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

VIII. OSMOTIC AND IONIC REGULATION ON FRESHWATER AND SALINE DRINKING REGIMENS

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Summary

Crabs supplied with deionized water (DIW) and food maintained constant haemolymph osmolality, [Na] and [Cl]. Drinking of 300, 600 or 1000mosmolkg^{-1} sea water (300SW, 600SW, 1000SW) elevated Na and Cl concentrations, which restabilized by 12 days on the 300SW and 600SW regimens but continued to rise on the 1000SW regimen. [Ca] increased slightly on all regimens. [K] changed only on the 1000SW regimen. Haemolymph [Mg] was poorly regulated on all saline regimens, doubling after 47 days on the 1000SW regimen.

Urine was always isosmotic to haemolymph, had twice the [K] and a lower [Ca]. Changes in urinary ion concentrations paralleled those in the haemolymph, indicating that the antennal organs are unimportant in ionic regulation. Changes in ionic composition of the branchially modified urine (P) reflected drinking water concentrations on a mole-for-mole basis (except for [Mg]), confirming the regulatory role of P. Water and ion budgets indicate reingestion of 70–90% of filtered urine. Branchial ion uptake and reingestion allow variable reclamation of 70–99.9% of filtered Na, Ca, Mg and Cl and 37–96% of K.

Crabs drank DIW daily (mean rate, 16.2 g kg^{-1} day^{-1}) and released P intermittently (intervals ranged from less than 1 to more than 6 days; mean 5.8 g kg^{-1} day^{-1}). Provision of 300SW and 600SW doubled drinking rate and trebled P output. Intense initial drinking of 1000SW (58.1 g kg^{-1} day^{-1}) was not maintained. Return to DIW after 1000SW stimulated very high drinking rates initially (119 g kg^{-1} day^{-1}).

Key words: terrestrial crab, *Birgus latro*, osmoregulation, ionic regulation, water balance, excretion, urine reprocessing.

Birgus can vary P composition rapidly and widely (less than 100mosmolkg^{-1} and 20mmoll^{-1} [NaCl] to more than 1000mosmolkg^{-1} and 500mmoll^{-1} [NaCl] within 1 day) and sometimes produce P that is hyperosmotic to the haemolymph. Production of P hyperosmotic to the drinking water and increased drinking rate permit a gain of osmotically free water to balance evaporation when drinking 300SW and 600SW, but not 1000SW. Adjustments to the volume and composition of the P appear to be more important than 'behavioural osmoregulation'.

Introduction

Behavioural osmoregulation, which involves choices between drinking water sources of appropriate salinity, has been considered to be an important component of normal osmoregulation by terrestrial coenobitids (Gross, 1955; de Wilde, 1973). When allowed access to both fresh water and sea water, *Birgus latro*, *Coenobita perlatus* and *C. clypeatus* maintained haemolymph osmolality somewhat hypo-osmotic to sea water (Gross, 1955; Gross and Holland, 1960; de Wilde, 1973). Changes in osmolality of the haemolymph, following salt depletion and loading, were restored by adjusting the frequency and duration of drinking from the two sources. In *Coenobita clypeatus*, such selection of drinking water is used to adjust the composition of the shell water which, in effect, forms the animal's external medium (de Wilde, 1973). However, *Birgus* lacks a shell except in the glaucothoe and first crab stages (Harms, 1932; Reese and Kinzie, 1968) and must be subject to greater evaporative losses.

The urine of anomuran land crabs, like that of brachyurans, is approximately isosmotic with the haemolymph and is produced at rates comparable with those of other crabs (Gross and Holland, 1960; Gross, 1964; de Wilde, 1973; Greenaway *et al.* 1990). Without additional conservation mechanisms, compensation of the salt loss from this route alone would require sustained seawater drinking at rates averaging at least 5% of body mass per day.

It is unclear whether many individual *Birgus* have regular access to saline water for osmoregulatory purposes. On small islands, sea water would certainly be readily available but, on large islands, such as Christmas Island in the Indian Ocean, these crabs are found many kilometres from the shore (Hicks *et al.* 1984; H. H. Taylor, P. Greenaway and S. Morris, unpublished observations). Adult *Birgus* visit the sea for reproduction (Hicks *et al.* 1984) but there is no evidence that they make other regular visits to the sea. They are able to drink from small rainwater puddles, picking up water on their chelipeds and transferring it to their maxillipeds (Lister, 1888; Gross, 1955). However, high rainfall and porous substrata beneath island forest habitats must cause drinking water sources to be ephemeral and dilute. The crabs' predominantly herbivorous diet (Hicks *et al.* 1984) is likely to be relatively low in ions (Wolcott and Wolcott, 1988).

Recently, it has been demonstrated that *Birgus* (Greenaway and Morris, 1989; Greenaway *et al.* 1990; Morris *et al.* 1991) and several species of brachyuran terrestrial crabs (Wolcott and Wolcott, 1985, 1988, 1991; Greenaway and Nakamura, 1991) are able to release to the exterior a dilute final excretory fluid, P, formed by post-renal modification of the urine. In *Birgus*, the nephropores on the antennal bases are directed

posteriorly and lie within the branchial chambers, where they are covered by anterior extensions of the branchiostegites (Morris *et al.* 1991). The inner branchiostegal integument, the scaphognathites, the mouthparts and the nephropores all bear hydrophilic hairs that permit conduction of the urine either towards the mouth or towards the gills. It has been shown that P production involves both reingestion of urine and branchial ion-transport processes (Greenaway *et al.* 1990; Morris *et al.* 1991).

In considering the potential contributions of extrarenal processing of the urine to salt and water homeostasis, it is important to examine the direction, magnitude and speed of responses to an applied change. It is also of interest to examine the handling of individual ions and, in particular, to consider the relative importance of renal or branchial transport mechanisms for control of their output. In this paper we examine ionic regulation of the haemolymph, the time course of changes in the composition of the urine and P, and the rates of drinking and P formation in crabs experimentally switched between freshwater and saline-water regimens.

Materials and methods

Animal collection and maintenance

Birgus latro L. (300–550g) were collected from rainforest on the Australian Territory of Christmas Island in the Indian Ocean under permits from the Australian National Parks and 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, at least 75% relative humidity (RH) and under a 12h/12h light/dark cycle. Crabs were fed on dog biscuits, various fruits and sweet corn and supplied with deionised water for drinking.

Experimental procedures

General procedures were as described by Greenaway *et al.* (1990). Metabolism cages were constructed from 15l plastic buckets with lids, the bottoms being replaced with stainless-steel mesh, to provide a platform for the crab and to trap faeces. A plastic bag was taped over the bottom of the bucket to contain fluid (P) 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 firmly secured within a second larger plastic container which contained any spillage. Four different concentrations of drinking water were used: deionized water (DIW), and sea water diluted to $300\,\mathrm{mosmol\,kg^{-1}}$, $600\,\mathrm{mosmol\,kg^{-1}}$ and $1000\,\mathrm{mosmol\,kg^{-1}}$. The dye Trypan Blue was added to the drinking waters ($0.33\,\mathrm{g\,l^{-1}}$) to allow detection of spillage in fluid sampled from the plastic bag. In practice, spillage was rare and the dye did not affect drinking behaviour. Crabs were placed individually in the buckets and supplied with 50 or 100ml of drinking water and two small cat biscuits (approximately 0.5 g drymass; Na 665, K 141, Ca 255, Mg 59, Cl $111\,\mu\mathrm{mol\,g^{-1}}$ drymass). They invariably ate all of the food supplied. Each day, the water was renewed, new food was supplied and the faeces were removed.

