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## Review

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# Ammonia excretion in aquatic and terrestrial crabs

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### Summary

The excretory transport of toxic ammonia across epithelia is not fully understood. This review presents data combined with models of ammonia excretion derived from studies on decapod crabs, with a view to providing new impetus to investigation of this essential issue. The majority of crabs preserve ammonotelically regardless of their habitat, which varies from extreme hypersaline to freshwater aquatic environments, and ranges from transient air exposure to obligate air breathing. Important components in the excretory process are the Na<sup>+</sup>/K<sup>+</sup>(NH<sub>4</sub><sup>+</sup>)-ATPase and other membrane-bound transport proteins identified in many species, an

exocytotic ammonia excretion mechanism thought to function in gills of aquatic crabs such as *Carcinus maenas*, and gaseous ammonia release found in terrestrial crabs, such as *Geograpsus grayi* and *Ocypode quadrata*. In addition, this review presents evidence for a crustacean Rhesus-like protein that shows high homology to the human Rhesus-like ammonia transporter both in its amino acid sequence and in its predicted secondary structure.

Key words: ammonia excretion, ammonia transporter, crab, exocytosis, Rhesus-like protein, Na<sup>+</sup>/K<sup>+</sup>-ATPase.

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### The ammonia problem

Ammonia<sup>†</sup> (i.e. the total of NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup>: T<sub>Ammon</sub>) is highly toxic in most animals. Hydrated NH<sub>4</sub><sup>+</sup> and K<sup>+</sup> ions have the same ionic radius (Knepper et al., 1989) and due to their K<sup>+</sup>-like behavior, ammonium ions affect the membrane potential for example, in the giant axon of *Loligo pealei* (Binstock and Lecar, 1969) and of mammalian neurons (Cooper and Plum, 1987). In mammals, elevated ammonia causes major damage in the central nervous system, including changes in blood-brain barrier morphology (Laursen and Diemer, 1997). In addition, elevated ammonia levels in mammals have been related to Alzheimer disease (Alzheimer Type II astrocytosis) due to toxic accumulation of glutamine in astrocytes, which leads to cell swelling and cell death (for review see Butterworth, 2002). Also, in microglia and astroglia cell lines, ammonia affects major functional activities, such as phagocytosis and endocytosis. Ammonia also modifies the release of cytokines and increases the activity of lysosomal hydrolases (Atanassov et al., 1994, 1995). Marcaida et al. (1992) speculated that ammonia toxicity is mediated by excessive activation of *N*-methyl-D-aspartate (NMDA)-type

glutamate receptors in the brain. As a consequence, cerebral ATP depletes while intracellular Ca<sup>2+</sup> increases with subsequent increases in extracellular K<sup>+</sup> and finally cell death.

In crustaceans, for example in the lobster *Homarus americanus* (Young-Lai et al., 1991) and the crayfish *Pacifastacus leniusculus* (Harris et al., 2001), elevated ammonia levels in low-salinity media disrupt ionoregulatory function. Exposure of the green shore crab *Carcinus maenas* to 1 mmol l<sup>-1</sup> total ammonia leads to increased ion permeability and salt flux across the gill; higher concentrations reduce both variables (Spaargaren, 1990). In fish, branchial gas exchange and oxidative metabolism are disturbed by excess ammonia (Wilkie, 1997).

An effective ammonia detoxification or excretion system is, therefore, essential to maintain cellular functions, and to keep cellular and body fluid ammonia levels within a tolerable range. In most species, including mammals (Cooper and Plum, 1987), fish (Wood et al., 2002) and aquatic crabs (Cameron and Batterton, 1978; Weihrauch et al., 1999), the ammonia concentration of the body fluids is typically low (50–400 μmol l<sup>-1</sup>; Table 1). Concentrations exceeding 1 mmol l<sup>-1</sup> total ammonia (NH<sub>3</sub>+NH<sub>4</sub><sup>+</sup>) are usually toxic to mammalian cells (Hrnjez et al., 1999). In crustaceans,

<sup>†</sup>In this review, NH<sub>3</sub> refers to molecular ammonia, NH<sub>4</sub><sup>+</sup> to ammonium ions, and ammonia to the sum of both.

Table 1. *The concentration of ammonia in the hemolymph of selected crab species*

Species	Ammonia (mmol l <sup>-1</sup> )	Source
Aquatic		
<i>Callinectes sapidus</i>	0.83	Kormanik and Cameron (1981)
<i>Cancer pagurus</i>	0.22	Regnault (1994)
	0.08	Weihrauch et al. (1999)
<i>Carcinus maenas</i>	0.08	Durand and Regnault (1998)
<i>Eriocheir sinensis</i>	0.12	Weihrauch et al. (1999)
<i>Maia squinado</i>	0.05–0.07	Durand et al. (2000)
<i>Necora puber</i>	0.12	Durand and Regnault (1998)
Air-breathing		
<i>Austrothelphusa transversa</i>	0.3–0.5	Linton and Greenaway (1995)
<i>Cardisoma carnifex</i>	1.6	Wood et al. (1986)
<i>Discoplax hirtipes</i>	1.66–2.31	Dela-Cruz and Morris (1997)
<i>Gecarcoidea natalis</i>	1.46	Greenaway and Nakamura (1991)
<i>Geograpsus grayi</i>	1.92	Greenaway and Nakamura (1991)
	1.18–2.07	Varley and Greenaway (1994)
<i>Ocypode quadrata</i>	0.86	De Vries et al. (1994)
<i>Potamonautes warreni</i>	0.2–0.3	Morris and van Aardt (1998)

environmental exposure of ammonia is lethal at relatively low doses. For instance, LC<sub>50</sub> after 96 h of exposure was determined in the crayfish *Orconectes nais* at 186 µmol l<sup>-1</sup> NH<sub>3</sub> (Hazel et al., 1982), in the Sao Paulo shrimp *Penaeus paulensis* at 19 µmol l<sup>-1</sup> NH<sub>3</sub> and 0.307 mmol l<sup>-1</sup> total ammonia (Ostrensky et al., 1992) and in the redbtail prawn *Penaeus penicillatus* 58 µmol l<sup>-1</sup> NH<sub>3</sub> and 1.39 mmol l<sup>-1</sup> total ammonia (Chen and Lin, 1992).

Mammals accrue ammonia both from metabolism and as an influx to the hepatocytes from the gastrointestinal tract. This ammonia is detoxified in the urea cycle, an energy-consuming process, by incorporation into the less-toxic urea. Crustaceans are largely ammonotelic, aquatic species exclusively so, and in water excrete their nitrogenous waste directly to the environment as highly soluble ammonia.

### Origin of ammonia in crustaceans

The vast majority of the excreted ammonia originates from the catabolism of proteins and amino acids. There is little evidence to support nitrogenous excretion as amino-nitrogen because consumable amino acids released by crustaceans are more likely a result of passive loss *via* amino-acid-permeable structures (Dagg, 1976) and *via* feces (Claybrook, 1983). Some additional ammonia is produced in reactions involving purine and pyrimidine bases (Claybrook, 1983) (Fig. 1). However, ammonia derived *via* this uricolytic pathway is considered to contribute only a small portion of the total ammonia excreted compared with the predominant production from amino acids (Hartenstein, 1970; Schoffeniels and Gilles, 1970). Ammonia

derives, for the most part, from deamination of glutamine, glutamate, serine and asparagine by the specific enzymes glutaminase, glutamate dehydrogenase, serine dehydrogenase and asparaginase, respectively (King et al., 1985; Krishnamoorthy and Srihari, 1973; Greenaway, 1991, 1999).

