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THE EFFECT OF CALCIUM ON SODIUM INFLUX AND LOSS IN *GAMMARUS* (AMPHIPODA)

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INTRODUCTION

Comparative studies of sodium influx and loss have been made on a series of gammarid crustaceans from different environments, ranging from marine through to fresh water (Shaw & Sutcliffe, 1961; Sutcliffe, 1968, 1970). In this work sodium influx and net uptake of sodium were measured in sodium chloride solutions made with de-ionized water, and sodium loss was determined by measuring net sodium loss into small volumes of de-ionized water. Some unpublished experiments carried out early on in these studies had indicated that the addition of calcium salts to these media, giving a calcium concentration of 1 mM/l., did not affect either sodium influx or sodium loss in *Gammarus pulex* and *G. duebeni*. This was in accord with the observations of Shaw (1960) on the effect of calcium on sodium influx in the crayfish *Astacus pallipes*. Recently, however, Morris & Bull (1968) reported that the addition of calcium salts lowered the rate of sodium loss into de-ionized water in ammocoetes of the river lamprey, *Lampetra planeri*. They also suggested that the presence of calcium was responsible for a much faster sodium uptake in dilute Ringer solution compared with the uptake rate from pure sodium chloride. The effects of calcium on sodium movements in *Gammarus* were therefore examined again, using three species from freshwater and brackish-water habitats.

MATERIAL AND METHODS

Gammarus pulex (L.) was obtained from Crag Lough, Northumberland, and from Esthwaite Lake and from a small stream at Outgate, Lancashire. *G. duebeni* Lilljeborg was obtained from fresh water on the Stranraer peninsula, Wigtown (Sutcliffe, 1970) and from salt-marsh pools at Warton, Lancashire. *G. zaddachi* Sexton was collected in the tidal zone of a small stream near Barrow-in-Furness, Lancashire.

Most of the experiments were carried out at temperatures of 9 ± 1 °C or 10 ± 1 °C with animals acclimatized to these temperatures for at least 1 week. Some experiments on sodium loss in *G. pulex* were carried out at 20 ± 1 °C.

Sodium influx was determined with $^{22}NaCl$ as the tracer. In experiments where calcium was added to NaCl solutions containing the tracer this was brought about by adding small amounts (usually less than 0·15 ml.) of a concentrated solution of $CaCO_3$ or $CaCl_2$. Sodium loss was measured in de-ionized water or in a solution of $CaCl_2$. All salt solutions were made up with de-ionized water. The techniques for measuring sodium influx and loss were described previously (Shaw & Sutcliffe, 1961; Sutcliffe, 1967*a*).

RESULTS

Experiments on Gammarus pulex (L.)

Effect of calcium on sodium influx

Some earlier experiments (1964) were carried out with *G. pulex* from Northumberland. The animals were divided into two batches; (A) acclimatized to 0·1 mM/l. NaCl and (B) acclimatized to 0·1 mM/l. NaCl containing 1 mM/l. $CaCO_3$. Groups of animals from each batch were used to determine sodium influx from NaCl solutions ranging in concentration from 0·06 to 0·5 mM/l. NaCl at 10 °C. The results are shown