Series I

To examine changes in water and ion status, crabs were transferred from the holding conditions (DIW for drinking) to the four regimens for 9 days (DIW, N=7; 300mosmolkg^{-1}, N=7) or for 12 days (600mosmolkg^{-1}, N=7; 1000mosmolkg^{-1}, N=8). At daily intervals, the crabs were weighed, the volume of water drunk was recorded, the excretory fluid (P) was collected from the bag and weighed and a pericardial haemolymph sample (1ml; less than 1% of haemolymph volume) was taken. Urine samples were taken on the final day as described by Greenaway *et al.* (1990). Fluid samples were stored frozen before analysis of ions and osmolality. Chloride (Radiometer CMT10 chloride titrator after pretreatment with an equal volume 0.4mol l^{-1} H_2SO_4) and osmolality (Wescor 5100C vapour pressure osmometer) were measured on all daily samples. Osmolalities below 100mosmolkg^{-1} could not be measured with precision and are plotted as 100mosmolkg^{-1}. A few individual values of osmolality of the P which were less than 100mosmolkg^{-1} (1 day and 9 day values, DIW crabs, Table 1) were estimated from [Cl] and [NH_4] as described below. Initial and final samples of haemolymph, urine and P, and the drinking water, were analysed for [Na], [K], [Ca] and [Mg] by atomic absorption spectroscopy (Varian AA175 AB). Some P samples from the DIW group were also analysed for ammonia/ammonium (Boehringer Mannheim urea test kit no. 4788 omitting the urease step). Further details of the analytical procedures are given by Greenaway *et al.* (1990).

Series II

These crabs were maintained on the above regimens for 14 days (DIW, N=12; each saline, N=6). Drinking rates, rates of primary and final urine production and P flow of these crabs have been reported previously (Greenaway *et al*. 1990). Here we combine detailed ionic analyses of the haemolymph, urine and P for this group with the previous data to construct a budget for each ion and for water.

Series III

Eight crabs from the 1000mosmolkg^{-1} regimens (drawn from series I and II) were returned to a DIW regimen. Their water balance, and the osmolality and [Cl] of the haemolymph and P, were monitored for a further 7 days.

Series IV

Five crabs from each of the 600mosmolkg^{-1} and 1000mosmolkg^{-1} regimens (drawn from series I and II) were returned to maintenance conditions, as described above (i.e. with varied food *ad libitum*), but continuing the same saline drinking water regimens for a total of 47 days. Haemolymph ionic composition was measured at the end of this time.

Statistics

Means are given with the standard errors of the means (S.E.M.). Where sample sizes (crabs and/or days) are not stated, they are given in the protocols above. Statistical

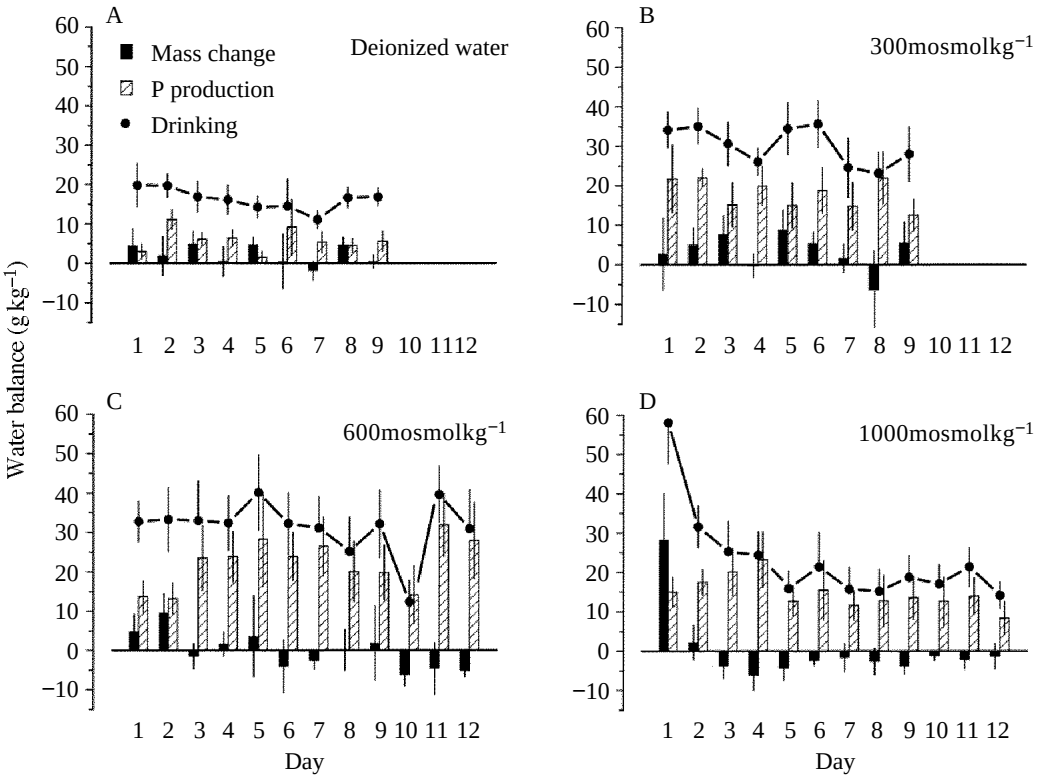

Fig. 1. Daily water (or mass) balance in *Birgus latro* supplied on day zero with deionized water (A) or 300mosmolkg^{-1} (B), 600mosmolkg^{-1} (C) or 1000mosmolkg^{-1} (D) sea water for drinking (series I crabs). Prior to this crabs were maintained on fresh water. Means ± S.E.M.

analyses were performed using the SOLO package (BMDP software). These included paired and unpaired Student's *t*-tests, analysis of variance (ANOVA, general linear model), with Fisher's least significant difference (LSD) test for *post hoc* comparisons of means, and linear regression. Further details are given in the text and figures.

Results

Effects of drinking water salinity on salt and water balance (series I)

Drinking rates

Crabs drank daily on all four regimens. The mean rate of drinking of DIW (all crabs, all days) was 16.17±2.75 g kg^{-1} day^{-1} (Fig. 1A). Provision of 300 and 600mosmolkg^{-1} salines (Fig. 1B,C) elevated drinking rate to about twice the DIW level for the whole 9- to 12-day period (overall means, 30.12±1.63 and 31.22±2.05 g kg^{-1} day^{-1} respectively; differences from the DIW group were highly significant on all days, P<0.001, ANOVA). Crabs initially drank even larger volumes of 1000mosmolkg^{-1} sea water (Fig. 1D). The means on day 1 (58.06±10.56 g kg^{-1} day^{-1}) and day 2 (31.54±5.36 g kg^{-1} day^{-1}) were

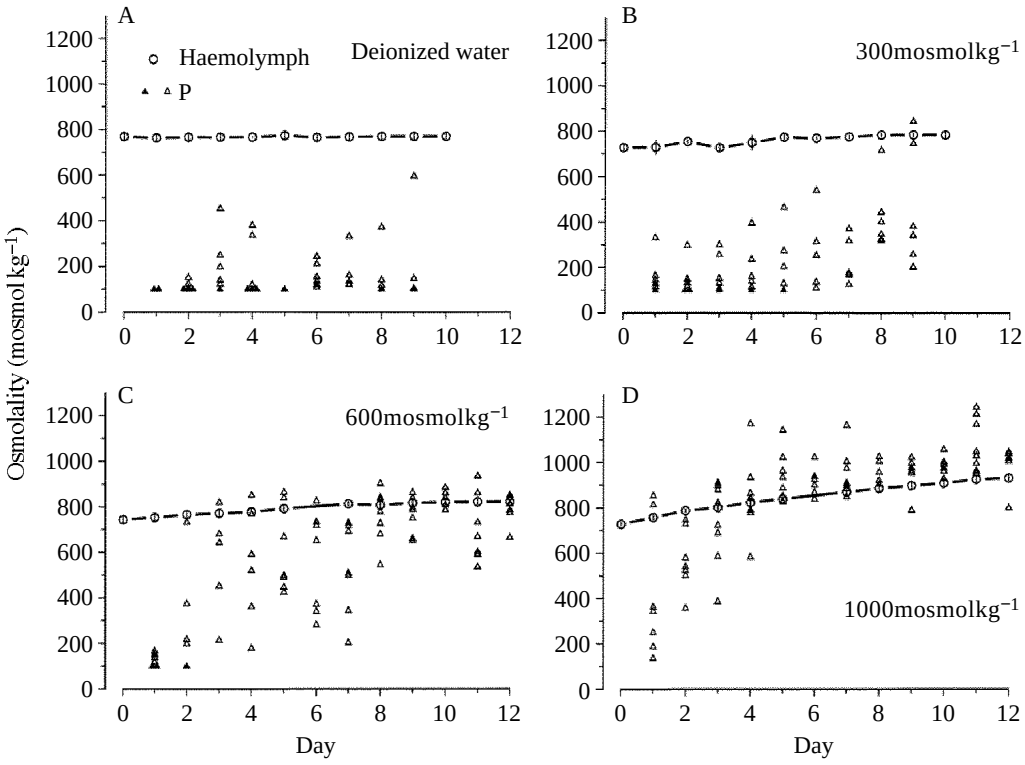

Fig. 2. Osmolality of the haemolymph and the P in *Birgus latro* supplied on day zero with deionized water (A) or 300mosmolkg^{-1} (B), 600mosmolkg^{-1} (C) or 1000mosmolkg^{-1} sea water (D) for drinking (series I crabs). Haemolymph values are means ± S.E.M.; P values are individual daily samples; osmolalities below 100mosmolkg^{-1} are plotted at 100mosmolkg^{-1} (filled triangles); some points are displaced slightly for clarity.

both significantly greater than overall DIW rates (P<0.01 and P<0.05 respectively), but the increase was not sustained and drinking quickly fell to DIW rates [statistically similar to DIW; day 1 was significantly greater than on each subsequent day (P<0.001)].

