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NEUROENDOCRINE REGULATION OF OSMOREGULATION AND THE EVOLUTION OF AIR-BREATHING IN DECAPOD CRUSTACEANS

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Summary

Gills are the primary organ for salt transport, but in land crabs they are removed from water and thus ion exchanges, as well as CO_2 and ammonia excretion, are compromised. Urinary salt loss is minimised in land crabs by redirecting the urine across the gills where salt reabsorption occurs. Euryhaline marine crabs utilise apical membrane branchial Na^+/H^+ and Cl^-/HCO_3^- exchange powered by a basal membrane Na^+/K^+-ATPase, but in freshwater crustaceans an apical V-ATPase provides for electrogenic uptake of Cl^- in exchange for HCO_3^-. The HCO_3^- is provided by carbonic anhydrase facilitating CO_2 excretion while NH_4^+ can substitute for K^+ in the basal ATPase and for H^+ in the apical exchange. Gecarcinid land crabs and the terrestrial anomuran *Birgus latro* can lower the NaCl concentration of the urine to 5 % of that of the haemolymph as it passes across the gills. This provides a filtration–reabsorption system analogous to the vertebrate kidney.

Crabs exercise hormonal control over branchial transport processes. Aquatic hyper-regulators release neuroamines from the pericardial organs, including dopamine and 5-hydroxytryptamine (5-HT), which via a cAMP-mediated phosphorylation stimulate Na^+/K^+-ATPase activity and NaCl uptake. Freshwater species utilise a V-ATPase, and additional mechanisms of control have been suggested. Crustacean hyperglycaemic hormone (CHH) has now also been confirmed to have effects on hydromineral regulation, and a putative role for neuropeptides in salt and water balance suggests that current models for salt regulation are probably incomplete.

In a terrestrial crabs there may be controls on both active uptake and diffusive loss. The land crab *Gecarcoidea natalis* drinking saline water for 3 weeks reduced net branchial Na^+ uptake but not Na^+/K^+-ATPase activity, thus implying a reduction in diffusive Na^+ loss. Further, in *G. natalis* Na^+ uptake and Na^+/K^+-ATPase were stimulated by 5-HT independently of cAMP. Conversely, in the anomuran *B. latro*, branchial Na^+ and Cl^- uptake and Na^+/K^+-ATPase are inhibited by dopamine, mediated by cAMP. There has been a multiple evolution of a kidney-type system in terrestrial crabs capable of managing salt, CO_2 and NH_3 movements.

Key words: land crab, ion regulation, air-breathing, monoamine, osmoregulation.

Introduction

The maintenance of salt and water balance is clearly a different challenge for air-breathing animals compared to aquatic species, while for aquatic species, living in fresh water presents far greater ionic and osmotic problems than life in sea water. The evolution to life on land must pass through a transitional phase in which the animals are truly amphibious species and must survive alternately in air and water.

Crustaceans provide a spectrum of extant study species, from fully aquatic through a range of amphibious to fully terrestrial. The evolution of air-breathing has occurred in several crustacean lineages including both the brachyuran and anomuran decapod crabs (Hartnoll, 1988). Amphibious crustaceans are found in intertidal regions, brackish water estuaries and in freshwater lakes and rivers, and it appears that crabs have moved into the terrestrial habitat both directly from the ocean, via the intertidal, and through a variety of freshwater habitats (Greenaway, 1988). However, even the most terrestrial crustaceans remain relatively permeable to water (Greenaway, 1994) and are restricted in their activity to periods of relatively high humidity.

Wolcott observes and comments: “Because little has appeared on the endocrine control mechanisms for water and salt balance since the work of Dorothy Bliss and her colleagues, my focus will be at the whole-animal level” (Wolcott, 1992; see Bliss, 1968, for a review). There were important studies in that interim period (e.g. Savage and Robinson, 1983; Mantel, 1985; Charmantier et al., 1984) but during the 10 years since Wolcott made this observation there have been notable investigations into the possible control mechanisms, and both generalisations and newly defined questions have emerged, especially in relation to the radiation of crabs onto land.

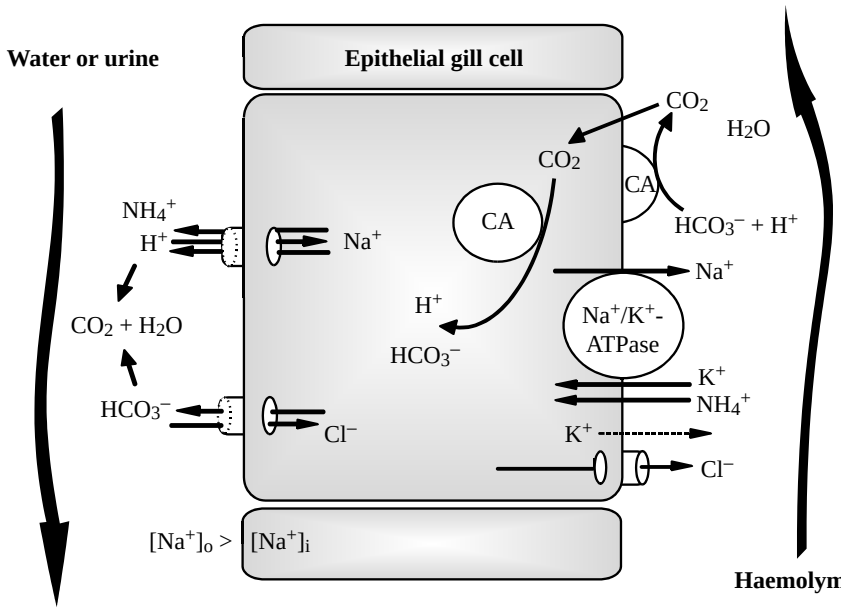

Fig. 1. Model of gill epithelial ion exchange mechanisms typical for a marine euryhaline crab, but also the basic model for terrestrial crustaceans of marine ancestry, showing the apical exchange of HCO_3^- for Cl^- and H^+ for Na^+ and linkage between the CO_2 and ammonia excretion systems and salt uptake. CA, carbonic anhydrase.

In marine crabs the gills represent the primary organ for salt transport, while in freshwater species this branchial pumping is supplemented by salt reclamation from the urine within the antennal organs. The gills of freshwater crabs must work considerably harder than those of marine crabs at maintaining body salt concentrations against a high outward gradient for diffusive salt loss. Removing the gills from contact with water, by moving into air, prevents any branchial ion exchange with water. In addition, the other branchial exchanges linked to the ion pumping, such as CO_2 and NH_3/NH_4^+ excretion, and thereby acid–base balance, are also severely compromised. Terrestrial crabs must either satisfactorily deal with branchial ion exchange so that these other processes can continue, or separate them from their linkage with salt balance.

