

## ADAPTATIONS TO A TERRESTRIAL EXISTENCE BY THE ROBBER CRAB *BIRGUS LATRO*

### VI. THE ROLE OF THE EXCRETORY SYSTEM IN FLUID BALANCE

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#### Summary

Primary urine is formed by filtration in the antennal organ of *Birgus latro* L. Urine isosmotic with the haemolymph is released into the anterior branchial chambers where substantial reabsorption of water and ions may occur. Some of the branchial fluid is ingested and the remainder (final excretory fluid, P) is released.

Crabs supplied with fresh water have a low drinking rate ( $1.82 \text{ ml } 100 \text{ g}^{-1} \text{ day}^{-1}$ ). Primary urine is partially reabsorbed (27%) in the antennal organ and urine flow ( $4.48 \text{ ml } 100 \text{ g}^{-1} \text{ day}^{-1}$ ) is significantly lower than filtration rate ( $5.77 \text{ ml } 100 \text{ g}^{-1} \text{ day}^{-1}$ ). The volume of P released is small in crabs drinking fresh water ( $0.45 \text{ ml } 100 \text{ g}^{-1} \text{ day}^{-1}$ ) and the fluid is dilute ( $\approx 25 \text{ mmol l}^{-1} \text{ NaCl}$ ). The difference between P flow and drinking rate ( $1.37 \text{ ml } 100 \text{ g}^{-1} \text{ day}^{-1}$ ) represents evaporative and faecal water losses.

Provision of saline drinking water (300, 600 or  $1000 \text{ mosmol kg}^{-1}$  sea water) doubles rates of drinking, filtration and urine flow and increases P flow fourfold. Evaporative/faecal water loss remains constant. Reabsorption of salts from the P rapidly decreases when saline media are provided for drinking.

#### Introduction

The water available to terrestrial animals is generally dilute and ionic requirements are filled primarily from the food. Whilst intake of water can usually be accurately controlled *via* drinking, ion intake depends on the ion content and rate of ingestion of the food. Control of salt intake is, therefore, poor and the chief effector of internal homeostasis is expected to be on the output side, i.e.

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principally involving regulation of the flow rate, concentration and composition of the urine.

Early work on terrestrial crabs indicated a considerable degree of internal homeostasis although, surprisingly, the urine was near-isosmotic with the haemolymph (reviewed in Greenaway, 1988). Consequently, it was suggested that osmoregulation was achieved by controlling ion input rather than output, i.e. by selecting the amount and salinity of water drunk to match requirements (Gross, 1955; Gross and Holland, 1960; de Wilde, 1973). Few species of land crabs, however, have access to the range of saline and fresh water bodies necessary to permit this type of regulation and over most of their range many species have access only to fresh water. Under these conditions the ability to produce dilute urine seems essential.

For several species of terrestrial brachyuran crabs, it is now known that isosmotic urine is not released from the crab but directed to another site (gills or gut) where salts are reabsorbed as required (Wolcott and Wolcott, 1982, 1984, 1985). A final, often dilute, excretory fluid, referred to as 'P' by Wolcott and Wolcott (1985), is then released. By this mechanism, *Gecarcinus lateralis* Freminville maintains haemolymph concentration on a low-salt diet whilst drinking fresh water (Wolcott and Wolcott, 1988). Although the existence of extra-renal reprocessing of urine has been conclusively demonstrated in terrestrial gecarcinids and ocypodids, there is currently no information on the mechanisms involved.

Amongst the anomuran land crabs, the shell-carrying species *Coenobita clypeatus*, *C. rugosus* and *C. brevimanus* and the robber crab *Birgus latro* range far inland and frequently have access only to fresh water (Gross and Holland, 1960; Gross, 1964; de Wilde, 1973; Vaninni, 1975, 1976). A high degree of control over urine production and composition would again be expected under such conditions, but data for these species are extremely limited. There are no measurements of the rate of urine flow in any coenobitids, although the filtration rate has been measured in *Birgus latro* (Kormanik and Harris, 1981). The few measurements indicate that the urine of *Birgus latro* and *Coenobita* are near-isosmotic with the haemolymph (Gross and Holland, 1960; Gross, 1964). *C. perlatus* has some control over the ionic composition of urine but not its overall concentration (Gross and Holland, 1960). This limited information suggests that the antennal organs of the coenobitids are not a major site of osmotic and ionic regulation. Nevertheless, both *Birgus latro* and *Coenobita* can regulate the concentration and composition of body fluids when provided with food and fresh water (Gross, 1955; Gross and Holland, 1960; de Wilde, 1973) and similar regulation is apparent in field situations where only fresh water is available (Gross, 1964; Greenaway, 1988). Where a seawater/freshwater choice is available the blood concentration is elevated somewhat and with access to sea water alone a further increase is seen, with the new levels then maintained (Gross, 1955). Kormanik and Harris (1981), although unable to collect urine from *Birgus latro*, provided evidence that sodium efflux from animals drinking fresh water was considerably lower than from those drinking sea water. This strongly suggests some control over the output of salts and

the presence of an extra-renal regulatory site. In support of this, Greenaway and Morris (1989) reported the release of a dilute excretory fluid by *Birgus latro*, although neither the site of formation nor that of release was identified.