P flow

On the DIW regimen, the volume of P produced was small (Fig. 1A) and its release was erratic (intervals varied from less than 1 day to more than 6 days; Fig. 2A shows actual numbers of crabs that produced P each day). Mean production rate (5.80±0.97 g kg^{-1} day^{-1}) was about one-third of the rate of drinking of DIW (Fig. 1A).

Crabs supplied with saline produced P daily and immediately increased the volume released more than threefold (Figs 1B–D, 2B–D). Overall mean outputs by the 300, 600 and 1000mosmolkg^{-1} groups were 17.94±1.22, 22.15±1.77 and 14.71±1.14 g kg^{-1} day^{-1} respectively. Each was significantly higher than the DIW rate (P<0.001 in each case). Production of P was maximal in the 600mosmolkg^{-1} group (significantly greater than in each of the other groups; P<0.001).

Mass changes

At an individual level, there were large daily mass changes reflecting episodic drinking and P production. The DIW and the 300mosmolkg^{-1} groups steadily increased in mean mass over 9 days by 18.05 and 30.20 g kg^{-1} respectively (mean masses on days 5–9 were each significantly greater than on day zero, $P<0.001$ and $P<0.01$ respectively). The mean mass of the 600 and 1000mosmolkg^{-1} groups peaked on day 2 and declined thereafter (initial and final masses were not significantly different; mass on days 2–4 was significantly greater than on day 0 and day 12 in both groups, $P<0.05$).

The daily mass deficits (drinking minus mass gain minus P), which approximate evaporative water losses, were calculated from the data in Fig. 1. These deficits were statistically similar in the four groups, reflecting similar conditions in the chambers. With increasing salinity of the drinking water, the overall mean deficits were, respectively, 8.18±2.37, 8.86±1.65, 9.33±3.09 and 8.45±2.80 g kg^{-1} day^{-1}. Comparison with the rates of P production given above suggests that evaporation was the main mechanism of water loss in the DIW crabs whereas release of P was the main route in the saline groups.

Osmolality and [Cl] of haemolymph and P

The mean osmolality and [Cl] of daily haemolymph samples from all series I crabs supplied with DIW were 766.7±5.0mosmolkg^{-1} and 361.2±2.5mmol l^{-1} respectively (Figs 2A, 3A). Although there were significant differences among individual crabs (ranges of individual means: 678–812mosmolkg^{-1}; 333–382mmol l^{-1} [Cl]; $P<0.001$, ANOVA), there were no significant trends with time in this group. All P samples in the DIW group were hypo-osmotic to the haemolymph, the majority (26 of 40) being less than 100mosmolkg^{-1}, but ranging up to 594mosmolkg^{-1}. Mean [Cl] of the 40 P samples was 32.0±1.8mmol l^{-1}. Remarkably, the osmolality of the P was not significantly correlated with [Cl] in the DIW group ($r^2=0.02$). Sixteen of these samples spanning the range of osmolalities were analysed for ammonia/ammonium ([NH_4]). These concentrations ranged from 6 to 194mmol l^{-1}. Together, [NH_4] and [Cl] satisfactorily predicted the osmolality (OP) of these samples (multiple regression, $OP=2.03[NH_4]+3.3[Cl]-2.0$, $r^2=0.92$). Because *Birgus* are not considered normally to release ammonium in the P (Greenaway and Morris, 1989), it is possible that breakdown of urate in faecal contamination had occurred in some samples.

The osmolality and [Cl] of the haemolymph did not change in the crabs supplied with DIW (Figs 2A, 3A) but slowly increased in those supplied with salines (Figs 2B–D, 3B–D; statistically significant, $P<0.01$ to $P<0.001$, ANOVA, comparing first day and last 3 days). The average rates of rise in haemolymph osmolality over 9 days (linear regression) were (DIW first) 0.34±1.58, 6.47±1.54, 7.76±1.15 and 17.31±1.01mosmolkg^{-1} day^{-1} respectively. Corresponding rates of increase in haemolymph [Cl] were 0.21±0.79, 3.49±0.75, 4.48±0.70 and 9.13±0.61mmol l^{-1} day^{-1}.

The osmolality and [Cl] of the P rose when saline drinking water was provided (Figs 2B–D, 3B–D). Individual responses were highly variable. Some crabs responded immediately and produced P nearly isosmotic with the drinking water during the first day whereas, in others, there was a delay of several days before any increase in concentration

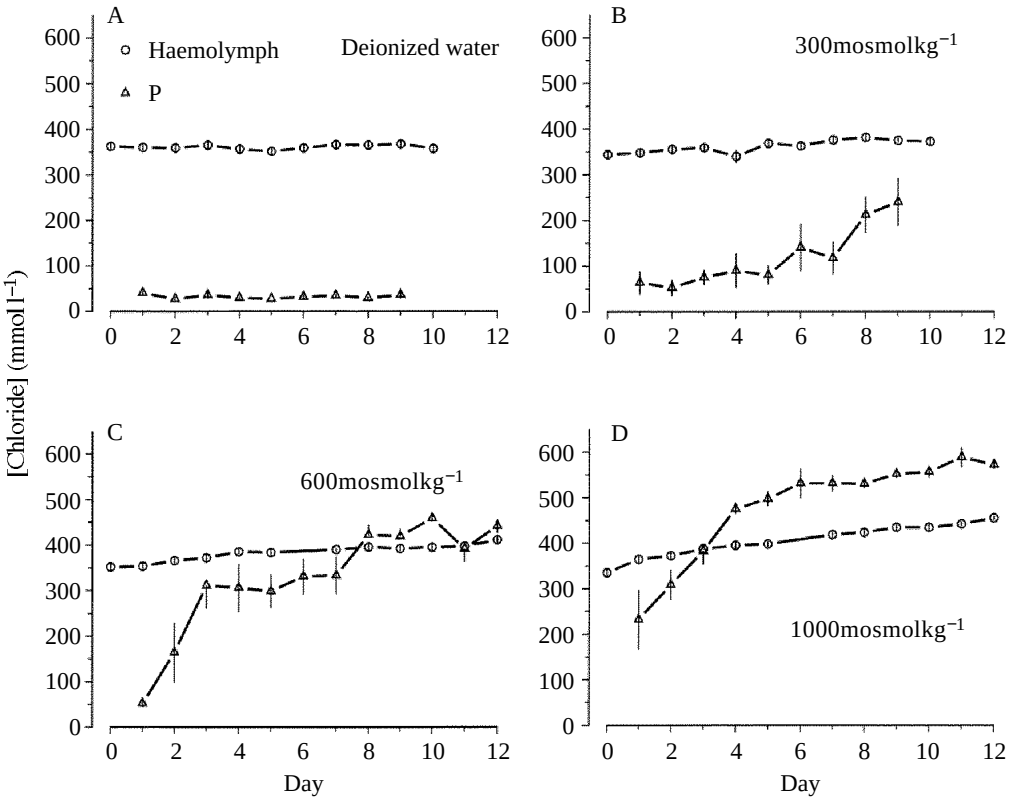

Fig. 3. Chloride concentration of the haemolymph and the P in *Birgus latro* supplied on day zero with deionized water (A) or 300mosmolkg^{-1} (B), 600mosmolkg^{-1} (C) or 1000mosmol kg^{-1} sea water (D) for drinking. Means ± S.E.M.

occurred. P concentrations rose more rapidly, and with shorter delay, when the higher-salinity drinking waters were supplied. Within a week, P concentrations reached a plateau at the highest salinities and, thereafter, rose more slowly. Mean osmolality and [Cl] of the P eventually exceeded that of the drinking water in all four groups of crabs and, in the 600mosmolkg^{-1} group and 1000mosmolkg^{-1} group, exceeded those of the haemolymph. In the latter case, the mean difference between P and haemolymph for days 5–12 was 100.5±18.3mosmolkg^{-1}, 122.2±7.2mmol l^{-1} [Cl] (P<0.001, paired t-tests on all haemolymph and P samples from days 5–12).