The different physical properties of air and water as respiratory media require morphological and physiological adaptations for gas exchange. Terrestrial crabs have developed progressively more elaborate lungs while their gill area has decreased from near $1000\,mm^2\,g^{-1}$ body mass to less than $200\,mm^2\,g^{-1}$ body mass. These lungs are formed by the elaboration and vascularisation of the lining of the branchial chamber (e.g. Farrelly and Greenaway, 1987; Farrelly and Greenaway, 1993). Gills are retained in air-breathing crabs as an important part of the ion-regulatory system. Some of the more terrestrial crab species are capable of redirecting their urine from the antennal gland to the branchial chambers where salt reabsorption occurs across the gills (Wolcott and Wolcott, 1985; Wolcott and Wolcott, 1991; Greenaway and Morris, 1989; Wolcott, 1992; Morris et al., 1991; Morris et al., 2000). Ghost crabs additionally utilise the ion-exchange mechanisms within the antennal gland to retain salts (DeVries and Wolcott, 1993; DeVries et al., 1994). A variety of mechanisms have been adopted to maintain CO_2 and nitrogen excretion, involving both the antennal gland (e.g. DeVries and Wolcott, 1993) and the gills (e.g. Varley and Greenaway, 1994) in different species (see below).

Branchial mechanisms in aquatic species

Marine crustaceans are essentially isosmotic with sea water and the primary osmolytes in the haemolymph are Na^+ and Cl^-; water and ion fluxes are thereby minimised. Euryhaline marine species are capable of penetrating into estuaries and brackish water and employ branchial pumping mechanisms to maintain ion balance (Lucu, 1990). The generally accepted model for the pump system in the gill epithelial cells includes Na^+/H^+ and Cl^-/HCO_3^- exchangers that direct Na^+ and Cl^- into the cell over the apical membrane (Fig. 1) (see Péqueux and Gilles, 1988, for a review). In circumstances where the external Na^+ concentrations are greater than those of the cytoplasm, hyper-regulation in diluted sea water is apparently powered by a basal membrane Na^+/K^+-ATPase (Fig. 1).

A number of recent studies have confirmed the importance and role of the Na^+/K^+-ATPase. For example, in *Carcinus maenas* transferred to dilute sea water the activity of Na^+/K^+-ATPase in the posterior gills increased fourfold, partly due to synthesis of new ATPase protein (Lucu and Flik, 1999). Similarly, in the intertidal *Hemigrapsus nudus* and *Leptograpsus variegatus* the ATPase approximately doubled in activity when the crabs were exposed to 50 % sea water (Corotto and Holliday, 1996; Cooper and Morris, 1997). Thus there is a clear link between the extent of hyper-regulation and the activity of the basal membrane pump.

The mechanisms for branchial Na^+ and Cl^- transport in freshwater crustaceans are less clear. When the external NaCl concentration becomes significantly less than that of the epithelial cells then simple apical exchange of H^+ for Na^+ must cease, thereby making an electrogenic motive force necessary. However, recent models (Fig. 2) seem to agree on some basic components. For example, studies of the Chinese mitten crab

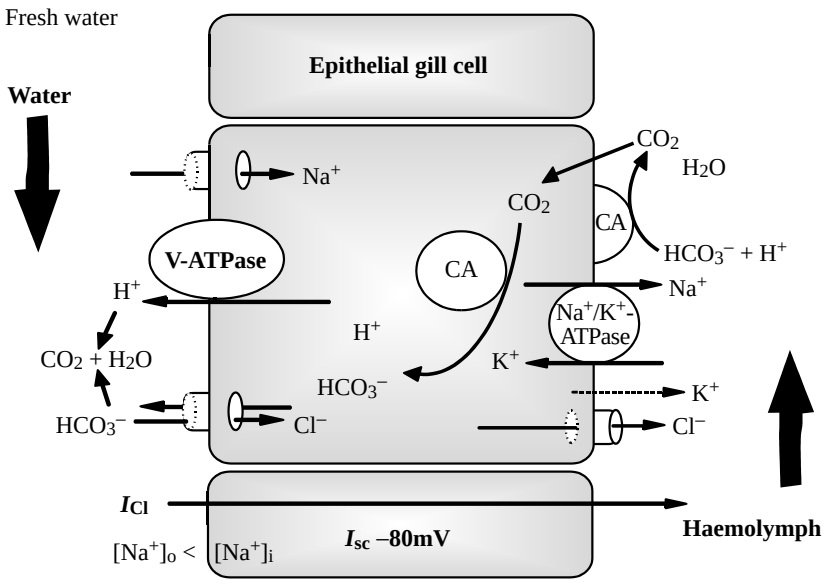

Fig. 2. Generalised model for branchial transport mechanisms in freshwater decapods, based largely on findings for *Eriocheir sinensis* (Onken and Putzenlechner, 1995), showing the requirement for an apically located V-ATPase to actively extrude H^+ into water low in Na^+. The model creates an electrical gradient for Cl^-, which exchanged for HCO_3^- facilitates CO_2 excretion. CA, carbonic anhydrase.

Eriocheir sinesis (Onken et al., 1991; Riestenpatt et al., 1994; Onken and Putzenlechner, 1995) all concluded that Cl^- transport was electrogenic (I_{Cl}=−80 mV) and driven by an apical V-ATPase pumping H^+ into the water independently of Na^+ uptake. These investigators all agreed that the basal Na^+/K^+-ATPase was important in extruding into the haemolymph Na^+ that entered into the branchial epithelial cells via apical Na^+ channels (Fig. 2). There have been worthwhile attempts to produce models for V-ATPase-supported Na^+ transport, for example in crayfish (Zare and Greenaway, 1998). However, these incorporate spatial separation, into separate cellular compartments, of apical Cl^- and Na^+ transport and of H^+ extrusion and HCO_3^- exchange, and thus require considerably more experimental support.