This study was designed to reveal how regulation of the output of salts and of water are achieved in the anomuran land crab *Birgus latro*. In this paper, fluid balance is investigated providing data on filtration, urine production and flow of final excretory fluid. Additionally, the osmotic concentrations of urine and excreted fluid are determined. Experimental animals were supplied with drinking water ranging in concentration from deionized water to sea water, in order to test regulatory ability over the full range of salt intake to which the crabs are exposed in the field. Subsequent papers will examine ionic regulation and the mechanisms of ion reabsorption.

## Materials and methods

### *Materials*

*Birgus latro* L. (300–550 g) were collected from rain forest on the Australian Territory of Christmas Island (Indian Ocean) under permits from the Australian National Parks & Wildlife Service and the Department of Primary Industry (Quarantine Service). They were flown to Sydney and maintained at the University of New South Wales in individual containers at 25°C on a 12 h/12 h light/dark cycle. Crabs were fed on dog biscuit (Green's Complete Dog Food, Yennora, NSW, Australia), various fruits and sweetcorn and supplied with fresh water for drinking. This was supplied weekly and proved adequate to sustain growth and eventually promoted moulting.

### *Methods*

#### *Experimental protocols*

Experiments were carried out in metabolism cages made from 15 l plastic buckets with tight-fitting lids. The bottoms of the buckets were removed and replaced with stainless-steel mesh, firmly wired in place, which provided a platform for the crab and trapped faeces. A plastic bag taped over the bottom of the bucket collected fluid falling through the mesh. Mineral oil in the bottom of the bag prevented evaporation from collected fluid until it was removed through a small hole in the plastic by means of a syringe and catheter tubing. Drinking water was supplied in a plastic beaker attached within a second larger plastic container which contained any spillage. Both containers were firmly wired to the mesh bottom of the bucket.

The same basic regimen was used in all experiments but four different concentrations of drinking water were used: deionised water and sea water diluted to 300, 600 and 1000 mosmol kg<sup>-1</sup>. The dye Trypan Blue was added to the drinking waters (0.33 g l<sup>-1</sup>) to allow detection of spillage in fluid sampled from the plastic bag. In practice spillage was rare and immediately obvious without the use of a spectrophotometer. The dye did not affect drinking behaviour.

Crabs were placed individually in the buckets and supplied with 50 ml of drinking water and two small cat biscuits (Whiskettes, Seafood Recipe, Uncle Ben's, Raglan, NSW, Australia). Each day the water was renewed, new food was supplied and the faeces removed. All animals invariably ate all the food supplied. The crabs were also weighed daily, the amount of water drunk was recorded and the excretory fluid released (P) was collected from the bag. Measurements made on control chambers without animals indicated that evaporation from drinking water was negligible over a 24 h period. After measurement of volume, P samples were frozen for future analysis.

Preliminary experiments indicated that 7 days of exposure to the above regimen allowed new osmotic and ionic steady states to be reached in all groups. The rates of filtration of the haemolymph and of urine flow were then measured over the succeeding 7 days, using  $^{51}\text{Cr-EDTA}$ . A measured volume of  $^{51}\text{Cr-EDTA}$  (Amersham) was injected slowly into the pericardial cavity through a pre-drilled hole at a dose rate of approximately  $1 \mu\text{l g}^{-1}$  wet mass ( $3.7 \text{ kBq g}^{-1}$ ). After 1 h, a  $100 \mu\text{l}$  sample of haemolymph was withdrawn and subsamples were analyzed for radioactivity, osmotic pressure and  $[\text{Cl}^-]$ . Further samples were taken at daily intervals thereafter, for 1 week. EDTA space (assumed to be equal to extracellular volume) was determined from the initial dilution of  $^{51}\text{Cr-EDTA}$  in the haemocoel [EDTA space (ml) = amount of radioactivity injected / specific activity of the haemolymph]. The rate of clearance of EDTA from the haemolymph (filtration) was determined from the slope ( $b$ ) of a plot of the natural logarithm of the percentage of the original radioactivity level remaining in the haemolymph *versus* time (EDTA clearance ( $\text{ml h}^{-1}$ ) =  $b \times$  EDTA space). These logarithmic plots showed a high degree of linearity in all individuals. Determination of the ratios between specific activity of the haemolymph and final urine (U:H), which were measured at the end of the experiment, allowed calculation of the rate of urine flow (urine flow = clearance rate  $\div$  U:H). This calculation relies on the assumption that  $^{51}\text{Cr-EDTA}$  is filtered freely into the antennal organs and is neither reabsorbed from nor secreted into the urine.

Samples of drinking water (2 ml) were taken daily during the clearance experiments and their radioactivity was measured as above. This allowed values for drinking rate and P flow to be corrected in instances where P was released into the drinking water. This behaviour was rare and for most animals no correction was necessary or the adjustment was less than 5% of P flow. Several individuals habitually released some of their excretory fluid into their water, however, and corrections of 5–20% were made in these cases. In two groups of experimental animals (one on fresh water and one on  $600 \text{ mosmol kg}^{-1}$  sea water) faeces were also collected daily, and their radioactivity measured.

