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Regulation of urine reprocessing in the maintenance of sodium and water balance in the terrestrial Christmas Island red crab *Gecarcoidea natalis* investigated under field conditions

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Summary

Land crabs produce isosmotic urine but reduce salt loss by reabsorbing salt *via* the gills to produce a dilute excretory fluid (P). This branchial salt reclamation is regulated in response to changes in dietary salt availability. The regulation of branchial Na reabsorption and osmotic status was investigated in the terrestrial crab *Gecarcoidea natalis* on Christmas Island. Confinement within field enclosures had no general effect on salt and water balance compared with crabs free in the rainforest but there were seasonal effects. Extracellular fluid volume was 27.9% body mass during the wet season but only 22.7% in the dry season. Urine production was 53 ml kg^{-1} day^{-1} in the dry season but 111 ml kg^{-1} day^{-1} in the wet season, while water flux rates were 140 ml kg^{-1} day^{-1} and 280 ml kg^{-1} day^{-1}, respectively. Serotonin but not dopamine increased urine production by at least 16% but only during the dry season when rates were seasonally lowered. Crabs acclimated to drinking 50% seawater increased haemolymph osmotic pressure and downregulated branchial reabsorption of salt. Net Na flux (J_{net}) and unidirectional Na influx (J_{in}) were investigated in branchial perfusion experiments. In red crabs acclimated to drinking freshwater, J_{in}, J_{net} and the activity of the Na^+/K^+-ATPase were increased by serotonin, indicating that the increase of sodium absorption was due to a stimulation of the ATPase. Red crabs drinking 50% seawater reduced J_{net} primarily due to increased passive loss (J_{out}), since both J_{in} and Na^+/K^+-ATPase were unchanged. Serotonin and dopamine abolished the increased diffusive loss and re-established J_{net} with no change in J_{in}. *G. natalis* exhibits different regulatory systems. Branchial salt uptake can be adjusted *via* the leak component when adequate salt is available but also by stimulated active uptake under diluting conditions. The gills are important sites of ion pumping in euryhaline aquatic crabs, and the upregulation of J_{net} in red crabs is reminiscent of that in marine crabs. Serotonergic stimulation of branchial uptake, independent of cAMP, and hormonally modulated ion leakage are presently unique to terrestrial species.

Key words: osmoregulation, water flux, J_{net}, Na flux, branchial uptake, serotonin, red crab, *Gecarcoidea natalis*.

Introduction

The primary urine of crabs is isosmotic with the haemolymph. Since terrestrial crabs must replenish body water loss by drinking mainly freshwater, the urine is a potential and important route for salt loss. Gills are retained in air-breathing crabs as a post-renal ion-regulatory system (reviewed by Morris, 2002). Brachyuran and anomuran species are capable of directing their urine from their nephropores into their branchial chambers where salt reabsorption occurs across the gills to produce a dilute end product, P (e.g. black back crab *Gecarcinus lateralis*, ghost crab *Ocypode quadrata*: Wolcott and Wolcott, 1985, 1991; Wolcott, 1992; robber crab *Birgus latro*: Greenaway and Morris, 1989; Greenaway et al., 1990; Morris et al., 1991; Taylor et al., 1993; little nipper *Geograpsus grayi*: Varley and Greenaway, 1994; red crab *Gecarcoidea natalis*: Morris, 2001; Taylor and Greenaway, 2002; Greenaway, 2003). Salt reclamation during branchial transit has been measured as ion absorption from artificial urine passed through the branchial chambers of *B. latro* (Morris et al., 1991, 2000), *G. grayi* (Varley and Greenaway, 1994) and *G. natalis* (Morris, 2001; Taylor and Greenaway, 2002). This ion uptake is driven by ATPases in at least some of the gills in all species studied (reviewed by Morris, 2001).

Land crabs could adjust the salt loss in the P by adjusting both the volume ultimately voided and the salt composition of the P (Wolcott and Wolcott, 1985, 1991; Taylor et al., 1993). The crabs could both lower urine filtration rate and/or re-ingest some urine (Wolcott, 1992; Greenaway et al., 1990; Taylor et al., 1993; Greenaway, 1994), providing for an ion-regulatory role for the gut (e.g. Bliss, 1968; Ahearn et al., 1999). Exactly how the extent of urine modification is

regulated in land crabs is far from clear. There is growing evidence of hormonal control but considerably more information is required.

In the terrestrial anomuran *B. latro*, dopamine, a biogenic amine released from the pericardial organs, downregulates branchial Na uptake and Na^+/K^+-ATPase in the gill epithelial cells (Morris et al., 2000; Greenaway, 2003). By contrast, evidence from osmoregulating aquatic brachyuran crabs supports a hormonal upregulation of branchial Na^+/K^+-ATPase and ion uptake (reviewed by Morris, 2001). Biogenic amines, including octopamine and dopamine, have been linked to an elevation of cAMP in increasing the Na uptake in diverse marine species (e.g. Kamemoto and Oyama, 1985; Lohrmann and Kamemoto, 1987; Sommer and Mantel, 1988, 1991). In Chinese mitten crab *Eriocheir sinesis*, bioamines promote protein phosphorylation *via* a cAMP-dependent protein kinase in the gill tissue (Trausch et al., 1989) and stimulate Na flux (Bianchini and Gilles, 1990; Detaille et al., 1992; Mo et al., 1998). This mechanism is also present in freshwater crayfish (Mo and Greenaway, 2001) and is thus ubiquitous in aquatic decapods. Neuropeptides may also prove important, since, most recently, crustacean hyperglycaemic hormone has been shown to have marked effects on Na^+ transport in crustacean gills (Spanings-Pierrot et al., 2000; Serrano et al., 2003).

The possibility that net branchial salt uptake may be acutely adjusted by alterations in permeability, and thereby the rate of salt loss to a hypo-osmotic environment, has received relatively little consideration (e.g. Onken, 1999; Onken and Reistenpatt, 2002). Crabs clearly do adjust overall permeability in response to environmental circumstances (Péqueux, 1995). Adjustment of paracellular conductance would alter leak permeability, which may be altered as much as 10-fold by salinity acclimation (Onken, 1999; Tresguerres et al., 2003). Paracellular channels are generally under complex control (e.g. Anderson and van Itallie, 1995). In the insect Malpighian tubule, control of paracellular permeability includes neuropeptide messengers (Wang et al., 1996). Hormonal adjustments to diffusive efflux have apparently been detected in at least one decapod (Tullis, 1975).

The present study considered the putative hormonal regulation of salt reclamation from the urine by the gills in the terrestrial red crab from Christmas Island, *Gecarcoidea natalis* (Brachyura: Gecarcinidae), under field conditions. *G. natalis* is endemic to the rainforest of Christmas Island in the Indian Ocean, where the great majority of the crabs live away from the ocean and do not have ready access to seawater to replenish body salts (Greenaway, 1994; Adamczewska and Morris, 2001a). In the laboratory, red crabs produce a very dilute P when given freshwater to drink (Greenaway and Nakamura, 1991) and in the field manage salt and water balance in response to the availability of water and dietary salt (Greenaway, 1994). Furthermore, *G. natalis* held in the laboratory behaved in many ways as if under semi-xeric conditions. For example, in the field, Na and water turnover were much higher than in the laboratory whereas filtration rate was lower, perhaps due to the greater and more diverse availability of water and salt within the rainforest environment (Greenaway, 1994).

Preliminary assessment of data from *in situ* studies suggested that the bioamine serotonin, rather than dopamine, has an important stimulatory role in branchial ion pumping and Na uptake in *G. natalis* (Morris, 2001). Subsequent to the completion of the present study, Taylor and Greenaway (2002) provided laboratory data for regulation of salt balance in *G. natalis*. That study, of Cl rather than Na uptake, confirmed modulated branchial Cl uptake from urine in response to changes in Cl availability and haemolymph concentration, provided evidence of drinking urine as a potential mechanism of volume and salt regulation and showed that dopamine can stimulate Cl uptake, but only in crabs acclimated to drinking 70% seawater. This final finding is consistent with biogenic amines stimulating branchial ion uptake and with a role in post-renal urine modification (Taylor and Greenaway, 2002). However, the ecological and physiological significance of a mechanism that promotes net salt uptake only into already salt-replete crabs needs clarification.