Respiratory gas exchange by aquatic crustaceans is driven primarily by O_2 uptake, and CO_2 excretion occurs primarily as HCO_3^- through the apical HCO_3^-/Cl^- exchanger (see Mantel and Farmer, 1983; Truchot, 1983; Henry and Wheatly, 1992, for reviews). In marine crabs the acid–base regulation is carried out by the same posterior gills as employed in ion regulation, while freshwater crabs use all gills (Henry and Wheatly, 1992). The excretion of CO_2 from the haemolymph over the branchial epithelia is facilitated by carbonic anhydrase (CA) (Figs 1, 2). Two populations of CA are involved: one is on the basal membrane to accelerate the dehydration of HCO_3^- to CO_2, which diffuses into the cytoplasm, where a second CA population assists the rehydration to HCO_3^- and H^+ (Henry, 1984; Burnett et al., 1985; Henry, 1988). Thus CO_2 excretion is achieved but also provides the counterions for the Cl^- and Na^+ exchangers, and manages acid–base balance. There have been a number of demonstrations of this linkage. For example, Henry and Cameron (Henry and Cameron, 1982a; Henry and Cameron, 1982b) showed firstly that *Callinectes sapidus* exposed to dilute water increased haemolymph pH and HCO_3^- concentration, and secondly, that the activity of CA was elevated in the posterior gills of crabs in dilute water. *C. sapidus* living in 85 % sea water contained CA within gill number 6 capable of producing approximately 13 mmol CO_2 g^{-1} min^{-1}, whereas those acclimated to 10 % sea water could produce almost 28 mmol CO_2 g^{-1} min^{-1} (Henry and Cameron, 1982b). Essentially the same results were obtained from hyper-regulating *Callinectes similis* in which both the Na^+/K^+-ATPase and the CA content of posterior, but not anterior, gills increased markedly (Piller et al., 1995). Thus, hyper-regulation requires not only elevated Na^+/K^+-ATPase but also increased CA to provide counterions, with consequences for acid–base status.

The situation is somewhat different in freshwater crustaceans since not only are all the gills involved in branchial ion exchange but the antennal gland also reabsorbs ions to produce a hypo-ionic urine (Fig. 3). The antennal gland of crayfish is rich in Na^+/K^+-ATPase and CA (Wheatly and Henry, 1987), which facilitates the ion reabsorption up to threefold greater than unidirectional branchial influx (Wheatly and Toop, 1989). Freshwater brachyurans also show pronounced activity in the antennal gland and, for example, *Potamomautes warreni* excretes less than 5 % of the urine produced as a filtrate in the antennal gland and reclaims up to 95 % of the urinary salts (Morris and van Aardt, 1998).

Aquatic crustaceans are ammonotelic and excrete NH_3/NH_4^+, primarily across the gills (see Greenaway, 1991, for a review). At physiological pH, approximately 99 % of the ammonia in the haemolymph is as the ion, NH_4^+, and thus the partial pressure of NH_3 is normally very low. Excretion of nitrogenous waste across the gills as NH_4^+ has immediate implications for both branchial ion exchange and for CO_2/acid–base status (Fig. 1). The NH_3 is highly soluble and would diffuse easily outward over the gills, but the partial pressure gradient is insufficient to drive significant gaseous excretion. More likely is the substitution of NH_4^+ for K^+ in the basal membrane Na^+/K^+-ATPase (Towle and Holleland, 1987) so that NH_4^+ is actively moved into the epithelial cell, linking nitrogen excretion to Na^+ regulation (Fig. 1). There is some evidence that the cytosolic NH_4^+ can also substitute for H^+

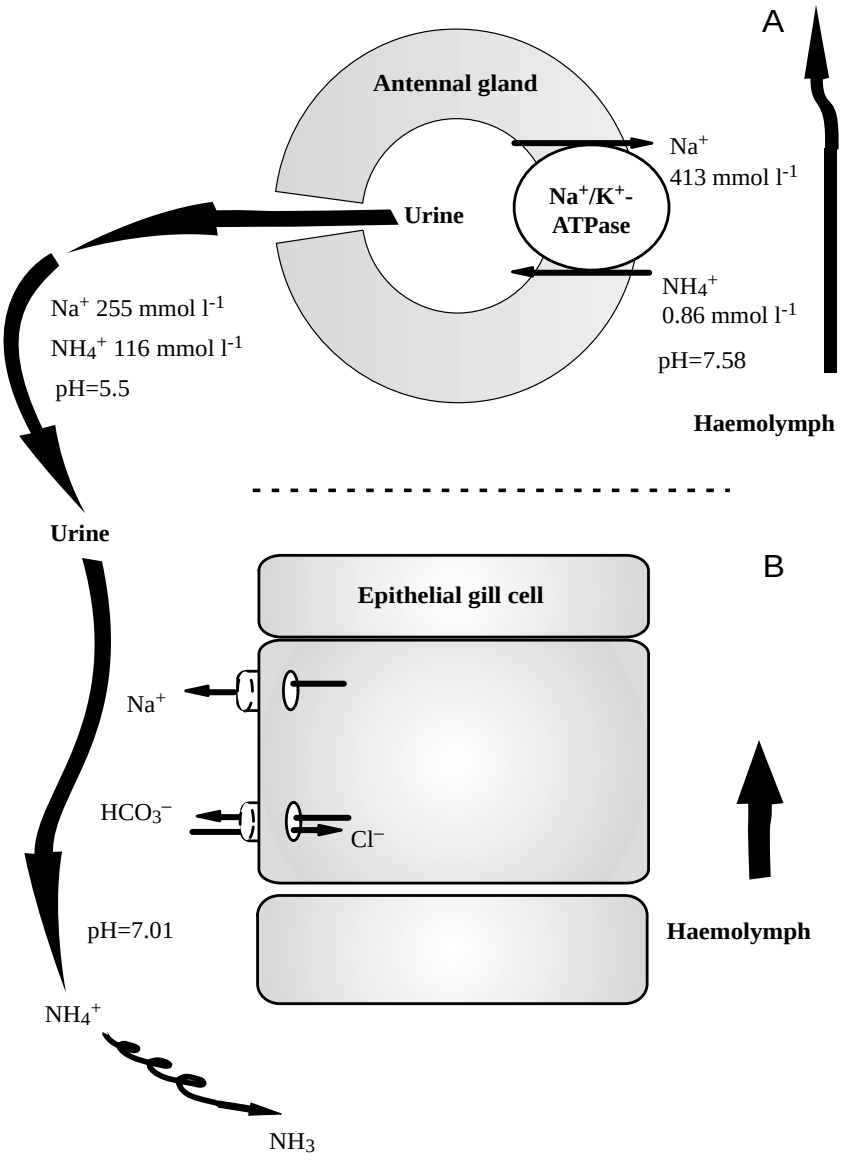

Fig. 3. Two part model. (A) The antennal gland and (B) the branchial epithelial layer. In many freshwater decapods the antennal gland apparently utilises a Na^+/K^+-ATPase to reclaim Na^+ from the urine and thereby other ions, to produce a hypo-ionic urine. In species such as ghost crabs NH_4^+ substitutes for the K^+ and facilitates NH_3 excretion into an acidic urine, which traps NH_4^+. During passage over the gills the transport of base equivalents into the urine raises the pH and promotes the volatilisation of a large part of the NH_4^+ as NH_3 at the same time as facilitating CO_2 excretion (DeVries and Wolcott, 1993; DeVries et al., 1994).

