J. exp. Biol. (1976), 64, 645–663
With 4 figures
Printed in Great Britain

OSMOTIC AND IONIC REGULATION IN DIFFERENT POPULATIONS OF THE NEW ZEALAND FRESHWATER CRAYFISH *PARANEPHROPS ZEALANDICUS*

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(*Received* 24 *October* 1975)

SUMMARY

1. Some features of the osmoregulatory mechanism are compared in four populations of *Paranephrops zealandicus* White collected from freshwaters of different ionic concentrations.

2. Crayfish from freshwaters of *ca.* 2·0 mM-NaCl concentration show a sustained decrease in blood concentration of *ca.* 8% when placed in 0·2 mM-NaCl.

3. Populations from freshwaters of *ca.* 0·2–0·4 mM-NaCl show lower rates of net salt loss in distilled water and higher rates of net salt uptake from dilute NaCl solutions than do populations from freshwaters of *ca.* 0·8–2·0 mM-NaCl.

4. Renal salt losses over the first 24 h in distilled water account for *ca.* 18% of the total salt loss.

5. It is suggested that *P. zealandicus* from environments of lowest concentration shows a similar degree of adaptation to freshwater as do crayfish of the northern hemisphere. It differs in possessing a substantially higher blood concentration.

INTRODUCTION

Freshwater crayfish maintain the concentration of their blood appreciably higher than the concentration of the freshwaters in which they live. This hyperosmotic regulation is aided by a relatively low permeability of their external surfaces to salts and to water (Potts & Parry, 1964, Tables V.5 and V.13), by the possession of ion transporting systems with a high affinity for specific ions (Lockwood, 1962), and by the production of urine which is hyposmotic to the blood (Riegel, 1970). These factors act together to restrict the amount of salt lost to the dilute external medium through extra-renal and renal sites, to replace these salt losses by active uptake from the external medium, and to rid the animal of water which is continuously driven into the body by the osmotic gradient between the body fluids and the dilute external medium. Progressive degrees of adaptation to freshwater shown by many crustaceans may include also a lowering of the blood concentration, thereby reducing the gradients for loss of salts and uptake of water (Lockwood, 1962).

Many previous studies have demonstrated the operation of these factors in

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freshwater crayfish of the northern hemisphere. Following the classic work of Krogh (1939), permeability to salt has usually been measured by observing the rate of appearance of salt in the external medium over the first few hours after placing the crayfish in distilled or deionised water (Wikgren, 1953; Shaw, 1959*a*; Bryan, 1960*b*). Exposing these salt-depleted crayfish to dilute salt solutions has enabled estimations to be made of the rate of uptake of specific ions (Krogh, 1939; Maluf, 1940; Wikgren, 1953; Shaw, 1959*a*, 1960*a*, *b*, *c*; Bryan, 1960*a*, *b*, *c*). Permeability to water has been assessed from the rate of urine production or by uptake of water into isolated gills (Bergmiler & Bielawski, 1970), or by the rate of movement of tritiated water (Rudy, 1967).

There have been no comparable studies on freshwater crayfish of the southern hemisphere. Two species of freshwater crayfish occur in New Zealand (Hopkins, 1970). *Paranephrops planifrons* is found in the North Island and in the north west of the South Island, and *P. zealandicus* occurs in the east and south of the South Island. It has recently been shown that each of these species maintains a constant blood concentration, even though occurring in lakes and streams which differ greatly in ionic concentration (Wong & Freeman, 1976*a*). In the case of *P. zealandicus*, populations found in inland, high altitude streams draining into the lakes of Central Otago inhabit freshwaters which differ by 14- to 17-fold in sodium and chloride concentrations from the freshwater inhabited by populations from coastal streams draining into tidal lagoons. It appears likely, therefore, that there may be some qualitative or quantitative differences in some aspects of osmoregulatory functioning between crayfish inhabiting the extremes of these different environments. In this paper are presented the results of experiments which were devised to compare *P. zealandicus* from four freshwater localities chosen to represent a wide range in environmental concentration. First, crayfish collected from a coastal population were exposed to an external medium of similar concentration to that inhabited by an inland population to assess whether they were able to maintain their normal blood concentration in this more dilute medium. Secondly, measurements were made on crayfish from all four populations to compare the rates of salt loss into distilled water and rates of uptake of sodium and chloride from dilute NaCl solutions. Measurements were made also on rates of urine flow and on urine concentrations, and this information is used to assess salt losses in the urine during salt depletion in distilled water. A detailed consideration of the role of the antennary glands in osmoregulation in *P. zealandicus* will be presented elsewhere.

MATERIALS AND METHODS

Acclimation experiment

Twenty hard-shelled non-ovigerous crayfish were collected in July from a creek draining into a tidal lagoon in coastal Otago. This creek is the station D of Wong & Freeman (1976*b*) and has a NaCl concentration of approximately 2 mM. The crayfish were marked, weighed and measurements made of carapace length. They were maintained in the laboratory for one week prior to the experiment in 30 l of dechlorinated tap water (*ca.* 0·4 mM-NaCl) to which NaCl had been added to bring the concentration up to 2·0 mM-NaCl. At the end of the week 10 animals (group 1)

were placed in fresh 2·0 mM-NaCl medium and the other 10 (group 2) were placed in dechlorinated tap water diluted with distilled water to bring the NaCl concentration to 0·2 mM.

After 72 days in these experimental media the 9 surviving animals of group 1 were subdivided into two sub-groups. Sub-group 1A (5 animals) was retained in 2·0 mM-NaCl and sub-group 1B (4 animals) was transferred to the 0·2 mM-NaCl solution. At the same time, a similar reciprocal transfer was carried out on the 8 surviving animals of group 2. Four animals (sub-group 2A) were retained in 0·2 mM-NaCl, and the other 4 animals (sub-group 2B) were transferred to 2 mM-NaCl. The experiment was terminated after a further 36 days, at which time all the animals were killed and measurements made of any gastroliths present.

The concentrations of all media were checked every 2–3 days and held at the stated values by addition of calculated amounts of 1 M-NaCl or distilled water. The solutions were completely renewed each week. Temperature was maintained at 5 ± 1 °C and all solutions were aerated throughout the experiment. Blood samples of *ca.* 0·4 ml were taken from each animal by inserting a hypodermic needle into the ventral sinus through the arthrodial membrane at the base of a walking leg. Samples were taken 2 days before day 0 (5 days after capture) and at appropriate intervals throughout the 108 days of the experiment. Blood samples were transferred to 1 ml glass ampoules and stirred until a clot formed. The clot was removed and the ampoule flame-sealed. The ampoule was immersed in a beaker of water at 55–65 °C for 5–10 min to prevent gelation of the serum (Leon-Fredericq, cited in Florkin, 1960), and stored at 0–3 °C to await analysis.