### Organs of ammonia excretion in aquatic crabs

In aquatic crabs, primary urine is formed *via* ultrafiltration in the antennal gland, which is thought to play the key role in regulation of body water and divalent cations (e.g. Mg<sup>2+</sup>, Ca<sup>2+</sup>; Mantel and Farmer, 1983), but not to contribute significantly to the excretion of nitrogenous waste products (Regnault, 1987). For instance, in the blue crab *Callinectes sapidus*, <2% of total ammonia is excreted in the urine *via* the antennal gland system (Cameron and Batterton, 1978).

The main site for ammonia excretion by aquatic crabs is the phyllobranchiate gill (Claybrook, 1983; Kormanik and Cameron, 1981; Regnault, 1987), featuring a single-cell-layered epithelium covered by an ion-selective cuticle (Avenet and Lignon, 1985; Lignon, 1987; Onken and Riestenpatt, 2002; Weihrauch et al., 2002). The gills of aquatic crabs are multifunctional organs. In addition to their function in excretion of nitrogenous waste products, they are also responsible for respiratory gas exchange (Burnett and McMahon, 1985), regulation of acid–base balance (Henry and Wheatly, 1992) and osmoregulatory ion transport (Towle, 1981; Lucu, 1990; Riestenpatt et al., 1996; Towle and Weihrauch, 2001). Several transporters and enzymes putatively linked and involved in ammonia transport have been shown to be present in the branchial epithelium of crabs, as summarized in Fig. 2 and Table 2.

### Ammonia excretion in aquatic crabs

In solution, both forms of ammonia, non-ionic ammonia (NH<sub>3</sub>) and the ammonium ions (NH<sub>4</sub><sup>+</sup>) exist in a pH-dependent equilibrium. As a weak base (pK ≈ 9.48 at 20°C and NaCl=250 mmol l<sup>-1</sup>; Cameron and Heisler, 1983) and at a physiological pH of pH 7.8, 98% of total ammonia exists in the ionic form NH<sub>4</sub><sup>+</sup>, whereas only 2% is present as non-ionic NH<sub>3</sub>. However, the higher lipid solubility of NH<sub>3</sub> makes it more diffusible through phospholipid bilayers. Kormanik and Cameron (1981) reported that ammonia excretion of seawater adapted blue crabs *Callinectes sapidus* occurred mainly by diffusion of non-ionic NH<sub>3</sub>. An excretion mechanism based predominately on NH<sub>3</sub> diffusion is not likely, however, because membrane permeability of NH<sub>3</sub> is much lower than that of CO<sub>2</sub> (Knepper et al., 1989). Indeed, some plasma membranes of animal epithelia are relatively impermeable to NH<sub>3</sub> as shown for frog oocytes (Burckhardt and Frömter, 1992), the renal proximal straight tubules (Garvin et al., 1987) and colonic crypt cells (Singh et al., 1995). Accordingly, other authors have obtained experimental evidence for at least partial excretion of ammonia in its ionic form (NH<sub>4</sub><sup>+</sup>) in *Callinectes sapidus* (Pressley et al., 1981) and *Carcinus maenas* (Lucu, 1989; Siebers et al., 1995).

Studies on isolated perfused gills of several aquatic crabs

showed that ammonia can be excreted actively against a 4–8-fold inwardly directed ammonia gradient across both the anterior and the posterior gills to a similar degree despite their different morphological and physiological characteristics (Copeland and Fitzjarrell, 1968; Goodmann and Cavey, 1990; Weihrauch et al., 1998, 1999; Towle and Weihrauch, 2001) (Fig. 3). Under physiologically relevant conditions, the potential for active branchial ammonia excretion is significantly greater in the marine *Cancer pagurus* than in freshwater-acclimated Chinese mitten crabs *Eriocheir sinensis*, despite the much larger ionic conductance of *Cancer pagurus* gills (~250–280 mS cm<sup>-2</sup>) compared with that of *Eriocheir sinensis* gills (~4 mS cm<sup>-2</sup>) (Fig. 4). It is noteworthy that the posterior gills of *Carcinus maenas* (thought to play the dominant role in osmoregulatory NaCl uptake) and also the anterior gills (thought to be primarily responsible for gas exchange) are equally capable of active ammonia excretion (Weihrauch et al., 1999).

### Ecological relevance of active ammonia excretion in aquatic crabs

The finding that at least three different crab species with disparate ionic regulatory requirements can actively excrete ammonia raises issues about the metabolic costs involved. The ability to excrete ammonia against a gradient has significant ecological implications with regard to habitats that would, thus, be available to these crabs. Traditionally, ammonia excretion in aquatic animals has been considered to be a passive process driven by diffusion along the partial pressure gradient of NH<sub>3</sub> (P<sub>NH<sub>3</sub></sub>). Such a model requires environmental concentrations to be kept low, normally by bacterial nitrification of ammonia to nitrite and nitrate. This view is probably justified in pelagic animals colonizing the water column of aquatic habitats because, according to Koroleff (1983), the amounts of NH<sub>4</sub><sup>+</sup> rarely exceed 5 μmol l<sup>-1</sup> in oxygenated, unpolluted seawater.

By contrast, benthic and interstitial animals are often faced with higher ambient ammonia concentrations. High ammonia is especially prevalent in anoxic, deep stagnant water and pore water during periods of high mineralization following collapse of phytoplankton blooms. For example, several investigations of pore water composition in the North Sea showed considerable concentrations of ammonia in a range between 100 and 300 μmol l<sup>-1</sup>, but also up to 2500 μmol l<sup>-1</sup> in 4–9 cm sediment depth (Enoksson and Samuelsson, 1987; Lohse et al., 1993).

Like most aquatic crab species, *Carcinus maenas*, *Cancer pagurus* and *Eriocheir sinensis* are benthic-living animals,

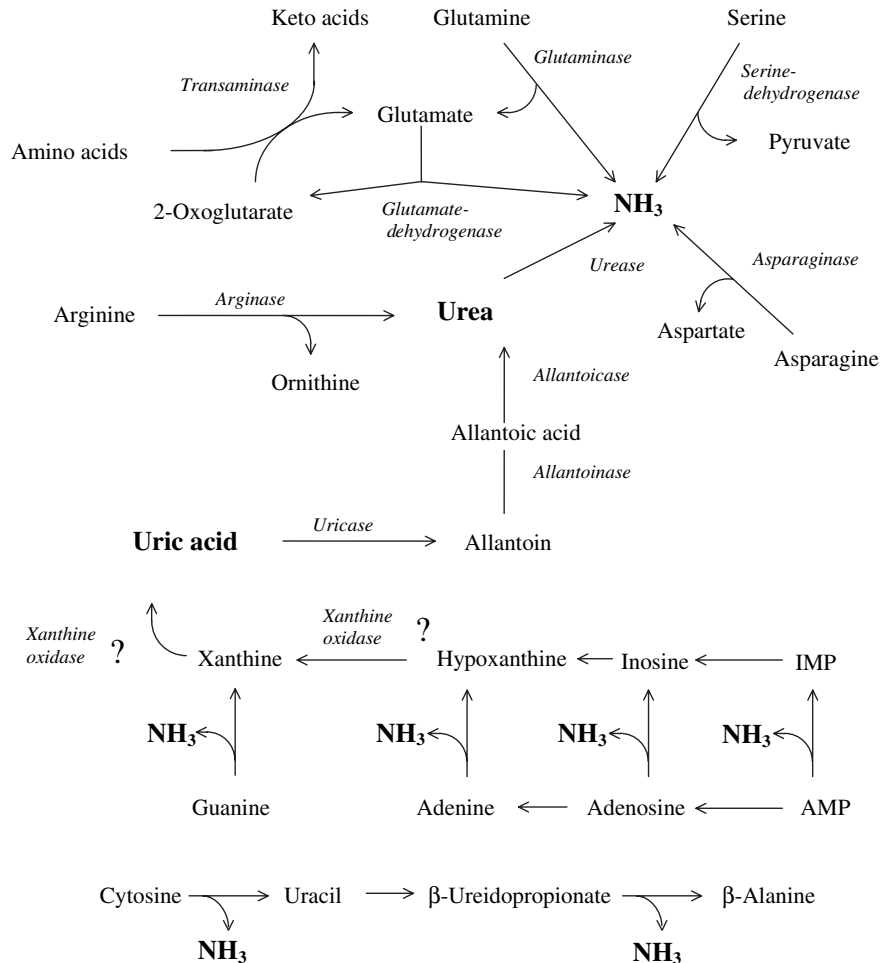


Fig. 1. Metabolic formation of nitrogen excretion products in Crustacea. Modified after Claybrook (1983). Existence of xanthine oxidase is unclear, as indicated by ‘?’.

hiding under stones or burying themselves in the sediment for long periods, for example, during low tide or in the winter season. Under conditions where crabs are situated at sites with low rates of ambient water exchange, plus the fact that the animals produce and excrete metabolic ammonia, the concentration of the ambient ammonia can reach high values.