A second drinking experiment was conducted using the standard protocol but supplying the crabs with deionized water labelled with  $^{51}\text{Cr-EDTA}$  ( $44 \text{ kBq ml}^{-1}$ ). Drinking was recorded daily and P and faeces were collected for assay of radioactivity. Samples of haemolymph were taken at intervals over the 8 day experiment and also assayed for radioactivity. At the end of the experiment one

animal was killed, the extensive midgut gland dissected out, blotted dry and its radioactivity measured.

#### *Haemolymph and urine sampling*

Samples of haemolymph (100–200  $\mu\text{l}$ ) were taken from the pericardial cavity, in iced syringes, through a predrilled hole which was sealed with silicone grease between sampling. The samples were dispensed into 1.5 ml centrifuge tubes and, if not analyzed immediately, were frozen for later action. Samples normally remained fluid under the conditions described.

Urine samples were taken from the nephropores at the base of the second antennae. Owing to the inaccessibility of the pores, and the difficulty of handling such powerful crabs, a procedure similar to that used by Gross (1964) and Gross and Holland (1960) was followed. Crabs were kept at 4°C for 30 min, by which time they were torpid. It was then relatively simple to sample urine direct from the nephropores with a Pyrex glass pipette bent at the tip. Chilling relaxed the urine retention mechanism and volumes up to 1 ml could often be collected. The crabs rapidly recovered at room temperature but urine collection was always the last step of the experiments.

#### *Analyses*

Concentrations of inorganic elements in samples of haemolymph, urine and P were determined after suitable dilution with 7.4 mmol l<sup>-1</sup> CsCl (Na and K) or 7.2 mmol l<sup>-1</sup> LaCl<sub>3</sub> (Ca and Mg) using a Varian model AA175 AB atomic absorption spectrophotometer. Chloride was measured with a Radiometer CMT 10 chloride titrator. With haemolymph samples the large amount of protein present interfered with measurement and in order to obtain reproducible data it was necessary to pretreat the samples. Haemolymph samples were diluted 10-fold with 0.4 mol l<sup>-1</sup> sulphuric acid and the mixture agitated vigorously and then centrifuged. Analyses performed on the supernatant gave consistent results. Osmotic pressures were measured with a Wescor 5100C vapour pressure osmometer.

For measurement of radioactivity (<sup>51</sup>Cr-EDTA) samples were diluted to 2 ml in gamma counting tubes and counted with a Packard Autogamma counter. At least 10<sup>4</sup> counts were recorded for each sample and all samples were corrected for radioactive decay and background radiation.

Between-group comparisons were made using one-way analysis of variance and means were compared using Tukey's HSD test at a probability  $\leq 0.05$ . Other comparisons were made using dependent or independent *t*-tests, again with a probability  $\leq 0.05$  being the accepted fiducial limit. Values are reported as means  $\pm$  s.d. unless otherwise stated.

## **Results**

### *Body mass and EDTA space*

Animals in holding conditions produced a large quantity of faeces owing to the

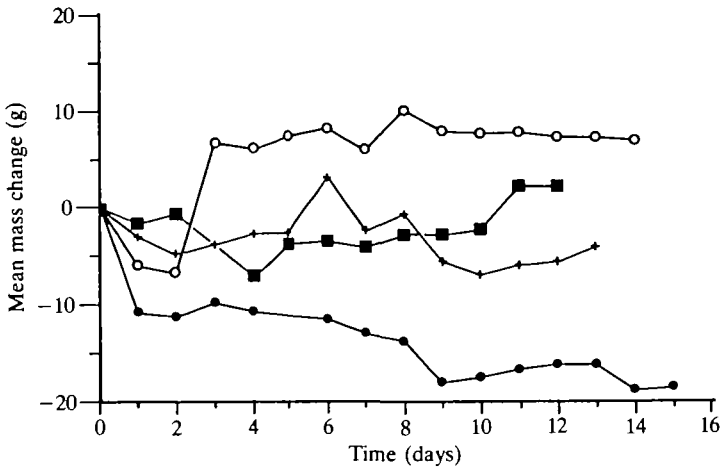


Fig. 1. Mass change of *Birgus latro* during  $^{51}\text{Cr}$ -EDTA clearance experiments on several different drinking regimens. Data points represent the mean change in body mass for each group of crabs. Within each group, no significant change in mean body mass occurred from day 1 until the end of the experiment (ANOVAR). s.d. values have been omitted from the figure for clarity but the range of s.d. for each group follow: fresh water,  $\pm 2.28$ – $6.57$  g;  $300 \text{ mosmol kg}^{-1}$ ,  $\pm 5.16$ – $15.98$  g;  $600 \text{ mosmol kg}^{-1}$ ,  $\pm 4.5$ – $12.94$  g;  $1000 \text{ mosmol kg}^{-1}$ ,  $\pm 16.2$ – $23.2$  g.  $N=6$  for saline groups and 12 for the freshwater group. ○ fresh water; ■  $300 \text{ mosmol kg}^{-1}$ ; +  $600 \text{ mosmol kg}^{-1}$ ; ●  $1000 \text{ mosmol kg}^{-1}$ .

high fibre content and *ad libitum* nature of their diet. On transfer to experimental regimens most animals initially continued the high faecal production. As food intake on the experimental diet was reduced (two cat biscuits  $\approx 0.7 \text{ g day}^{-1}$ ), this resulted in an initial loss of body mass in many individuals. Mass changes during the course of the experiments were small in all groups and mean maximal change from day 1 onwards was less than 5% of body mass (Fig. 1). No significant changes in mean body mass were found for any group at any time interval from day 1 onwards. There was considerable individual variability in drinking patterns and ability to maintain body mass between individuals and this is discussed further below.