Sodium and potassium were estimated by flame photometry (EEL), chloride by electrochemical titration (Aminco-Cotlove chloride titrator), and total osmotic pressure by a Fiske G.66 osmometer reading directly in milliosmoles per kilogram water (m-osmoles).

Salt depletion and reuptake experiments

Two series of experiments were carried out. The first involved the use of unrestrained animals and yielded data on net loss rates in distilled water and net uptake rates from various dilute NaCl solutions. The second series involved the use of animals which had been cannulated to provide information on urine flow rates and concentrations, as well as information on net loss and net uptake rates.

Animals

Only hard-shelled non-ovigerous crayfish of weight 20–38 g were used in all experiments. They were collected from four stations, chosen to represent a wide range of external concentrations. These were stations A, B, C and D described by Wong & Freeman (1976*b*). During two years of field study, station A had sodium concentrations of 0·18–0·23 mM and chloride concentrations of 0·15–0·23 mM; station B had 0·28–0·57 mM-Na and 0·24–0·43 mM-Cl; station C had 0·63–0·78 mM-Na and 0·50–0·69 mM-Cl; and station D had 2·00–2·42 mM-Na and 1·95–2·46 mM-Cl. During initial acclimation in the laboratory, and in subsequent experiments in which crayfish were exposed to concentrations approximating those of their normal habitat, animals from station A were placed in 0·2 mM-NaCl, those from

station B in 0·4 mM-NaCl, those from station C in 0·8 mM-NaCl, and those from station D in 2·0 mM-NaCl. In the account which follows, these solutions will be referred to as 'normal media' for crayfish from the appropriate stations. In each case, the normal medium was made from dechlorinated tap water diluted with distilled water or with NaCl added.

Sampling methods

(1) *Medium.* Samples of about 10 ml of the experimental medium were collected in Pyrex test-tubes, which were then sealed with parafilm. Estimations were made in duplicate of the sodium, chloride and potassium by the methods listed above.

(2) *Blood.* Serial blood samples were taken from each crayfish at intervals of 1–4 days over periods of up to 21 days. It was, therefore, essential that only small volumes of blood be taken for each sample. In the case of unrestrained crayfish, the animal was removed from its medium and clamped with its dorsal surface against a perspex plate by means of a cross bar fitted across its ventral surface between the base of the chelae and the first walking legs. Animals which were cannulated for urine collection were already attached to the plate. A hole in the perspex plate exposed a circular central area, *ca.* 1 cm diameter, of the dorsal surface of the carapace. Before taking a blood sample, this exposed surface was rinsed with distilled water and dried with acetone. A nickel-plated sewing needle was used to pierce the carapace at a point slightly anterior to the heart. By partially withdrawing the needle, a drop of blood was allowed to well onto the carapace. Fifteen microlitres of blood were taken up in a drawn glass capillary tube connected by a short length of polyethylene tubing to a 100 μl Hamilton Aliquanter micropipette. The blood sample was transferred to a glass ampoule and washed down with 35 μl of distilled water which had previously been drawn into the syringe. Two further 50 μl aliquots of distilled water were added, making a total sample volume of 150 μl (blood diluted 1:10). Once calibrated, the settings of the Aliquanter syringe were unchanged. The ampoules were flame-sealed and stored at 0–3 °C until the samples were analysed. After the blood sample had been taken, the needle was withdrawn and the small hole sealed with silicone grease. The surface was flushed with distilled water and the animal returned to the medium. The same hole was used for subsequent sampling, after wiping off the excess grease.

(3) *Urine.* The method used for continuous collection of urine from each nephropore was modified from that of Kamemoto & Ono (1968). The modifications were directed towards avoiding clamping the crayfish over the lateral aspects of the anterior part of the carapace, i.e. those regions housing the antennary glands, and also avoiding the removal of the chelae. The crayfish were held throughout the experiment (up to 21 days) against the perspex plate described above. A similar holding device is described by Bryan (1960*a*) who found that his crayfish remained in good condition for up to 25 days. Initial preparation involved the rigid clamping of the crayfish to the plate, after first lightly drying them. A cotton pellet was inserted into the anterior aspect of the branchial chamber, and the chelae were restrained by a rubber band. An inclined perspex platform, adjustable for horizontal position and angle, was manoeuvred into a position which prevented any of the thoracic limbs coming into contact with the renal papillae. The flared ends of polyethylene cannula tubing

(Portex 120) were then applied to the dried surfaces of the renal papillae, and the junctions initially sealed with dental cement (de Treys Poly-C lining cement) to form a base for the application of Eastman 910 adhesive, which formed a permanent seal. This procedure of lining the junction between the cannula tubing and the renal papillae with dental cement before applying the Eastman 910 prevented the labile adhesive creeping inside the junction and onto the membrane covering the renal pore, and occluding it. When the adhesive had dried completely (15–20 min) the cotton pellets were removed, the inclined platform was readjusted until the maxillipeds could move freely, and the rubber band was removed from the chelae, which were, however, still prevented by the inclined platform from reaching the area of the cannulae and renal pores. The clamps connecting the supporting bar to the dorsal plate were slackened slightly until the crayfish was merely supported and restrained.

After cannulation, the preparation was returned to normal medium for recovery. Urine flow often ceased for up to 4 h after cannulation, and experimental collection was not made until after at least 12 h. Urine from each nephropore passed into a polyethylene tube (i.d. 2·89 mm) with one end drawn out so that it made a tight fit onto the cannula tubing. The cannula itself (*ca.* 8 cm long) formed a dead space from which urine was not actually collected, and there was, therefore, a time lapse between the voiding of urine and the time it was collected. This lapse was approximately 1–2 h, and since urine was collected over periods of 24 h, this is not considered to be important. The contents of the collecting tubes were drained into glass tubes of known weight, and estimations made of volume and concentration. The progressive accumulation of urine in the collecting tubes until the level was above that of the renal opening could possibly lead to the development of back pressure affecting urine flow. This effect was lessened by the use of collecting tubes with relatively large diameters. In a preliminary test on 4 animals, urine flow continued until the height of the fluid column reached *ca.* 40 cm. In all experiments reported here, the level of urine accumulated in the collecting tubes never exceeded 4 cm above the horizontal level of the renal pore.

A preliminary experiment was carried out to assess the effect of clamping and cannulation on the osmoregulatory abilities of the crayfish. Three animals were cannulated and clamped as described above, another three animals were clamped but not cannulated, and a further three animals were unrestrained in any way. These three groups were maintained for 30 days during which blood samples were taken from each crayfish at intervals of 3–5 days. All animals maintained essentially the same blood concentrations over the experimental period. The ability of an osmoregulating animal to maintain a fairly constant blood concentration over long periods can be taken as evidence that the animal is functioning normally, and it is concluded that clamping and cannulation as described here has no adverse effect on the osmoregulatory ability of the crayfish.