Considering hemolymph ammonia concentrations of ~100 μmol l<sup>-1</sup> (Weihrauch et al., 1999; Table 1) of which less than 5 μmol l<sup>-1</sup> exist in the gaseous form NH<sub>3</sub>, these crabs may encounter ambient NH<sub>3</sub> and/or NH<sub>4</sub><sup>+</sup> concentrations exceeding those in their hemolymph. While NH<sub>3</sub> diffuses along its partial pressure gradient across the exposed epithelia, NH<sub>4</sub><sup>+</sup> follows its electrochemical gradient by either paracellular diffusion or NH<sub>4</sub><sup>+</sup> permeable channels and transporters (see Table 2). An adaptive protection against net ammonia influxes (i.e. an active mechanism for excretion of metabolic ammonia against an inwardly directed gradient, tolerances for high hemolymph ammonia concentrations or efficient detoxification mechanisms) must, therefore, have evolved.

### Branchial ammonia excretion mechanisms in aquatic crabs

In the blue crab *Callinectes sapidus*, ammonia excretion

rates are correlated with  $\text{Na}^+$  absorption (Pressley et al., 1981). The same result was obtained both for the Chinese crab *Eriocheir sinensis* (Péqueux and Gilles, 1981) and for the shore

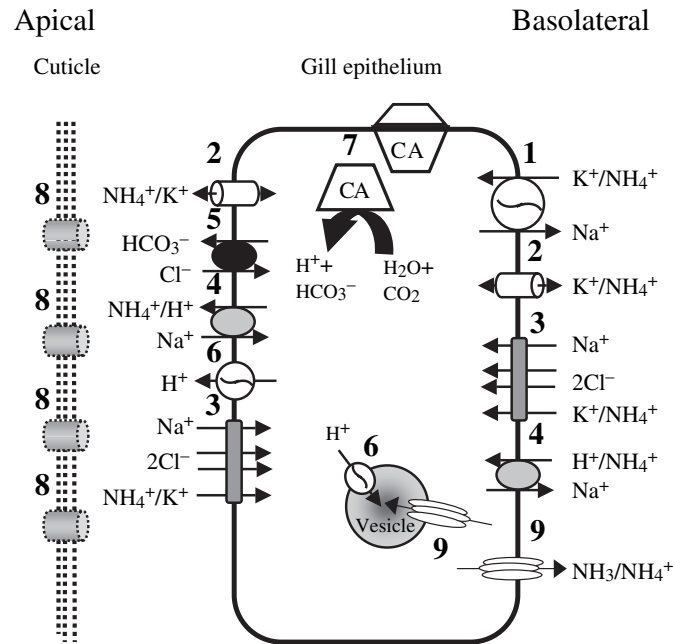


Fig. 2. Transporters and enzymes putatively involved in the process of ammonia excretion in crabs. Note: composition and localization of transporters may vary between crab species. (1)  $\text{Na}^+/\text{K}^+$ -ATPase; (2)  $\text{K}^+$  channels; (3)  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  co-transporter; (4)  $\text{Na}^+/\text{H}^+$  exchanger; (5)  $\text{HCO}_3^-/\text{Cl}^-$  exchanger; (6) V-type  $\text{H}^+$ -ATPase; (7) Carbonic anhydrase (CA); (8) amiloride-sensitive cation-permeable channel-like structures of the cuticle; (9) Rhesus-like protein (RhCM), putative ammonium transporter with unknown localization. For further information please refer to Table 2.

crab *Carcinus maenas* (Lucu et al., 1989). Studies employing membrane vesicles from gill epithelia (Towle and Hølleland, 1987) and isolated, perfused gills (Lucu et al., 1989) indicated that  $\text{NH}_4^+$  substitutes for  $\text{K}^+$  in activation of the ouabain-sensitive  $\text{Na}^+/\text{K}^+$ -ATPase. In gill sections from *Callinectes sapidus*, this  $\text{Na}^+/\text{K}^+$ -ATPase was demonstrated to be located in the basolateral membranes of the branchial epithelial cells (Towle and Kays, 1986; Towle et al., 2001). Complete or partial cDNA sequences for the  $\alpha$ -subunit of  $\text{Na}^+/\text{K}^+$ -ATPase from crab gills have been published in GenBank (see Table 2) thus confirming both its presence in branchial epithelia and its similarity to  $\alpha$ -subunits of other species.

Recently, Masui et al. (2002) showed that the branchial  $\text{Na}^+/\text{K}^+$ -ATPase from *Callinectes danae* is synergistically stimulated by  $\text{NH}_4^+$  and  $\text{K}^+$ , increasing its catalytic activity by up to 90%. Masui et al. (2002) came to the conclusion that the two ions bind to different sites of the branchial  $\text{Na}^+/\text{K}^+$ -ATPase. This observation was also attributed to the branchial  $\text{Na}^+/\text{K}^+$ -ATPase of the freshwater shrimp *Macrobachium olfersii* by Furriel et al. (2004), who suggested for this species that at high  $\text{NH}_4^+$  concentrations the pump exposes a new binding site for  $\text{NH}_4^+$  which, after binding to  $\text{NH}_4^+$ , modulates the activity of the  $\text{Na}^+/\text{K}^+$ -ATPase independently of  $\text{K}^+$  ions.

In the marine crab *Cancer pagurus*, active branchial excretion of ammonia is completely inhibited by ouabain, a specific inhibitor of the  $\text{Na}^+/\text{K}^+$ -ATPase (Weihrauch et al., 1999), suggesting this pump is the only driving force for excretion. However, in the gills of *Carcinus maenas* acclimated to brackish water, both gradient-driven (Lucu et al., 1989) and active ammonia excretion (Weihrauch et al., 1998) are only partially inhibited by ouabain, consistent with a second active mechanism responsible for branchial ammonia extrusion in this species.