Extracellular fluid spaces were measured with  $^{51}\text{Cr}$ -EDTA after 7 days of the experimental regime. In groups supplied with  $600 \text{ mosmol kg}^{-1}$  sea water, EDTA space ( $25.53 \pm 1.533 \text{ ml } 100 \text{ g}^{-1}$ ) was significantly elevated with respect to fresh-water controls (EDTA space =  $22.69 \pm 2.803 \text{ ml } 100 \text{ g}^{-1}$ ) and animals drinking  $300 \text{ mosmol kg}^{-1}$  sea water (EDTA space =  $21.96 \pm 1.076 \text{ ml } 100 \text{ g}^{-1}$ ) (Table 1). EDTA space in animals drinking  $1000 \text{ mosmol kg}^{-1}$  sea water ( $23.87 \pm 1.062 \text{ ml } 100 \text{ g}^{-1}$ ) did not differ significantly from that of any other group.

#### Drinking rate

The data presented for drinking rates (Fig. 2) represent the mean daily intake of

drinking water over the period of measurement of EDTA clearance and urine flow (i.e. days 7–14 of each experiment). Drinking by crabs supplied with deionised water was quite low at  $1.82 \pm 0.603 \text{ ml } 100 \text{ g}^{-1} \text{ day}^{-1}$ . The rate was significantly higher in animals supplied with 300 mosmol  $\text{kg}^{-1}$  sea water ( $3.3 \pm 0.86 \text{ ml } 100 \text{ g}^{-1} \text{ day}^{-1}$ ), 600 mosmol  $\text{kg}^{-1}$  sea water ( $3.61 \pm 0.902 \text{ ml } 100 \text{ g}^{-1} \text{ day}^{-1}$ ) and

Table 1. Extracellular fluid space in *Birgus latro* on different drinking regimens measured with  $^{51}\text{Cr}$ -EDTA

Drinking regimen	Extracellular space (ml $100 \text{ g}^{-1}$ )
Fresh water	$22.69^{\text{a}} \pm 2.803$ (12)
300 mosmol $\text{kg}^{-1}$ SW	$21.96^{\text{a,b}} \pm 1.706$ (6)
600 mosmol $\text{kg}^{-1}$ SW	$25.53^{\text{c}} \pm 1.533$ (6)
1000 mosmol $\text{kg}^{-1}$ SW	$23.87^{\text{a,b,c}} \pm 1.062$ (6)

Values are mean  $\pm$  s.d. (N).

Means without shared superscripts differed significantly at the 5% probability level. SW, sea water.

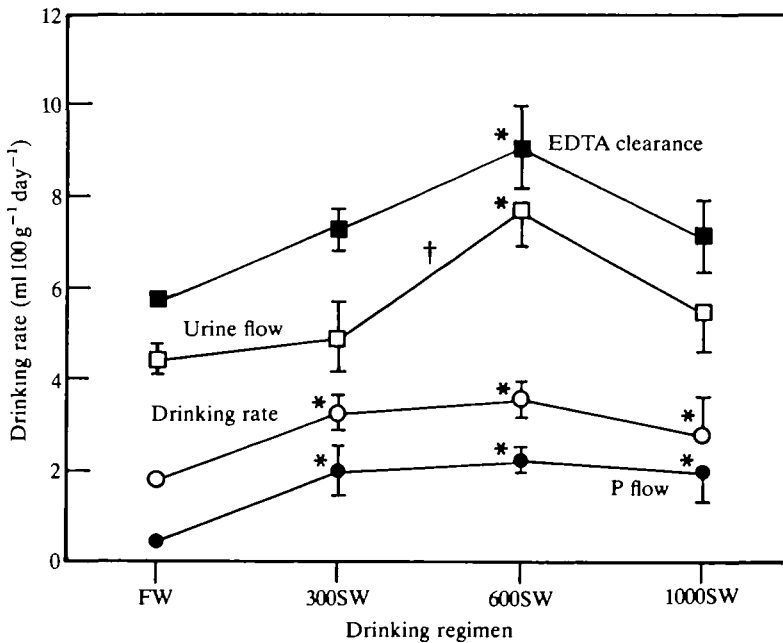


Fig. 2. Water intake and production of excretory fluid by *Birgus latro* on several different drinking regimens. All values are for the steady-state period in the second week of the experiment. For groups drinking saline  $N=6$  and for the freshwater group  $N=12$ . Values are mean  $\pm$  s.e.m. \* represents a significant difference from the control value (fresh water) for each parameter; † represents a significant difference between adjacent means for a given parameter. 300SW, 300 mosmol  $\text{kg}^{-1}$  sea water.

1000 mosmol kg<sup>-1</sup> sea water ( $2.86 \pm 1.85$  ml 100 g<sup>-1</sup> day<sup>-1</sup>) ( $P < 0.001$ ). The drinking rates of animals on different saline water regimens were statistically similar.