It is important to resolve the roles of monoamines. The present study was conducted entirely *in situ* on Christmas Island during which different pharmacological approaches to determining the regulatory steps of branchial salt reclamation were used. The study also addressed the quasi-xeric response shown by red crabs in the laboratory by examining the effect of confinement and the availability of drinking water.

Materials and methods

Water and sodium balance were investigated in red crabs *Gecarcoidea natalis* Pocock 1888 on Christmas Island, Indian Ocean. All work was carried out using crabs collected directly from the rainforest and performed within the environs of the plateau rainforest (for detailed descriptions of Christmas Island, see Adamczewska and Morris, 2001a). Water and sodium balance of free-ranging red crabs, fitted with radio transmitters, was compared with that of crabs confined within enclosures in the forest. The assessment was performed during a wet season (February 1997) and the succeeding dry season (June 1997). The role of biogenic monoamines in the regulation of salt and water in *G. natalis* was also investigated in the following wet season (February 1998). Temperature in the rainforest varied from 23°C to 26°C and there was no rainfall during the June 1997 dry season. Consequently, the only available free water was dew that occurred shortly before dawn when temperatures briefly declined below the dew point. Crabs were observed each morning foraging and drinking for 1–2 h in the high humidity conditions. Rainfall averaged 10.0±2.8 mm day^{-1} and 3.0±1.8 mm day^{-1} in the wet seasons of 1997 and 1998, respectively, but in 1998 there were several successive days with no rain at all. The respective dew points were 22.8±0.1°C and 23.4±0.2°C; consequently, humidity was always close to 100% and, at times, the air was supersaturated with water vapour.

The studies of isotopically labelled crabs were carried out in

remote areas within Christmas Island National Park under permit from Parks Australia and the Health Department of Western Australia. All isotopes were shipped directly by Amersham Biosciences (Sydney, Australia).

Seasonal water and salt turnover under natural and confined conditions

Two treatment groups of crabs were established in each season; free-ranging animals fitted with radio transmitters and crabs confined in field enclosures. Crabs were collected from the forest, weighed and fitted with radio-transmitters (Titley Electronics, Ballina, NSW, Australia) glued to the carapace, as described previously (Adamczewska and Morris, 2001a). The crabs were injected through the arthrodial membrane at the base of the penultimate walking limb with the appropriate isotope, and 2 h was allowed for equilibration in the body. Dosages of isotopes in all experiments were: $^{3}H_2O$, 46.25 Bq g^{-1} wet mass; $^{22}NaCl$, 52.0 Bq g^{-1} wet mass; ^{51}Cr-EDTA, 7.4 kBq g^{-1} wet mass, and the largest fluid volume injected was 1 μl g^{-1}. After equilibration, a blood sample (~0.5 ml) was removed and stored in a sealed vial, and the crabs were released at the point of capture. Dilution of the radiolabels allowed the calculation of total body water, extracellular fluid volume (ECFV) and Na space. During the wet season, crabs moved within an extended 'home range' and it was necessary to locate the crabs each day.

The confined animals were treated similarly except that they were returned to enclosures at the point of capture inside the rainforest. The enclosures were made from fuel drums (JET A1 aviation fuel; 200 litre) that had been cut in half midway and from which the ends had been removed to produce open tubes. The steel walls were firmly inserted into the forest floor, so that the animals could contact the normal soil substrate and leaves, and the top was enclosed with wire mesh to prevent marauding robber crabs, *B. latro*, from preying on the experimental animals. Drinking water vessels were excluded from some enclosures so that the effect of availability of water could be assessed. Haemolymph samples for isotope analysis where taken on days 1, 3 and 6 after release. This required that animals be weighed using a calibrated spring balance in the field (Salter, Bury St Edmunds, UK) and ultimately with an electronic balance. The initial and final samples were in some cases also analysed for major ions and osmotic pressure. The radioactivities of the samples were measured using liquid scintillation counter (Packard Instrument Company, Meridan, USA) or, for ^{51}Cr, using a gamma counter (LKB Wallac Clini Gamma Counter, Turku, Finland).

The daily rate exchange fraction (K) and the biological half-life ($t_{½}$) of sodium and water were derived as described previously for crustaceans (Greenaway, 1980; Morris and van Aardt, 1998):

$$K = \log_e(H_0/H_1)/t$$

and

$$t_{½} = \log_e(2/K)\,, \qquad (1)$$

where t is the elapsed time in hours and H is the specific activity at time t. Extrapolation of this plot to the y intercept was used to derive the dilution of label in body water at time zero, which allowed calculation of total body water and sodium. The total body sodium was derived from the calculated Na space and the haemolymph Na concentration. The unidirectional efflux rates for sodium and tritium were calculated according to Nagy and Costa (1980):

$$E_T = \frac{2000\,(W_2 - W_1)\log_e(H_1W_1/H_2W_2)}{(M_1 + M_2)\log_e(W_2/W_1)/t}\,, \qquad (2)$$

where E_T is tritium or sodium efflux (ml kg^{-1} day^{-1}), M is body mass (g), W is the total body water (ml), and subscripts 1 and 2 refer to initial and subsequent values, respectively. Providing the crabs remain in water and sodium balance, the efflux will be equalled by influx and the rates will represent balanced turnover (Greenaway, 2001).

Rates of ^{51}Cr-EDTA clearance, urine production and fluid resorption within the urinary system were determined where possible. Clearance (ml h^{-1}) was calculated as $K \times$ EDTA space, determined from dilution of the injected ^{51}Cr-EDTA (Greenaway et al., 1991; Morris and van Aardt, 1998). Urine samples were obtained by gently deflecting the nephropore flap and drawing released urine into a fire-drawn pipette. The ratio of specific ^{51}Cr activity between urine and haemolymph (U:H) allowed calculation of fluid resorption within the antennal gland.

Drinking rates and the production rate of the final excretory product, P, were determined at the end of the trial periods as well as in some crabs collected directly from the rainforest. This required the recaptured animals to be individually transferred to large plastic chambers (P-chambers) suspended in the lower canopy of the rainforest. Each chamber comprised a bucket that had the base replaced by stainless steel mesh through which fluid but not faeces could pass to be collected in a hydrophobic polyethylene trap. A drinking vessel was included in each chamber. Evaporative loss of water from the drinking containers was determined in the same chambers containing dampened paper towel to simulate crabs. The crabs were weighed before and after their sojourn in the chambers, which was between 18 h and 23 h. The importance of drinking water was further assessed by including a further treatment in which the crabs were provided with empty drinking vessels.

Haemolymph and urine salt composition

The osmotic pressure of the haemolymph and urine, where required, was determined using a vapour pressure osmometer (Wescor, Logan, USA) or, for especially dilute solutions, Osmomat (Gonotek; Berlin, Germany) – freezing point depression osmometer. The remaining sample was denatured by mixing 1:1 with 0.1 mmol l^{-1} HNO_3 and was transported to the laboratory for further analysis. The Cl concentration was determined using a chloride titrator (CMT10; Radiometer, Copenhagen, Denmark), and Mg, Ca, K and Na were measured by atomic absorption spectrophotometry (AAS; GBC 906, GBC Melbourne, Australia). To suppress interference, samples for measurement of Na and K were diluted with 5.9 mmol l^{-1}

$CsCl_2$, while for Mg and Ca measurements samples were diluted with 7.2 mmol l^{-1} $LaCl_3$.

Pharmacological investigations – water and sodium balance

Dopamine and serotonin (5-hydroxytryptamine) were administered to crabs by infusion through the arthrodial membrane of the walking legs at 10^{-10} mol g^{-1}, and cAMP was administered as the membrane-permeable dibutyryl-cAMP (db-cAMP) at 10^{-9} mol g^{-1}. Infused volumes did not exceed 220 μl and were composed of an appropriate carrier saline (below) that had been sterilised by boiling. To avoid oxidation, dopamine was dissolved 10 s prior to infusion in saline deoxygenated by boiling. A saline control infusion group was included in all determinations. The saline was modified from that of Greenaway (1994) and comprised: NaCl, 376 mmol l^{-1}; $NaHCO_3$, 1 mmol l^{-1}; $CaCl_2$, 12.9 mmol l^{-1}; KSO_4, 6.1 mmol l^{-1} and $MgSO_4$, 9.24 mmol l^{-1}. The rate of clearance of ^{51}Cr-EDTA and the efflux of ^3H_2O and Na was determined for treated crabs in the forest, and the effects on Na flux rates were determined also in branchial perfusion of crabs held within P-chambers (above).