Regulation and handling of individual ions

A more detailed ionic analysis (osmolality, [Na], [K], [Ca], [Mg] and [Cl]) was conducted for certain samples from series I crabs (Table 1). These samples were the drinking water, initial (day 0) and final (9 days or 12 days) haemolymph, final urine samples, and the first and last P samples (not necessarily obtained on the first and last days, as not all crabs produced P daily). Statistical data supporting the trends described below are presented in Table 1.

Osmolality, sodium and chloride

As described above, the haemolymph osmolality and [Cl] of the crabs supplied with salines were elevated at the end of the experimental period, compared with their initial values. The differences were greater at the higher salinities. The same trends were also observed in the urine. The urine was isosmotic with the haemolymph sampled at the same time but, in all four groups, urine [Cl] was about 60mmol l^{-1} higher than the [Cl] of the haemolymph (highly significant). The behaviour of the other major ion, sodium, mirrored that of chloride and osmolality. Haemolymph [Na] did not change in the group given DIW (overall mean of initial and final [Na]=389mmol l^{-1}). However, urine [Na] was elevated above haemolymph [Na] by a mean of 36mmol l^{-1} in the DIW group. The difference decreased as drinking water salinity increased and, in the 1000mosmol kg^{-1} group, urine and haemolymph [Na] were not significantly different. The osmolality, [Na] and [Cl] of the P increased rapidly in the crabs that drank salines and differences were apparent, even on day 1; the values finally exceeded those of the drinking water in all cases and of the haemolymph in the 1000mosmol kg^{-1} group.

Potassium

Potassium was well regulated in the haemolymph (overall mean 9.2mmol l^{-1} in DIW crabs), and showed a small, but statistically significant, rise of 1.4mmol l^{-1}, only in the 1000mosmol kg^{-1} group. Urine [K] was approximately twice the haemolymph concentration but was not further elevated in the saline groups. Indeed, urine [K] of the 600mosmol kg^{-1} crabs was significantly lower than that of the DIW group. The [K] of the P was higher than that of the haemolymph and the drinking water in all groups. While [K] of the P was lower than that of the urine in the freshwater and 300mosmol kg^{-1} groups, it rose to exceed that of the urine in the 600 and 1000mosmol kg^{-1} groups ($P<0.05$, paired *t*-tests).

Calcium

A small rise in mean haemolymph [Ca] occurred in crabs on all of the regimens, including DIW. Overall mean [Ca] in the latter group was 16.9±0.6mmol l^{-1}. Haemolymph [Ca] was unaffected by increased salinity of the drinking water. The [Ca] of the urine was consistently 5–6mmol l^{-1} lower than that of the haemolymph (highly significant) and also was unchanged by consumption of salines. The P, in contrast, responded strongly to calcium loading in the drinking water, increasing its mean [Ca] from 2–4mmol l^{-1} initially (lower than in both haemolymph and urine; $P<0.001$) to 14.5mmol l^{-1} in the 1000mosmol kg^{-1} group (not significantly different from values in haemolymph and urine).

Magnesium

Initial and final [Mg] of the haemolymph in the DIW group averaged 19.8±0.7mmol l^{-1}. No significant change was observed in haemolymph [Mg] of crabs drinking DIW and 300mosmol kg^{-1} saline but it increased markedly in the 600 and 1000mosmol kg^{-1} groups. Haemolymph [Mg] increased by 51% after 12 days on the

Table 1. *Changes in osmolality and ionic composition of the haemolymph, urine and final excretory fluid of* Birgus latro *after transfer from fresh to saline drinking water*

Drink		Osmolality (mosmolkg^{-1}) and ion concentrations (mmoll^{-1}) Haemolymph			Urine	Final excretory fluid (P)	
Osmolality	[Ion]	0 days	9–12 days	47 days	9–12 days	1 day	9–12 days
Osmolality							
0	–	748±18	768±17^{NS,a}	–	787±9^{NS,a}	110±6^{a}	119±13***^{,a}
300	–	727±15	783±17**^{,a,b}		790±13^{NS,b}	143±33^{a}	444±99*^{,b}
600	–	743±16	821±9**^{,b,c}	834±17^{NS}	815±19^{NS,b}	232±98	786±25^{NS,c}
1000	–	728±9	930±11***^{,d}	1093±29**	945±13^{NS,b}	434±97^{b}	1065±50*^{,d}
			$\Delta[OP]_H/\Delta[OP]_D$		$\Delta[OP]_U/\Delta[OP]_D$		$\Delta[OP]_P/\Delta[OP]_D$
			=0.17±0.02***		=0.16±0.02***		=0.94±0.08***
			r^2=0.71		r^2=0.67		r^2=0.84
Sodium							
0	0	384±7	393±4^{NS,a}	–	429±4***^{,a}	26±4^{a}	38±8***^{,a}
300	141	368±7	395±7**^{,a}		426±6***^{,a}	49±17^{a}	213±49**^{,b}
600	282	360±8	407±3***^{,a}	415±7^{NS}	426±11**^{,a}	83±47^{a}	398±7^{NS,c}
1000	470	351±9	462±7***^{,b}	523±16*	476±13^{NS,b}	194±46^{b}	510±26^{NS,d}
			$\Delta[Na]_H/\Delta[Na]_D$		$\Delta[Na]_U/\Delta[Na]_D$		$\Delta[Na]_P/\Delta[Na]_D$
			=0.15±0.02***		=0.10±0.03**		=1.01±0.08***
			r^2=0.67		r^2=0.33		r^2=0.84
Chloride							
0	0	362±9	358±8^{NS,a}	–	424±15**^{,a}	31±5^{a}	42±11***^{,a}
300	161	344±7	372±8**^{,a}		433±8***^{,a,b}	63±25^{a}	225±52*^{,b}
600	322	352±9	411±6***^{,b}	424±8*	460±8***^{,b}	104±52^{a}	443±12*^{,c}
1000	536	335±6	455±4***^{,c}	544±15**	532±8***^{,c}	238±56^{b}	601±19***^{,d}
			$\Delta[Cl]_H/\Delta[Cl]_D$		$\Delta[Cl]_U/\Delta[Cl]_D$		$\Delta[Cl]_P/\Delta[Cl]_D$
			=0.19±0.02***		=0.21±0.03***		=1.06±0.07***
			r^2=0.84		r^2=0.71		r^2=0.89
Potassium							
0	0	9.3±0.3	9.1±0.2^{NS,a}	–	21.5±1.4***^{,a}	12.9±2.6	16.5±2.3*^{,a}
300	3	8.6±0.2	8.3±0.3^{NS,a}		19.7±2.0**	16.9±1.5	12.7±2.3^{NS,a}
600	6	8.9±0.2	9.0±0.4^{NS,a}	9.6±0.4^{NS}	15.1±1.3**^{,b}	21.3±4.3	17.4±1.5**^{,a}
1000	10	9.3±0.2	10.7±0.3**^{,b}	11.4±0.4*	19.4±1.3***	24.3±6.1	26.5±4.1**^{,b}
			$\Delta[K]_H/\Delta[K]_D$		$\Delta[K]_U/\Delta[K]_D$		$\Delta[K]_P/\Delta[K]_D$
			=0.17±0.04***		=−0.26±0.22^{NS}		=1.10±0.38**
			r^2=0.35		r^2=0.05		r^2=0.23
Calcium							
0	0	16.4±0.8	17.3±0.7**	–	11.5±0.7***	2.4±0.5	4.3±1.7***^{,a}
300	3.6	15.5±1.3	17.4±0.5^{NS}		12.4±1.0***	2.7±1.0	5.7±1.3***^{,a}
600	7.2	13.1±0.7	16.6±0.4***	16.9±0.4^{NS}	10.3±0.4***^{,a}	1.6±0.3	8.7±0.5***^{,a}
1000	12	14.8±0.7	17.4±0.5*	19.5±0.6**	12.8±0.6***^{,b}	4.5±1.6	14.5±2.9^{NS,b}
			$\Delta[Ca]_H/\Delta[Ca]_D$		$\Delta[Ca]_U/\Delta[Ca]_D$		$\Delta[Ca]_P/\Delta[Ca]_D$
			=0.02±0.06^{NS}		=0.05±0.09^{NS}		=0.87±0.21***
			r^2=0.01		r^2=0.01		r^2=0.40