in the apical H^+/Na^+ exchangers of marine crabs (e.g. Hunter and Kirschner, 1986) and thereby escape to the water. However, this relies on the entry of Na^+, via Na^+ channels, over the apical membrane. Clearly this is impossible without any extra-corporeal water and at least very difficult in fresh or very dilute sea water with low Na^+ concentration. Greenaway (Greenaway, 1991) hypothesised on the basis of the findings of Krippeit-Drews et al. (Krippeit-Drews et al., 1989) that the apical H^+ pump, the V-ATPase, might have a role in freshwater crabs. Thus, the H^+ excretion required to drive salt uptake could lower the cytosolic availability of H^+ to the extent that NH_4^+ could become deprotonated and the diffusive excretion of NH_3 promoted.

Consequences of breathing air and the significance of urine reprocessing

The adoption of air-breathing removes the gills from water and compromises the processes of, and the linkages between, salt balance, CO_2 excretion, acid–base balance and nitrogen excretion. Freshwater crustaceans are in many ways 'pre-adapted' to life on land (e.g. Wolcott, 1992). The use of the antennal gland to produce of a hypoionic urine minimises salt loss, while low urine volumes can conserve water (Fig. 3), both of which seem useful attributes for animals moving into air (e.g. Morris and van Aardt, 1998). There remain substantial problems, however, with respect to CO_2 excretion and the removal of nitrogenous wastes. Marine crabs with their almost complete reliance on branchial processes face additional difficulties of water and salt loss when moved into air.

There is now quite good evidence that progressively more terrestrial crustaceans possess lungs with increasingly higher concentrations of carbonic anhydrase (Randall and Wood, 1981; Morris and Greenaway, 1991; Henry, 1990; Morris et al., 1996). The lungs of crabs are formed from the branchiostegal linings, which have become progressively more vascularised to act as gas exchange organs (see Burggren and McMahon, 1988;

Greenaway and Farrelly, 1990, for reviews). The CA occurs within the membrane (Morris and Greenaway, 1990) and apparently also the cytosol of pulmonary epithelia (Henry, 1991). Terrestrial crabs have elevated haemolymph P_{CO_2} compared to aquatic species (see Burggren and McMahon, 1988, for a review), but this results from the increased ventilation requirement for CO_2 excretion into air compared to water rather than representing an increased gradient for improved diffusive loss. The rate-limiting step in pulmonary CO_2 excretion would appear to be the conversion of HCO_3^- to molecular CO_2, the process catalysed by CA. For example, the CA in cytosol and the cell membranes of the branchiostegal lining of *Callinectes sapidus* turned over approximately 270–290 μmol l^{-1} CO_2 min^{-1}, but in *Gecarcinus lateralis* these rates increased to nearly 3000 and 1800 μmol l^{-1} CO_2 min^{-1}, respectively, and to over 3000 μmol l^{-1} CO_2 min^{-1} in *Birgus latro* (Henry, 1991). In *Birgus latro*, at least, there is good evidence that CO_2 excretion is moved, in large part, away from the gills to the lungs (Greenaway et al., 1988).

The retention of nitrogenous waste until such time as the crab can immerse is one possibility, and seems to be adopted by species of both marine and freshwater origins. In amphibious river crab *P. warreni* the excretion of nitrogen is clearly as branchial NH_4^+ transport, and during several days of air-breathing N excretion was minimal (Morris and van Aardt, 1998). Similar results have been obtained for *Cardisoma hirtipes* (Dela-Cruz and Morris, 1997) and *Cardisoma carnifex* (Wood et al., 1986). All these species show a pulse of elevated N excretion when immersed. Although the urine of *Cardisoma* may initially contain as much as 5 mmol l^{-1} NH_4^+, the release of urine quickly slows to near zero, as part of water conservation, preventing any further excretion. This is not a viable strategy for long-term terrestrial excursions.

Urine represents a major source of salt loss in land crabs (see Greenaway, 1988; Wolcott, 1992, for reviews). Gercarcinid land crabs such as *Gecarcinus lateralis* (Wolcott and Wolcott, 1984) and *Gecarcoidea natalis* and the anomuran *Birgus latro* (Table 1), as well as ghost crabs *Oycpode quadrata* (Wolcott and Wolcott, 1985) all reprocess their primary urine to produce an altered, hypo-osmotic final product 'P'. The urine is passed from the opening of the antennal gland into the branchial chambers where branchial uptake mechanisms reabsorb the required salt (Morris et al., 1991; Taylor et al., 1993; Morris et al., 2000), often lowering the NaCl concentration to 5% of that of the haemolymph. Essentially the ion-exchange mechanisms that were employed by their aquatic ancestors have been modified to conduct similar exchanges with their own urine (Fig. 1).

The primary filtration in the antennal gland and reabsorption in the branchial system is reminiscent of the vertebrate kidney and at least creates the possibility of both CO_2 and NH_3^+ excretion into the urine passing over the gill epithelia (Fig. 1). This strategy is in fact adopted by both *G. lateralis*, which increased the NH_4^+ content of the urine by tenfold as it passed over the gills (Wolcott, 1991), and *G. natalis*, which increased more than 25-fold during urine reprocessing (Greenaway and Nakamura, 1991). Thus, NH_4^+ clearance and salt reclamation are simultaneously facilitated. The same apical gill epithelial exchange systems seem to be used by other terrestrial species such the grapsid *Geograpsus grayi* (Fig. 4). In this species Na^+/NH_4^+ exchange is employed to excrete ammonia into the branchial fluid, the pH of which is then raised, apparently by HCO_3^- exchanged for Cl^-, thereby volatilising the ammonia as NH_3 at the same time as excreting CO_2 (Varley and Greenaway, 1994).