Pharmacological treatment of confined crabs

G. natalis were held for 24 h in enclosures and then infused with either a monoamine, db-cAMP or saline, as described above. At the same time, the animals were injected with either ^{51}Cr-EDTA or ^3H_2O (as described previously). Crabs were re-infused with the pharmacological trial compound or saline as a control every 8 h over 3 days and sampled for haemolymph every 24 h. At the end of the 3 days, the ^{51}Cr-EDTA-infused animals were sampled for urine, which was also assessed for ^{51}Cr activity and osmotic pressure. During the dry season, crabs were assessed for ^{51}Cr-EDTA clearance only, since dry season water flux rates had been extremely low. During the dry season only, an extra group was included to test the effects of octopamine. The entire experiment was duplicated for the dry season except that the animals were deprived of drinking water. Estimates of drinking and P production could be obtained only by holding the animals in metabolism chambers, i.e. P-chambers, within the rainforest. Therefore, additional estimates of water flux were made over the 24 h period that animals were held in the P-chambers.

Branchial perfusion and Na flux

The branchial perfusion studies determined the net flux of Na from the urine into the haemolymph (J_{net}), the unidirectional flux into the crab by utilizing ^{22}Na tracer (J_{in}), and J_{out} as the difference between the two ($J_{out}=J_{in}-J_{net}$).

To detect up- and/or downregulation of branchial Na uptake, *G. natalis* were either acclimated to drinking 50% seawater (SW) for 2 weeks or provided with freshwater (FW) (Morris, 2001). The branchial perfusions were carried out using a modified method from Morris et al. (1991, 2000). The mean mass of the FW group was 265.8±14.05 g and of the SW group was 288.2±10.90 g. The carapace (anterior branchiostegite) of the crabs was drilled through into the branchial chamber with a fine dental drill (battery hobby drill; Dremel) to allow the insertion of polyethylene cannulae (0.97 mm i.d., 1.27 mm o.d.). The opening was cauterized, and the 5 cm-long cannula fixed in place with cyanoacrylate adhesive. The animals were then left to recover for at least 24 h.

The perfusion experiments were carried out at ambient temperature within the environs of the plateau rainforest. The chambers were those used previously for collecting P (above) but in which the animal rested above a funnel to collect overflowing branchial perfusate. Saline solution of ionic composition close to that of the urine [artificial urine (AU)] was pumped to both branchial chambers at 0.8 ml min^{-1} using a peristaltic pump. The composition of the two different AU used were, for FW and SW respectively, NaCl, 400 mmol l^{-1} and 416 mmol l^{-1}; KCl, 16 mmol l^{-1} and 12.5 mmol l^{-1}; $MgCl_2$, 7.8 mmol l^{-1} and 12 mmol l^{-1}; and $CaCl_2$, 27 mmol l^{-1} and 17.5 mmol l^{-1}; both AU contained 50 mmol l^{-1} Na_2SO_4 and 1 mmol l^{-1} $NaHCO_3$. Both AU were labelled with ^{22}NaCl as a tracer for unidirectional Na influx. The use of a small fixed extra-corporeal volume allowed the ready application of Shaw's solutions for unidirectional ^{22}Na flux calculations (Shaw, 1963).

To initiate the perfusion experiments for each animal, a ~20 ml volume of AU was circulated for 20 min to fill the interstices of the branchial chambers and was then drained to waste. At the same time as the initial perfusate was supplied, the crab was infused with either sterile saline as a sham treatment or with saline containing either dopamine at 2×10^{-4} mol l^{-1} or db-cAMP at 6.1×10^{-4} mol l^{-1}. The infusion of the monoamines very occasionally caused the crabs to regurgitate and, if so, this always occurred during the pre-perfusion, allowing the perfusate to be replaced or, more often, for the animal to be excluded from the experiment. After the 20 min preliminary perfusion, the perfusate was drained and a second measured volume (~20 ml) was supplied and recirculated. Samples of 0.3 ml were taken shortly after the initial start time and every 15 min thereafter up to 90 min. The recirculating system can require the judicious inclusion of monitoring procedures. ^{51}Cr-EDTA injected as a urinary tracer has a short half-life of 27.7 days, and decay within ^{22}Na-labelled samples was revealed by counting over several weeks. Very few animals were discarded, *post hoc*, from the data set. The crabs ingested a small volume of perfusate. The amount of Na and ^{22}Na counts thereby removed during the 90 min perfusion was readily calculated and subtracted from the branchial uptake rates. Samples were assayed in triplicate for Na and ^{22}Na. The net rate of Na uptake was calculated as described previously (Morris et al., 1991). The rate of ^{22}Na uptake was calculated from the linear relationship of the decreased $\log_e$ c.p.m. (counts per minute) in a fixed volume over time to provide a rate constant (Morris and van Aardt, 1998). The requirement to exclude some data eventually produced a balanced design with N=8 for each of the treatments.

Table 1. *The osmotic pressure and concentration of major ions in the haemolymph and urine of* Gecarcoidea natalis *in the rainforest during the wet season (February 1997)*

	Haemolymph		Urine	
	Free ranging	Confined	Free ranging	Confined
Osmotic pressure (mOsm)	753.3±28.3	798.8±6.0	768.7±30.2	816.4±6.2
Na (mmol l^{-1})	338.3±23.4	331.3±6.7	302.8±14.4	269.6±9.6
K (mmol l^{-1})	6.1±0.4	5.9±0.9	5.9±0.7	6.3±0.8
Mg (mmol l^{-1})	6.6±0.5	5.7±1.2	5.6±0.6	5.9±1.4
Cl (mmol l^{-1})	315.5±11.7	316.3±4.7	299.3±10.7	386.0*,†±5.3
Ca (mmol l^{-1})	18.4±0.9	16.8±1.2	9.5†±1.3	16.1*±2.0

Crabs were either free ranging in the rainforest or held within open-topped enclosures that allowed rainfall to enter and contact with the forest floor. Values are means ± S.E.M. (N=8–12 in each group). *, significantly different to free-ranging crabs at $P<0.05$; †, urine significantly different to haemolymph at $P<0.05$.

Determination of branchial Na^+/K^+-ATPase activity and the effects of serotonin

Na^+/K^+-ATPase activity was determined in the gill tissue of *G. natalis* previously infused with serotonin or saline (control). 30 min after the infusion, the crabs were cooled at 4°C until completely torpid, the gills were removed from one side of the animal, and the animals were then rapidly frozen. The gills were weighed and homogenized in 25 mmol l^{-1} Tris/acetate buffer containing phenylmethylsulphonyl fluoride (0.2 mmol l^{-1}), dithiothreitol (0.1 mmol l^{-1}) and aprotinin (100 units ml^{-1}). Protein concentration was determined for the gill homogenate using a test kit (protein assay 500-0001 Kit 1; Bio-Rad, Hemel Hempsted, UK) calibrated using bovine gamma globulin in the concentration range of 0–1.57 mg ml^{-1}. Each homogenate assay was replicated, and absorbances were determined at 595 nm. For assay, the homogenates were 8–9 mg ml^{-1}. ATPase activity was determined in (1) a buffer of the following composition – $MgCl_2$, 6 mmol l^{-1}; NaCl, 100 mmol l^{-1}; KCl, 10 mmol l^{-1}; Tris, 25 mmol l^{-1}, adjusted to pH 7.4 with acetic acid – and (2) in the same buffer without KCl but containing 3.5 mmol l^{-1} ouabain, which specifically inhibits Na^+/K^+-ATPase. The difference between the ATPase activity in the two buffers could then be attributed to Na^+/K^+-ATPase activity. The reaction was started by the addition of vanadium-free ATP (9.1 mmol l^{-1}) and stopped after 20 min at 25°C by the addition of trichloracetic acid (0.6 mol l^{-1}). After centrifugation at 10 000 ***g*** for 10 min, the inorganic phosphate (P_i) concentration was determined in the supernatant using the method of Fiske and Subbarow (kits 661-11 and 661-8; Sigma). Absorbance was determined at 660 nm using a transportable spectrophotometer (Novaspec II; PharmaciaBiotech, Uppsala, Sweden).