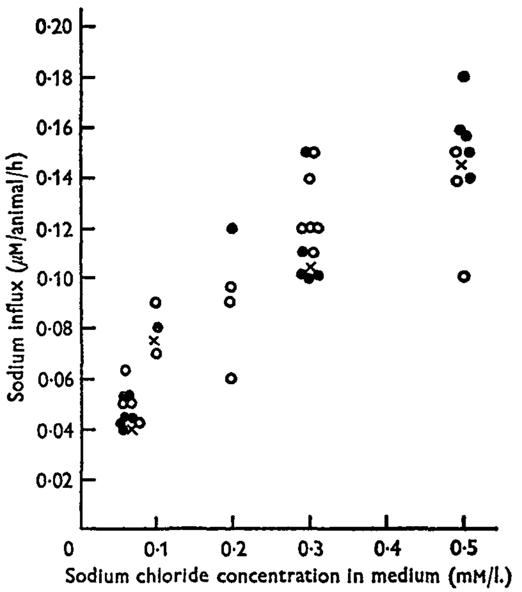

Fig. 1

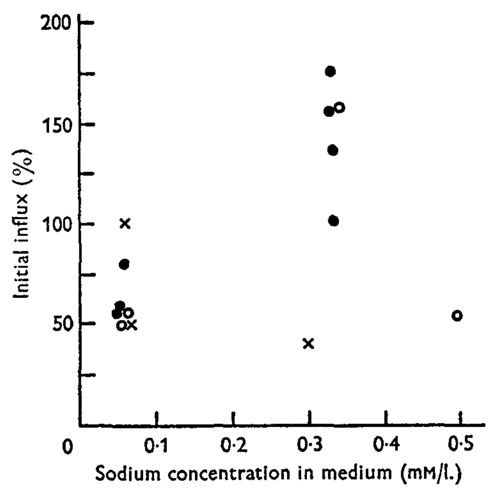

Fig. 2

Fig. 1. Sodium influx in *G. pulex* at 10 °C from a series of NaCl solutions. Open circles represent sodium influx in animals acclimatized to 0·1 mM/l. NaCl (batch A). Closed circles represent sodium influx in animals acclimatized to 0·1 mM/l. NaCl + 1 mM/l. $CaCO_3$ (batch B). The crosses represent sodium influx from a simulated 'fresh water' medium. See text for further explanation.

Fig. 2. Relative changes in sodium influx in *G. pulex* from NaCl solutions at 10 °C following the addition of 1 mM/l. calcium. Open circles represent sodium influx after addition of $CaCl_2$, closed circles after addition of $CaCO_3$, in animals acclimatized to 0·1 mM/l. NaCl + 1 mM/l. $CaCO_3$. Crosses represent sodium influx after addition of $CaCO_3$ in animals acclimatized to 0·1 mM/l. NaCl.

in Fig. 1. Sodium influx from NaCl solutions took place at the same rate in animals from (A) and from (B), so it appeared that sudden exposure to a calcium-free medium did not affect the influx in animals acclimatized to 1 mM/l. $CaCO_3$ in (B). The latter animals were then acclimatized to a simulated 'freshwater' medium containing 0·1 mM/l. NaCl, 1 mM/l. $CaCO_3$ and 0·25 mM/l. $MgSO_4$. Sodium influx in these animals was determined at NaCl concentrations of 0·06–0·5 mM/l. in the presence of 1 mM/l. $CaCO_3$ and 0·25 mM/l. $MgSO_4$ (Fig. 1). Again prior acclimatization to this simulated 'fresh water' did not affect the influx, which was the same as from a solution of NaCl.

However, some changes in sodium influx were observed when *G. pulex* from (A) and from (B) were suddenly exposed to calcium ions during sodium uptake from a solution of NaCl. For this the influx was measured over a period of about 30 min. A small amount of $CaCO_3$ or $CaCl_2$ was then added to the NaCl medium to give a calcium concentratoin of 1 mM/l., and the influx was measured again during a further period of 30–60 min. The results of a few determinations are given in Fig. 2, where sodium influx is expressed as a percentage of the initial influx before the addition of $CaCO_3$ or $CaCl_2$. In a total of fourteen measurements of sodium influx two were not

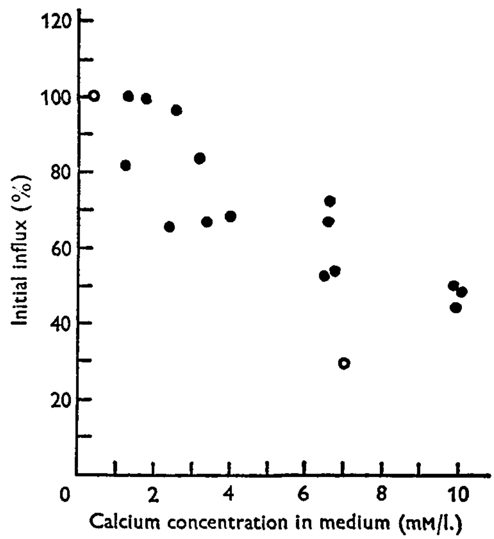

Fig. 3. Relative changes in sodium influx from 0·15 mM/l. NaCl in *G. pulex* at 9 °C following the addition of calcium as $CaCl_2$ (closed circles) and $CaSO_4$ (open circles).

altered, four showed an increase and eight showed a decrease. The main conclusion from these results is that addition of calcium does not consistently raise the influx of sodium. This was confirmed by later experiments on *G. pulex* obtained from Esthwaite, Lancashire.