Table 1. *The osmotic pressure in the haemolymph, urine and final excretory product of the anomuran* Birgus latro *and brachyuran* Gecarcoidea natalis *land crabs after a week of drinking either fresh water or diluted sea water*

		Osmotic pressure (mosmol l^{-1})		
	Drinking water	Haemolymph	Urine	Excretory product
Birgus latro[a]	FW	768±17	787±9	119±13‡,§
	60% SW	821±9*	815±19*	786±25*
Gecarcoidea natalis[b]	FW	825±17	855±19	73±18‡,§
	50% SW	948±9*	992±21*	392±67*,‡

FW, fresh water; SW, sea water.

Values are means ± S.E.M.; N=7 or 8.

*Effect of drinking water salinity; ‡difference compared to haemolymph; §difference compared to urine.

[a]Taylor et al., 1993; [b]S. Morris, unpublished observations.

While the gecarcinid, grapsid and even freshwater Potamoidea conform to the principle that crabs rely on branchial excretion of NH_3/NH_4^+, it appears that the ocypodids do not (Fig. 3) (DeVries and Wolcott, 1993; DeVries et al., 1994). Concentrations of NH_4^+ in excess of 100 mmol l^{-1} have been measured in the urine of *Ocypode quadrata* (DeVries and Wolcott, 1993), which had a pH 5.5 and clearly worked as an acid NH_4^+ trap (DeVries et al., 1994). Furthermore, the Na^+/K^+-ATPase activity within the antennal gland exceeded that of the gills, while urine Na^+ was markedly lower than Cl^-, leading DeVries et al. (DeVries et al., 1994) to suggest a Na^+-dependent trapping of NH_4^+ in the urine (Fig. 3). Subsequent urine reprocessing by the gills resulted in both increased pH and CO_2, presumably by HCO_3^-/Cl^- exchange, since Cl^- concentrations were approximately halved, promoting the excretion of CO_2 and the volatilisation of NH_3 (Fig. 3; DeVries and Wolcott, 1993).

The anomuran Robber crab, *Birgus latro*, also employs branchial reprocessing of urine for salt reclamation (Table 1), but has managed to unlink nitrogen excretion from either branchial or antennal gland processes by becoming almost entirely purinotelic, with faeces containg large amounts of urate and guanine (Greenaway and Morris, 1989; P. Greenaway, personal communication).

Control of branchial exchange and the mechanism of regulating urine reprocessing

A number of features of the branchial exchange and excretions processes of air-breathing crabs imply important control and regulation mechanisms. For example, the

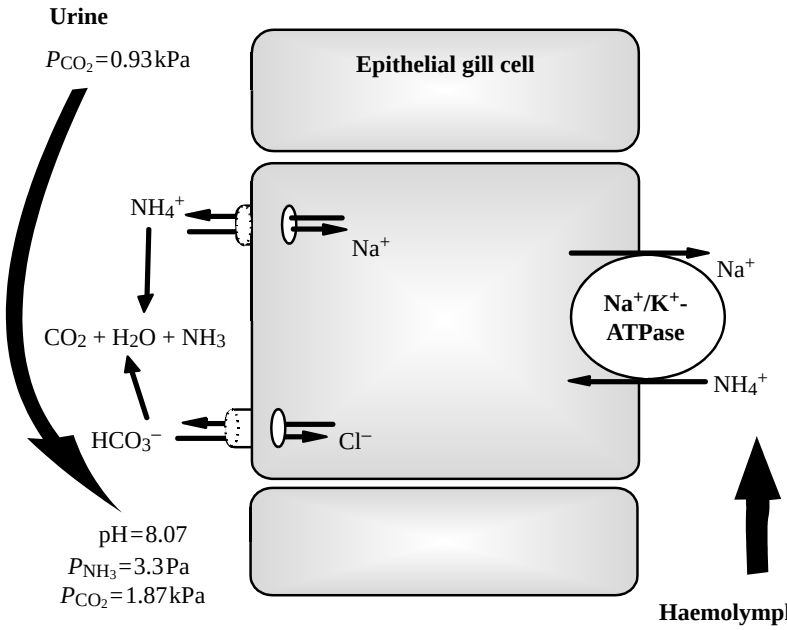

Fig. 4. Demonstrating the putative NH_3 excretion mechanism in the terrestrial carnivore *Geograpsus grayi*. Urine passed over the gills receives NH_4^+ in exchange for Na^+; the urine is then alkalinised by the addition of HCO_3^-, which combines with the NH_4^+ to volatilise both NH_3 and CO_2. The net result is a final excretory fluid with a high P_{CO_2} and P_{NH_3} (Varley and Greenaway, 1994).

volatilisation of NH_3 by *Geograpsus grayi* (Fig. 4) is an acutely discontinuous process (Varley and Greenaway, 1994). Bursts of excretion lasted from 3 h to 3 days, during which ammonia excretion rates exceeded 200 μmol l^{-1} kg^{-1} h^{-1} compared to the overall mean rate of 74 μmol l^{-1} kg^{-1} h^{-1}. Varley and Greenaway (Varley and Greenaway, 1994) speculate that the nitrogen may be stored as non-toxic amino acids and/or purines, and that its release may be dependent on the provision of Na^+ and Cl^- for branchial exchange. Two conclusions may be drawn: that the process is under control and that N^+ excretion in this land crab is dependent on the production of a finite volume of urine that is passed over the gills.

The kidney analogue system of land crabs is also under fine control since anomuran and brachyuran land crabs are able to vary the extent of salt reabsorption from the urine as it passes over their gills (Table 1). *B. latro* and *G. natalis* drinking fresh water reclaimed, respectively, 85 % and 95 % of the salt in their urine (Table 1); but given approximately half-strength sea water to drink, these values decline to 4 % and 54 %. Clearly the crabs must be sensitive to their internal ionic status and modify salt reclamation accordingly.

Since Mantel (Mantel, 1985) and Wolcott (Wolcott, 1992) alerted us to a deficit in our understanding of the controls on branchial exchange and salt regulation there have been further concerted efforts to address this question. The nature of the primary and second messengers have been investigated, as have their targets and mechanisms of action, primarily on aquatic species but more recently with respect to the special problems of terrestrial species.