Statistical analyses

Homogeneity of variances was verified using Bartletts χ^2 test prior to one- and two-way analysis of variance (ANOVA). *Post hoc* testing was by Tukey's test for one-way analyses and by A Matrix Contrast analyses when significant differences were indicated by two- and three-way ANOVA. Where it was required to determine if means differed from zero, a one-sample *t*-test was employed. Analysis used Systat packages.

Results

Osmotic and ionic status

Confinement of *G. natalis* in the field enclosures during the wet season had virtually no effect on either haemolymph or urine composition, which were essentially the same (Table 1). The exceptions were elevated Cl concentration in the urine of confined crabs, and Ca concentration that was lowered in the urine of free-ranging crabs only (Table 1). Confinement in the wet season presented no significant overall osmotic stress. Confining red crabs in the enclosures during the dry season decreased the osmotic pressure in both the haemolymph and urine and also with respect to the osmotic pressure of the crabs in the wet season (Table 2). This seasonal difference in osmotic pressure could not be otherwise correlated with changes in the measured osmolytes.

Providing drinking water in the enclosures made no difference to urine composition, and the only effect was a dilution of Na concentration of the haemolymph of crabs with access to water (Table 2). However, in contrast to the wet season, confinement during the dry season had further effects. Most noticeable was a general reduction in Mg concentrations in confined crabs but also a tendency to a specific increase in urinary Mg not seen in the wet season (Table 2). The lower urinary Ca concentration (9.5±1.3 mmol l^{-1}) seen in free-ranging crabs during the wet season was also observed during the dry season (6.6±2.1 mmol l^{-1}). Apparently, Ca was always reabsorbed from the urine of free-ranging crabs but not from that of crabs confined in enclosures.

Water turnover

The crabs lost some mass and, although the largest relative loss of 8.14% was in the crabs confined during the dry season, the loss could not be correlated with season or generally with any effect of confinement (Table 3). Curiously, the lowest loss

Table 2. *The osmotic pressure and concentration of major ions in the haemolymph and urine of* Gecarcoidea natalis *in the rainforest during the dry season (June 1997)*

	Haemolymph			Urine		
		Confined			Confined	
	Free ranging	Drinking water	No drinking water	Free ranging	Drinking water	No drinking water
Osmotic pressure (mOsm)	805.8±12.9^{W}	696.0±22.5^{W,*}	734.9±13.9^{W,*}	834.8±21.7	722.1±9.7^{W,*}	738.8±9.3^{W,*}
Na (mmol l^{-1})	482.8±15.9^{W}	396.5±17.7^{W,*,‡}	453.8±13.7^{W}	488.8±22.1^{W}	429.5±8.1^{W,*}	440.9±18.4^{W,*}
K (mmol l^{-1})	9.7±0.2^{W}	8.1±0.4^{W}	9.0±0.2^{W}	8.0±0.9	8.1±1.1	7.8±2.0
Mg (mmol l^{-1})	11.4±0.5^{W}	8.8±0.5^{W,*}	9.2±0.2^{W,*}	13.2±0.2^{W,†}	11.6±0.6^{W,*,†}	10.6±2.5^{W,*,†}
Cl (mmol l^{-1})	328.7±13.4	297.0±10.1	319.0±8.3	370.3±20.2^{W,†}	348.9±11.5^{W,†}	298.5±66.1^{W}
Ca (mmol l^{-1})	21.6±0.4^{W}	17.9±0.7	20.9±0.7^{W}	6.6±2.1†	16.6±2.3*	16.0±4.9*

Crabs were either free ranging in the rainforest or one of two groups held confined within enclosures in the rainforest. One of the confined groups was provided with drinking water and the other was dependent on water naturally available within the enclosures. The enclosures were open at the top and allowed crabs contact with the forest floor. Values are means ± S.E.M. (N=8–12 in each group). *, significantly different to free-ranging crabs at P<0.05; †, urine significantly different to haemolymph at P<0.05; ‡, effect of including drinking water; ^{W}, different to corresponding value for free-ranging or confined crabs in the preceding wet season – see Table 1.

Table 3. *Changes in body mass and water space of confined or free-ranging* G. natalis *during 6 days in the rainforest*

	Dry Season		Wet Season	
	Confined	Free ranging	Confined	Free ranging
Change in body mass (g kg^{-1} day^{-1})	–24.23±0.92†	–8.68±0.58*	–17.69±2.45	–20.09±4.52*
Change in water space (ml kg^{-1} day^{-1})	–28.9±6.43	–19.87±14.97	–4.89±5.25	–35.89±16.97

Since the crabs were of different mass, the changes are normalized per kg. Values are means ± S.E.M. (N=8–9 in each group). * indicates a seasonal difference; † indicates a single case where confinement had an effect.

rates were in free-ranging crabs in the dry season (Table 3). There were also some significant decreases in the water space, but again these were not correlated with season or treatment (P=0.24) and could not simply account for any mass loss (Table 3). There was no difference in the half-life for water turnover of confined animals compared with free-ranging *G. natalis*, which in the dry season was 5.29±0.55 days and 4.81±0.47 days, respectively. In comparison, there was a very marked decrease in the half-life of water in *G. natalis* in the wet season, to 1.75±0.21 days for confined crabs and to 2.24±0.39 days for free-ranging animals. The rate of water efflux in wet season animals was approximately double that of dry season animals, with overall mean rates of 272.13±34.97 ml kg^{-1} day^{-1} and 140.34±10.31 ml kg^{-1} day^{-1} (Fig. 1). The water efflux rates were completely unaffected by confinement in field enclosures.

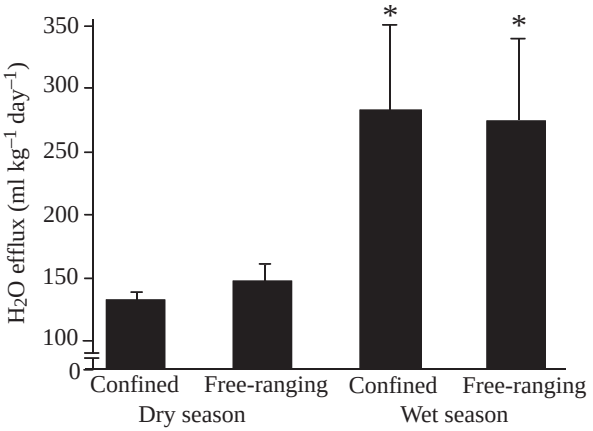

Fig. 1. Water efflux determined from $^{3}H_2O$ clearance from *G. natalis* during the dry season and the wet season in the rainforest of Christmas Island. Determinations were made in both seasons for free-ranging crabs (N=8) and for crabs in field enclosures (N=9). Asterisks indicate a significant increase in wet season compared with dry season animals.

^{51}Cr-EDTA clearance and urine flow

The ^{51}Cr-EDTA space was significantly smaller in the dry season crabs (22.7% body mass) than in those in the wet season (27.9%) but did not vary between crabs confined in enclosures and those ranging in the surrounding forest. The most remarkable result was the more than doubling of the clearance rate during the wet compared with the dry season (Fig. 2). In free-ranging crabs, the rate increased from 53.45±8.74 ml kg^{-1} day^{-1} in the dry season to 111.26±23.76 ml kg^{-1} day^{-1} in the wet season. Despite an apparent reduction in the dry season, confinement had no significant effect on clearance rate or, as far as could be determined, on urine flow rate (Fig. 2). Urine flow rate was