G. pulex from Esthwaite were acclimatized to 0·15 mM/l. NaCl at 9 °C. Sodium influx from 0·15 mM/l. NaCl was determined before and after the addition of $CaCl_2$ to give calcium concentrations of 1–10 mM/l. The results are shown in Fig. 3. At calcium concentrations greater than 2 mM/l. the influx was consistently lowered, and it was reduced to about 60% of the initial rate at 6·7 mM/l. calcium, and to 50% at 10 mM/l. calcium. A single measurement of the influx following addition of 7 mM/l. $CaSO_4$ showed a decrease to 30% of the initial rate. The influx was unaffected by 0·1 mM/l. $CaSO_4$ (Fig. 3). In the crayfish *Astacus*, Shaw (1960) found that sodium influx from 0·05 mM/l. NaCl occasionally was reduced to 60% of the initial rate at external calcium concentrations of 2–5 mM/l., but in the majority of cases the influx remained unchanged. This is interesting in view of the fact that in Shaw's experiments with *Astacus* the ratio of calcium/sodium in the external medium was three times greater than the ratio in the present experiments with *G. pulex*. Further experiments are necessary to explore the possibility that the influx is reduced by competition between calcium and sodium ions at the transporting sites.

Effect of calcium on sodium loss

Earlier experiments with *G. pulex* from Northumberland had shown that net sodium loss occurred at the same rate in de-ionized water and in a solution of 1 mM/l. $CaCl_2$. This was confirmed by recent experiments on *G. pulex* from Outgate, Lancashire.

Table 1. *Concentrations of major ions in Windermere water (mM/l.)*

Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	SO_4^{2-}
0·20	0·015	0·16	0·03	0·23	0·20	0·07

One hundred and twenty animals with a mean wet weight of 41 mg were acclimatized to Windermere lake water containing 0·2 mM/l. sodium and 0·16 mM/l. calcium (Table 1) at a temperature of 20 ± 1 °C. Using groups of 15 animals, four groups were placed in 75 ml. de-ionized water and four groups were placed in 75 ml. of 0·5 mM/l. $CaCl_2$ solution. Sodium loss was measured at 10 min. intervals during the first hour. The rate of sodium loss was similar in both media (Fig. 4). After 2·5 h sodium loss had raised the sodium concentration of the medium to 33–38 μM/l. in the case of groups in de-ionized water and to 34–38 μM/l. in the case of groups in $CaCl_2$ solution. The respective groups were then transferred to 50 ml. de-ionized water or 0·5 mM/l. $CaCl_2$ solution and the loss rates were measured during a period of 1 h. Again the sodium loss rates in the two media were very similar (Fig. 4) but the rates had fallen to about 60% of the initial loss rate (*c.* 0·1 μM/h) measured during the first period of sodium loss. The lower loss rates during the second period of sodium loss are associated with a 5% reduction in total body sodium, calculated from unpublished data on the total body sodium content in *G. pulex*. After a further period (*c.* 20 h) of net sodium loss the total body sodium of animals in both media had fallen to about 85% of the initial sodium content. A third determination of the sodium loss rate was then made in 50 ml of the appropriate medium. The loss rates were again very similar in de-ionized water and in $CaCl_2$ solution, with a mean rate of about 0·06 μM/h (Fig. 4). It is interesting to note that a reduction in body sodium from 95% to 85% of the initial body sodium did not lead to a further decrease in the loss rate during the third period of sodium loss.