Table 1. *Continued*

Drink		Osmolality (mosmolkg^{-1}) and ion concentrations (mmoll^{-1}) Haemolymph			Urine	Final excretory fluid (P)	
Osmolality	[Ion]	0 days	9–12 days	47 days	9–12 days	1 day	9–12 days
Magnesium							
0	0	20.7±1.0	19.3±0.9^{NS,a}	–	19.6±1.4^{NS,a}	9.3±1.4^{a}	8.9±1.3***^{,a}
300	16.5	19.2±1.1	20.6±0.6^{NS,a}		20.6±0.6^{NS,a}	11.0±2.3^{a}	17.3±1.3^{NS,b}
600	33	20.4±0.6	27.4±0.9***^{,b}	34.4±1.4**	26.5±1.0^{NS,b}	11.9±2.1^{a}	27.3±1.0^{NS,c}
1000	55	22.2±0.5	33.6±0.9***^{,c}	47.0±2.1***	33.5±1.5^{NS,c}	23.0±4.9^{b}	43.0±3.2*^{,d}
			$\Delta[Mg]_H/\Delta[Mg]_D$ =0.28±0.02*** r^2=0.85		$\Delta[Mg]_U/\Delta[Mg]_D$ =0.27±0.03*** r^2=0.76		$\Delta[Mg]_P/\Delta[Mg]_D$ =0.62±0.05*** r^2=0.86

Values are means ± standard error of mean; N=7 or 8, except 47 days where N=5.

Where no P was produced on the required day, the first P sample after day 1 and the last sample before day 9 or 12 was used.

Haemolymph osmolalities and ion concentrations after 9–12 days on each saline regimen are contrasted (paired *t*-test, two-tailed) with the initial values (0 days) from the same crabs. 47 day haemolymph values are contrasted with 9–12 day values. Urine and P concentrations are contrasted with haemolymph values for the same crabs measured at the same time. NS, not significantly different; asterisks denote a significant difference (*P<0.05, **P<0.01, ***P<0.001).

Statistically significant differences in the composition of corresponding fluid samples, among groups of crabs supplied with different salines, are indicated by different alphabetical superscripts (ANOVA, Fisher's LSD test, P<0.05). Absence of superscripts indicates no significant differences from the other groups at that time.

$\Delta[Na]_H/\Delta[Na]_D$, etc., are slopes of regressions of haemolymph (H), urine (U) or final excretory fluid (P) ion concentrations (or osmolality) on the concentration of the same component of the drinking water (D) after 9–12 days. Standard errors and probabilities that slopes do not differ from zero (symbols as above) are given.

latter regimen. Urine [Mg] levels were closely similar to those measured in the haemolymph at the same time in all crabs. [Mg] in the P of the DIW group was only half that of the urine. Drinking saline caused a marked increase in the [Mg] of the P; the concentration exceeded haemolymph and urine levels in the 1000mosmolkg^{-1} group.

Relative changes in haemolymph, urine and P in response to ion loading in the water

Indices, termed here 'sensitivities' were calculated from series I data after 9–12 days (Table 1). The sensitivity is the ratio of the change in the concentration of an ion in the haemolymph, the urine or the P to the change in concentration of the same ion in the drinking water, and was obtained by linear regression (the slopes $\Delta[ion]_H/\Delta[ion]_D$, $\Delta[ion]_U/\Delta[ion]_D$ and $\Delta[ion]_P/\Delta[ion]_D$). The index is useful in assessing the degree of ionic regulation of the haemolymph and the relative importance of the urine and the P in the regulatory response. Values of 0.15–0.19 for this index for osmolality, [Na], [K] and [Cl] in the haemolymph indicate that only 15–19% of the increases in the drinking waters were reflected in increases in these constituents of the haemolymph at this time. For [Ca], the slope was zero (no correlation with drinking water [Ca]), indicating good haemolymph regulation of this ion. Haemolymph [Mg] was the most sensitive to changes

Table 2. *Urine:haemolymph (U:H) and P:haemolymph (P:H) ratios for ^{51}Cr-EDTA, ions and osmolality in* Birgus latro *on several different saline drinking regimens (quasi-steady state)*

	U:H							P:H		
	Na	K	Ca	Mg	Cl	OP	EDTA	Cl	OP	EDTA
Fresh water										
Mean	1.04	2.32*	0.52*,^{a}	0.93	1.10*	0.98	1.31	0.07*,^{c}	–	1.69
S.E.M.	0.05	0.32	0.06	0.10	0.03	0.01	0.09	0.01		0.19
N	12	12	12	12	12	12	11	4		9
300mosmolkg^{-1}										
Mean	1.04*	1.77*	0.60*,^{a}	0.96	1.14*	0.97*	1.59	0.47*,^{d}	0.46*,^{f}	1.41
S.E.M.	0.01	0.15	0.02	0.02	0.03	0.01	0.17	0.06	0.06	0.27
N	6	6	6	6	6	6	6	6	6	6
600mosmolkg^{-1}										
Mean	1.04*	1.65*	0.78^{b}	0.94	1.13*	0.98	1.20	0.89^{e}	0.84*,^{g}	1.17
S.E.M.	0.01	0.06	0.07	0.03	0.02	0.01	0.06	0.10	0.07	0.15
N	6	6	6	6	6	6	6	5	5	6
1000mosmolkg^{-1}										
Mean	1.06*	1.73*	0.83*,^{b}	1.09*	1.14*	1.00	1.36	1.13*,^{e}	1.07^{g}	1.37
S.E.M.	0.01	0.13	0.06	0.03	0.02	0.01	0.10	0.04	0.04	0.29
N	5	5	5	5	5	5	6	4	4	4

Different alphabetical superscripts within a column indicate that the means differ significantly (ANOVA, Fisher's LSD test, $P<0.05$). Absence of superscripts in a column indicates that none of the means within that column is significantly different from any other.

* indicates a significant difference between the concentration of a substance in the haemolymph and that in P or urine (paired *t*-test, $P<0.05$).

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

in drinking water concentration (0.28). Sensitivities of the urine to drinking water ion concentrations were of similar magnitude to the corresponding haemolymph values, except that [Ca], and also [K], showed no significant correlation with their concentration in the drinking water. Changes in the P were much greater. Sensitivities of approximately 1.0 for most ions show that the composition of the P directly reflects changes in the drinking water on a near mole-for-mole basis, despite much smaller changes in the haemolymph. Only the value for magnesium (0.62) was significantly different from 1.0 ($P<0.001$, *t*-test).