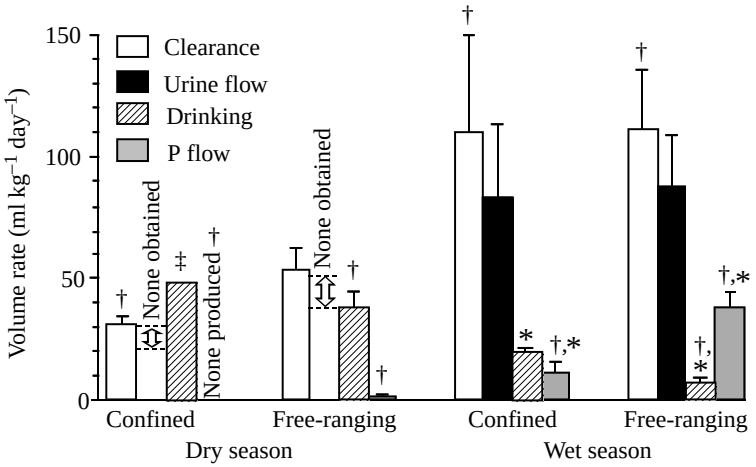

Fig. 2. The volume rates of ^{51}Cr-EDTA clearance, urine flow, drinking and production of the final excretory product P by *G. natalis* either free ranging in the rainforest or after 6 days confinement in field enclosures. Clearance was determined over 6 days, and urine flow using the appropriate U:H ratio of ^{51}Cr in the urine and haemolymph. Drinking and P production rates were determined on separate animals over a 24 h period after 6 days in the chambers described in the Materials and methods, which were held within the rainforest on Christmas Island. In the dry season, the crabs provided no urine at all and thus ^{51}Cr measurements in the urine could not be made. The vertical arrows indicate the range of possible urine flow calculated using a U:H ratio of 1.04 from Greenaway (1994) or the values obtained from free-ranging and confined animals in the wet season. Values are means + S.E.M. (N=6–9). * indicates an effect of confinement compared with free-ranging crabs; † indicates a difference between dry and wet season values; ‡ indicates that the animals confined during the dry season drank the entire volume of water provided and thus a minimum rate is available.

Table 4. *Sodium turnover in* Gecarcoidea natalis *free-ranging or confined within field enclosures in the rainforest on Christmas Island in 1997*

	Dry season		Wet season	
	Confined	Free ranging	Confined	Free ranging
Sodium space (% initial mass)	37.8±3.91^{f}	34.3±2.18^{e}	30.71±1.10	26.46±1.81^{e,f}
$t_{½}$ (day^{-1})	11.91±1.58^{a}	8.68±2.28	8.20±1.41	5.54±0.51^{a}
Rate constant (day^{-1})	0.0581±0.0090^{b}	0.0876±0.0288	0.0990±0.0141	0.1320±0.0113^{b}
Total body sodium (mmol)*	30.43±1.70^{c,h}	36.44±2.96^{c,d,h}	24.78±2.21^{d}	16.53±4.01^{h}
Sodium efflux (mmol kg^{-1} day^{-1})	8.37±1.67	11.45±2.01	9.82±1.66	10.58±1.03

*Scaled to 250 g body mass; see Greenaway (1994) for mass scaling exponent.

Measurements were carried out in both the wet season (February) and dry season (June). Values are means ± S.E.M. (N=8–12 in each treatment). Values sharing similar superscript letters were significantly different at P<0.05 (note that ANOVA of sodium efflux was P=0.21).

determined from the urine-to-haemolymph ratio (U:H) for ^{51}Cr-EDTA, which in wet season crabs was 1.23±0.07 in free-ranging animals and 1.44±0.46 in confined animals. These values were not different from each other (P=0.14) nor were they different from values of one (*t*-test). Thus, no significant concentration of the urine occurred within the antennal gland. It was impossible to obtain urine from any of the crabs in the dry season and thus to determine urine ^{51}Cr-EDTA. A range of urine flow values was estimated for dry season crabs by using the values of U:H=1.04 from Greenaway (1994) and from the wet season animals above (Fig. 2). In any case, urine flow rates were not different from the corresponding clearance rate (Fig. 2).

The dry season crabs drank considerably more than the crabs in the wet season, and the animals that had been confined drank all of the available water, more than 48 ml kg^{-1} day^{-1} (Fig. 2). For example, free-ranging crabs in the dry season drank 38.4±7.07 ml kg^{-1} day^{-1} whereas those in the wet season drank only 7.17±1.94 ml kg^{-1} day^{-1}. The mean drinking rate of free-ranging crabs in the wet season was not significantly different from a rate of zero, i.e. not drinking at all (one-sample *t*-test, P=0.058). However, the seasonal difference in drinking of approximately 31 ml kg^{-1} day^{-1} was a small fraction of the difference in urine flow (Fig. 2) or, especially, the 127 ml kg^{-1} day^{-1} difference in overall water efflux (Fig. 1). The rate of P flow was reciprocal to the trend in drinking rate so that crabs that drank the largest amount during the dry season produced little, or more often, no P (Fig. 2). At the other extreme, during the wet season free-ranging crabs produced P at a rate (37.81±7.4 ml kg^{-1} day^{-1}) approaching 50% of the urine flow rate (88.0±21.12 ml kg^{-1} day^{-1}) and, importantly, more than five times greater than the drinking rate (Fig. 2). *G. natalis* that had been confined had both higher drinking rates and lower rates of P release than the free-ranging animals.

Sodium turnover

In the wet season of February 1997, there was rainfall on every day of the trials, with 52 mm falling on the second day. There were few significant effects of confinement alone on the Na status but some changes were associated with the season (Table 4). The Na space was clearly largest in animals confined in enclosures during the dry season (37.8% body mass) and smallest in *G. natalis* ranging in the forest during the wet season (26.5% body mass), which was associated with a

Table 5. *The rate of ^{51}Cr-EDTA clearance from the haemolymph, extracellular fluid volume and body mass (% loss) of* Gecarcoidea natalis *during the 1997 dry season*

	^{51}Cr-EDTA clearance (ml kg^{-1} day^{-1})		Extracellular fluid volume (% body mass)		Change in body mass (%)	
Dry Season	Water	No water	Water	No water	Water	No water
Saline	130.02±12.10	90.57±4.88†	19.15±0.76	19.00±0.85	3.61±0.74	3.70±0.91
Dopamine	123.44±5.62	93.25±5.84†	22.94±1.69	20.05±0.92†	3.24±0.83	3.60±0.78
db-cAMP	125.32±14.07	103.20±16.8†	21.99±1.57	17.60±0.48†	4.24±0.57	4.06±0.51
Serotonin	151.11±10.72*	108.72±13.36*,†	21.29±0.99	17.69±1.36	4.50±0.82	4.00±0.83
Octopamine	123.16±7.37	90.33±9.26†	19.22±1.08	20.31±0.81	4.43±0.46	3.90±0.80

The crabs were treated by infusion of saline, dopamine, serotonin, octopamine (all at 10^{-10} mol g^{-1}) or db-cAMP (10^{-9} mol g^{-1}). Infusions in saline carrier at 1 µl g^{-1} were performed at 8 h intervals for 3 days. Crabs were held within enclosures that were open at the top and allowed crabs contact with the forest floor. Values are means ± S.E.M. (*N*=12 in each group). *, effect of drug; †, effect of removing drinking water. Extracellular fluid volume was measured from EDTA space, expressed as % body mass. The change in % body mass was tested by ANOVA of the arcsin-transformed values; there were no significant differences between the values.

Table 6. *The rate of ^{51}Cr-EDTA clearance from the haemolymph, extracellular fluid volume (ECFV), urine flow, water efflux and half-life of* Gecarcoidea natalis *during the 1997 wet season*

				Water efflux	
Wet season	Clearance (ml kg^{-1} day^{-1})	ECFV (% body mass)	Urine flow (ml kg^{-1} day^{-1})	$t_{½}$ (h)	(ml kg^{-1} day^{-1})
Saline (control)	132.68±13.92	27.37±2.62	168.00±51.44	50.03±1.06	192.59±9.22
Dopamine	165.81±9.26	33.34±1.65	146.04±26.60	51.44±1.58	228.77±19.52
db-cAMP	187.15±17.98	33.19±1.94	179.73±23.91	44.44±2.41	226.63±5.47
Serotonin	170.85±36.01	32.80±1.42	180.71±31.34	48.51±6.37	234.08±19.15

The crabs were treated by infusion of either saline, dopamine, serotonin (both at 10^{-10} mol g^{-1}) or db-cAMP (10^{-9} mol g^{-1}). Infusions, in saline carrier at 1 µl g^{-1} were performed at 8 h intervals for 3 days. Crabs were held within enclosures that were open at the top and allowed crabs contact with the forest floor. Values are means ± S.E.M. (*N*=12 in each group).