The above experiment was terminated by replacing the animals in Windermere water for 48 h. The experiment was then repeated, but on this occasion the sodium loss rates into de-ionized water or 0·5 mM/l. $CaCl_2$ solution were determined over three successive periods of 1 h, using 50 ml. aliquots of the respective media for each determination. The results are shown in Fig. 5. The loss rates were higher than in the previous experiment but there was no significant difference between sodium losses in the presence or absence of calcium ions. In this experiment the sodium loss rate during the second period of sodium loss was slightly lower than the initial loss rate, following an estimated 5% reduction in total body sodium. But during the third period of sodium loss the loss rate was reduced to approximately 70% of the initial rate, when the total body sodium had fallen by 8–10% (Fig. 5). The loss rate then remained unchanged during a fourth period of sodium loss although the total body sodium had fallen to below 85 % of the initial sodium content.

During these experiments with *G. pulex* it was noted that the external 'balance' concentration, achieved when sodium uptake from the medium equals the sodium loss rate, was similar in both de-ionized water and 0·5 mM/l. $CaCl_2$ solution.

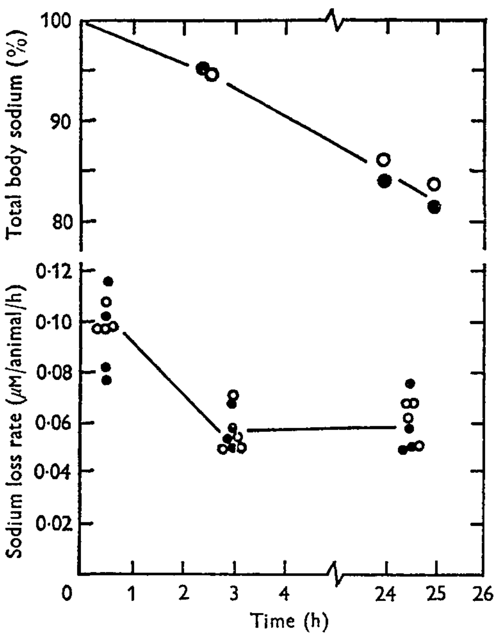

Fig. 4

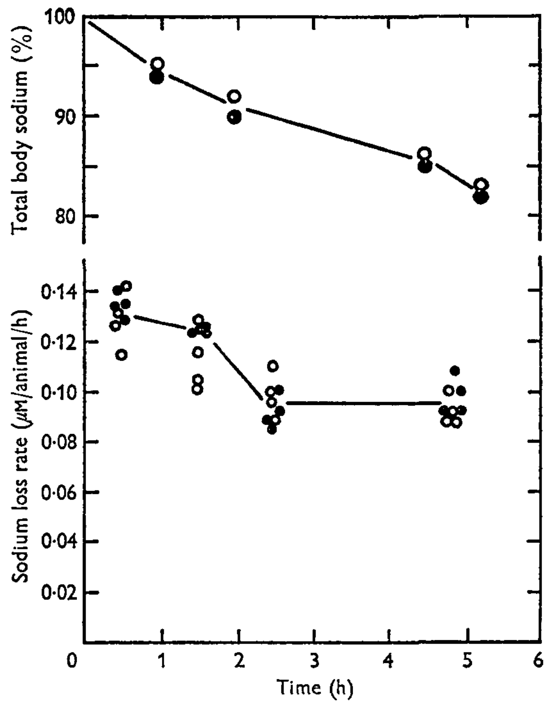

Fig. 5

Fig. 4. Progressive changes in total body sodium and sodium loss rate in *G. pulex* acclimatized to Windermere lake water at 20 °C. Open circles represent groups of animals in 0·5 mM/l. $CaCl_2$, closed circles represent groups of animals in de-ionized water.

Fig. 5. Progressive changes in total body sodium and sodium loss rate in *G. pulex* acclimatized to Windermere lake water at 20 °C. Symbols as in Fig. 4.

Experiments on Gammarus duebeni *Lilljeborg*

Effect of calcium on sodium influx

This was tested on *G. duebeni* from Stranraer. Animals with a mean wet weight of 75 mg were successively acclimatized to a series of media containing various concentrations of sodium and calcium at 9 °C. The media were Windermere lake water with the sodium concentration raised by addition of NaCl, and NaCl solutions with or without the addition of $CaCl_2$. Sodium influx was determined in a series of similar media. The results are shown in Table 2. For easy reference the acclimatization media are numbered (1)–(4).