Among the aquatic crabs the marine *C. maenas* and the euryhaline freshwater *E. sinensis* have become almost exclusive models – a situation that needs addressing in wider, more comparative investigation. Pioneering work on *Callinectes sapidus* by Kamemoto and colleagues (e.g. Kamemoto, 1982; Kamemoto and Oyama, 1985; Lohrmann and Kamemoto, 1987) as well as Mantel (Mantel, 1985) led to a clear appreciation that ionic regulation and the activity of the branchial exchange mechanisms was under neurohormonal control, and most importantly, that the pericardial organs (PO) may be of primary importance (Kamemoto and Oyama, 1985). The POs are neurosecretory axons closely adjacent to the heart, which secret a variety of monoamines, including dopamine, 5-hydroxytryptamine (5-HT) and octopamine as well as peptide hormones such as proctolin (Stangier et al., 1986) and cardioactive peptide (Stangier et al., 1987) into the haemolymph circulatory system. These same authors (Kamemoto and Oyama, 1985) concluded that dopamine and octopamine might stimulate Na^+-uptake by increasing cAMP levels, an effect that has been demonstrated (Lohrmann and Kamemoto, 1987). Neuropeptides may prove to be important, however, since crustacean hyperglyceamic hormone has recently been shown to have marked effects on Na^+ transport in *C. maenas* gills (Spanings-Pierrot et al., 2000).

In *C. maenas* the injection of dopamine (10^{-5} mol l^{-1}) significantly increased the rate constant (*K*) for Na^+ uptake by 0.09, whereas the injection (10^{-6} mol l^{-1}) of the membrane-permeable cAMP analogue dibutyryl cAMP resulted in an elevation in *K* of 0.134, compared to PO extract, which gave a similar increase (0.127) (Sommer and Mantel, 1988). Sommer and Mantel subsequently confirmed the linkage between dopamine and cAMP, since injecting 10^{-5} mol l^{-1} dopamine promoted an increase of 94 % in the cAMP of *C. maenas* gill tissue (Sommer and Mantel, 1991). Importantly, they also showed 75 % and 135 % increases in cAMP concentration in the gills of *C. maenas* acclimated to 40 % sea water compared to 100 % sea water (Sommer and Mantel, 1991), substantiating neuroendocrine involvement as part of the acute hyper-regulatory response of euryhaline crabs. Similarly, injection of dopamine or dibutyryl-cAMP (db-

cAMP) into the intertidal *Leptograpsus variegatus* increased branchial Na^+/K^+-ATPase 67 % and 63 %, respectively (Morris and Edwards, 1995). Pre-injection with IMBX (a phosphodiesterase inhibitor) increased the effect of cAMP, consistent with the role cAMP as second messenger, while pre-injection of the dopamine antagonist, butaclamol hydrochloride, reduced Na^+/K^+-ATPase activity from 0.32 to 0.18 nmol P_i mg^{-1} min^{-1} (Morris and Edwards, 1995). *L. variegatis* also appears to rapidly increase Na^+/K^+-ATPase activity utilising dopamine as a primary and cAMP as the intracellular second messenger and it may be that this feature is ubiquitous in the marine brachyurans. However, while recent work (Lucu and Flik, 1999) also established a link between cAMP and gill membrane Na^+/K^+-ATPase in *C. maenas*, it established a negative correlation between cAMP and ATPase activity, which almost doubles in crabs transferred to dilute sea water. These authors (Lucu and Flik, 1999) concluded that for shore crabs "cAMP is involved in Na^+/K^+-ATPase regulation, yet in a diametrically opposite mode in seawater crabs compared to freshwater crabs". This conclusion is generally inconsistent with the previous studies (described above) with respect to the mechanism of action in freshwater crabs.

The studies of the euryhaline Chinese mitten crab, *Eriochier sinesis*, have provided insight into the mechanism of action, but unfortunately do not reach any general consensus. Detailed investigation of *E. sinesis* gill tissue (Trausch et al., 1989) revealed that the membrane fraction containing Na^+/K^+-ATPase also included dopamine and 5-HT receptors, and that protein phosphorylation, stimulated by the bioamines, occurred only in the presence of the soluble fraction containing a cAMP-dependent protein kinase. Dopamine added to the perfusate of isolated perfused *E. sinesis* gills stimulated Na^+ flux (Bianchini and Gilles, 1990; Detaille et al., 1992). Bianchini and Gilles demonstrated conclusively that cAMP is implicated in transepithelial NaCl transport with the involvement of a protein kinase (Bianchini and Gilles, 1990). The application of protein kinase C inhibitors increased transepithelial Na^+ flux by 250 % and since there was no effect on basal membrane Cl^- flux, this was suggested to be due to effects on the Na^+/K^+-ATPase (Asselbourg et al., 1991). Thus, the prevailing model appeared similar for both hyper-regulating marine and freshwater species (e.g. Fig. 6 of Asselbourg et al., 1991), in which the primary site of action was the basal membrane Na^+/K^+-ATPase, with apical exchange of Na^+ for H^+ and Cl^- for HCO_3^-. However, Bianchini and Gilles recognised some difficulty with this model (Bianchini and Gilles, 1990) since it should have promoted K^+ transport and depolarisation of the epithelium, not the observed hyperpolarisation.

Introducing an apical V-ATPase for H^+ excretion in freshwater crabs provides a different indirect control of Na^+ and Cl^- uptake in *E. sinesis*. Riestenpatt et al. (Riestenpatt et al., 1994; Riestenpatt et al., 1995) proposed that net Cl^- flux is independent of Na^+ flux and is an electrogenic transport driven by the V-ATPase. Application of db-cAMP to isolated perfused *E. sinesis* gills promoted an increase in transcellular Na^+ conductance, not only via increased affinity of apical Na^+ transport channels but apparently also in their number, without any change in the electromotive force (Riestenpatt et al., 1994). At the same time they (Riestenpatt et al., 1994) suggested that Cl^- uptake through Cl^-/HCO_3^- exchange and through the basal Cl^- channels is enhanced by the H^+ extrusion. This addressed the difficulties alluded to by Bianchini and Gilles (Bianchini and Gilles, 1990), but most importantly completely removed the basal Na^+/K^+-ATPase as an important regulatory process and fundamentally questioned the cAMP-mediated phosphorylation of the basal membrane pump as a control mechanism. The nature of the control remains unclear since reexamination of the perfused gills of *E. sinensis* has shown that in Cl^--free media, dopamine or db-cAMP still stimulate Na^+/K^+-ATPase and promoted Na^+ influx (Mo et al., 1998). This leads to the uncomfortable situation where cAMP may act to induce electrogenic Cl^- transport, promote increased affinity and numbers of apical Na^+ channels, and also stimulate basal Na^+/K^+-ATPase and even basal Cl^- channels. Thus, currently the situation remains incompletely resolved.