There was no significant effect of any pharmacological treatment.

reciprocal trend in both the biological half-life of Na in the animal and the rate constant for Na turnover (Table 4). For example, in crabs confined in enclosures during the dry season, $t_{½}$ was 11.9 days but was only half this (5.5 days) in free-ranging crabs during the wet season. The same trend was less obvious in the total body Na of *G. natalis* (Table 4), despite scaling to a standard mass of 250 g. Nonetheless, the lowest value of 16.53 mmol was recorded in the wet season, and the highest value of 36.44 mmol during the dry season. Consequently, there were no differences in Na efflux between seasons or as a result of confinement (Table 4), and the overall mean efflux was 10.49±0.96 mmol kg^{-1} day^{-1}.

Pharmacological trials – Cr-EDTA clearance and water flux

The large decrease in clearance rate when the crabs were deprived of drinking water was confirmed but there was no effect of dopamine, octopamine or db-cAMP on mass, ECFV or Cr-EDTA clearance (Table 5). By contrast, during the dry season serotonin induced an increase in clearance rate of 16–17%. However, in wet season trials, neither dopamine (10^{-10} mol g^{-1}), serotonin (10^{-10} mol g^{-1}) nor db-cAMP (10^{-9} mol g^{-1}) produced any significant change in the clearance of ^{51}Cr-EDTA, the flow of urine or the water efflux rates of *G. natalis* (Table 6).

Neither serotonin, dopamine nor db-cAMP altered water efflux in either the freshwater (FW) or seawater (SW) groups. Acclimating the crabs to drinking 50% SW increased the water flux by 113% and at the same time also reduced P production, in some cases to zero (Table 7). There was no effect of either acclimation (*P*=0.26) or drug infusion (*P*=0.24). Both dopamine and serotonin caused marked reductions in P production by FW-acclimated crabs to 4.5 ml kg^{-1} day^{-1} and 4.4 ml kg^{-1} day^{-1}, respectively, as compared with 17.6 ml kg^{-1} day^{-1} in the saline-infused controls. This effect was apparently not mediated by cAMP (Table 7). In these crabs, water flux was determined during the 24 h period that crabs were held in the P-chambers, and the rates were much lower than water flux and clearance rates otherwise determined *in situ.*

Branchial modification of urine

Different results of pharmacological infusions were obtained from *G. natalis* that had been drinking FW compared with those acclimated to drinking 50% SW (Figs 3, 4). In the FW

Table 7. *The effect of tested pharmacological compounds on the rates of water efflux, P production and drinking of* Gecarcoidea natalis *during the 1998 wet season*

	Water efflux (ml kg^{-1} day^{-1})		Drinking (ml kg^{-1} day^{-1})		P production rate (ml kg^{-1} day^{-1})	
Wet season	FW	SW	FW	SW	FW	SW
Saline (control)	29.5±9.30	41.4±9.90†	6.00±2.45	6.09±2.49	17.6±3.94	1.8±2.03†
Dopamine	21.3±12.92	66.7±15.98†	7.02±2.87	14.17±5.78	4.5±0.70*	NP†
db-cAMP	22.5±11.91	64.0±15.28†	10.52±4.29	8.19±3.34	11.0±2.27	NP†
Serotonin	21.7±8.59	30.4±13.10†	7.69±3.14	11.14±4.55	4.4±2.99*	2.7±1.93

The rate determinations were made over a 24 h period inside P-chambers (see Materials and methods). The crabs were either acclimated to drinking 50% seawater (SW) or assigned to a control group provided with normally available freshwater (FW), as for the branchial perfusion determinations. Crabs from each of the SW and FW groups were treated by infusion of either saline, dopamine, serotonin (both at 10^{-10} mol g^{-1}) or db-cAMP (10^{-9} mol g^{-1}). Infusions, in saline carrier at 1 µl g^{-1}, were performed at 8 h intervals for 3 days. Crabs were held prior to determinations within enclosures that were open at the top and allowed crabs contact with the forest floor. Values are means ± S.E.M. (N=8 in each group). *, effect of drug infusion; †, effect of drinking saline water; NP, none produced (in the case of NP values, the significance of the SW values was derived by testing the FW values in a one-tailed t-test against a value of zero – i.e. none produced).

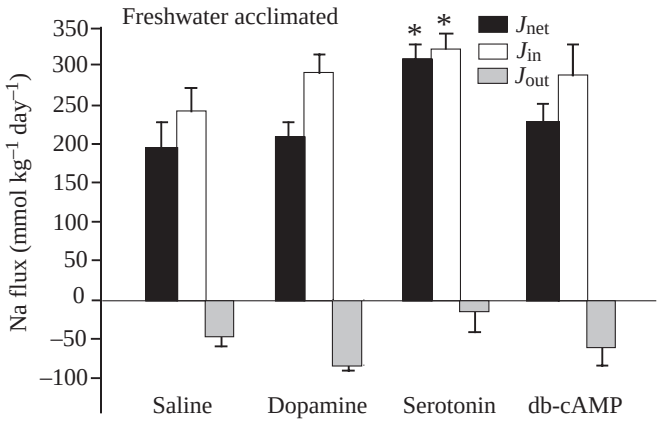

Fig. 3. Branchial sodium flux in *G. natalis* acclimated to drinking freshwater and then infused with either a saline carrier (control), dopamine or serotonin at 2×10^{-4} mol l^{-1} or dibutyryl cyclic AMP (db-cAMP; membrane-permeable cAMP) at 6.1×10^{-4} mol l^{-1}. The branchial chambers were perfused with artificial urine labelled with ^{22}NaCl sufficient to provide 6000 c.p.m. in a 400 µl sample. Flux rates were determined over 90 min. Changes in total [Na] were used to provide rates of net uptake (J_{net}); uptake of ^{22}Na provided rates of unidirectional influx (J_{in}); and unidirectional loss was calculated for each crab as J_{in}–J_{net}=J_{out}. For details, see Materials and methods. N=8 for each treatment. * indicates significant elevation compared with saline-infused control crabs.

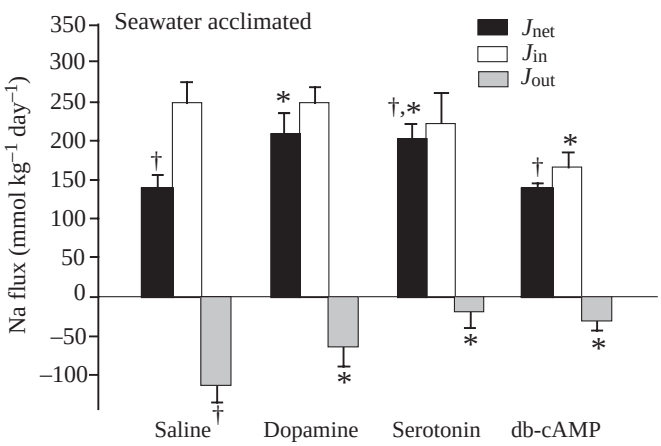

Fig. 4. Branchial sodium flux in *G. natalis* acclimated to drinking seawater (50% SW) and then infused with either a saline carrier (control), dopamine or serotonin at 2×10^{-4} mol l^{-1} or dibutyryl cyclic AMP (db-cAMP; membrane-permeable cAMP) at 6.1×10^{-4} mol l^{-1}. The branchial chambers were perfused with artificial urine labelled with ^{22}NaCl sufficient to provide 6000 c.p.m. in a 400 µl sample. Flux rates were determined over 90 min. Changes in total [Na] were used to provide rates of net uptake (J_{net}); uptake of ^{22}Na provided rates of unidirectional influx (J_{in}); and unidirectional loss was calculated for each crab as J_{in}–J_{net}=J_{out}. For details, see Materials and methods. N=8 for each treatment. * indicates significant depression compared with saline-infused control crabs; † indicates significant difference to corresponding rate in crabs acclimated to freshwater in Fig. 3.