(1) *Acclimatized to lake water containing* 0·5 *mM/l. sodium.* The mean sodium influx from 2 mM/l. NaCl was slightly greater than the mean influx from lake water containing 2 mM/l. sodium, but the difference is not regarded as significant.

(2) *Acclimatized to* 2 *mM/l. NaCl.* Sodium influx from 2 mM/l. NaCl fell to a mean of 0·30 μM/h compared with a mean of 0·44–0·49 μM/h in (1) when acclimatized to 0·5 mM/l. sodium in lake water. However, a reduction in the sodium influx of animals moved to a higher external sodium concentration is a normal feature of sodium regulation in gammarids, effectively reducing the sodium uptake rate in response to an in-
ed internal sodium content.

Table 2. *The effect of calcium on sodium influx in* Gammarus duebeni *from Stranraer (mean wet weight* 75 *mg.) at* 9 °*C*

Acclimatization medium	Na influx from 2·0 mM/l. NaCl (μM/animal/h)	Na influx from media containing calcium (μM/animal/h)	
Lake water containing 0·5 mM/l. Na, 0·16 mM/l. Ca		0·43	Sterilized lake water containing 2·0 mM/l. Na, 0·16 mM/l. Ca
	0·43	0·41	
	0·63	0·50	
	0·42	0·42	
	Mean 0·49	Mean 0·44	
(2) 2·0 mM/l. NaCl	0·34	—	
	0·30	—	
	0·27	—	
	Mean 0·30	—	
(3) Lake water containing 2·0 mM/l. Na, 0·16 mM/l. Ca		0·23	2·0 mM/l. NaCl + 0·5 mM/l. $CaCl_2$
	0·34	0·21	
	0·30	0·19	
	0·25	0·57	
	0·28	0·43	
	0·28	0·24	
	Mean 0·29	Mean 0·31	
(4) 2·0 mM/l. NaCl + 0·25 mM/l. $CaCl_2$	0·52	0·42	2·0 mM/l. NaCl + 0·25 mM/l. $CaCl_2$
	0·52	0·68	
	0·44	0·44	
	0·47	0·42	
	0·45	0·45	
	Mean 0·48	Mean 0·48	
		0·41	2·0 mM/l. NaCl + 10 mM/l. $CaCl_2$
		0·53	
		0·60	
		Mean 0·51	

(3) *Acclimatized to lake water containing* 2 *mM/l.* sodium. The sodium influxes from 2 mM/l. sodium (0·29–0·31 μM/h) were not affected by the presence of 0·5 mM/l. calcium. In addition, since the influxes were at the same level as in (2), it appears that sodium influx was not affected by prior acclimatization in a medium containing 0·16 mM/l. calcium.

(4) *Acclimatized to* 2 *mM/l. NaCl* + 0·25 *mM/l.* $CaCl_2$. Sodium influx from 2 mM/l. NaCl was not affected by the addition of low (0·25 mM/l.) or high (10 mM/l.) concentrations of calcium.

The levels of sodium influx in (4) were much higher than in (3). When the influxes in (4) were determined the batch of *G. duebeni* had been used for measuring sodium influx and loss over a period of 6 weeks. During this period mortality reduced the number of animals in the batch from 90 in (1) to 70 at the end of (3). At the start of

(4) only 36 animals remained alive. The higher sodium influxes in (4) were therefore probably the result of higher than average influxes in the survivors remaining after a period of heavy mortality. An increased mean sodium influx following heavy mortality in *G. duebeni* has been observed before (Shaw & Sutcliffe, 1961).

Effect of calcium on sodium loss

After determination of sodium influx in (3) with animals acclimatized to lake water containing 2 mM/l. sodium, the sodium loss rate into de-ionized water and into 1 mM/l. $CaCl_2$ solution was compared using groups of 14 animals in 50 ml. of medium. The animals were returned to the acclimatization medium for 24 h after each determination of the loss rate. The results are given in experimental order in Table 3. On the first occasion the loss rate in the presence of calcium was lower than on subsequent occasions where the loss rate was similar in the presence or absence of calcium. This was the only occasion where a lower sodium loss rate has been observed in *G. duebeni* in the presence of calcium.