#### *EDTA clearance, urine flow and P flow*

The rate of clearance of <sup>51</sup>Cr-EDTA from the haemolymph was followed over a 7 day period (days 7–14 of each experiment) and the values presented (Fig. 2) are, therefore, averages for this period. In fact, clearance was more or less constant over the 7 day period of measurement. The mean clearance rate measured in *Birgus* supplied with deionised water ( $5.77 \pm 1.306$  ml 100 g<sup>-1</sup> day<sup>-1</sup>) was significantly lower than the value for crabs drinking 600 mosmol kg<sup>-1</sup> sea water ( $9.09 \pm 2.031$  ml 100 g<sup>-1</sup> day<sup>-1</sup>). The mean rates of clearance in groups supplied with 300 mosmol kg<sup>-1</sup> sea water ( $7.27 \pm 1.009$  ml 100 g<sup>-1</sup> day<sup>-1</sup>) and 1000 mosmol kg<sup>-1</sup> sea water ( $7.19 \pm 1.755$  ml 100 g<sup>-1</sup> day<sup>-1</sup>) did not differ significantly from each other or from other groups.

The rates of urine flow in *Birgus* drinking fresh water ( $4.48 \pm 0.956$  ml 100 g<sup>-1</sup> day<sup>-1</sup>) and 300 mosmol kg<sup>-1</sup> sea water ( $4.97 \pm 1.877$  ml 100 g<sup>-1</sup> day<sup>-1</sup>) were significantly lower than the rate in animals drinking 600 mosmol kg<sup>-1</sup> sea water ( $7.79 \pm 2.049$  ml 100 g<sup>-1</sup> day<sup>-1</sup>). There was no significant difference in mean rate of urine flow between animals on 1000 mosmol kg<sup>-1</sup> sea water ( $5.57 \pm 2.212$  ml 100 g<sup>-1</sup> day<sup>-1</sup>) and other groups, nor between freshwater and 300 mosmol kg<sup>-1</sup> sea water groups. Urine:haemolymph ratios were similar in all groups (Table 2) and, in consequence, urine flow showed a response to changing salinity of drinking water very similar to that seen for EDTA clearance (Fig. 2). However, as the mean U:H in each group was greater than 1, urine flow was lower than clearance in each group ( $P < 0.025$ ).

The rate of release of excretory fluid (P) from the animals followed the same general pattern seen for rates of drinking, urine flow and clearance (Fig. 2). Thus, the rate of P production in animals drinking fresh water ( $0.45 \pm 0.422$  ml 100 g<sup>-1</sup> day<sup>-1</sup>) was minimal, being significantly lower than for animals drinking 300 mosmol kg<sup>-1</sup> sea water ( $2.03 \pm 1.375$  ml 100 g<sup>-1</sup> day<sup>-1</sup>), 600 mosmol kg<sup>-1</sup> sea water ( $2.29 \pm 0.701$  ml 100 g<sup>-1</sup> day<sup>-1</sup>) or 1000 mosmol kg<sup>-1</sup> sea water ( $2.03 \pm 1.623$  ml 100 g<sup>-1</sup> day<sup>-1</sup>). Mean P flow in the latter three groups did not differ significantly. On each drinking regimen, the mean rate of P production was lower than the mean rates of clearance ( $P < 0.001$ ), urine flow ( $P < 0.001$ ) and drinking rate ( $P < 0.005$ ) (Fig. 2).

#### *Reabsorption of fluid from primary urine*

On each drinking regimen a proportion (14–26%) of the fluid filtered from the haemolymph (=primary urine) was reabsorbed in the antennal organ (Fig. 3) After release of urine into the branchial chambers further reabsorption occurred, bringing the total recovery of fluid to 74–75% in crabs drinking saline media and 93% in crabs drinking fresh water (Fig. 3). The mean values for the total fraction of the filtrate reabsorbed did not differ significantly ( $P = 0.063$ ). The branchial



Table 2. U:H and P:H ratios for  $^{51}\text{Cr}$ -EDTA, chloride and osmotic pressure in *Birgus latro* after 7 days on several different drinking regimens

Drinking regimen	U:H			P:H		
	Chloride	OP	EDTA	Chloride	OP	EDTA
FW	1.10*	0.98	1.31	0.07 <sup>a</sup> *	—	1.69
±s.d.	0.09	0.03	0.30	0.02		0.56
N	12	12	11	4		9
300 mosmol kg <sup>-1</sup>	1.14*	0.97*	1.59	0.47 <sup>b</sup> *	0.46 <sup>d</sup> *	1.41
±s.d.	0.07	0.02	0.42	0.15	0.14	0.65
N	6	6	6	6	6	6
600 mosmol kg <sup>-1</sup>	1.13*	0.98	1.20	0.89 <sup>c</sup>	0.84 <sup>c</sup> *	1.17
±s.d.	0.04	0.03	0.14	0.23	0.16	0.36
N	6	6	6	5	5	6
1000 mosmol kg <sup>-1</sup>	1.14*	1.00	1.36	1.13 <sup>c</sup> *	1.07 <sup>c</sup>	1.37
±s.d.	0.05	0.03	0.24	0.08	0.07	0.58
N	5	5	6	4	4	4

U:H and P:H for EDTA did not differ either within or between groups.

Different alphabetical superscripts within a column indicate that the means differ significantly ( $P \leq 0.05$ ).

The absence of superscripts in a column indicates that none of the means within that column differed significantly.