Experimental procedure

In both series of experiments, involving unrestrained or cannulated animals, the crayfish were first maintained in normal medium at 16 ± 1 °C for 1 week after collection. They were then immersed in distilled water for 4–5 days, the distilled water being replaced if necessary to ensure continuing salt depletion (as assessed by falling

blood concentration and increasing concentrations of salt in the surrounding water). The crayfish were then placed in 0·05, 0·15, 0·25 or 0·5 mM-NaCl solutions. Net uptake of sodium and chloride was measured by serial sampling of the medium. If no uptake was apparent from a particular NaCl solution after 48 h, the crayfish was transferred to a medium of higher concentration. Finally, the crayfish were returned to their normal medium for a few days before being killed and measurements made of any gastroliths present.

RESULTS

Acclimation experiment

Only the results for the 17 crayfish which survived the 108 days of the experiment are considered. Seven of these animals were found to have gastroliths at the termination of the experiment. The ratio Gastrolith length/Gross carapace length (G.L./G.C.L.) was between 0·10 and 0·15. In crayfish, the onset of a moult is known to be associated with a decrease in blood concentration, but, for reasons discussed by Wong & Freeman (1975*b*), it was considered that these 7 animals with gastroliths had not advanced in the moult cycle to a stage where their blood concentration would be affected. The changes in blood concentration in these crayfish were generally the same as those in crayfish without gastroliths, and the data from these animals are included in calculations for the groups to which they belonged. The presence of gastroliths in crayfish which had been maintained for over 16 weeks at 5 °C suggests that gastrolith development in *P. zealandicus* is not inhibited by this low temperature. Roberts (1957) found that the moulting cycle of *Pachygrapsus crassipes* was inhibited below 8·5 °C.

It can be seen from Fig. 1 that the blood concentration of those crayfish maintained in 2·0 mM-NaCl was held constant throughout the period of exposure to this external concentration (108 days for sub-group 1A; 72 days for sub-group 1B). On transfer to 0·2 mM the concentrations of sodium and chloride and the total osmotic pressure of the blood of sub-group 1B decreased, and thereafter the concentrations were maintained at this lower level. There was little change in the potassium concentration of the blood, but there had been only a twofold change in the potassium concentration of the experimental media, from 0·04 to 0·02 mM.

Figure 2 shows that crayfish which had been held in the stock tank in 2·0 mM-NaCl for one week and were then transferred to 0·2 mM on day 0 (group 2) showed a gradual decrease in blood sodium, chloride and osmotic pressure. A new low level had been achieved in the samples taken on day 22, and this level was maintained throughout the rest of the period in 0·2 mM-NaCl medium (to day 108 in sub-group 2A; to day 72 in sub-group 2B). To this extent these group 2 animals showed similar responses to those of sub-group 1B discussed above. In both cases, the blood concentrations of animals in 0·2 mM-NaCl stabilized at *ca.* 8% below their levels in 2·0 mM-NaCl. The four crayfish which were transferred back to 2·0 mM-NaCl after 72 days (sub-group 2B) showed marked increases in blood sodium, chloride and osmotic pressure. There was little change in blood potassium in response to changes of concentration of the medium in either direction.

It is evident from these results that populations of *P. zealandicus* which normally inhabit a medium of NaCl concentration of 2·0 mM are not able to maintain their

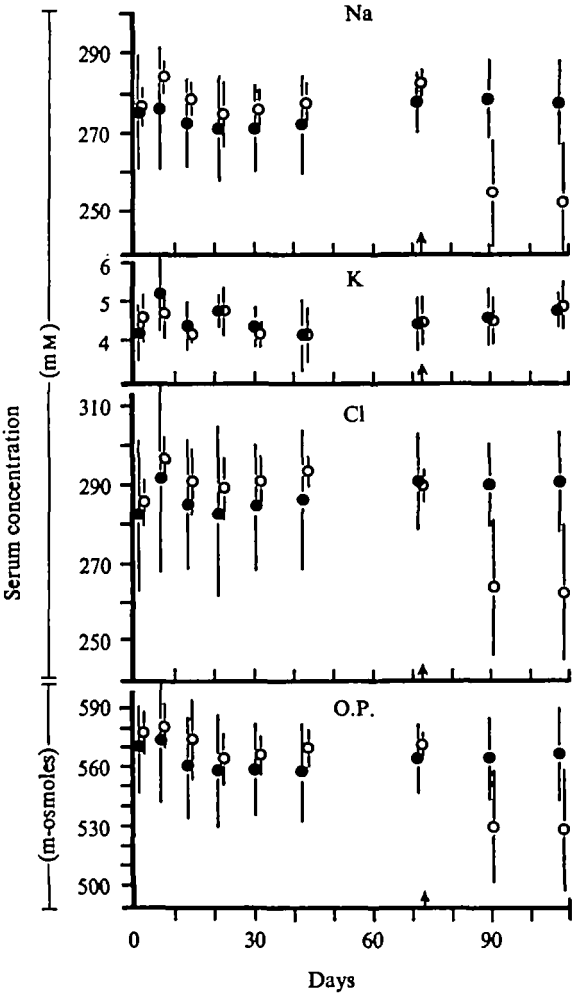

Fig. 1. The concentrations of sodium, potassium, chloride and the osmotic pressure of the blood of *P. zealandicus* maintained for 72 days in 2·0 mM-NaCl, and then either retained in 2·0 mM-NaCl for a further 36 days (●) or transferred to 0·2 mM-NaCL for 36 days (○). Mean ± 1 standard deviation presented.

normal blood concentration when placed in a medium in which NaCl concentration is 1/10 of that present in their natural environment. There is a lowering of the blood concentration by about 8%, and there is no evidence of recovery to the previous level throughout periods of exposure of 15 weeks. The blood concentration does, however, regain its initial level if the animals are returned to 2·0 mM-NaCl.

The lowering of blood concentration in response to a lowering of the external concentration below a critical limit is a general osmoregulatory phenomenon (Shaw, 1959*a*; Bryan, 1960*c*; Shaw & Sutcliffe, 1961; Sutcliffe, 1968; Sutcliffe & Shaw, 1968; Potts & Parry, 1964; Lockwood, 1968). It has the effect in hyperosmotic freshwater animals of reducing the gradients for passive salt loss and water entry, and it also stimulates the mechanism for active uptake of salts (Shaw, 1959*a*; Bryan, 1960*b*). In the case of the population of *P. zealandicus* studied in this experiment, it would