Table 3. *Sodium loss rates into de-ionized water and calcium chloride solutions in* Gammarus duebeni *from Stranraer (mean wet weight 75 mg) at 9 °C*

Medium	Mean Na loss rate (μM/animal/h)	No. of groups
1 mM/l. $CaCl_2$	0·17	5
De-ionized water	0·24	5
De-ionized water	0·23	5
1 mM/l. $CaCl_2$	0·23	4

Table 4. *The effect of calcium on sodium influx and loss in* Gammarus duebeni *from Stranraer (mean wet weight 44 mg) at 9 °C*

Acclimatization medium	Na influx from 2·0 mM/l. NaCl (μM/animal/h)	Na influx from 0·5 mM/l. NaCl (μM/animal/h)	Na loss rate in de-ionized water (μM/animal/h)	Na loss rate in 1·0 mM/l. $CaCl_2$ (μM/animal/h)
2 % sea water (9 mM/l. Na, 0·2 mM/l. Ca)	0·26 (5)	0·13 (5)	0·14 (5)	0·12 (6)
2·0 mM/l. NaCl	0·25 (4)	0·13 (1)	—	—
	*0·24 (3)	*0·13 (1)	—	—
2·0 mM/l. NaCl + 0·5 mM/l. $CaCl_2$	0·21 (5)	—	0·14 (5)	0·13 (5)
2·0 mM/l. NaCl	0·20 (2)	—	—	—
1·0 mM/l. NaCl	0·42 (5)	0·19 (5)	—	—
	—	*0·20 (3)	—	—
1·0 mM/l. NaCl + 0·5 mM/l. $CaCl_2$	—	0·21 (4)	—	—

* Sodium influx from NaCl + 0·5 mM/l. $CaCl_2$.

Some results obtained with a batch of smaller *G. duebeni* from Stranraer are given in Table 4, where the number of groups used to determine the influx or loss rate are given in parenthesis. Again there was no evidence of any consistent change in the influx or loss rate when the animals were acclimatized to media containing calcium or when calcium was added to the media used to determine sodium influx and loss. The higher sodium influx (0·42 μM/h) from 2 mM/l. NaCl in animals acclimatized to media containing 1 mM/l. sodium (Table 4) was expected from previous work on sodium regulation in *G. duebeni* (Shaw & Sutcliffe, 1961; Sutcliffe, 1967*b*; Sutcliffe & Shaw, 1968).

A few measurements of sodium influx were made on *G. duebeni* obtained from a brackish-water locality. The results on animals with a mean wet weight of 31 mg are given in Table 5. Sodium influx from 2 mM/l. NaCl was not affected by the addition of 0·5 mM/l. $CaCl_2$ and it remained unaltered after prior acclimatization to a NaCl solution containing 0·5 mM/l. calcium.

Table 5. *The effect of calcium on sodium influx in* Gammarus duebeni *from Warton salt-marsh at* 9 °*C*

Acclimatization medium	Na influx from 2·0 mM/l. NaCl (μM/animal/h)	Na influx from 2·0 mM/l. NaCl + 0·5 mM/l. $CaCl_2$ (μM/animal/h)
2·0 mM/l. NaCl	0·25	0·26
	0·25	0·20
	0·26	0·19
	0·25	0·26
	0·30	0·28
		0·24
	Mean 0·26	Mean 0·24
2·0 mM/l. NaCl + 0·5 mM/l. $CaCl_2$	0·25	—
	0·25	—
	0·25	—
	0·25	—
	Mean 0·25	—