\* indicates a significant difference between the concentrations in the haemolymph and in the P or urine.

OP, osmotic pressure.

component of reabsorption by crabs on 600 mosmol kg<sup>-1</sup> sea water was significantly greater than in 300 and 1000 mosmol kg<sup>-1</sup> sea water groups.

Although the rate of production of P was much lower than urine flow, the P:H ratio for EDTA was similar to U:H in each group (Table 2). Removal of fluid and marker from the branchial chamber, therefore, proceeded at similar rates. In two groups of animals (freshwater and 600 mosmol kg<sup>-1</sup> sea water) the radioactivity excreted in the faeces was measured during clearance experiments. In both groups radioactivity was present in the faeces, suggesting that fluid reabsorption might occur across the gut following ingestion of P (see Discussion). The amount of radioactivity recovered in the faeces was less than 10% of that in the P reabsorbed.

#### *Drinking of labelled water*

To investigate further the fate of ingested  $^{51}\text{Cr}$ -EDTA, and to examine the reliability of the compound as a volume marker for P, crabs were supplied with drinking water labelled with  $^{51}\text{Cr}$ -EDTA.

Detectable radioactivity appeared in the faeces and P of some individuals within

1 day of provision of labelled water, but there was considerable variability between individuals in the amounts of P, faeces and radioactivity produced. In all animals that drank significant amounts of water, radioactivity was detectable in the haemolymph within 1–3 days and continued to rise with time (Fig. 4). The

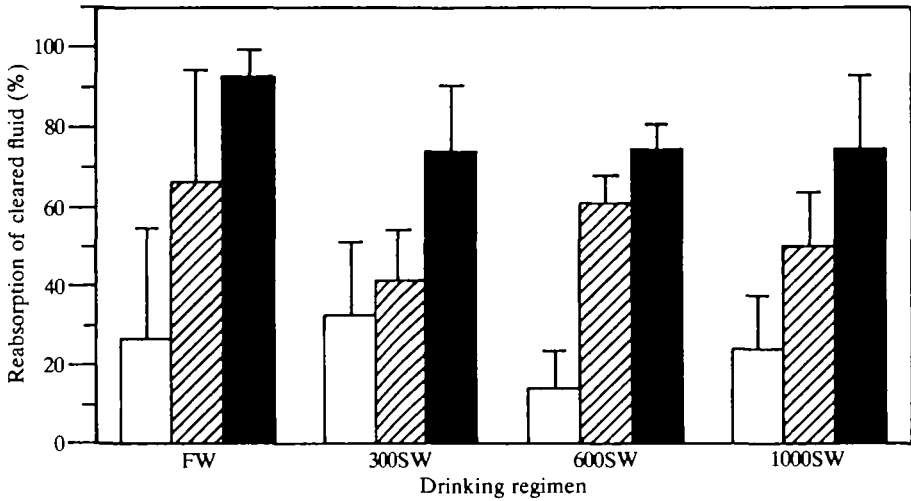


Fig. 3. Reabsorption of water from primary urine and branchial chambers/gut in *Birgus latro* drinking fresh or saline water.  $N=6$  for groups drinking saline and 12 for the group drinking fresh water. The percentage of fluid not reabsorbed represents P flow. Values are mean+s.d. Unfilled bars, reabsorption in antennal organ; hatched bars, reabsorption in branchial chamber; filled bars, total reabsorption. 300SW, 300 mosmol  $\text{kg}^{-1}$  sea water.

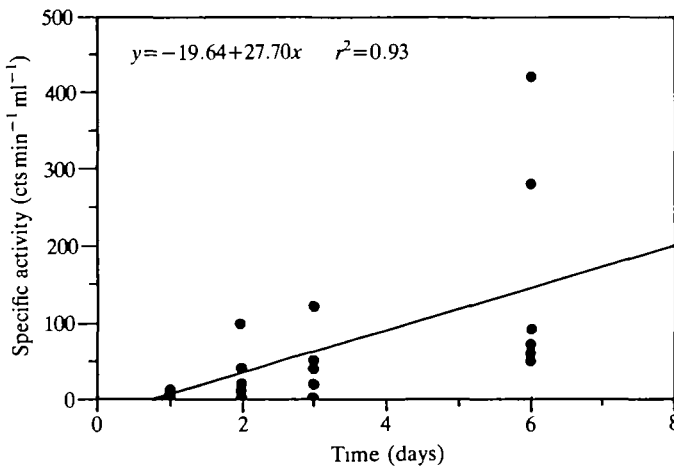


Fig. 4. The appearance of radioactivity in the haemolymph of *Birgus latro* drinking fresh water containing  $^{51}\text{Cr-EDTA}$  ( $44 \text{ kBq ml}^{-1}$ ). Points represent values for seven crabs and the regression line was calculated using the mean values at each sampling time.