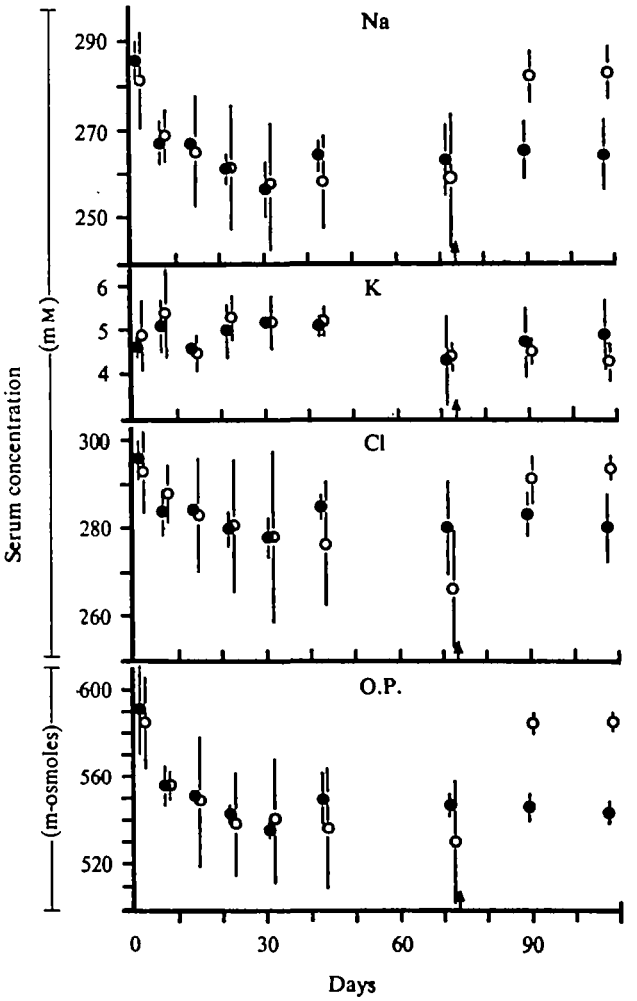

Fig. 2. The concentrations of sodium, potassium, chloride and the osmotic pressure of the blood of *P. zealandicus* maintained for 72 days in 0·2 mM-NaCl, and then either retained in 0·2 mM-NaCl for a further 36 days (●) or transferred to 2·0 mM-NaCL for 36 days (○). Mean ± 1 standard deviation presented.

Fig. 3. Blood concentration, medium concentration, urine flow rate and concentration, and renal salt loss in *P. zealandicus* in external media of different concentrations. (*a*) Concentration of sodium (△) and chloride (▲) in the blood. (*b*) Concentration of sodium (△) and chloride (▲) in the medium when this was other than 'normal' medium (0·2 mM-NaCl). (*c*) Urine flow rates from left kidney (▥) and from right kidney (□). (*d*) Concentration of urine from right kidney; sodium (▥), potassium (□), chloride (■). (*e*) Concentration of urine from left kidney; symbols as in (*d*). (*f*) Rates of losses of sodium, potassium and chloride from left and right kidneys. Symbols as in (*d*) and (*e*). Values for left kidney below cross bar; values for right kidney above cross bar. Height of entire column represents total for the animal. Time axis is common for all graphs and histograms. The crayfish was successively exposed to 0·2 mM-NaCl (6 days), distilled water (5 days), 0·5 mM-NaCl (5 days) and 0·2 mM-NaCl (5 days). Arrows indicate times of transfer.

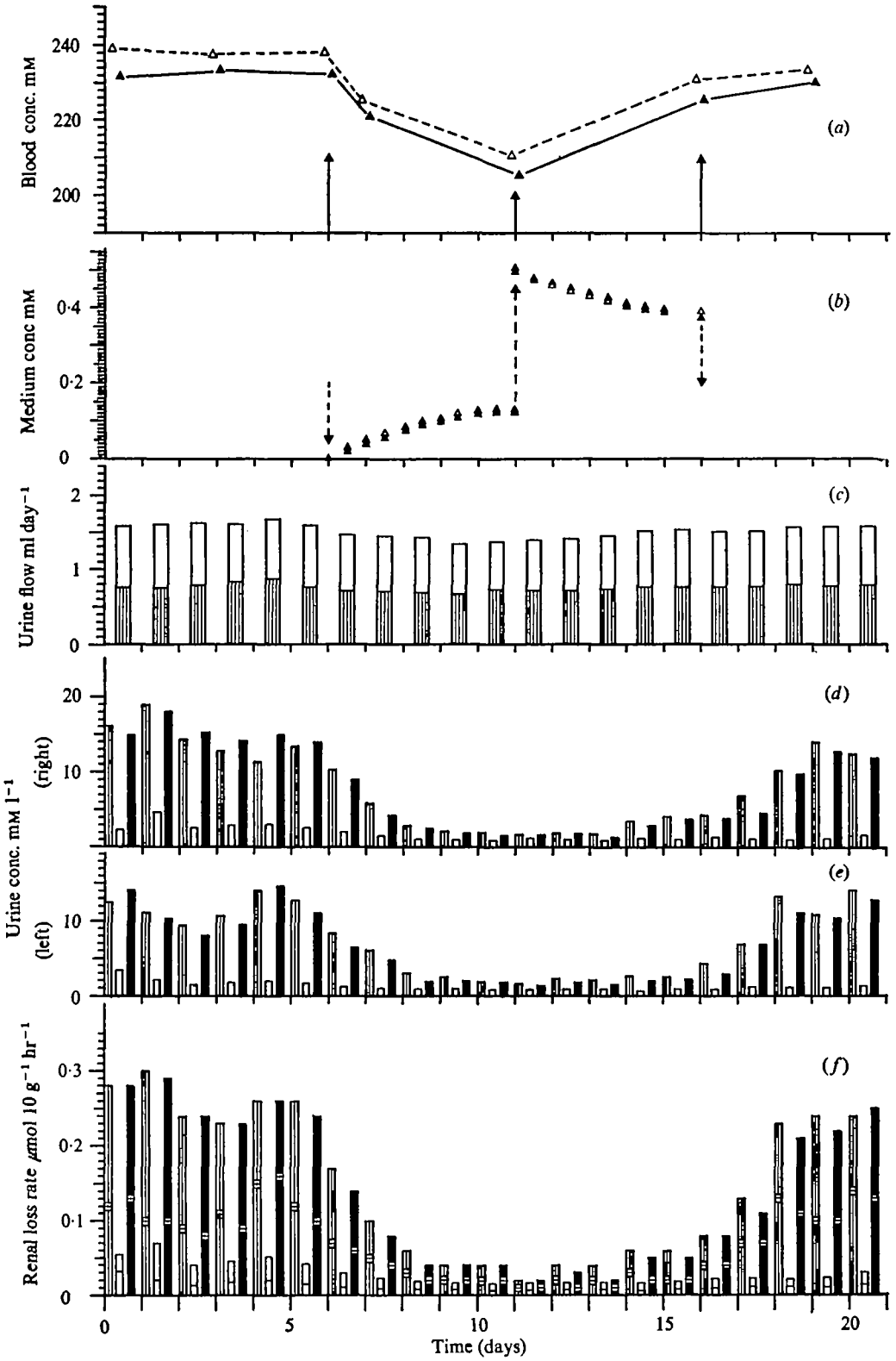

Fig. 3. For legend see opposite.

appear that the crayfish are unable to take up sodium and chloride from 0·2 mM-NaCl at a rate sufficient to maintain the level of blood concentration which they achieve in 2·0 mM-NaCl.