Experiments on Gammarus zaddachi *Sexton*

Effect of calcium on sodium influx

G. zaddachi were acclimatized to 0·5 mM/l. NaCl at 9 °C. Sodium influxes in 0·5 and 0·15 mM/l. NaCl were measured during a period of about 30 min. A small amount of $CaCl_2$ solution was then added to the medium to give concentrations of 2–53 mM/l. calcium. Sodium influx measured after the addition of $CaCl_2$ is expressed as a percentage of the influx determined beforehand. The effect of calcium on the influx is shown in Fig. 6. Sodium influx was increased at calcium concentrations of 2–4 mM/l. In one instance the influx was increased to 160% of the initial influx for a period of 25 min; it then returned to its original rate. In six other instances the increased influx remained at the new rate during measurements made over periods of 40–60 min. At calcium concentrations of 7–10 mM/l. the influx either remained unaltered or else showed a slight increase or decrease. A marked reduction in the influx was evident at 20–30 mM/l. calcium and at 53 mM/l. calcium the influx was reduced to one-half the initial rate. It is possible that at these high calcium concentrations where the ratio of calcium to sodium was 40–100:1 the calcium ions were competing with sodium ions at the sites for ion transport. On the other hand it is difficult to reconcile this with an increased sodium influx under conditions where the ratio of calcium to sodium was as high as 26:1 (4 mM/l. calcium, 0·15 mM/l. sodium). Whatever the reason, both increases and decreases in the influx followed immediately after addition of $CaCl_2$, and the new rate was invariably established within 5–10 min. The results are also of interest because it appears possible that sodium uptake may be influenced by calcium ions at the concentrations encountered in the low-salinity regions of estuaries occupied by *G. zaddachi*.

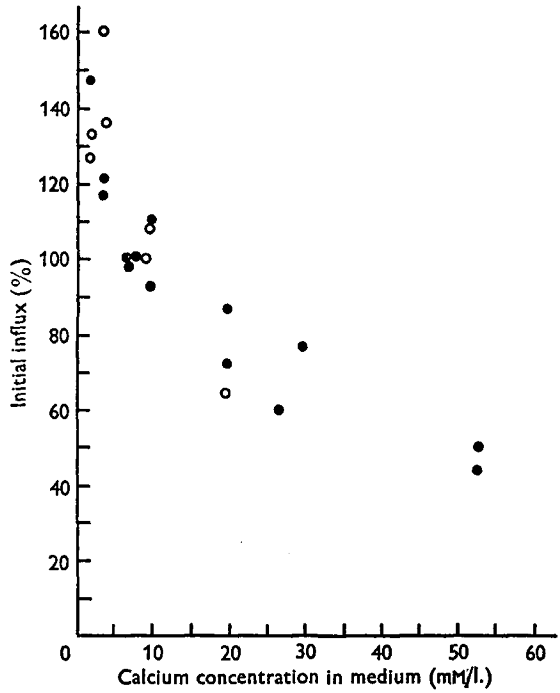

Fig. 6. Relative changes in sodium influx from NaCl solutions in *G. zaddachi* at 9 °C. following the addition of calcium as $CaCl_2$. Open circles represent sodium influx from 0·15 mM/l. NaCl, closed circles from 0·5 mM/l. NaCl.

DISCUSSION

In the case of *Gammarus pulex* and *G. duebeni* measurements of sodium influx from pure sodium chloride solutions and measurements of the sodium loss rate in de-ionized water produced results comparable with those obtained when calcium was present at concentrations below 2 mM/l. This covers the range of calcium concentrations normally encountered in the majority of fresh waters in Britain. Moreover, prior acclimatization to media containing calcium, including natural lake water, had no noticeable effect, in that sudden exposure to a calcium-free medium and exposure to a medium containing calcium following acclimatization in a calcium-free medium, produced no change in the influx or loss rate of sodium. From this it is concluded that, for at least some purposes, when studying sodium movements in these two species it is not necessary to add calcium to the experimental media.

Under certain circumstances, however, the sodium influx is affected by the presence of calcium. In *G. pulex* the influx was lowered following the addition of calcium, even at concentrations of only 2–4 mM/l. In contrast, the same concentrations of calcium raised the influx in *G. zaddachi* but then depressed the influx at concentrations above

10 mM/l. calcium. In *G. duebeni* the influx was not affected by calcium at concentrations up to 10 mM/l., although in the experiments on this species calcium was added to the experimental medium *before* determination of the influx. The possibility that addition of calcium *during* the determination may affect the influx temporarily requires further study, although it seems unlikely since there is no obvious difference between this procedure and that of suddenly exposing the animals to a medium containing calcium at the start of the influx determination.