Table 3. The radioactivity (RA) ingested by seven crabs drinking fresh water labelled with  $^{51}\text{Cr-EDTA}$  and the proportion recovered in P, faeces and haemolymph over an 8 day period

	RA ingested (cts min <sup>-1</sup> )	RA recovered (cts min <sup>-1</sup> )	Recovery of ingested RA (%)
Mean	1 634 903	163 922	10.7
±s.d.	829 211	223 260	14.1

radioactivity that appeared in the blood (sum of the total radioactivity in haemolymph and radioactivity in the P) was estimated as a percentage of the radioactivity ingested. This value was small and reached only  $4.97 \pm 5.87\%$  ( $N=7$ ) after 6 days. Of the total amount of radioactivity ingested during the experiment only 10.7% could be located in faeces, P and haemolymph (Table 3). The bulk of the  $^{51}\text{Cr}$  (89.3%) was, therefore, sequestered elsewhere in the body. At the end of the experiment the midgut gland of one crab (which had ingested a large amount of  $^{51}\text{Cr-EDTA}$ ) was assayed and found to contain about half of the missing radioactivity. The assay was performed 1 day after the end of the experiment, during which time the crab would have voided (undetected) further radioactivity. Only one animal could be spared for this analysis.

The total radioactivity in the blood at day 6 was only loosely correlated with the volume of water drunk, whilst faecal radioactivity showed no apparent correlation with ingestion of radioactivity. This again suggested that movement of the radioactive label from gut lumen to haemolymph was not a direct transfer.

## Discussion

### *Freshwater regimen*

Extracellular fluid volume measured as  $^{51}\text{Cr-EDTA}$  space ( $22.69 \text{ ml } 100 \text{ g}^{-1}$ ) was somewhat lower than reported previously for animals drinking fresh water ( $28.3 \text{ ml } 100 \text{ g}^{-1}$ ) (Kormanik and Harris, 1981). The abdomen of *Birgus latro* undergoes considerable volume change as a result of different nutritional states and moult stages and this may be accompanied by changes in extracellular fluid volume. The abdomens of the animals used in these experiments were noticeably small and this may well account for the differences seen between the two studies.

It was apparent from the experiment involving crabs drinking water labelled with  $^{51}\text{Cr-EDTA}$  that much of the  $^{51}\text{Cr}$  entering the gut was stored in the midgut gland, presumably intracellularly, and was only slowly released into the haemolymph (see below). It is evident that drinking of P containing  $^{51}\text{Cr-EDTA}$ , during clearance experiments, would also result in the addition of small amounts of radioactivity to the haemolymph, with a consequent underestimation of the rate of clearance. The entry of  $^{51}\text{Cr}$  into the haemolymph during drinking of  $^{51}\text{Cr-EDTA}$  was very slow, however, and during the 7 day clearance experiments is likely to

have caused only a minimal error. This is supported by the individual clearance data, where plots of the natural logarithm of the percentage of  $^{51}\text{Cr}$ -EDTA remaining vs time showed a high degree of linearity over the whole period (7 days). Additionally, the measured rate of  $^{51}\text{Cr}$ -EDTA clearance ( $5.8\text{ ml } 100\text{ g}^{-1}\text{ day}^{-1}$ ) was close to that obtained with inulin under similar conditions ( $6.6\text{ ml } 100\text{ g}^{-1}\text{ day}^{-1}$ ) by Kormanik and Harris (1981). Although this compound would also have been ingested in P it is unlikely that it subsequently behaved in the same way as  $^{51}\text{Cr}$ .

The mean U:H ratios of EDTA were greater than unity in all four groups, indicating that urine flow was lower than the EDTA clearance ( $P=0.006$ ). Single-injection clearance studies, however, tend to overestimate the true U:H, particularly if the bladder is large or bladder residence time is long (Riegel *et al.* 1974; Cornell, 1983). In consequence, apparent reabsorption of fluid from the antennal organ (26%) was probably overestimated slightly. Similarly, P:H ratios will also have been slightly overestimated.

Urine flow rate has not previously been measured in *Birgus latro* owing to the failure of earlier workers to collect urine (Kormanik and Harris, 1981; Harris and Kormanik, 1981). The nephropores of the crab are within the anterior extensions of the branchiostegites and fluid released is rapidly drawn into the anterior portions of the branchial chambers by hydrophilic hairs on the mouthparts and the branchiostegal walls. Drinking of fluid is also quite feasible owing to the proximity of the mouth and surrounding mouthparts (S. Morris, H. H. Taylor and P. Greenaway, in preparation). Thus, urine released from the antennal organs is not immediately jettisoned by the animal but enters the branchial chambers, offering the possibility for further processing before the final excretory fluid is released. The rate of urine production in *Birgus latro* fell within the range measured for several species of terrestrial and amphibious brachyuran crabs (summarised in Greenaway, 1988).

Of the filtered fluid, only about 8% was finally lost as P and approximately 26% was reabsorbed in the antennal organ. The remaining 67% must have been reclaimed from the urine after it left the nephropores but before the residual P was released. As this decrease in volume was not accompanied by a significant increase in the concentration of EDTA (Table 2) both marker and water were removed at similar rates. This would most readily be achieved by ingestion of the fluid, and this contention was supported by the appearance of  $^{51}\text{Cr}$  in the faeces during the clearance experiments and by the data for ingestion of labelled drinking water. The 'branchial reabsorption' of fluid, therefore, must occur largely by drinking of P.

Only 25% of the water drunk was collected as P and in the absence of any mass gain during the experiment (Fig. 1) the other 75% must have been lost by other routes. Whilst underestimation of P production due to losses on the walls and mesh of the animal chambers would account for some of this difference, the major part must have been due to evaporative loss from the body surface and some loss of free water in the faeces.