crabs, serotonin increased Na J_{net} significantly by 57% from 197.2±31.1 mmol kg^{-1} day^{-1} to 309.9±17.6 mmol kg^{-1} day^{-1}, which was primarily the result of an increase in active uptake so that unidirectional Na influx (J_{in}) increased from 242.6±29.7 mmol kg^{-1} day^{-1} to 321.1±22.2 mmol kg^{-1} day^{-1} (Fig. 3). By contrast, dopamine infusion resulted in a J_{in} of 210.4±20.5 mmol kg^{-1} day^{-1}, which was not significantly different from the control, and neither was the rate in cAMP-infused crabs (Fig. 3). There were no significant fluctuations in the J_{in} of SW-drinking *G. natalis.*

Changes in the overall loss component (J_{out}) were important in the SW-acclimated crabs (Fig. 4). Acclimation of *G. natalis* to drinking 50% SW reduced the J_{net} rate compared with FW-infused control crabs by 29% to 139.7±16.5 mmol kg^{-1} day^{-1} (Fig. 4). The decline in J_{net} was due not to any change in the active uptake component (J_{in}) but instead to a significantly greater passive loss, as shown by J_{out}=–111.9±24.6 mmol kg^{-1} day^{-1} as compared with only

–45.4±12.8 mmol kg^{-1} day^{-1} in the FW control crabs. In the SW *G. natalis*, both dopamine and serotonin increased J_{net} (247.8±21.5 mmol kg^{-1} day^{-1} and 223.5±38.4 mmol kg^{-1} day^{-1}, respectively) and re-established a net Na uptake rate similar to that in FW saline control crabs (Figs 3, 4). J_{in} remained unaffected and similar to that of the control crabs (Fig. 4). Rather, the primary effect of dopamine and especially serotonin in SW *G. natalis* was to reduce the relatively elevated loss such that J_{out} was lower (dopamine, –62.9±26.6 mmol kg^{-1} day^{-1}; serotonin, –21.0±19.8 mmol kg^{-1} day^{-1}) than that of SW control crabs (Fig. 4). Infusion of db-cAMP also had effects in SW crabs but these were not apparent in J_{net}, which remained unchanged due to a decrease in J_{in} that balanced a lower J_{out} (Fig. 4).

The rate of drinking and thus the uptake of Na by ingestion was highly variable between individual crabs and it was not possible to discern any difference with respect to either the salinity of drinking water acclimation (FW, 230.6 mmol kg^{-1} day^{-1}; SW, 239.9 mmol kg^{-1} day^{-1}; P=0.61) or pharmacological infusion (P=0.36).

Na^+/K^+-ATPase and serotonin

Serotonin infusion of *G. natalis* drinking FW promoted a 92% increase in Na^+/K^+-ATPase activity to 1.71±0.32 μmol P_i mg^{-1} protein h^{-1} compared with control crabs (0.89±0.16 μmol P_i mg^{-1} protein h^{-1}). In view of the similarity between the J_{in} values for SW and FW crabs perfused with saline only (Figs 3, 4), it would seem important that the Na^+/K^+-ATPase activity of SW crabs (0.79±0.03 μmol P_i mg^{-1} protein h^{-1}) was unchanged compared with that of FW *G. natalis*.

Discussion

Seasonally responsive changes in the management of salt and water balance by *Gecarcoidea natalis* were often substantial and were generally more important than any smaller changes associated with *in situ* confinement.

Seasonal water balance

Water turnover of red crabs doubled from ~140 ml kg^{-1} day^{-1} in the dry season to ~280 ml kg^{-1} day^{-1} in the wet season, demonstrating a considerable flexibility in water management in the field. The dry season water turnover rate *in situ* was almost identical to the 138 ml kg^{-1} day^{-1} previously measured on Christmas Island at the end of the dry season but was considerably greater than the 71 ml kg^{-1} day^{-1} in red crabs driven by the dry season into burrow retreats (Greenaway, 1994). Significantly, the flux rates of crabs measured in P-chambers rapidly declined to 30 ml kg^{-1} day^{-1} (21% of the dry season rate *in situ*). In the laboratory, *G. natalis* took up water at a rate between 13% and 22% of that in crabs in the field (Greenaway, 1994). The water turnover rates of *Birgus latro* in the laboratory were as little as one-third of those measured in the field (Greenaway et al., 1990; Taylor et al., 1993; Greenaway, 2001). The difference might be variously explained by re-ingestion of urine (Greenaway, 2001), recycling the tritium label, and by depriving the crabs of the water in their food. For laboratory-held red crabs, urine flow was 85 ml kg^{-1} day^{-1} while the final P flow was less than 10 ml kg^{-1} day^{-1} (Greenaway, 1994). It is apparent that confining the crabs in P-chambers induced a semi-xeric response similar to that seen in laboratory-held crabs.

Red crabs drank from the branchial perfusate during the current investigation and also in other studies during which oral flow increased from 37 ml kg^{-1} day^{-1} to 184 ml kg^{-1} day^{-1} (Taylor and Greenaway, 2002). In *B. latro*, EDTA clearance in the field was similar to clearance rates determined in the laboratory, leading Greenaway (2001) to speculate that primary urine formation might be held constant and water balance managed by regulated drinking of the urine. In red crabs on Christmas Island, the reduced dry season water turnover was managed by a significantly reduced clearance: ≤50 ml kg^{-1} day^{-1} compared with >100 ml kg^{-1} day^{-1} in the wet season as well as an almost complete cessation of P production. Drinking rate was similar to urinary flow rate and thus water from the food made little contribution during the dry season.

During the wet season, red crabs drank less and had high urine flow and water turnover. Greenaway (1994) comments on the large water content of food during the wet season and this, together with direct absorption of water during heavy downfalls (red crabs shelter from heavy rain; S. Morris and M. D. Ahern, personal observation), probably leads to the excessive water uptake requiring the production of large volumes of a dilute P. Water turnover rates were higher than clearance rates in both confined and free-ranging crabs, and the possibility of some isotopic exchange during the wettest weather contributing to elevated rates must be recognised (Greenaway, 1994). Lowered activity of semi-fossorial red crabs during the dry season may allow both generally lowered metabolic rate (Adamczewska and Morris, 2000, 2001a,b) and water conservation (Greenaway, 1994). Under dry season circumstances in which almost no P is produced there is thus no opportunity for post-renal modification of urine.

Seasonal salt balance

Haemolymph osmotic pressure was approximately 50 mOsm higher in free-ranging *G. natalis* crabs during the dry season (805 mOsm) compared with those in the wet season. This was not a large difference when compared with ~1100 mOsm in red crabs during an unseasonably dry breeding migration (Greenaway, 1994). Osmotic pressure in red crabs on Christmas Island typically ranges between 670 mOsm and 810 mOsm (Adamczewska and Morris, 2000, 2001b) and is somewhat below this range in laboratory-held crabs (Taylor and Greenaway, 2002).

The antennal glands are of low importance in ion regulation except for some Ca reabsorption. The dry season increase in osmotic pressure was generally reflected in increased haemolymph ion concentration. The lower osmotic pressure, [Na] and [Mg] in both haemolymph and urine of confined

versus free-ranging red crabs may be explained by a relatively higher drinking rate without a corresponding increase in clearance in the former.

The Na space of dry season crabs tended to be greater than that in the wet season crabs, particularly in the confined crabs, but this was not reflected in the total body Na. While the Na space values were similar, the trend is opposite to that previously found when comparing wet and dry conditions (Greenaway, 1994). It is significant that red crabs in the dry season of 1997 had a mean ECFV of 21.9%, similar to the dry season value of 24.3% reported by Greenaway (1994), while in the wet season the ECFV value was much higher at 31.7% and much closer to that of the freshwater amphibious crabs *Holthuisana transversa* (Taylor and Greenaway, 1994) and *Potamonautes warreni* (Morris and van Aardt, 1998). Thus, the Na space is enlarged in the wet season by increases in the relatively Na-rich ECFV. Confined crabs in the dry season had the greatest biological half-life for Na of $t_{½}$=12 days, which is nonetheless considerably shorter than reported previously (Greenaway, 1994). The consequence of the greater Na body content but greater half-life of Na in dry season animals is an efflux that was constant at all times. Greenaway (1994) reported a doubling of Na efflux from 2.31 mmol kg^{-1} day^{-1} to 4.39 mmol kg^{-1} day^{-1} when comparing sampling periods with and without rainfall. The lowest rate in the present study was 8.37 mmol kg^{-1} day^{-1}. The rate of 2.31 mmol kg^{-1} day^{-1} was associated with a water turnover rate of 71 mmol kg^{-1} day^{-1}, which was also lower than the *in situ* rates reported here, as too are the clearance rates. If the urine represents a major route for Na loss, then reduced clearance and increased urine ingestion would promote Na retention (reduced efflux). Crabs during the dry season appear to be primarily in water conservation mode and to balance evaporative water loss by drinking whatever water is available in addition to almost all of their own urine. The evidence is that crabs held under laboratory conditions are most like dry season crabs but even more like those held in P-chambers. Red crabs in the wet season appear to experience an excess of water that must be excreted without excessive loss of ions. A situation of unavoidable high water throughput, and thus high clearance and thereby high potential salt loss, would be that most requiring branchial urine reprocessing; i.e. during the wet season.