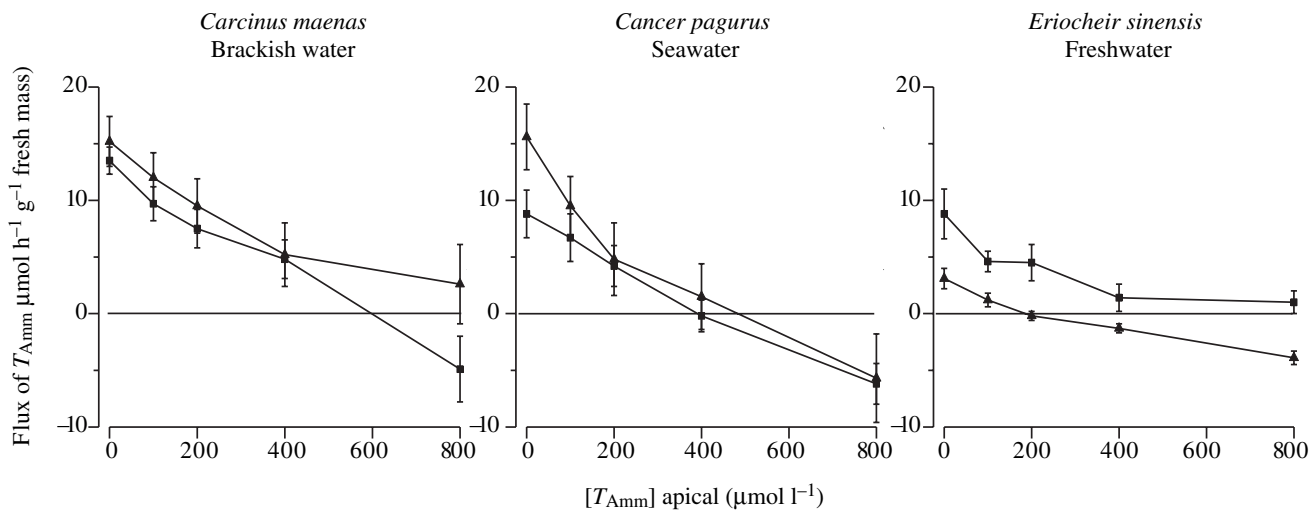


Fig. 3. Fluxes of total ammonia ( $T_{\text{Amm}}$ ) across anterior (triangles) and posterior (squares) gills of seawater-adapted *Cancer pagurus*, brackish water-adapted *Carcinus maenas*, and freshwater-adapted *Eriocheir sinensis*. Gills were perfused with salines containing  $100 \mu\text{mol l}^{-1} \text{NH}_4\text{Cl}$ . Concentrations of  $\text{NH}_4\text{Cl}$  in the bathing saline increased stepwise from 0 to  $800 \mu\text{mol l}^{-1}$ . Positive and negative values represent net effluxes and influxes from/into perfusate, respectively. Data represent means  $\pm$  S.E.M. *Carcinus maenas*:  $N=7$  (anterior) and  $N=9$  (posterior gills). *Cancer pagurus*:  $N=7$  (anterior) and  $N=8$  (posterior gills). *Eriocheir sinensis*:  $N=7$  (anterior) and  $N=6$  (posterior gills). (Modified after Weihrauch et al., 1999.)



The presence of an apically located amiloride-sensitive  $\text{Na}^+/\text{NH}_4^+$  exchanger, transporting  $\text{NH}_4^+$  from the epithelial cell into the ambient medium in exchange for  $\text{Na}^+$ , has been suggested for *Callinectes sapidus* (Pressley et al., 1981) and for *Carcinus maenas* (Lucu et al., 1989; Siebers et al., 1995). Indeed, branchial mRNA expression of a  $\text{Na}^+/\text{H}^+$ -antiporter, putatively transporting also  $\text{NH}_4^+$  ions, was demonstrated in *Carcinus maenas* (Towle et al., 1997) and in *Eriocheir sinensis* (Weihrauch and Towle, 2000). However, experiments employing the isolated cuticle from *Carcinus maenas* have

shown that cuticular  $\text{Na}^+$  and  $\text{NH}_4^+$  conductances ( $G_{\text{cut}}$ ) are inhibited by apically applied amiloride in a dose-dependent manner, with an inhibitor constant  $K_{\text{amiNa}^+}=0.6 \mu\text{mol l}^{-1}$  for sodium ions and  $K_{\text{amiNH}_4^+}=20.4 \mu\text{mol l}^{-1}$  for ammonium ions, respectively (Onken and Riestenpatt, 2002; Weihrauch et al., 2002). Differences in  $K_{\text{amiNH}_4^+}$  and  $K_{\text{amiNa}^+}$  are not understood yet. One can speculate that amiloride blocks the passage of cations in the cuticle in a mechanical way like a plug, rather than by blocking a general cation-binding site. Passage of smaller ions (like  $\text{Na}^+$ ) that carry a larger coat of water

Table 2. Type and location of ion transporters in various species of crab

Protein/transporter	Species	Localization	References	GenBank*
$\text{Na}^+/\text{K}^+$ -ATPase	<i>Callinectes sapidus</i>		1, 2	AF327439
	<i>Callinectes danae</i>		3	AY035550
	<i>Carcinus maenas</i>		1, 4	AF548369
	<i>Chasmagnathus granulatus</i>	General	5	AF409119
	<i>Dilocarcinus pagei</i>	Basolateral	6	AF375957
	<i>Eriocheir sinensis</i>		7, 8	
	<i>Hemigrapsus nudus</i>		9	
	<i>Pachygrapsus marmoratus</i>		10	
	<i>Uca sp.</i>		11, 12	
$\text{K}^+$ channel	<i>Carcinus maenas</i>	Basolateral	4	
		Apical		
$\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter	<i>Callinectes sapidus</i>	Unknown	13	AF190129
	<i>Carcinus maenas</i>	Apical (?)	4	AY035548
	<i>Chasmagnathus granulatus</i>	Unknown	8	AF548368
	<i>Eriocheir sinensis</i>	Unknown		AF301160
$\text{Na}^+/\text{H}^+$ exchanger	<i>Callinectes sapidus</i>	Apical (?)	14	
	<i>Carcinus maenas</i>	Apical (?)	15	U09274
	<i>Eriocheir sinensis</i>	Unknown	8	AF301159
$\text{Cl}^-/\text{HCO}_3^-$ exchanger	<i>Carcinus maenas</i>	Apical (?)	16	–
V-Type $\text{H}^+$ ATPase	<i>Callinectes sapidus</i>	Unknown	17	AF189780
	<i>Cancer irroratus</i>	Unknown	17	AF189781
	<i>Carcinus maenas</i>	Vesicular	17	AF247971
	<i>Chasmagnathus granulatus</i>	Unknown	17	AF189783
	<i>Dilocarcinus pagei</i>	Unknown	17, 18	AF409118
	<i>Eriocheir sinensis</i>	Apical		AF189782
	<i>Pachygrapsus marmoratus</i>	Unknown		AF375958
Carbonic anhydrase	<i>Callinectes sapidus</i>	Cytoplasmatic and membrane-bound	19, 20, 21	
	<i>Carcinus maenas</i>	Unknown	22, 23	
	<i>Chasmagnathus granulatus</i>	Unknown	8	
	<i>Eriocheir sinensis</i>			
Cation-channel-like structures	<i>Carcinus maenas</i>	Cuticle	24–26	
Rh-like protein (putative ammonia transporter)	<i>Carcinus maenas</i>	Unknown	27	AF364404

\*All cDNA sequences submitted by the authors of the present paper.

References for source data: (1) Towle and Kays (1986); (2) Towle et al. (2001); (3) Masui et al. (2002); (4) Riestenpatt et al. (1996); (5) Luquet et al. (2002); (6) Onken and McNamara (2002); (7) Péqueux et al. (1984); (8) Weihrauch and Towle (2000); (9) Corotto and Holliday (1996); (10) Schleich et al. (2001); (11) D'Orazio and Holliday (1985); (12) Lin et al. (2002); (13) Towle and Weihrauch (2001); (14) Burnett and Towle (1990); (15) Towle et al. (1997); (16) Lucu (1989); (17) Weihrauch et al. (2001a); (18) Onken and Putzenlechner (1995); (19) Henry (1988); (20) Böttcher and Siebers (1993); (21) Henry et al. (2003); (22) Vitale et al. (1999); (23) Lopez Mananes et al. (2000); (24) Lignon (1987); (25) Onken and Riestenpatt (2002); (26) Weihrauch et al. (2002); (27) Weihrauch et al. (2001b).