Ionic regulation in series II crabs

Ionic analyses of fluids from crabs established on the same four regimens for 14 days are summarized as urine:haemolymph (U:H) and P:haemolymph (P:H) ratios (Table 2). An approximate steady state was established in this time (Figs 1–3). These data were entirely consistent with the data from series I (Table 1) and thus provide independent support for the interpretations given above. Summarizing: (1) urine was isosmotic with

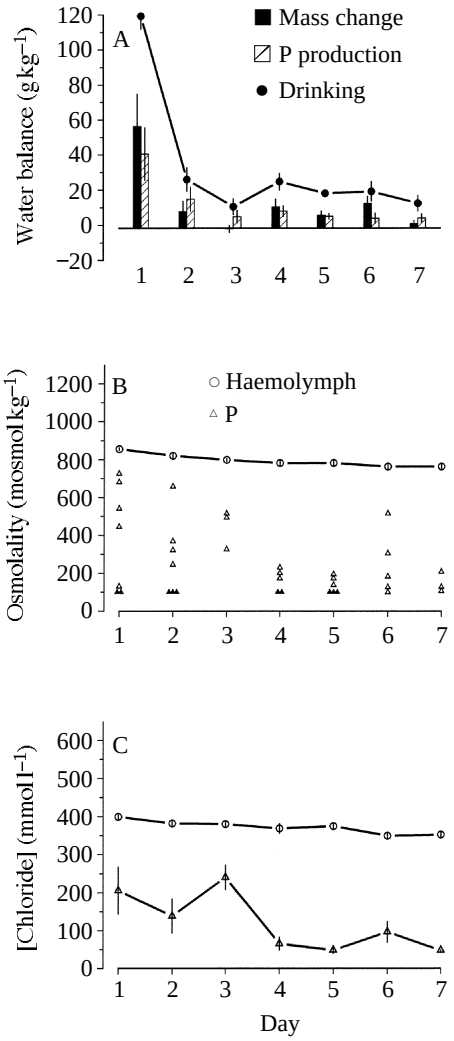

Fig. 4. Daily water (or mass) balance (A) and changes in the osmolality (B) and chloride concentration (C) of the haemolymph and of the P in *Birgus latro* returned to deionized drinking water after 2 weeks on the 1000mosmolkg^{-1} seawater regimen. Details as Figs 1–3.

haemolymph on all regimens; (2) sodium and chloride were slightly, and potassium strongly, concentrated in the urine; (3) calcium was markedly depleted in the urine; (4) urine magnesium concentrations were similar to haemolymph values; (5) crabs drinking DIW produced very dilute P; (6) the osmolality and [Cl] of the P increased with drinking water salinity and, in crabs drinking 1000mosmolkg^{-1} sea water, [Cl] of the P exceeded that of the haemolymph (P<0.001).

Return to drinking DIW after sea water (series III)

Seven crabs from the 1000mosmolkg^{-1} groups were returned to the DIW regimen and their drinking rates, mass, haemolymph and P were monitored as before (Fig. 4). On the

first day that DIW was given, all crabs drank avidly (mean 119.2±7.6 g kg^{-1}, range 96–154 g kg^{-1}). Part of this water was excreted in a very high initial P production, but it also contributed to a large weight gain. This gain was augmented slightly during the following week (Fig. 4A). Drinking and P production rates were restored to the normal range of DIW values by day 2 (compare Figs 1A and 4A). After a week, haemolymph and P concentrations and water balance were also similar to those of the DIW group (compare Figs 2A, 3A with Fig. 4B,C). Three crabs returned to production of dilute P (less than 100mosmol kg^{-1} and less than 40mmol l^{-1} Cl) on the first day whereas, in the others, P concentration declined progressively. The initially high haemolymph concentrations (856.0±9.2mosmol kg^{-1}, 399.5mmol l^{-1} Cl) quickly fell to DIW levels (763.7±12.5mosmol kg^{-1}, 352.4±9.6mmol l^{-1}; differences from initial values were highly significant, $P<0.001$, paired *t*-test).

Long-term changes in the haemolymph (series IV)

After 47 days on the 1000mosmol kg^{-1} regimen, osmolality and ion concentrations of the haemolymph were all significantly elevated compared with their values after 12 days (Table 1), although rising more slowly (osmolality 4.7mosmol kg^{-1} day^{-1}, [Cl] 2.5mmol l^{-1} day^{-1}, during days 12–47). Haemolymph [K] was least affected by drinking sea water, rising 15% after 12 days and a total of 23% after 47 days. Magnesium was poorly regulated, the corresponding rises being 51% and 112%. Prolonged exposure to the 600mosmol kg^{-1} regimen barely raised haemolymph osmolality and ion concentrations above 12-day values. Magnesium was the only ion to be conspicuously further elevated (34% and 69% after 12 and 47 days). These regimens were finally terminated because some individuals appeared to be experiencing severe water retention. Their abdomens were stretched taut, indicating great internal pressure, and motor coordination problems were evident.

Discussion

Urine formation

The regulatory role of the antennal organs in *Birgus* is clearly minor and their main function appears to be the generation of a flow of primary urine. The near doubling in clearance and urine flow noted in the 600mosmol kg^{-1} animals (Greenaway *et al.* 1990) may assist ion and water balance as discussed below. However, the urine was isosmotic to the haemolymph at all drinking water concentrations (Tables 1, 2). Potassium and, to a lesser extent, sodium and chloride were concentrated in the urine but their urinary concentrations on the different regimens do not imply an active role in haemolymph regulation. Secretion of potassium into the urine would assist the excretion of this ion, which is abundant in the normally vegetarian diet. However, the decrease in U:H ratio for potassium in the crabs that drank salines up to 600mosmol kg^{-1} is in the wrong direction to be regulatory and perhaps may simply be a consequence of the reported increase in urine flow (Greenaway *et al.* 1990) and unchanging K reabsorption. Urine calcium concentrations about one-third lower than haemolymph values would help calcium

balance in crabs supplied with low-salinity drinking water but not high-salinity water. However, a proportion of the haemolymph calcium of crustaceans is often bound to organic molecules (Greenaway, 1985), and renal modification of the filtrate cannot be inferred without knowledge of ionized calcium levels.

Magnesium was handled passively by the antennal organs and the U:H ratio for this ion was always close to 1.0. This situation contrasts with that seen in most marine, brackish-water and terrestrial crabs (including *Coenobita perlatus*), which typically maintain haemolymph magnesium levels below those of sea water and actively secrete magnesium into the urine (Robertson, 1949, 1953; Lockwood and Riegel, 1969; Mantel and Farmer, 1983; Greenaway, 1988).

Final excretory product, P

In contrast to urine formation, the processes leading to the formation of P make rapid adjustments to rate and composition as required in an osmo-controller. Within 1 day, P [Cl] could be increased, or decreased, more than 20-fold between less than 25 and more than $500 mmol l^{-1}$, and the rate of release of P could be varied between zero and more than $150 ml kg^{-1} day^{-1}$. The concentrations in the P of Na, K, Ca, Mg and Cl could be reduced below their concentration in the urine (DIW group) or elevated above urinary levels ($1000 mosmol kg^{-1}$ group) (Table 1). Furthermore, this extrarenal system appears to be able to respond selectively to individual ionic requirements. Thus, the proportions of individual ions in the P were closely similar in all three groups of crabs drinking seawater dilutions (despite large absolute differences), but very different from those in the crabs drinking fresh water. From Table 1, the molar ratios Na:Cl:K:Ca:Mg in the final P were, for the $1000 mosmol kg^{-1}$ crabs, 1.00:1.18:0.05:0.03:0.08, and, in the DIW group, the ratios were 1.00:1.11:0.35:0.09:0.19. Potassium, calcium and magnesium were each enhanced, by different factors, over sodium and chloride in the latter group.

Sensitivities of haemolymph, urine and P to ion loading

The greater importance of branchial than renal processes in the regulation of haemolymph ions is illustrated by the sensitivities of the ion concentrations in the haemolymph, urine or P to changes in their concentrations in drinking water (Table 1). After 9–12 days of exposure to the four regimens, haemolymph osmolality, [Na] and [Cl] each changed by 0.15–0.19 of the corresponding change in the drinking water. The urinary sensitivities were similar, implying no regulatory role. In contrast, the sensitivities of the P to changes in these ions were each close to 1.0, i.e. 5–7 times greater, which is consistent with the involvement of P formation in negative feedback regulation of the haemolymph ion concentrations. Regulatory responses in the P of similar magnitude are also evident for K, Ca and Mg, despite sometimes inappropriate changes in urinary [K] and [Ca]. Magnesium was the worst-regulated ion in the haemolymph, and this was associated with the smallest response in the P. Calcium was regulated best (haemolymph and urinary sensitivities both zero).

Dilution and concentration of the final excretory fluid

Animals on the freshwater regimen strongly absorbed ions from urine released into the

branchial chambers so that the final P was very dilute. With increasing concentration of the drinking water, ion concentrations in the P rose and, in crabs drinking full-strength sea water, the concentrations of all ions except calcium exceeded those in the haemolymph. The similarity of the U:H and the P:H ratios for ^{51}Cr-EDTA, a volume marker, in each of the experimental groups in series II (Table 2) suggests that little water reabsorption accompanied ion reabsorption, despite strong osmotic gradients between the P and the haemolymph at the lower salinities. It appears that the gills and other surfaces within the branchial chambers that contact the excretory fluids are quite impermeable to water.