Pharmacological trials

Pharmacological trials on confined *G. natalis* had few effects on whole-body indices of water and salt management. The elevation of clearance rate promoted by serotonin was apparent only during the dry season but occurred even when the crabs were deprived of drinking water. It seems likely that very high clearance rates during the wet season (>150 ml kg^{-1} day^{-1}) represent maximum rates to deal with super-availability of water. If so, the capacity for serotonin to promote an increase in clearance will exist only in dry season crabs when clearance rate is inherently lower.

Water flux over 24 h in crabs in P-chambers was low [29.5 ml kg^{-1} day^{-1} (FW) and 41.4 ml kg^{-1} day^{-1} (SW)] compared with control crabs (132.7 ml kg^{-1} day^{-1}). In the SW group, the total body water declined to 53.9% compared with 67.1% in free-ranging crabs and to 63.0% in FW crabs. These lowered flux rates were very similar to those of Greenaway (1994) and indicate a rapid response by red crabs on being moved into the experimental chambers. Resolving this response will require further *in situ* field investigations of water movements in unconfined crabs. Nonetheless, acclimating the crabs to drinking 50% SW resulted in almost zero P production and thus removed any scope for further downregulation of branchial ion reabsorption. In the laboratory, increased salinity of the drinking water of red crabs produced a dynamic change in the volume of P over 5 days (fig. 1 in Taylor and Greenaway, 2002). In the FW crabs, both serotonin and dopamine also reduced the volume of P released, presumably partly due to re-ingestion of the urine. The present *in situ* study was unable to establish any additional effect of dopamine on urine production (Taylor and Greenaway, 2002; but see discussion of passive permeability below).

Branchial mechanisms

The branchial perfusion experiments confirmed FW-acclimated red crabs to be in a very different physiological state to those acclimated to drinking 50% SW. The marked effect of serotonin in increasing net branchial Na uptake by FW *G. natalis* (Morris, 2001) was confirmed, as was the absence of any effect of either dopamine or db-cAMP (Taylor and Greenaway, 2002). The upregulation by serotonin in red crabs is similar in principle to that in osmoregulating marine brachyuran crabs (Morris, 2001 for review) but differs in detail. A dopaminergic, cAMP-mediated upregulation of branchial ion pumping appears ubiquitous in the aquatic brachyuran species (Lohrmann and Kamemoto, 1987; Sommer and Mantel, 1988, 1991; Bianchini and Gilles, 1990; Detaille et al., 1992; Morris and Edwards, 1995). Other than the work of Trausch et al. (1989) on *Eriocheir sinesis*, there has been little suggestion of a role for serotonin but this too was dependent on a cAMP-activated protein kinase. Red crabs without access to seawater utilize serotonin as a primary messenger, but independently of cAMP, and thus appear quite different to their marine ancestors.

The serotonin-induced elevation in branchial J_{net} for Na was due to an increase in J_{in}, and none of the treatments altered J_{out} in FW crabs. The importance of the serotonin message to the branchial Na pumps in *G. natalis* is clear in the large response of the branchial Na^+/K^+-ATPase. There is little doubt as to the pivotal regulatory role of the branchial pumping system (e.g. Asselbourg et al., 1991; Mo et al., 1998; Towle et al., 2001; Henry et al., 2002).

G. natalis almost completely cease branchial Cl uptake when given 70% seawater to drink (Taylor and Greenaway, 2002). A similar response was evident in the Na uptake of *B. latro* drinking seawater (Taylor et al., 1993). On Christmas Island, *G. natalis* showed a less extreme downregulation, possibly since the water was less saline and also because there was some

rainfall during the acclimation period. The reduced uptake was clearly not as any result of decreased J_{in} since, somewhat unexpectedly, the pumping component of the branchial epithelia remained unchanged. Instead, the lowered J_{net} was consequent on increased Na leak permeability, i.e. in J_{out}. The branchial Na^+/K^+-ATPase activity was likewise undiminished by SW acclimation, which strongly supports this conclusion.

A relatively small upregulation of Cl uptake by *G. natalis* was reported by Taylor and Greenaway (2002). The upregulation of branchial Na J_{net} by dopamine in *G. natalis in situ* was not due to any change in unidirectional influx of Na since J_{in} remained at the rate found in FW- and SW-acclimated control crabs. The elevation in J_{net} was entirely due to amelioration of the elevated Na leakage (J_{out}) in SW crabs and most likely due to effects on paracellular conductances. An almost identical response was determined for serotonin in SW red crabs. The net result in both cases was to return J_{net} to close to that in FW control crabs. This rate was well below the elevated rate caused by serotonin in FW crabs. In the SW crabs there was some evidence that the effects on J_{out} may be mediated by cAMP but that cAMP may also be involved in downregulation of Na influx, but in such way that J_{net} is unchanged.

The mechanisms of salt reclamation from the urine of Christmas Island red crabs are considerably more complex than described by preliminary models (Morris, 2001; Greenaway and Taylor, 2002). Serotonin has a potentially adaptive role in stimulating branchial Na^+/K^+-ATPase and branchial Na uptake from the urine in crabs with low Na intake. *G. natalis* drinking freshwater and with a low Na diet can produce a very dilute P (Greenaway, 1994), suggesting that the branchial uptake of Na should be close to maximum with little capacity for serotonin to stimulate uptake. The experimental crabs in the rainforest had access to the normal leaf-litter diet and reduced the osmotic pressure of the P from 392 mOsm in SW crabs to 73 mOsm in FW crabs (~90% absorption relative to the haemolymph) compared with more than 97% Cl reabsorption exhibited in the laboratory (Taylor and Greenaway, 2002). Thus, in the present study, the wet season animals, with their high turnover rates, apparently still had significant unutilized uptake capacity that could be activated by serotonin.

In SW-acclimated animals, branchial uptake mechanisms would be expected to be proportionately less active. In other words, uptake would be 'turned down' and thus increase the capacity for biogenic stimulation and upregulation. The mechanisms revealed are inconsistent with this simple model. Decreased net uptake in SW-acclimated crabs is not achieved by any acclimatory downregulation of Na^+/K^+-ATPase or of Na J_{in} but by an increased loss. The stimulatory effect of serotonin is blocked in SW red crabs and replaced by modulation of leak permeability. In this regard, improving net salt uptake by reducing loss with no increase in pumping work seems advantageous.

There appear to be two suites of responses. Small alterations in leak permeability and thus J_{net} might represent very straightforward mechanisms for assisting the voiding of excess salt gained perhaps from the diet. The serotonergic responses of FW-acclimated animals seem useful if they are elicited in addition in crabs with very low dietary Na uptake and in crabs that must promote urinary salt reclamation beyond that routinely required.

The terrestrial gecarcinid was established to be quite different to the anomuran *B. latro* and this is consistent with a separate evolution to life on land (Morris, 2001, 2002; Taylor and Greenaway, 2002; Greenaway, 2003). However, the branchial reprocessing system in *G. natalis* is not otherwise very similar to that in marine crabs or, consequently, to that of a putative marine ancestor. The multilevel response and the complex interaction of changes in branchial Na pumping and modulated Na leakage may well be derived from a marine species but provide features especially suited to life on land where salt is usually limiting and water must be conserved without excessive salt loss. Work on other terrestrial species and their nearest aquatic relatives is urgently required to resolve both the function and phylogenetic origins of these control mechanisms.

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