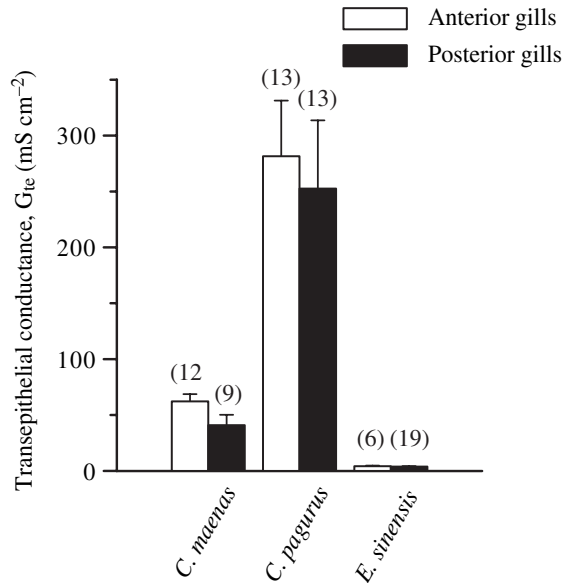


Fig. 4. Transepithelial conductance in isolated half lamellae of gills of seawater-adapted *Cancer pagurus*, brackish water-adapted *Carcinus maenas*, and fresh water-adapted *Eriocheir sinensis*. For the electrophysiological measurements, half lamellae of anterior and posterior gills were mounted in a modified Ussing chamber. Data represent means  $\pm$  S.E.M. with the number of experiments given in brackets. (From Weihrauch et al., 1999.)

molecules is, therefore, possibly easier to block out by lower amiloride concentrations than  $K^+$  or  $NH_4^+$  ions, which carry only about half the number of water molecules around their core. However, according to these observations, some of the results obtained by applying amiloride to crab gills (apical) should be interpreted with caution and with special attention to the concentration of this particular inhibitor.

Further studies on the branchial ammonia excretion mechanism in *Carcinus maenas* employing the  $K^+$  channel blocker  $Cs^+$  (10 mmol l<sup>-1</sup>) revealed that basolateral (but not apical)  $K^+$  channels play a role in the excretory process (Weihrauch et al., 1998). In addition, experiments inhibiting the branchial V-Type  $H^+$ -ATPase by adding bafilomycin A<sub>1</sub> resulted in a reduction of active ammonia transport by 66%, identifying the  $H^+$ -ATPase as the second active component in the excretory mechanism of the shore crab (Weihrauch et al., 2002). While in *Eriocheir sinensis* a V-Type  $H^+$ -ATPase has been localized to the apical membrane of the gill epithelium (Onken and Putzenlechner, 1995), in *Carcinus maenas* this pump was found predominantly in the cytoplasm, probably associated with vesicles (Weihrauch et al., 2001a). This latter finding led to the suggestion (Weihrauch et al., 2002) of a vesicular ammonia-trapping mechanism, in which cellular  $NH_3$  diffuses into acidified vesicles to be transformed into its membrane-impermeable ionic form,  $NH_4^+$ . For a directed excretion, these  $NH_4^+$ -loaded vesicles would then be transported to the apical membrane for exocytotic release. Such an excretion mechanism was supported by data showing total inhibition of active ammonia excretion by blockers of the

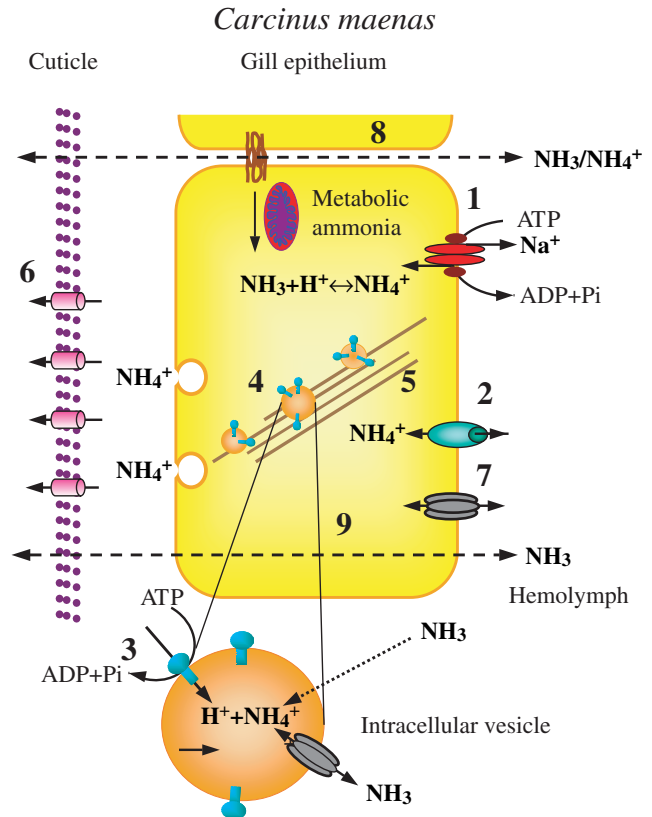


Fig. 5. Proposed hypothetical model of active ammonia excretion across gills of the shore crab *Carcinus maenas*. According to this model,  $NH_4^+$  is pumped across the basolateral membrane by  $Na^+/K^+$ -ATPase (1) or traverses the membrane *via*  $Cs^+$ -sensitive channels (2). Dissociation of cytosolic  $NH_4^+$  to  $H^+$  and  $NH_3$  is accompanied by diffusion of  $NH_3$  into vesicles acidified by V-type  $H^+$ -ATPase (3). The ammonia-loaded vesicles (4) then are moved *via* microtubules (5) to the apical membrane where vesicles fuse with the external membrane, releasing  $NH_4^+$  into the subcuticular space. The  $NH_4^+$  then is believed to diffuse across the cuticle, *via* amiloride-sensitive structures (6). Role and localization of the putative ammonia transporter (RhCM) identified in *Carcinus maenas* gill epithelium (GenBank Accession number: AF364404) are presently uncharacterized (7). Paracellular ammonia diffusion (8) and non-ionic transcellular diffusion of  $NH_3$  (9) is considered to be low under physiologically meaningful transepithelial ammonia gradients (Weihrauch et al., 1998). (Modified after Weihrauch et al., 2002.)

microtubule network, including colchicine, thiabendazole and taxol (Weihrauch et al., 2002). The resulting hypothetical model of the ammonia excretion in *Carcinus maenas* is described in detail in Fig. 5.

For crabs (such as the partially limnic Chinese crab *Eriocheir sinensis*) that utilize a proton gradient across the apical membrane of the epithelial cell to accomplish NaCl uptake from highly diluted media, it is likely that  $NH_3$  diffuses across the apical membrane along its partial pressure gradient, as shown in freshwater rainbow trout *Oncorhynchus mykiss* (Wilson et al., 1994).