The significance of this observation for *P. zealandicus* is that there are populations of this species which normally inhabit environments with a NaCl concentration of 0·2 mM-NaCl, and these populations maintain blood concentrations identical with those of populations from environments with a NaCl concentration of 2·0 mM (Wong & Freeman, 1976*a*, *b*). This suggests that there may be differences in some aspects of the osmoregulatory mechanisms between populations of this species of crayfish inhabiting environments of different concentrations. Such differences in the ability to maintain normal blood concentrations in dilute media have been established between populations of the amphipod *Gammarus duebeni*: brackish water populations show a sharp decrease in sodium concentration at external concentrations below 1 mM-NaCl, whereas freshwater populations can maintain normal blood concentrations at much lower external sodium concentrations (Shaw & Sutcliffe, 1961; Sutcliffe & Shaw, 1968).

Salt depletion and reuptake

None of the unrestrained animals possessed gastroliths when autopsied. Four of the cannulated crayfish possessed gastroliths $\left(\frac{\text{G.L.}}{\text{G.C.L.}} = 0{\cdot}10-0{\cdot}14\right)$. The data from these four animals are included in the results as the responses of these animals were essentially the same as those of animals without gastroliths. The general nature of the data obtained, upon which calculations of loss and uptake were made, can most conveniently be considered by reference to the results for one representative animal, a crayfish collected from station A and cannulated for collection of urine (Fig. 3). It can be seen that the blood concentration remained constant during the first 6 days in normal medium, decreased during the 5 days in distilled water, and increased again in 0·5 mM-NaCl. These changes in blood concentration were paralleled by changes in the opposite direction in the concentration of the external medium, indicating that the appearance of salts in the medium could reliably be attributed to loss from the animal, and conversely that the reduction in the concentration of salts in the medium was caused by uptake by the animal. The change in the concentration of the external medium over the first 24 h following transfer of the animal to that medium was used to calculate the rates of net flux of sodium and chloride. This was preferred to calculations based upon changes in blood concentration, as the external medium could be measured with far greater accuracy than the volume of the ionic space for each ion for each animal. It can be seen from Fig. 3 that the rate of change of concentration of the medium was still close to maximal at 24 h. Since urine was collected and analysed at 24 h intervals, this allowed convenient comparative estimations of renal and extra-renal rates of salt loss and calculations of rates of total loss.

Rates of net salt loss in distilled water

The data from the experiments on unrestrained animals yielded information only on total salt loss and uptake, whereas in the cannulated animals the renal loss

Table 1 A. *The initial blood sodium and chloride concentrations and rates of net loss of sodium and chloride in distilled water in four populations of* P. zealandicus

(Mean ± standard deviation presented; n = number of animals.)

Station	Weight of crayfish (g)	Blood concentration (mM) Na	Blood concentration (mM) Cl	Rate of net loss (μmol 10 g^{-1} hr^{-1}) Na	Rate of net loss (μmol 10 g^{-1} hr^{-1}) Cl	*t*-test for Na *v.* Cl loss rates t	*t*-test for Na *v.* Cl loss rates P
A (n = 18)	32·9 ± 9·3	235·7 ± 7·4	234·3 ± 7·0	2·31 ± 0·56	2·08 ± 0·55	1·2545	> 0·10
B (n = 13)	32·2 ± 14·5	235·4 ± 17·4	236·0 ± 21·7	2·38 ± 0·76	1·99 ± 0·61	1·4433	> 0·10
C (n = 13)	28·0 ± 6·2	240·4 ± 12·2	235·0 ± 15·9	3·40 ± 0·72	2·83 ± 1·01	1·6573	> 0·10
D (n = 11)	31·1 ± 7·3	242·1 ± 10·2	239·1 ± 13·5	3·38 ± 0·59	2·96 ± 0·71	1·5083	> 0·10

Table 1 B. *Comparison of rates of net salt loss in four populations of* P. zealandicus

(Values of Student's t for paired data for sodium losses given above the diagonal space; those for chloride below the space.)

Station	A	B	C	D
A		t = 0·30 P > 0·10	t = 4·78 P < 0·001	t = 4·95 P < 0·001
B	t = 0·43 P > 0·10		t = 3·51 P < 0·01	t = 3·49 P < 0·01
C	t = 2·66 P < 0·02	t = 2·57 P < 0·02		t = 0·07 P > 0·10
D	t = 3·74 P < 0·001	t = 3·60 P < 0·01	t = 0·36 P > 0·10	

component was isolated from the extra-renal loss. Within each population, there was no significant difference at the 5% level between the mean values of total net loss obtained by the two experimental procedures, and the data obtained by both methods are pooled for comparison of total net loss in the different populations (Table 1 A).

It can be seen that there are no significant differences between the weights nor between the initial blood concentrations of animals from the four stations. Within each population, the rate of net loss of sodium is not significantly different from the rate of net loss of chloride. There are, however, significant differences in the rates of net loss for each ion between some of the populations (Table 1 B). The rates of net loss in the crayfish from stations C and D, while not significantly different from each other, are both significantly higher than those recorded for crayfish from stations A and B. The differences between crayfish from stations A and B are not significant.

These results show that *P. zealandicus* which inhabit freshwaters of concentration 0·2–0·4 mM-NaCl show lower rates of salt loss over the first 24 h in distilled water than do members of the same species which inhabit freshwaters of concentration 0·8–2·0 mM-NaCl, although all crayfish normally maintain the same blood concentration when in their normal environmental medium. As total salt loss into distilled water is essentially a passive process, the rate of loss could be affected by the size of

Table 2A. *The rates of extra-renal salt loss in four populations of* P. zealandicus

(Mean ± S.D. presented. Number of animals used within parentheses.)

Station	Rate of extra-renal salt loss (μmol 10 g^{-1} hr^{-1})	
	Na	Cl
A (10)	1·91 ± 0·28	1·74 ± 0·21
B (5)	1·57 ± 0·44	1·51 ± 0·31
C (6)	3·33 ± 0·95	3·04 ± 0·99
D (4)	2·66 ± 0·71	2·60 ± 0·72

Table 2 B. *Comparison of rates of extra-renal salt loss in four populations of* P. zealandicus

(Values of Student's t for paired data for sodium losses given above the diagonal space; those for chloride below the space.)