Series I crabs, drinking full-strength sea water, produced P that was significantly hyperosmotic to the haemolymph and urine (Table 1). No volume marker was used in this group so that evaporative concentration during passage through the branchial chambers or water uptake across the gills cannot be excluded. Branchial transport of ions into the P might also take place. The ability to transport salts into the branchial chamber is associated with hypo-osmotic regulation in brachyuran terrestrial and semi-terrestrial crabs (Gross, 1955). *Coenobita clypeatus* is able to maintain its haemolymph slightly hypo-osmotic to shell water (de Wilde, 1973).

It is not known how the degree of dilution of the P is controlled. Potentially, this might be effected by modulating transporters in the branchial epithelium, by changing gill perfusion rates or routes, or by mechanically adjusting transit time and contact of urine with the exchange surfaces.

Water and ion budgets

The ion data for series II crabs (Table 2) were combined with mean clearances, urine and P production rates, drinking rates, dietary intake of ions and loss by haemolymph sampling for the same crabs (Greenaway *et al.* 1990) and a budget for each ion and water was constructed (Table 3). In all four groups, the quantities of water and ions voided in the P were a very small fraction of those in the primary urine. Some reclamation of water took place in the antennal organs (compare primary and final urine volumes in Table 3) and an unknown, probably small, fraction was lost from the P by evaporation and osmosis to the haemolymph. The rest is assumed to have been reingested and evidence for this process is presented elsewhere (Greenaway *et al.* 1990; Morris *et al.* 1991). Such bulk reingestion of urine (or of partially processed P) would permit the non-selective return of a major fraction of the filtered ions and water to the animal. Branchial absorption and secretion then result in differential excretion of ions in the final P. The combination of non-selective reabsorption of an ultrafiltrate followed by selective secretion/reabsorption mechanisms allows very flexible adjustment of the volume and composition of the urine and is a feature of the excretory systems of successful terrestrial animals (c.f. the proximal and distal sections of the vertebrate nephron). During the evolution of the terrestrial lifestyle in *Birgus* these features have been added by adaptation of extrarenal rather than renal features.

Crabs maintained on the freshwater regimen did not take up ions by drinking. The volume and ion concentration of the P were very low in this group and effectively all of the ions filtered into the primary urine were reabsorbed (more than 99% in most cases, Table 3). Net loss of ions from the excretory system (and the daily haemolymph sample)

Table 3. *Ion and water balance in* Birgus latro

Drinking regimen	Na	K	Ca	Mg	Cl	Water
Fresh water						
Gain from drinking water	0	0	0	0	0	1.821
Gain from food	150	32	58	13	25	
Loss in blood sample	35	1	1	2	34	
Filtered in primary urine	2048	54	86	104	1949	5.766
Output in final urine	1663	104	38	83	1670	4.481
Loss in P	1	2	0.1	0.2	1	0.45
Net gain (+) or loss (−)	+114	+29	+56.9	+10.8	−11	
Total recovered (%)	99.9	96.3	99.9	99.8	99.95	92.2
300mosmolkg^{-1} seawater						
Gain from drinking water	452	9.6	9.9	51.5	528	3.300
Gain from food	108	23	41	10	18	
Loss in blood sample	39	1	1.5	2.2	39	
Filtered in primary urine	2859	71	109	162	2815	7.268
Output in final urine	2026	85	44	106	2181	4.986
Loss in P	319	29	9.9	32	371	2.026
Net gain (+) or loss (−)	+202	+2.6	+39.5	+27.4	+136	
Total recovered (%)	88.8	59.2	90.9	80.2	86.8	72.1
600mosmolkg^{-1} seawater						
Gain from drinking water	991	20	21.6	112	1154	3.605
Gain from food	141	30	54	12	24	
Loss in blood sample	41	1	1.4	3	40	
Filtered in primary urine	3680	88	130	274	3674	9.087
Output in final urine	3240	122	80	225	3559	7.791
Loss in P	783	38	16	57	864	2.285
Net gain (+) or loss (−)	+308	+12	+58	+65	+274	
Total recovered (%)	78.7	56.8	87.7	79.2	76.5	74.9
1000mosmolkg^{-1} seawater						
Gain from drinking water	1177	24	25.7	134	1372	2.858
Gain from food	136	29	52	12	23	
Loss in blood sample	49	1	1.7	3.6	49	
Filtered in primary urine	3499	75	121	256	3530	7.187
Output in final urine	2873	101	77	214	3102	5.570
Loss in P	966	47	25	82	1131	2.032
Net gain (+) or loss (−)	+298	+6	+51	+60.4	+215	
Total recovered (%)	72.4	37.3	79.3	68.0	68.0	71.7

A summary of ion and water intakes (μmol100 g^{-1} day^{-1} and ml100 g^{-1} day^{-1} respectively), from food and drinking water, and outputs in the primary urine, in the final urine and in the excretory fluid actually released (P), for quasi-steady-state crabs. Also shown are the percentages of the total ions or water filtered in the primary urine, that were recovered by the crab (renally and extrarenally) before voiding the P. Output in the faeces and evaporative water loss are not estimated.

Values are means calculated from the ion data summarized in Table 2 and from water fluxes previously estimated for these crabs by Greenaway *et al.* (1990).

was more than balanced by ion intake in the food, except in the case of chloride. Therefore, when drinking fresh water, ion loss *via* the excretory system was minimised by maintaining a low water intake (and output) and by extensive reabsorption of ions before release of P. In the freshwater group, the filtration rate was also lower, minimising the filtered ion load and reducing the expenditure of energy in ion reabsorption. The apparent small net gain of ions may, in fact, be lost in faeces, accommodated by an increase in body volume or, in the longer term, excreted as the result of a rise in P concentrations.

The fraction of the filtered ion load reclaimed by ingestion and reabsorption was reduced with increasing concentration of drinking water, to about 70% for Na and Cl, in crabs drinking 1000mosmolkg^{-1} SW (Table 3). Potassium was reabsorbed least in these crabs (37% of the filtered load) and, in fact, was concentrated in the P. The greater net gain of ions in the crabs drinking salines (Table 3) presumably accounts for the rise in ion concentration of the haemolymph observed in these experiments (Fig. 2, Table 1).

Efficient mechanisms for conservation of salt in *Birgus* allow water and ion balance to be achieved with only minute input of ions from the diet and sufficient DIW to replace evaporative losses. The antennal organs and gills are not used for nitrogenous excretion (Greenaway and Morris, 1989) so that the volume of excretory product, P, can be very small. In the long term, the crabs are not able to maintain salt balance by drinking salines which approach or exceed haemolymph concentrations. However, the crabs certainly readily drink such salines and, in the short term, this is unlikely to be a problem. The changes in haemolymph ion concentrations are slow and, when fresh water is again available, any excess salt load is readily shed (Fig. 4) or diluted to augment the haemolymph volume in the distensible abdomen. *Birgus* appear healthy with a wide range of haemolymph osmolalities (present study and Gross, 1955) and, in an ion-poor environment, occasional bonuses of salts (from saline drinking water or from eating other crabs) may be put towards the haemolymph volume increase required at the next moult.

Compensation for evaporation

Survival of *Birgus* maintained on saline alone depends critically on the rate of evaporative water loss, the rate of drinking and on the production of an excretory fluid more concentrated than the drinking water for each ion. The minimal rate of drinking required for gain of osmotically free water to match evaporation may be calculated. For ion balance:

$$V_P = V_D/K, \quad (1)$$

where V_P is the rate of P production, V_D is the minimal rate of drinking and K is the highest concentration ratio achieved for any ion in the P compared with the drinking water. For water balance:

$$V_D = V_P + E, \quad (2)$$

where E is the rate of evaporative water loss. Substituting equation 1 in equation 2, and rearranging:

$$V_D = EK/(K - 1). \quad (3)$$

Clearly, as the supplied salinity is increased, the water turnover must increase sharply.