Recently, Weihrauch and others (D. Weihrauch, unpublished data) have sequenced a full-length cDNA coding

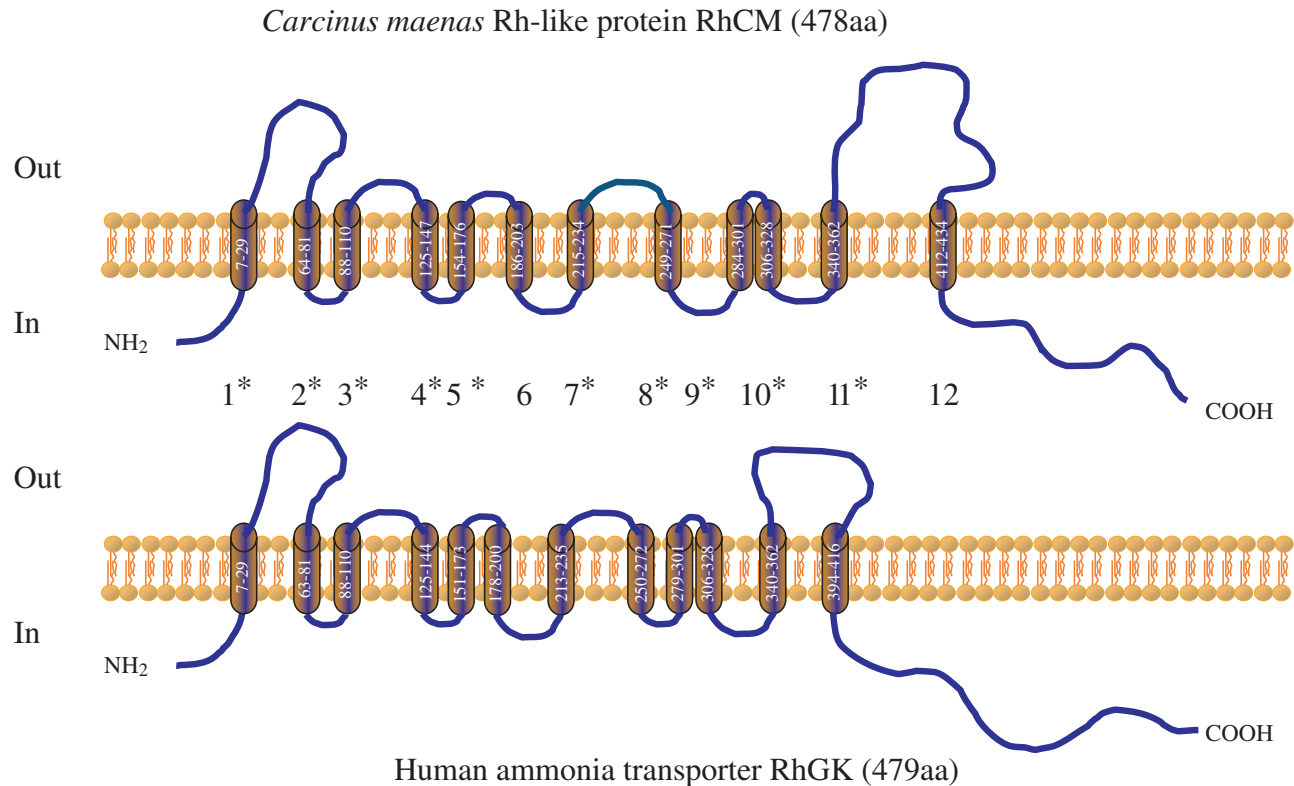


Fig. 6. Localization of the 12 predicted transmembrane domains <http://biowb.sdsc.edu> (TMHMM) of putative *Carcinus maenas* (RhCM, GenBank Accession number: AF364404) and human (RhGK, also called PDRC2, GenBank Accession number: AF081497) ammonia transporter. Asterisk indicates identical predicted sites of transmembrane domains in RhCM and the human ammonia transporter RhGK.

for a Rhesus-like protein from *Carcinus maenas* gills (GenBank Accession number: AF364404), named RhCM (Rhesus-related protein from *Carcinus maenas*). In mammals Rhesus-related proteins, such as RhGK, have been shown to mediate ammonia (NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>), but not K<sup>+</sup> or amino acid transport when functionally expressed in yeast mutants lacking endogenous ammonia transporters (triple  $\Delta$ Mep mutant; Marini et al., 2000). However, for this novel Rhesus-like ammonia transporter the detailed transport characteristics (such as mode of transport or kinetics) have not yet been defined. A comparison of the deduced secondary structure of the amino acid sequence of RhCM, and the human ammonia transporter RhGK, showed that 10 out of 12 predicted transmembrane domains are positioned at identical sites of the sequence (Fig. 6). The localization and role of RhCM in branchial ammonia excretion need to be investigated in detail in further studies. One can speculate that the putative ammonia transport of crabs is not localized in the apical membrane of the gill epithelium, because here ammonia (NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>) permeable structures would be of disadvantage allowing ammonia influxes when the animals are exposed to high external concentrations. The human Rhesus-like ammonia transporter RhGK (identical to RhCG), has been described to be localized in the distal tubule and the collecting duct of the kidney in co-localization with a V-type H<sup>+</sup>-ATPase (Eladari et al., 2002). RhCG expressed in *Xenopus* oocytes facilitates a

highly specific NH<sub>3</sub> diffusion via a complex electrogenic NH<sub>4</sub><sup>+</sup> transport (Bakouh et al., 2004). In addition, Eladari et al. (2002) suggested a secondary active mode of ammonia transport in the distal tubule by acid trapping. According to this assumption, RhGK would promote the transmembrane passage of NH<sub>3</sub>. A similar mechanism would be plausible in the gills of the shore crab *Carcinus maenas*, however, RhCM would be co-localized with the H<sup>+</sup>-ATPase within the membranes of intracellular vesicles to support the proposed vesicular acid-trapping mechanisms. Also, a basolateral localization cannot be excluded, where the putative ammonia transporter might serve as an overflow valve, transporting ammonia back in to the hemolymph, when crabs are exposed to high external ammonia concentrations. Under this condition, intracellular ammonia concentrations might rise to toxic levels due to a passive influx from the apical side, while the Na<sup>+</sup>/K<sup>+</sup>-ATPase is actively pumping NH<sub>4</sub><sup>+</sup> from the hemolymph space into the cytoplasm. Ammonia directed back into the hemolymph, via RhCM, could probably be buffered, at least for a short term, by incorporation into proteins, for instance glutamine or hemocyanins.

The high degree of conservation with ammonia transporters found in fungi, bacteria and archaeobacteria (~20%), as well as the striking homology to mammalian ammonia transporters (>40%), led to the suggestion that proteins of the Rh-family play a universal role in ammonia transport (Fig. 7).