There is some compelling evidence that the ion-regulatory mechanism may be even more complicated. The pericardial organs, while strongly implicated in hydromineral regulation, are not the only neurohaemal tissues in crabs, and sinus gland of the crustacean eyestalk is an important secretory tissue that has, until very recently, received little attention in this regard (Pierrot et al., 1994; Eckhardt et al., 1995; Spanings-Pierrot et al., 2000). Even simple experiments such as eyestalk ablation show that the sinus gland influences ion balance; for example, in the crayfish *Cherax destructor* the haemolymph $[Na^+]$ declined from 225.9±1.63 to 214±5.04 mmol l^{-1} following eyestalk removal (S. Morris, unpublished). The isolated perfused posterior gills from hyper-regulating *Pachygrapsus marmoratus*, when perfused with extracts from the sinus gland, showed in increase in transepithelial difference of approximately 50 % and elevated Na^+ influx by approximately 150 % (Pierrot et al., 1994; Eckhardt et al., 1995). In addition, perfusing the gills of *C. sapidus* with sinus gland extracts promoted elevated cGMP but not cAMP (Kamemoto and Oyama, 1985). Sinus gland extracts from *P. marmoratus* were deactivated by enzyme digestion and Eckhardt et al. concluded the active factor is a peptide(s) of >5000 Da that directly influences branchial function (Eckhardt et al., 1995). An osmoregulatory function has now been suggested for crustacean hyperglycaemic hormone (CHH), in addition to its role in regulating haemolymph sugar concentration (Spanings-Pierrot et al., 2000). The CHH fraction isolated from eyestalks of *E. sinensis* meets all the characteristics described previously for a putative peptide, and increased Na^+ influx by approximately 50 % (Spanings-Pierrot et al., 2000). These workers (Spanings-Pierrot et al., 2000) identified a second, much less active, compound and concluded that CHH may constitute a major factor involved in the control of osmoregulation in decapod crustaceans. Thus, there may be a separate and different neuroedocrine control system originating with the sinus gland in addition to that of the POs.

There are a considerable number of terrestrial brachyuran

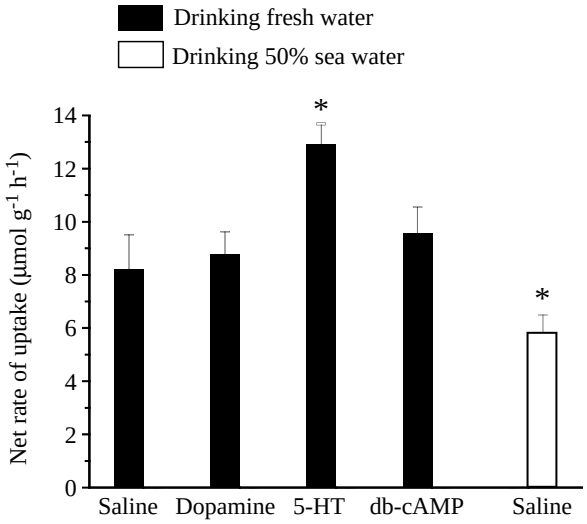

Fig. 5. Net Na^+ uptake by the gills of the Christmas Island red crab *Gecarcoidea natalis* determined by perfusing the branchial chambers with artificial urine (for methods, see Morris et al., 2000). Filled columns are for crabs with a history of drinking fresh water, the normal condition on Christmas Island, and the open column is the rate for crabs after drinking 50 % sea water for 3 weeks. Crabs were injected with saline or one of the following: dopamine, 5-hydroxytryptamine (5-HT) (10^{-4} mol l^{-1} at 1.5 μl g^{-1}) or dibutryl-cAMP (db-cAMP) (6×10^{-4} mol l^{-1} at 1.5 μl g^{-1}) as indicated (N=6). An asterisk indicates a value significantly different from saline control animals drinking fresh water. Values are means ± S.E.M.

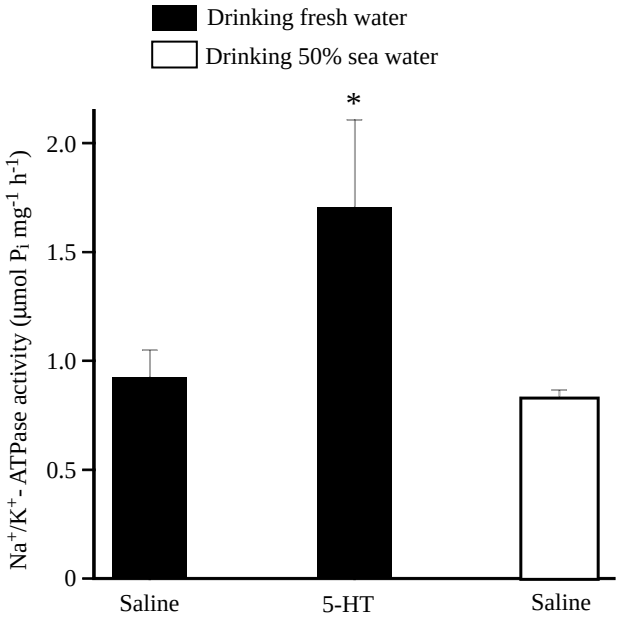

Fig. 6. Branchial Na^+/K^+-ATPase activity in *Gecarcoidea natalis* after 3 weeks of drinking fresh water (filled columns) or 50 % sea water (open columns). Crabs were injected with either saline or 5-hydroxytryptamine (5-HT) (10^{-4} mol l^{-1} at 1.5 μl g^{-1}) (N=6). An asterisk indicates a rate significantly greater than the control rate (for methods, see Morris et al., 2000). Values are means ± S.E.M.

crabs but *Birgus latro* is the sole truly terrestrial anomuran, since the coenobitid hermit crabs rely to a varying extent on the mollusc shells they inhabit. Contemporary investigations of neuroendocrine regulation of hydromineral balance are limited to the brachyuran Christmas Island land crab, *Gecarcoidea natalis* (S. Morris, unpublished) and *Birgus latro* (Morris et al., 2000). Clearly these animals can control ion resorbtion from their urine (Table 1) and in so much as they employ their gills for this purpose then the regulatory systems of the aquatic ancestors may remain available to them.