*Saline regimens*

After 7 days of drinking  $600 \text{ mosmol kg}^{-1}$  sea water the extracellular space was significantly elevated compared with freshwater controls. As body mass did not alter significantly during the 14 days of the experiment this elevation may have resulted from changes in distribution of body water or was perhaps hidden by the large individual variability in body mass.

Variability in data for body mass of crabs on various drinking regimens was attributable to several causes. Whilst each individual behaved consistently in terms of changes in body mass, not all crabs behaved similarly, e.g. in the  $1000 \text{ mosmol kg}^{-1}$  sea water group, individual response from day 1–14 of the experiment varied from a gain of 3 % of body mass to a loss of 17 %. This source of variability coupled with episodic drinking and P release statistically obscured any minor trends suggested by the data in Fig. 1.

The response of animals to saline drinking water was an immediate elevation of drinking rate to a maximal value ( $600 \text{ mosmol kg}^{-1}$  sea water group) twice that of the freshwater controls (Fig. 2). The high salt content of the saline drinking waters encouraged rather than deterred drinking, even when it was initially hyperosmotic to the haemolymph. The rate of filtration paralleled the drinking rate and, in the  $600 \text{ mosmol kg}^{-1}$  sea water group, it, too, was double the control level. Similar patterns of urine and P flow were evident (Fig. 2). The absolute differences between mean rates of P flow and drinking (=evaporative and faecal losses) were statistically similar for all drinking regimens ( $P=0.254$  ANOVAR, Fig. 2), a reflection of the constancy of the experimental conditions. P flow in the groups drinking saline water accounted for 60–70 % of the fluid drunk compared to 25 % in the group drinking fresh water.

*Mechanisms of osmoregulation*

The extra-renal reprocessing of urine in brachyuran crabs has been clearly established (Wolcott and Wolcott, 1982, 1984, 1985). However, whilst it is tacitly assumed that the extra-renal site is the branchial chambers and the gills are the effector organs, the evidence for this remains largely circumstantial and the gut may also be involved. The gut appears most suitable for total or volume reabsorption (fluid+ions), rather than for ion uptake alone, as the faeces of land crabs are invariably fairly dry and compact and are not a suitable vehicle for fluid excretion. The branchial chambers are suited for dilution of urine by ion reabsorption whilst maintaining an output of fluid.

In *Birgus latro*, the evidence suggests that both the gut and branchial chambers are utilised for urine reprocessing. A considerable amount of fluid is ingested, although whether this is urine, part-processed urine or P is not known. There is no indication from this study or a previous one (Greenaway and Morris, 1989) that the faeces are involved in volume regulation, so that ingested excretory fluid must pass into the haemolymph. There is good evidence that salts are reabsorbed from the urine in the branchial chambers and that the gills and the lining of the anterior

branchial chambers are probably responsible (Table 2). It is also clear that the fluid finally excreted (P) is derived from the branchial chamber fluid.

The excretory system of *Birgus latro* is potentially highly flexible. Depending on the requirements of the animal for particular ions and for water the P may be partitioned between the gut and the branchial chambers (Table 2) and retained in the latter only as long as necessary to achieve the degree of ion transport required. Adjustment of the proportion of urine reingested could enable the animal to accommodate to changes in evaporative water loss and drinking.

Owing to the risk of loss of urine from the branchial chambers during activity it is probable that *Birgus latro* only processes urine during periods of inactivity. The ability to retain urine by ingestion may be valuable during periods of prolonged or unexpected activity.

It is clear that measurements of urine flow are essentially irrelevant to salt and water balance in terrestrial crabs, as the urine is subjected to extra-renal resorptive processes which markedly alter its volume and salt content (Wolcott and Wolcott, 1985; Greenaway and Morris, 1989; this study). It is the flow rate and concentration of the excretory fluid released (P) that are important and the values given above for *Birgus latro* are the first data in the literature for these crucial parameters in land crabs on normal regimens.

The normal roles of a renal organ are in the regulation of body water and ionic content and in nitrogenous excretion and acid-base balance. In *Birgus latro* the antennal organs play only a minor role in water balance, reabsorbing a small portion of the filtered fluid, and the main control sites are the branchial chambers and the gut. The main ion regulatory role, too, is shifted to the branchial chamber. Acid-base adjustments of the urine by the antennal organ are also possible but, as the main nitrogenous excretory product is faecal uric acid (Greenaway and Morris, 1989) and the bulk of ion transport (and, thus, potential acid-base changes) occurs in the branchial chambers, little role is indicated for either acid-base balance or nitrogenous excretion. On present evidence, there is no clear indication of the importance of the antennal organ of *Birgus latro* other than in the formation and temporary storage of primary urine.

Salt loss in excretory fluid depends on both the concentration and flow rate of the fluid released and some salt loss by this route is inevitable, as salt concentrations are never reduced to zero and some flow is always necessary. Animals on low-salt diets and drinking fresh water must minimise excretory loss of salt and would be expected to produce a dilute fluid by reabsorbing ions and to minimise flow rate by restricting water intake. Such a pattern is seen clearly in *Birgus latro*, where drinking rate, P flow and P concentration are all minimal in crabs drinking fresh water, so that salt loss is very low.

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