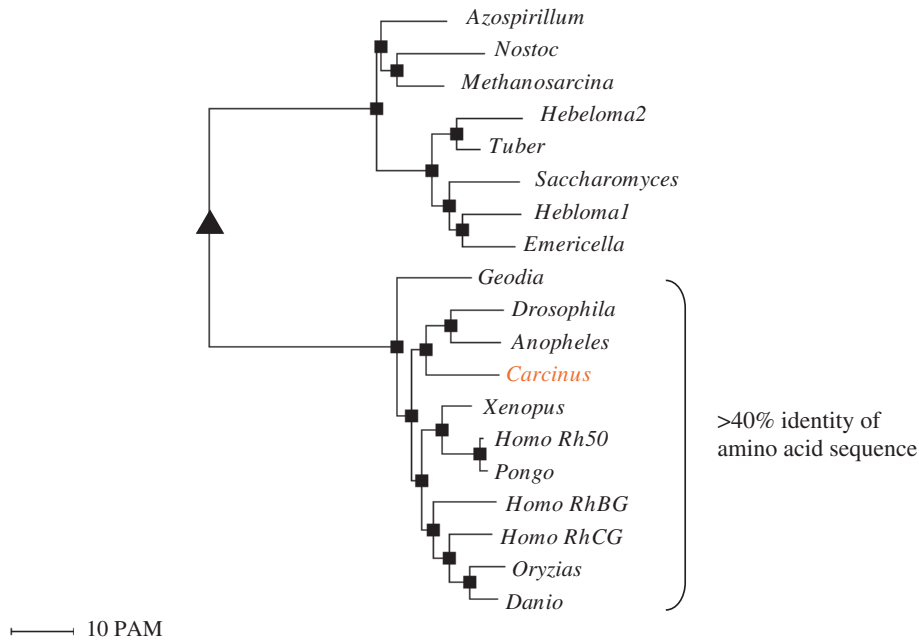


Fig. 7. Phylogenetic tree of published ammonia transporters. GenBank Accession numbers and type of ammonia transporter are given in parentheses. *Anopheles gambiae* (EAA01247, Rh-like protein); *Azospirillum brasilense* (AAC38548, AMT/MEP family); *Carcinus maenas* (AF364404, Rh-like protein); *Danio rerio* (AAM90586, Rh-like protein); *Drosophila melanogaster* (AF64673, Rh-like protein); *Emericella nidulans* (AAL73117, AMT/MEP family); *Geodia cydonium* (CAA73029, Rh-like protein); *Hebeloma cylindrosporium* 1 (AAK82417, AMT/MEP family); *Hebeloma cylindrosporium* 2 (AAM21926 AMT/MEP family); *Homo sapiens* 50 KDa (CAA45883, Rh-like protein), *Homo sapiens* RhBG (AAL05978, Rh-like protein); *Homo sapiens* RhCG (AAH30965, Rh-like protein); *Methanosarcina acetivorans* (AAM07268, AMT/MEP family), *Nostoc* sp. (BAB72949, AMT/MEP family); *Oryzias latipes* (BAB13346, Rh-like protein); *Pongo pygmaeus* (AAG00305, Rh-like protein), *Saccharomyces cerevisiae* (P53390, AMT/MEP family), *Tuber borchii* (AAL11032, AMT/MEP family); *Xenopus laevis* (BAB13345, Rh-like protein). Tree was constructed with Multalin (Corpet, 1988). PAM, percent accepted mutations (a measure of phylogenetic distance).

#### Ammonia excretion in terrestrial crabs – consequences of air exposure

The mechanisms and processes by which air-breathing crustaceans excrete nitrogenous waste into the terrestrial habitat have been subject to considerable scrutiny (Greenaway, 1988, 1991; Wolcott, 1991, 1992; O'Donnell and Wright, 1995). Terrestrial crabs appear to tolerate considerably greater hemolymph ammonia loads than do aquatic species (Table 1). Conversely, Wolcott (1992) points out that diluting ammonia to non-toxic levels in the urine might require an unsustainable water loss in a land crab.

The probability that branchial  $\text{NH}_4^+$  excretion is linked to sodium transport, both through apical ion exchangers and the basal membrane  $\text{Na}^+/\text{K}^+$ -ATPase, is of special importance to air-breathing crabs. In all land crabs examined to date, the gills have become adapted for reabsorption of salt from primary urine directed through the branchial chamber (Wolcott and Wolcott, 1985, 1991; Morris, 2001), allowing diffusive  $\text{NH}_3$  loss and  $\text{NH}_4^+$  extrusion in exchange for required ions from the urine. Ocypodid crabs seem exceptional in utilizing the

antennal gland for increasing urinary ammonia, although the gills are required to complete the excretory process (DeVries and Wolcott, 1993). Generally, while the gills are initially bathed with a fluid isosmotic with the hemolymph, osmotic concentration may decline by as much as 90% (Wolcott and Wolcott, 1985; Varley and Greenaway, 1994; Greenaway, 1999; Morris et al., 2000; Taylor and Greenaway, 2002; Morris and Ahern, 2003). Even very euryhaline aquatic species do not experience the same range of osmo-concentration as occurs in the extra-branchial fluid of some land crabs.

In terrestrial arthropods as a whole, the primary nitrogenous excretory products are generally purines, whereas in land crabs various mechanisms are employed to permit the continued excretion of ammonia. The single known exception is the terrestrial anomuran *Birgus latro*, which is purinotelic excreting urate (Greenaway and Morris, 1989) and guanine (Greenaway, 2003). The reasons for the general persistence of ammonotelism may be found by examining a continuum of extant species in the transition from aquatic to land crab.

#### Ammonia excretion and air exposure of aquatic crabs

Exposure to air of the aquatic crabs *Cancer pagurus* and *Cancer productus* caused hemolymph ammonia to increase by  $25 \mu\text{mol h}^{-1}$  (Regnault, 1992) and  $26 \mu\text{mol h}^{-1}$  (deFur and McMahon, 1984), respectively. However, in *Cancer pagurus* this rate of accumulation was between 15 and 30% of the rate expected by Regnault (1992) on the basis of basal aquatic rates, leading to the suggestion of nitrogen storage in tissues, to avoid toxic hemolymph ammonia loading. Many crustaceans store nitrogen (N) as solid urate (for review see Greenaway, 1999) but this seems to be formed primarily as a result of diet rather than being of any large significance in  $\text{NH}_3/\text{NH}_4^+$  detoxification (Linton and Greenaway, 1997a) – except possibly under desiccating conditions in land crabs (below). In any case, urate formation was ruled out as a significant contribution in *Cancer pagurus* (Regnault, 1992). Ammonia excretion during air exposure of *Cancer pagurus* was only 4% of the normal aquatic rate ( $170\text{--}190 \mu\text{mol kg}^{-1} \text{h}^{-1}$ ) but when re-immersed they exhibited a very large (50-fold within 5 min) but transient increase to  $8860 \mu\text{mol kg}^{-1} \text{h}^{-1}$  (derived from Regnault 1994). Thus, nitrogen storage as  $\text{NH}_4^+$ , or as some readily oxidized form, is apparently a normal response to transient air exposure, as is the subsequent pulsatile clearance



*Ocypode quadrata*

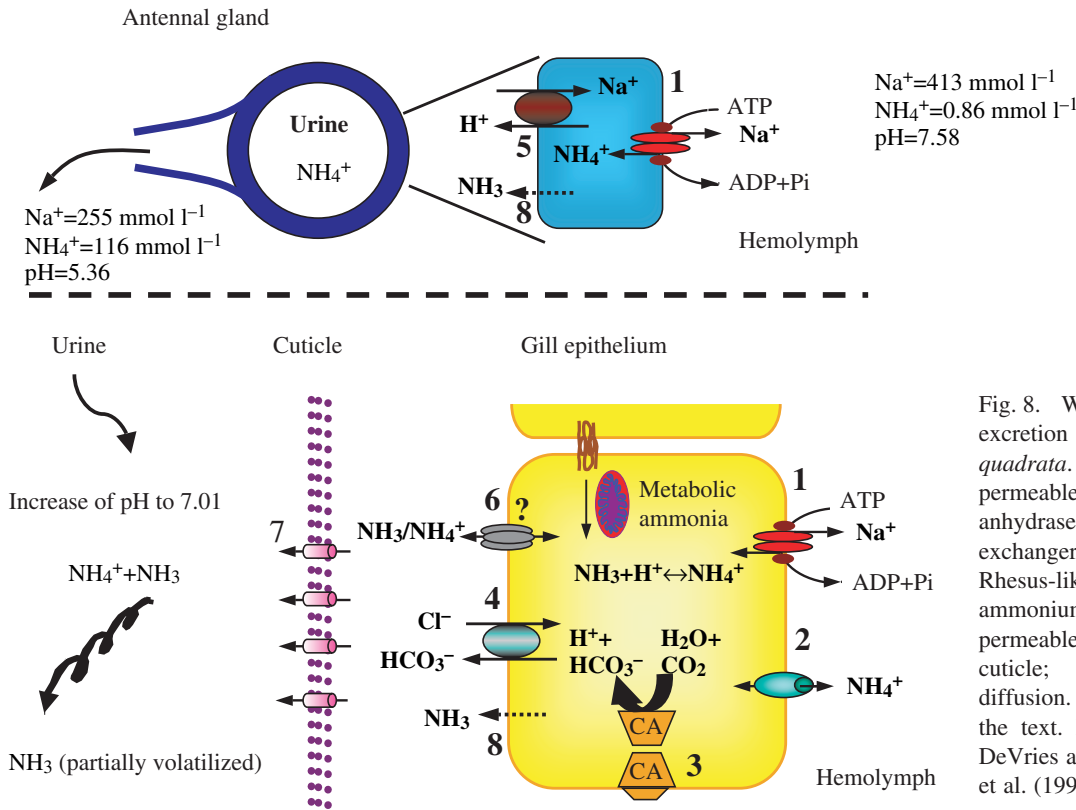


Fig. 8. Working model for ammonia excretion in the terrestrial crab *Ocypode quadrata*. (1)  $\text{Na}^+/\text{K}^+$ -ATPase; (2)  $\text{NH}_4^+$ -permeable  $\text{K}^+$  channels; (3) carbonic anhydrase (CA); (4)  $\text{HCO}_3^-/\text{Cl}^-$  exchanger; (5)  $\text{Na}^+/\text{H}^+$  exchanger; (6) Rhesus-like protein (RhCM), putative ammonium transporter; (7) cation-permeable channel-like structures of the cuticle; (8) transmembranous  $\text{NH}_3$  diffusion. For further details please refer to the text. Model compiled from data of DeVries and Wolcott (1993) and DeVries et al. (1994).

of ammonia on re-immersion. The possibility that urate can be so rapidly mobilized to  $\text{NH}_4^+$  seems unlikely and expensive. However, the vesicular sequestration of  $\text{NH}_4^+$  (see above) has hitherto not been considered in this role.