Station	A	B	C	D
A		$t = 1·96$ $P > 0·05$	$t = 4·56$ $P < 0·01$	$t = 3·07$ $P < 0·01$
B	$t = 1·86$ $P > 0·05$		$t = 3·78$ $P < 0·01$	$t = 4·30$ $P < 0·01$
C	$t = 4·16$ $P < 0·01$	$t = 3·28$ $P < 0·01$		$t = 1·20$ $P > 0·10$
D	$t = 3·78$ $P < 0·01$	$t = 3·15$ $P < 0·02$	$t = 0·75$ $P > 0·10$	

Table 3. *The rates of renal salt loss in four populations of* P. zealandicus

(Mean ± S.D. presented. Numbers of animals used within parentheses.)

Station	Rate of renal salt loss (μmol 10 g^{-1} hr^{-1})	
	Na	Cl
A (10)	0·23 ± 0·05	0·19 ± 0·06
B (5)	0·53 ± 0·23	0·52 ± 0·28
C (6)	0·48 ± 0·36	0·49 ± 0·34
D (4)	0·87 ± 0·67	0·89 ± 0·66

the animal (smaller crayfish having a relatively larger surface area) and by the concentration gradient between the blood and the external medium. Within the range of crayfish used in these experiments, there is no significant difference in size nor in initial blood concentration (Table 1 A). It is suggested that the differences in net loss rates between the four populations are attributable to differences in surface permeabilities to sodium and chloride, the crayfish from the inland stations of low external concentration being less permeable than animals from the coastal habitats of higher external concentration.

The results obtained from animals which were cannulated for urine collection enabled separate calculations to be made for salt losses from renal and extra-renal sites. Statistical testing by Student's t-test on paired data shows that extra-renal losses from animals from stations C and D are significantly higher than losses from

Table 4. *Mean rates of net uptake of sodium and chloride from dilute NaCl solutions by salt-depleted crayfish from four stations*

(Numbers of animals in parentheses. Negative values indicate net loss. Standard deviation listed where $n \geq 3$.)

External NaCl concentration	0·05 mM		0·15 mM		0·25 mM	
Net flux rate	Na (μmol 10 g^{-1} h^{-1})	Cl (μmol 10 g^{-1} h^{-1})	Na (μmol 10 g^{-1} h^{-1})	Cl (μmol 10 g^{-1} h^{-1})	Na (μmol 10 g^{-1} h^{-1})	Cl (μmol 10 g^{-1} h^{-1})
Station A	−0·66 (2)	−0·18 (2)	1·58 ± 0·53 (4)	1·38 ± 0·52 (4)	3·46 (2)	3·72 (2)
Station B	−0·50 (2)	−0·34 (2)	1·78 ± 0·28 (4)	1·75 ± 0·39 (4)	3·61 (2)	3·20 (2)
Station C	−1·03 ± 0·07 (3)	−0·99 ± 0·13 (3)	0·68 ± 0·22 (5)	0·60 ± 0·21 (5)	1·92 (2)	1·20 (2)
Station D	−1·08 (2)	−1·00 (2)	0·57 ± 0·16 (3)	0·51 ± 0·14 (3)	1·68 (2)	0·99 (2)

animals from stations A and B (Tables 2A and 2B). The differences between stations A and B, and between stations C and D, are not significant.

The rates of renal salt loss in animals from the four populations are given in Table 3. These differ only in detail from the pattern seen for total salt loss and extra-renal loss. The renal losses from crayfish from Station A are significantly lower than those of animals from the other three stations (station A *v.* station C, which shows the next lowest loss rates, for sodium $t = 2{\cdot}30$, $P < 0{\cdot}05$, for chloride $t = 2{\cdot}78$, $P < 0{\cdot}02$). There are no significant differences between animals from Stations B, C and D.

Comparison of the data for renal and extra-renal salt losses measured in the same animals (Tables 2A and 3) shows that the renal component accounts for only about 18% of the total loss. This is higher than the values of 8 and 10% recorded for *Austropotamobius pallipes* by Bryan (1960*a*) and by Shaw (1959*a*) respectively, but shows, nevertheless, that most of the net loss of sodium and chloride occurs through general body surfaces.

Rates of net salt uptake

The mean rates of uptake of sodium and chloride from dilute NaCl solutions are shown in Table 4. The crayfish had previously been exposed to distilled water for 5 days to effect salt depletion and stimulation of the active uptake mechanism. The rate of uptake was calculated from the decline in concentration of the external medium over the first 24 h. No crayfish was able to take up sodium or chloride from an external concentration of 0·05 mM-NaCl. All showed a net loss. The rates of net loss were greater in crayfish from stations C and D than in animals from stations A and B. Similar results were seen for salt loss in distilled water (Table 1A). Net uptake was effected from the other two external concentrations listed in Table 4. In all four populations the uptake of sodium was usually higher than that of chloride, but the differences were not statistically significant. From an external concentration of 0·15 mM-NaCl, the rates of net uptake of both sodium and chloride were significantly greater in animals from stations A and B than in animals from stations C and D;

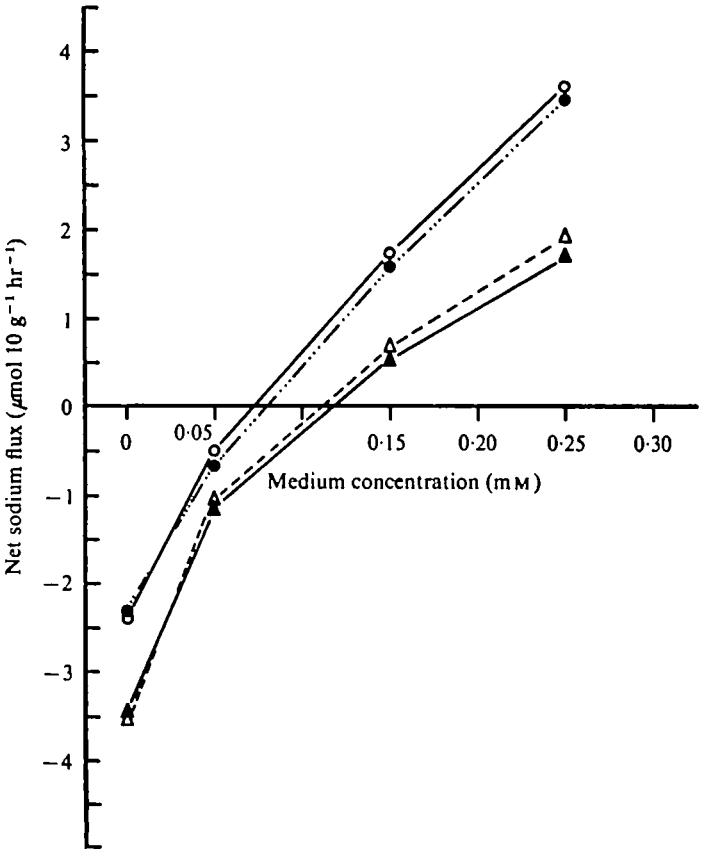

Fig. 4. The relationship between the net flux of sodium and the concentration of sodium in the external medium in four populations of *P. zealandicus*. ●, crayfish from station A, normal medium 0·2 mM-NaCl; ○, crayfish from station B, normal medium 0·4 mM-NaCl; △, crayfish from station C, normal medium 0·8 mM-NaCl; ▲, crayfish from station D, normal medium 2·0 mM-NaCl.

for example, station A *v.* station C gives $t = 3{\cdot}49$, $P < 0{\cdot}02$, for sodium, and $t = 3{\cdot}10$, $P < 0{\cdot}02$, for chloride. In an external medium of 0·25 mM-NaCl, the uptake rates, within each population, were greater than those from 0·15 mM-NaCl. The small sample size exposed to 0·25 mM-NaCl does not permit meaningful statistical analysis of the data but the mean uptake rates of crayfish from stations A and B are substantially higher than those of crayfish from stations C and D.

