maintain a low chloride concentration in the cells when the blood concentration is raised. The cell sodium

concentration also rises steeply in *G. pulex*, as in *Astacus* (Bryan, 1960), and this again stands in contrast to the situation found in the euryhaline gammarids. Here the intracellular sodium concentrations rise or fall by less than 50% in the face of much greater changes in the blood sodium concentration. In this respect sodium regulation resembles chloride regulation. In fact, if a major proportion of the intracellular sodium is situated outside the muscles, sodium regulation in the muscles of the euryhaline gammarids could resemble that of *Carcinus*, where the sodium concentration in the muscles is proportional to the sodium concentration in the blood (Shaw, 1955 b). In any case it appears that the regulation of cell sodium in *M. finmarchicus* differs from sodium
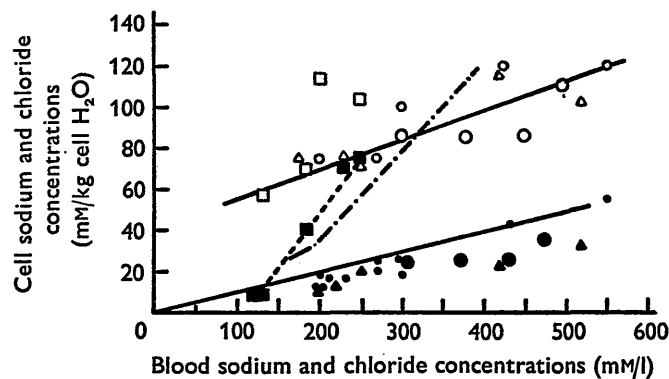

Fig. 5. A comparison of the intracellular concentrations of sodium and chloride in relation to the blood concentrations in a series of gammarids. O Na, ● Cl in cells of *Marinogammarus finmarchicus*. △ Na, ▲ Cl in cells of *Gammarus zaddachi*. o Na, ● Cl in cells of *G. duebeni* (Sutcliffe, 1971 a). □ Na, ■ Cl in cells of *G. pulex* (Sutcliffe, 1971 b). The solid line drawn through the origin of the Figure represents Na and Cl in the muscle fibres of *Carcinus* (Shaw, 1955 b) where the cell concentrations are proportional to the blood concentrations. The broken line (—·—·) represents Na concentrations in the muscles of *Astacus* (Bryan, 1960).

regulation (or the lack of it) in the muscles of *Limulus* (Robertson, 1970). Further speculation on this and related matters, such as the apparently close regulation of the proportions of body water in the blood space and intracellular space of *M. finmarchicus*, must wait until a more direct estimate of the blood space is available for these gammarids.

SUMMARY

1. A comparison was made of the body water contents and the concentrations of sodium, potassium and chloride in the blood and body water of *Gammarus zaddachi*, *G. locusta* and *Marinogammarus finmarchicus*.

2. *G. zaddachi* had a slightly higher body water content than *G. locusta* and *M. finmarchicus*.

3. In all three species the blood chloride concentration was lower than the external chloride concentration in 80–113% sea water, but the blood sodium concentration was equal to or slightly above the sodium concentration in the external medium.

4. The total body sodium concentration was always greater than the total body chloride concentration. In *M. finmarchicus* the ratio of body sodium/chloride increased from 1·2 to 1·3 over the salinity range 100–20% sea water. In *G. zaddachi* the ratio of

body sodium/chloride increased from 1·08 at 100% sea water to 1·87 in 0·25 mM/l NaCl.

5. The total body potassium concentration remained constant. The potassium loss rate and the balance concentration were relatively high in *G. zaddachi*.

6. The porportion of body water in the blood space was calculated from the assumption that a Donnan equilibrium exists between chloride and potassium ions in the extracellular blood space and the intracellular space. In *G. zaddachi* the blood space was equivalent to 60% body H_2O at 100% sea water, and equivalent to 50% body H_2O at 40% sea water down to 0·5 mM/l NaCl. In *M. finmarchicus* the blood space was equivalent to 38-44% body H_2O at salinities of 20-100% sea water.

7. The mean intracellular concentrations of sodium, potassium and chloride were also calculated. It was concluded that for each ion its intracellular concentration is much the same in the four euryhaline gammarids. The intracellular chloride concentration is roughly proportional to the blood chloride concentration. The intracellular sodium concentration is regulated in the face of large changes in the blood sodium concentration.

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