G. natalis provided with 50 % sea water to drink decrease the net branchial uptake of Na^+ from an artificial urine passed over their gills by 29 % (Fig. 5; for Materials and methods see Morris et al., 2000), but this appears to have little to do with any change in Na^+/K^+-ATPase activity, which remains constant (Fig. 6). The crabs in these experiments were acclimated for 3 weeks to the saline drinking water and a possible conclusion is that long-term changes in Na^+ uptake are governed by an adjustable Na-permeable of the gill epithelia, i.e. crabs with excess dietary salt become more leaky. The branchial uptake mechanisms of *G. natalis* do respond to neurohormonal stimulation, but unlike *Carcinus* (e.g. Sommer and Mantel, 1991) and *Eriochier* (e.g. Mo et al., 1998) neither dopamine (primary messenger) nor cAMP (secondary messenger) had any effect, whereas 5-HT markedly stimulated both net Na^+-uptake (Fig. 5) and Na^+/K^+-ATPase activity (Fig. 6). The influence of 5-HT is not limited to the branchial uptake mechanisms but also stimulates primary urine production, regardless of whether the crab has been provided with drinking water or not (Fig. 7). Depriving the crabs of drinking water reduced urine production by 30 %, but injecting 5-HT (10^{-4} mol l^{-1} at 1.5 μl g^{-1}; approximate initial circulating concentrations, 4×10^{-7} mol l^{-1}) stimulated clearance by 18 % in crabs with drinking water and by 21 % in crabs deprived of water for 3 days (Fig. 7). Thus, in this brachyuran land crab at least, acute adjustments in urine reprocessing can be induced by 5-HT, although the second messenger is not cAMP, which also stimulates urine production. Thus *G. natalis* branchial uptake is submaximal, even when on a normal freshwater drinking supply, but can be increased by stimulating the branchial ATPase. However, long-term adjustments to a relative surfeit of dietary salt seem to be managed by adjusting Na^+ loss rates across the gill epithelium into the urine.

The terrestrial anomuran *Birgus latro* reabsorbs urinary salts, apparently like *G. natalis*, but detailed investigations have revealed fundamental differences in the regulatory processes (Morris et al., 2000). Branchial Na^+ and Cl^- uptake across the gill epithelium of *B. latro* is regulated by dopamine, mediated by cAMP, but importantly this signal causes a decrease in uptake (Morris et al., 2000). For example, dopamine (2×10^{-4} mol l^{-1} at 1.5 μl g^{-1}) reduced Cl^- uptake by 45 % while db-cAMP (6×10^{-4} mol l^{-1} at 1.5 μl g^{-1}) depressed Na^+ uptake by 84 %. Injection of dopamine in *B. latro* elevated endogenous branchial cAMP by 132 % from 164 pmol g^{-1} fresh water to 381 pmol g^{-1} fresh water (Morris et al., 2000). While the mechanism of action was to modulate the activity of

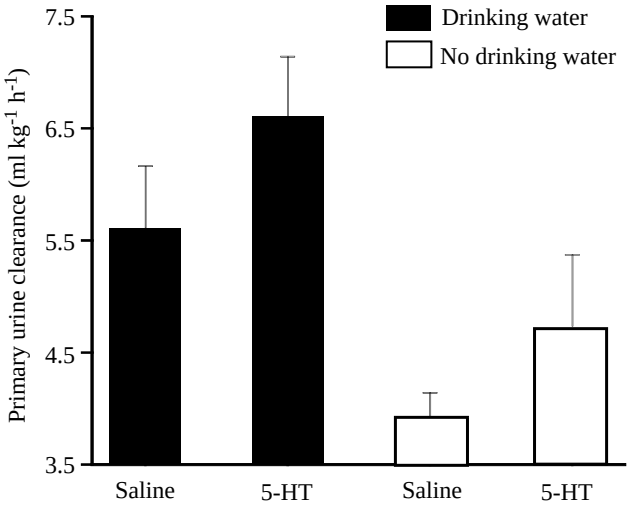

Fig. 7. Primary urine production by *Gecarcoidea natalis* provided with drinking water (filled columns) or deprived of water for 3 days (open columns). The crabs were maintained under field conditions on Christmas Island and were injected with saline or 5-hydroxytryptamine (5-HT) (6×10^{-4} mol l^{-1} at 1.5 µl g^{-1}) every 8 h for 3 days. The urine production rate was determined by the clearance of [^{57}Cr]EDTA (for methods see Morris and van Aardt, 1998) (N=8). Values are means ± S.E.M.

Na^+/K^+-ATPase, as found in aquatic brachyuran crabs (see above; but except for Lucu and Flik, 1999), the direction of modulation was negative. In *B. latro* db-cAMP reduced Na^+/K^+-ATPase activity by 63 % (Morris et al., 2000). Branchial salt uptake in *Birgus* on a normal regime of drinking fresh water is thus considerably above the minimal rate and when supplied by a surfeit of dietary salt, modulates uptake by a negative signal to the basal Na^+/K^+-ATPase and branchial pumping. Thus in the anomuran land crab, urine reprocessing occurs through branchial mechanisms apparently similar to brachyuran land crabs, but which at a cellular level operate in a direction generally opposite those of the other crabs.

Currently it appears that the brachyuran land crabs, of marine origin, have largely inherited and modified the branchial uptake systems of their aquatic ancestors, to provide a filtration–resorbtion system analogous to a kidney. This branchial exchange and salt regulation is linked in many air-breathing species to facilitate CO_2 excretion and both acid-trapping and volatilisation of excretory NH_3. The ion regulation systems of the freshwater Brachyura, notably the active role of the antennal gland, seem important in minimising both urinary water and salt loss but do not appear to provide a kidney analogue in freshwater land crabs. There are currently no published data suggesting hormonal control in antennal gland functioning. The anomuran land crabs with an evolutionary history separate from the brachyuran land crabs, typified by *Birgus latro*, employ a generally similar branchial regulation, but the second message determining ATPase activity is opposite to that in brachyuran land crabs. Importantly, the information obtained from these crustacean studies supports and reinforces the premise (the Malpighian tubule system of the uniramia notwithstanding) that successful evolution of life on land requires a kidney type system capable of managing CO_2 and NH_3 movements, as adjuncts to acid–base control – without excess loss of water and under fine hormonal regulation.

This was written as a tribute to Prof. Jean-Paul Truchot on the occasion of his birthday and retirement, and is dedicated to the memory of Dr Holger Rumpff, without whom and without his support, encouragement and friendship, much of the work on Christmas Island would never have been possible. Thanks go also to the Government Conservators and Staff of Parks Australia (Christmas Island).

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