The significant findings of this comparative study on rates of salt loss in distilled water and rates of uptake from dilute NaCl solutions by *P. zealandicus* from habitats of different external concentrations are shown in Fig. 4. As well as demonstrating that animals from stations A and B show lower rates of net salt loss in distilled water and in 0·05 mM-NaCl, and higher uptake rates in 0·15 and 0·25 mM-NaCl than do animals from stations C and D, Fig. 4 enables other conclusions to be drawn. Crayfish from Station D exposed to an external concentration of 0·20 mM-NaCl would be expected to show a net uptake rate of sodium of *ca.* 1·1–1·2 μmol 10 g^{-1} h^{-1}. It has already been demonstrated (Figs. 1 and 2) that these crayfish are unable, however, to maintain a normal blood concentration in an external medium of 0·20 mM-NaCl.

It can be concluded that uptake rates of this magnitude are insufficient to compensate for salt losses from crayfish from station D when their blood sodium concentration is at the normal level of *ca.* 275 mM and the external medium is 0·20 mM-NaCl. These crayfish were, however, able to maintain a lower blood sodium concentration of *ca.* 255 mM when transferred to 0·2 mM-NaCl (Fig. 1), and this lower level remained stable during 5 weeks of exposure to the medium. The rate of uptake is presumably adequate to compensate for salt loss when the gradient between the internal and external concentrations is reduced to this new level.

Fig. 4 shows also that the lines joining the net fluxes of sodium intersect the axis of zero net flux at different external concentrations. For crayfish from stations A and B this external concentration is *ca.* 0·08 mM-NaCl whereas for crayfish from stations C and D it is *ca.* 0·11 mM-NaCl. The estimates of net flux plotted in Fig. 4 are, of course, based on the ability of crayfish, which had been salt depleted by exposure to distilled water, to reduce the concentration of a dilute NaCl solution over the first 24 h in that dilute medium. In the actual experiment in which crayfish were placed in 0·15 mM-NaCl, the animals were able eventually to reduce the concentration to a point beyond which no further uptake (and hence no further reduction in external concentration) was shown. For the 8 crayfish from stations A and B listed in Table 4 this average external concentration of sodium below which uptake could not proceed was 0·081 mM. The average time, after transfer, to reach this point was 47 h. The 8 crayfish from stations C and D listed in Table 4 were able to reduce the Na concentration of 0·15 mM-NaCl to an average of 0·106 mM, but not below this point. The average time taken to lower the concentration to this level was 82 hours. These results further emphasize the different rates of net uptake shown by these different populations of crayfish.

DISCUSSION

It is evident that populations of *Paranephrops zealandicus* inhabiting the dilute freshwaters of Central Otago are better adapted to deal with low external concentrations than are populations from more coastal environments. These adaptations extend to many of the principal features of hyperosmotic regulation outlined in the Introduction. Populations inhabiting freshwaters of NaCl concentration from *ca.* 0·2 to 0·4 mM show significantly lower rates of salt loss into distilled water and higher rates of salt uptake from dilute NaCl solutions than do populations inhabiting freshwaters of NaCl concentration from 0·8 to 2·0 mM. The rates of renal salt loss are, moreover, lower in crayfish from station A than in crayfish from the other three stations, as measured over the first 24 h of exposure to distilled water.

The values obtained for rates of salt loss may be compared with those obtained for some other freshwater crustaceans under similar conditions of temperature and external medium. Sutcliffe (1974), in a comparative study of three species of *Asellus*, found a minimum value for sodium loss into deionised water of 260 μmol kg^{-1} h^{-1} at 20 °C, and compares this with similar values for *Potamon niloticus* and *Austropotamobius pallipes* as 'the least permeable of aquatic crustaceans'. Shaw (1959*a*) reports sodium losses of 150 μmol kg^{-1} h^{-1} for *Austropotamobius pallipes* at 12–13 °C, whereas his figure for *Potamon niloticus* at 20 °C is 800 μmol kg^{-1} h^{-1} (Shaw, 1959*b*), which led Potts & Parry (1964, p. 187) to observe that *Potamon* 'is more permeable to

salt than most other freshwater animals'. The mean value found here for the rate of total sodium loss at 16 °C in *P. zealandicus* collected from the freshwaters of lowest external concentration is 231 μmol kg^{-1} h^{-1}, and the rate of extra-renal loss in the same population is 191 μmol kg^{-1} h^{-1} (Tables 1 A and 2 A). These values suggest that sodium permeability in this population of *P. zealandicus* is very similar to those of the isopod *Asellus communis* and the crayfish *Austropotamobius pallipes*. The highest value measured for total sodium loss in a population of *P. zealandicus* (from station C) is 340 μmol kg^{-1} h^{-1}, which is approximately half the value obtained by Shaw for *Potamon niloticus*.

The essential aim of the present investigation was to compare the rates of salt loss and salt uptake in different populations of *P. zealandicus*. It is difficult to compare the absolute values of some of the parameters measured with those obtained, under somewhat different conditions, for other freshwater crayfish. For example, one measure of the degree of adaptation of an animal to freshwater is the minimum sodium balance concentration. This is the minimum external sodium concentration at which the sodium uptake mechanism, when stimulated to work at its fastest rate, can just balance sodium losses, so that there is no net movement of sodium between the animal and the external medium. No attempt was made to achieve the minimum sodium balance concentration as defined by Shaw (1959*a*), Shaw & Sutcliffe (1961) and Sutcliffe (1968, 1974), but the sodium concentration of the blood of the 29 crayfish represented in Fig. 4 had fallen by an average of 8·5% during 95–96 h of salt depletion. The results presented in Fig. 4, and discussed on page 659, show that, under these conditions, the crayfish from the habitats of lowest external concentration (stations A and B) exhibited zero net flux of sodium at an external concentration of *ca.* 0·08 mM-NaCl, whereas crayfish from the habitats of higher external concentration (stations C and D) showed zero net flux at an external concentration of *ca.* 0·11 mM-NaCl. These values are higher than the minimum sodium balance concentration of 0·04 mM determined for *Austropotamobius pallipes* by Shaw (1959*a*), but they appear to bear very favourable comparison with the results given in his Fig. 2 for individual crayfish achieving sodium equilibrium after being placed in deionized water. The results depicted in Shaw's figure were obtained under essentially the same conditions as those used here for *P. zealandicus*, and they show that the equilibrium concentration of three out of four of his crayfish after 96 h was 0·10–0·12 mM-NaCl.