Thus, if the P has twice the concentration of the drinking water, the crab must release P at the evaporation rate and drink at twice this rate; if the P is only 10% higher in concentration than the drinking water, minimal drinking and P production rates would be 11 and 10 times the evaporative loss rate, respectively. This, presumably, is the significance of the increased rates of drinking, clearance, urine production and P production observed in the 300 and 600mosmolkg^{-1} groups (Fig. 1, Table 3, Greenaway *et al.* 1990). Although *Birgus* was able to increase the P concentrations of some ions above haemolymph levels and even produced P significantly hyperosmotic to the haemolymph in the series I crabs, such concentrating ability was not marked. Thus, with haemolymph, urine and P osmolalities all around 800mosmolkg^{-1} (Table 1), drinking of 600mosmolkg^{-1} saline permitted long-term maintenance of salt and water balance, but drinking of 1000mosmolkg^{-1} saline did not and haemolymph ion concentrations continued to rise.

As the salinity of the drinking water is increased, the value of K in the above equations decreases towards 1.0 and either ion regulation or water balance must be compromised. As seen in Fig. 1, crabs supplied with 1000mosmolkg^{-1} sea water initially increased their drinking still further before abandoning this escalation. As haemolymph ion concentrations drifted upwards, the excreted concentrations also increased and, in principle, the crabs could ultimately have re-established balance conditions. It is unclear whether this state was achieved in the crabs provided with full-strength sea water for 47 days. Certainly, the rate of rise of haemolymph concentration was slowed. Gross (1955) reported the survival of a single *Birgus* on sea water alone for 78 days, after which the haemolymph osmolality was 118% of that of sea water. In our experiments, 47 days on sea water resulted in a mean haemolymph osmolality of 109% of that of sea water. However, several of our crabs were oedematous and uncoordinated. These effects may be associated with a loss of muscle tone related, perhaps, to the breakdown in [K] regulation or to the great rise in [Mg], the least well-regulated of the ions (Table 1). Magnesium has well-known neuromuscular inhibitory effects in crustaceans and is generally regulated well below seawater concentrations in the most active of decapods (Robertson, 1949, 1953; Lockwood and Riegel, 1969).

The extremely high initial rates of drinking exhibited by crabs returned to fresh water after the seawater regimen, and the rapid lowering of haemolymph osmolalities, suggest that the lower haemolymph electrolyte concentrations may be the preferred levels. These observations confirm that *Birgus* has a well-developed capacity to discriminate salt concentrations, as required for the salinity selection mechanism of osmoregulation (Gross, 1955) outlined in the Introduction, and also show that this crab develops an acute salt-induced thirst.

Ion regulation

In conclusion, *Birgus* possesses a repertoire of a devices that potentially regulate its salt and water balance. Besides having the ability to select drinking waters of appropriate salinity, when available, and to regulate drinking rates (Gross, 1955; present study), robber crabs are able to adjust rates of primary and final urine production (Greenaway *et al.* 1990), to secrete or reabsorb ions in the antennal organs (present study), to reingest

urine and to reabsorb ions from urine passed through the branchial chambers (Greenaway *et al.* 1990; Morris *et al.* 1991; this study). It is unlikely that behavioural osmoregulation as envisaged by Gross (1955) and Gross and Holland (1960) is an important mechanism by which robber crabs achieve ion balance under normal circumstances. Efficient ion conservation mechanisms effectively allow the production of a dilute excretory fluid. The crabs may osmoregulate and maintain ion balance on a low-salt diet, if they have regular access to fresh water. This is probably the situation facing most robber crabs and it is unlikely that they ever need actively to seek saline water. In the short term, *Birgus* is also able to balance ions and water quite accurately when only saline is available. In the longer term, where nearly full strength sea water is the principal supply, and there are also evaporative losses, *Birgus* does not appear to prevent the slow accumulation of ions, particularly magnesium, in the haemolymph. It appears that occasional drinking of fresh water would be required to facilitate the elimination of these ions in the P.

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References

DE WILDE, P. A. W. J. (1973). On the ecology of *Coenobita clypeatus* in Curaçao with reference to reproduction, water economy and osmoregulation in terrestrial hermit crabs. In *Studies on the Fauna of Curaçao and other Caribbean Islands*, vol. 44 (ed. P. W. Hummelinck and L. J. Van der Steen), pp 1–138. The Hague: Martinus Nijhoff.

GREENAWAY, P. (1985). Calcium balance and moulting in the Crustacea. *Biol. Rev.* **60**, 425–454.

GREENAWAY, P. (1988). Ion and water balance. In *Biology of the Land Crabs* (ed. W. W. Burggren and B. R. McMahon), pp. 211–248. Cambridge: Cambridge University Press.

GREENAWAY, P. AND MORRIS, S.(1989). Adaptations to a terrestrial existence by the robber crab *Birgus latro*. III. Nitrogenous excretion. *J. exp. Biol.* **143**, 333–346.

GREENAWAY, P. AND NAKAMURA, T. (1991). Nitrogenous excretion in two terrestrial crabs *Gecarcoidea natalis* and *Geograpsus grayi*. *Physiol. Zool.* **64**, 767–786.

GREENAWAY, P., TAYLOR, H. H. AND MORRIS, S. (1990). Adaptations to a terrestrial existence by the robber crab *Birgus latro*. VI. The role of the excretory system in fluid balance. *J. exp. Biol.* **152**, 505–519.

GROSS, W. J.(1955). Aspects of osmotic and ionic regulation in crabs showing the terrestrial habit. *Am. Nat*. **89**, 205–222.

GROSS, W. J. (1964). Water balance in anomuran land crabs on a dry atoll. *Biol. Bull. mar. Biol. Lab., Woods Hole* **126**, 54–68.

GROSS, W. J. AND HOLLAND, P. V.(1960). Water and ionic regulation in a hermit crab. *Physiol. Zool.* **33**, 21–28.

HARMS, J. W. (1932). Die Realisation von Genen und die consekutive Adaptation. II. *Birgus latro* (L.) als Landkrebs und seine Beziehungen zu den Coenobiten. *Z. wiss. Zool.* **140**, 167–190.

HICKS, J., RUMPFF, H. AND YORKSTON, H. (1984). *Christmas Crabs.* Christmas Island, Indian Ocean: Christmas Island Natural History Association.

LISTER, J. J. (1888). On the natural history of Christmas Island in the Indian Ocean. *Proc. Zool. Soc. Lond.* **1888**, 512–531.

LOCKWOOD, A. P. M. AND RIEGEL, J. A. (1969). The excretion of magnesium by *Carcinus maenas*. *J. exp. Biol.* **51**, 575–589.

MANTEL, L. H. AND FARMER, L. L. (1983). Osmotic and ionic regulation. In *The Biology of Crustacea*, vol. 5 (ed. L. H. Mantel), pp. 53–161. New York: Academic Press.

MORRIS, S., TAYLOR, H. H. AND GREENAWAY, P. (1991). Adaptations to a terrestrial existence by the robber crab *Birgus latro*. VII. The branchial chamber and its role in urine reprocessing. *J. exp. Biol.* **161**, 315–331.

REESE, E. S. AND KINZIE, R. A. (1968). The larval development of the coconut or robber crab, *Birgus latro* (L.) in the laboratory (Anomura, Paguridae). *Crustaceana* (Suppl.) **2**, 117–144.

ROBERTSON, J. A. (1949). Ionic regulation in some marine invertebrates. *J. exp. Biol.* **26**, 182–200.

ROBERTSON, J. A. (1953). Further studies on ionic regulation in marine invertebrates. *J. exp. Biol.* **30**, 277–296.

WOLCOTT, T. G. AND WOLCOTT, D. L. (1985). Extrarenal modification of urine for salt conservation in ghost crabs, *Ocypode quadrata* (Fabricius). *J. exp. mar. Biol. Ecol.* **91**, 93–107.

WOLCOTT, T. G. AND WOLCOTT, D. L. (1988). Availability of salts is not a limiting factor for the land crab, *Gecarcinus lateralis* (Freminville). *J. exp. mar. Biol. Ecol.* **120**, 199–219.

WOLCOTT, T. G. AND WOLCOTT, D. L. (1991). Ion conservation by reprocessing of the urine in the land crab *Gecarcinus lateralis* (Freminville). *Physiol. Zool.* **64**, 344–361.