In *G. pulex* the effect of calcium at concentrations above 2 mM/l. is independent of the accompanying anion, and the simplest explanation for its depressant effect on sodium influx is competition between calcium and sodium ions at the transporting sites. Lithium ions depress sodium influx in *Eriocheir* (Koch & Evans, 1956), and ammonium ions depress sodium influx in *Astacus* (Shaw, 1960), *G. pulex* and *G. zaddachi* (Sutcliffe, unpublished observations). Similarly, competition at relatively high concentrations of calcium could explain the depressant effect on sodium influx in *G. zaddachi*. On the other hand, the stimulation of sodium influx in *G. zaddachi* following the addition of calcium at low concentrations may not be due to the specific effects of calcium at all. In the mosquito larva *Aëdes aegypti*, addition of 2–5 mM/l. calcium also stimulates sodium influx but only when added as the chloride. This is due to increased net uptake of chloride from the raised chloride concentration in the medium, which in turn stimulates sodium uptake; calcium nitrate depressed the influx (Stobbart, 1965, 1967). Net uptake of chloride can also stimulate sodium uptake and sodium influx in *Astacus* (Shaw, 1964). In the experiments on *G. zaddachi* (Fig. 6) the stimulatory effect of adding 2–4 mM/l. calcium chloride may also be the result of net chloride uptake from the medium following the addition of a large excess of chloride, compared with the sodium concentrations of only 0·15 or 0·5 mM/l. It is suggested that the effects of competition at the transporting sites between calcium and sodium ions leading to depression of the influx then become dominant at higher concentrations of calcium chloride. A dual effect of this kind might be responsible for the conflicting results obtained on *G. pulex* following the addition of 1 mM/l. calcium (Fig. 2) except that, here, calcium carbonate had the same stimulatory and depressant effects on sodium influx as calcium chloride.

Relatively low concentrations of calcium chloride did not affect the sodium loss rate or 'balance' concentrations in *G. pulex* and *G. duebeni*, and the experiments shown in Fig. 4 and Fig. 5 demonstrate that the sodium loss rate is dependent solely on internal changes in sodium content. The reduction in sodium loss rate to its minimum rate following a 5–10% loss in total body sodium is in agreement with previous observations on changes in the loss rate associated with a 10–15% fall in the blood sodium concentration of *G. pulex* at low external concentrations (Shaw & Sutcliffe, 1961; Sutcliffe, 1967*a*), a feature common to *G. duebeni* and other species of *Gammarus* (Sutcliffe, 1967*b*, 1968; Sutcliffe & Shaw, 1967, 1968). In this respect *Gammarus* clearly differs from the lamprey ammocoete, where both net sodium loss and uptake are strongly influenced by calcium (Morris & Bull, 1968). In fact the ability to maintain sodium and chloride balance in media without calcium seems to be a general feature of freshwater arthropods, and some can tolerate prolonged exposure to glass-distilled or de-ionized water (Wigglesworth, 1938; Beadle & Shaw, 1950; Lockwood, 1959; Bryan, 1960; Parry, 1961; Stobbart, 1965, 1967). Some fishes, such as the eel

Anguilla, are also resistant to distilled water (Krogh, 1939) whereas others require the presence of calcium (Pickford *et al.* 1966; Stanley & Fleming, 1967). The role of calcium (in the external medium) in maintaining the internal steady states of sodium and chloride in these fishes is uncertain, and is complicated by the action of hormones like prolactin which affect gill permeability (Leatherland & Lam, 1969*a*, *b*).

SUMMARY

1. In *Gammarus pulex* and *G. duebeni* sodium influx from sodium chloride solutions and sodium loss into de-ionized water were not affected in a consistent manner by the presence of calcium ions at concentrations up to about 2 mM/l.

2. Sodium influx in *G. pulex* was depressed by the addition of 2–10 mM/l. calcium.

3. Sodium influx in *G. zaddachi* was stimulated by the addition of 2–4 mM/l. calcium chloride but depressed by the addition of 20–53 mM/l. calcium chloride.

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