Sutcliffe (1974) argues that the minimum sodium balance concentration provides an estimate of the lowest concentration that can be tolerated by an animal; a natural population would not be expected to occur where the ion concentration is below the minimum balance concentration. While this is almost certainly correct, the results presented in Figs. 1 and 2 show that *P. zealandicus* which normally live in external concentrations of *ca.* 2·0 mM-NaCl can achieve balance in an external concentration of 0·2 mM-NaCl only by undergoing a decrease in blood concentration of *ca.* 8%. The minimum sodium balance concentration for these crayfish is certainly well below 0·2 mM and, as discussed above, must be below 0·11 mM. The normal blood concentration of these crayfish (from station D) is the same as that of crayfish from station A (Wong & Freeman 1976*b*), which evidently achieve balance at an external concentration of 0·2 mM without any decrease in blood concentration.

Shaw (1959*a*) estimated also the rate of net sodium uptake from different external

sodium concentrations, using crayfish which had been allowed to come into balance with their minimum equilibrium concentrations. For his crayfish number 14, figured in his Fig. 5, the net uptake from an external concentration of 0·15 mM is *ca.* 1·5 μmol h^{-1} for an animal of 7·2 g body weight. This value of *ca.* 2·08 μmol 10 g^{-1} h^{-1} for *Austropotamobius pallipes* compares with mean values of $1{\cdot}58 \pm 0{\cdot}53$ ($n = 4$) and $1{\cdot}74 \pm 0{\cdot}28$ ($n = 4$) μmol 10 g^{-1} h^{-1} for *P. zealandicus* from those populations (from stations A and B) which showed the highest net uptake rates of sodium from 0·15 mM-NaCl. As explained above, these *P. zealandicus* had not attained their minimum sodium balance concentration before uptake commenced, but it is of interest to note that the highest individual uptake rate (for a crayfish from station A) was 2·36 μmol 10 g^{-1} h^{-1}.

It would appear that, even taking account of the differences in experimental conditions under which the measurements were made, *P. zealandicus* collected from habitats of low external concentration have similar values for salt loss into distilled water, sodium uptake from dilute NaCl solutions, and probably also for minimum sodium balance concentration to those of *A. pallipes*.

It has not been demonstrated that there are differences in the values of these osmoregulatory parameters between different populations of the same species of freshwater crayfish of the northern hemisphere, such as has been shown here for *P. zealandicus* from the South Island of New Zealand. In this regard, *P. zealandicus* compares with the isopod *Mesidotea entomon* (Lockwood & Croghan, 1957; Croghan & Lockwood, 1968) and with the amphipod *Gammarus duebeni* (Sutcliffe, 1967, 1971), even though the differences in external concentration between the natural habitats of the various populations of *P. zealandicus* are much smaller than those between the freshwater and brackish waters inhabited by *M. entomon* and *G. duebeni*. The adaptations to living in low external concentrations shown by *M. entomon* and *G. duebeni* are, in general, similar to those of *P. zealandicus*. The freshwater races of both *M. entomon* and *G. duebeni* show more efficient salt uptake mechanisms, *G. duebeni* exhibits lower renal salt losses in lower external concentrations, and *M. entomon* from Lake Malaren has a lower surface permeability to salt than do members of the same species from the Baltic. There were, however, no significant differences in surface permeability between the freshwater and brackish water races of *G. duebeni*.

It is possible to consider the degree of adaptation of *P. zealandicus* to freshwater in the light of the results presented in this study. Beadle & Cragg (1940) proposed that the invasion of freshwater involved two steps. Initially, there would have been the development of active uptake mechanisms to maintain blood concentrations at high levels. Secondly, the evolution of mechanisms for renal salt absorption would have been accompanied by a permanent lowering of the blood concentration. Shaw (1959*b*, 1961) has additionally argued that the maintenance of a high blood concentration involved also a reduction in the permeability of the body surface. In certain cases an overall reduction in surface permeability may be sufficient to reduce salt losses to such a level that the production of dilute urine is unnecessary. Within the framework of this hypothesis, freshwater crayfish of the northern hemisphere, as exemplified by *Austropotamobius pallipes*, are well adapted to their freshwater environment, and, as discussed above, those populations of *P. zealandicus* living in freshwaters of low concentration are probably not inferior to *A. pallipes* in their develop-

ment of a reduced surface permeability to salt and specific salt uptake mechanisms. *P. zealandicus*, like *A. pallipes*, also produces urine markedly hyposmotic to the blood. There is, however, one aspect in which *P. zealandicus* may be considered to be less well advanced in its adaptation to freshwaters than are northern hemisphere crayfishes such as *Austropotamobius*, *Orconectes*, *Procambarus* and *Pacifastacus*. The mean blood concentration of 159 individual *P. zealandicus*, collected from 13 stations in the South Island of New Zealand during the summer months, was 512 ± 18 m-osmoles (Wong & Freeman, 1976*a*). This compares with a range of 378–446 m-osmoles for northern hemisphere crayfishes (Scholles, 1933; Kerley & Pritchard, 1967; Sharma, 1969; Peterson & Loizzi, 1974). The osmotic concentration of the blood of *P. zealandicus* is, in fact, very similar to that of the freshwater crab *Potamon niloticus*, expressed as equivalent to 271 mM-NaCl by Shaw (1959*b*) and recalculated as 506 m-osmoles by Potts & Parry (1964). Shaw (1959*b*) points out that this places the blood concentration of *P. niloticus* in an intermediate position between the very high value for crabs such as *Eriocheir sinensis* and *Telphusa fluviatile*, which can live in full strength seawater, and the lower blood concentrations of freshwater crayfish. The only other species of freshwater crayfish in New Zealand, *Paranephrops planifrons*, has a blood concentration of 480 ± 17 m-osmoles ($n = 138$), which is significantly lower than that of *P. zealandicus* (Wong & Freeman, 1976*a*), although still higher than northern hemisphere crayfishes. The difference between the two New Zealand species is probably attributable to the difference in temperature between the geographical areas occupied by these species (Wong & Freeman, 1976*b*).

We wish to thank the Research Committee of the New Zealand University Grants Committee and the N.Z. Advisory Committee to the Nuffield Foundation for grants in support of this work. One of us (T.M.W.) was supported by a research scholarship provided by the New Zealand Government under the Colombo Plan.

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