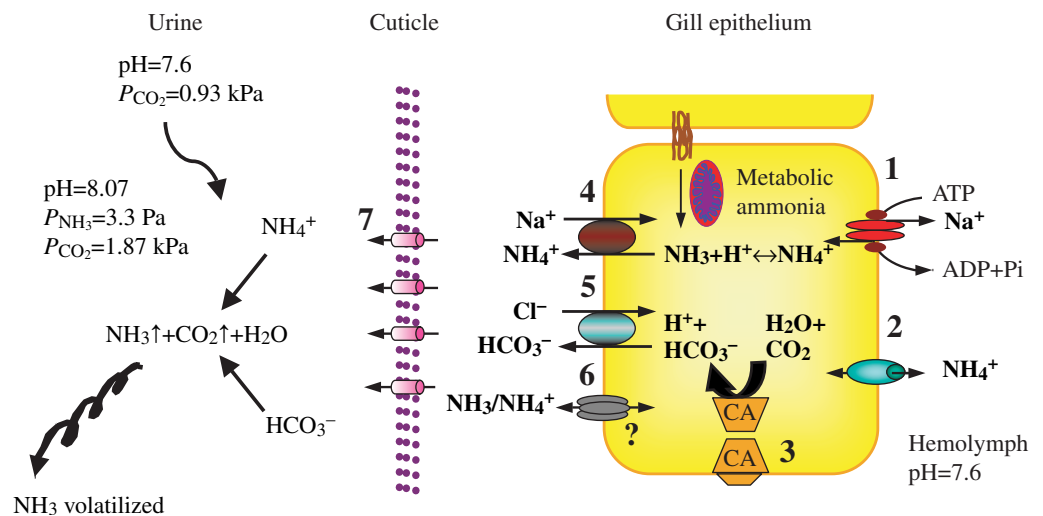
Ammonia excretion in land crabs that immerse

A similar 'storage-excretion' is seen in diverse air-breathing

crabs (e.g. *Potamonautes warreni*, Morris and van Aardt, 1998; *Austrohelphusa transversa*, Linton and Greenaway, 1995; *Discoplax hirtipes*, Dela-Cruz and Morris, 1997; *Cardisoma carnifex*, Wood et al., 1986). [Note: *Cardisoma hirtipes* has been revised to *Discoplax hirtipes* and *Holthuisana to Austrohelphusa* (Davie, 2002).] *Discoplax hirtipes* excretes 99% of its waste as ammonia, but when it is breathing air the

*Geograpsus grayi*

Fig. 9. Working model for ammonia excretion in the terrestrial crab *Geograpsus grayi*. (1)  $\text{Na}^+/\text{K}^+$ -ATPase; (2)  $\text{NH}_4^+$ -permeable  $\text{K}^+$  channels; (3) carbonic anhydrase (CA); (4)  $\text{Na}^+/\text{H}^+/\text{NH}_4^+$  exchanger; (5)  $\text{HCO}_3^-/\text{Cl}^-$  exchanger; (6) Rhesus-like protein (RhCM), putative ammonium transporter; (7) cation-permeable channel-like structures of the cuticle. For further details please refer to the text. Model compiled from data of Varley and Greenaway (1994).



rate of nitrogen loss in the urinary flow is only  $0.2 \mu\text{mol kg}^{-1} \text{h}^{-1}$  and  $\text{NH}_3$  is volatilized at a very slow rate ( $0.4 \mu\text{mol kg}^{-1} \text{h}^{-1}$ ). On re-immersion, the ammonia excretion rate is transiently elevated to  $1100 \mu\text{mol kg}^{-1} \text{h}^{-1}$  (compared with the normal  $300 \mu\text{mol kg}^{-1} \text{h}^{-1}$ ). *Potamonautes warreni* also does not excrete while in air but, on return to water, excretes ammonia across the gills at  $4900 \mu\text{mol kg}^{-1} \text{h}^{-1}$  (compared with the normal rate in water of  $70 \mu\text{mol kg}^{-1} \text{h}^{-1}$ ). Artificially irrigating the gills of air-breathing *Potamonautes warreni* sustained ammonia excretion (Morris and van Aardt, 1998). Gill irrigation appears to be a ubiquitous activity following excursions into the terrestrial environment (Dela-Cruz and Morris, 1997). The requirement to re-immense, albeit briefly, to accomplish ammonia excretion *via* the ancestral branchial mechanisms may ultimately limit the duration of air-breathing in these amphibious species. However, *Austrothelphusa transversa* can spend many months without access to water (Greenaway and MacMillan, 1978) and can forgo ammonia excretion during that time (Linton and Greenaway, 1995). Linton and Greenaway (1995) suggested that the near-cessation of nitrogen excretion in *A. transversa* implied reduced nitrogen catabolism and temporary nitrogen storage. The speed and brevity of the excretion pulse in *P. warreni* showed that wastes stored during terrestrial forays are rapidly excreted on return to water. However, it seems unlikely that this store is accumulated as  $\text{NH}_4^+/\text{NH}_3$  within the gill epithelium because hemolymph levels remain low and pH (and therefore  $P_{\text{NH}_3}$ ) remains unchanged (Adamczewska et al., 1997) although the required enzymes may be present (Linton and Greenaway, 1998). Further study is required to determine the storage product but an accessible intermediate is implicated (such as glutamine rather than, for example, urate). Apical  $\text{H}^+/\text{NH}_4^+$  exchange and V-ATPase-driven cell alkalization have been suggested as likely mechanisms of transbranchial ammonia transport (Linton and Greenaway, 1995), but experimental evidence is required to confirm this. Again, the involvement of a Rhesus-related ammonia transporter needs to be evaluated.

#### *Ammonia excretion in terrestrial crabs*

Gecarcinid land crabs recycle their urine over the branchial surfaces, producing a dilute fluid 'P' (Wolcott and Wolcott, 1985; for review Morris, 2002). *Discoplax hirtipes*, a crab that immerses from time to time, can reduce the NaCl concentration of the urine by 90% (Dela-Cruz and Morris, 1997) but this ion pumping does not allow ammonia excretion while in air. For example, while the  $\text{NH}_4^+$  content of 'P' of *Discoplax hirtipes* is significantly elevated ( $5 \text{mmol l}^{-1}$ ) compared with the hemolymph (Table 1), the rates of urinary and 'P' flow slow down to almost zero when the animals are in air (Dela-Cruz and Morris, 1997). Thus, ammonia excretion becomes severely limited by urine flow and consequent 'P' production rates.

In *Gecarcoidea natalis*, a gecarcinid land crab that does not routinely immerse or have access to pools of water, the primary urine contains  $0.36 \text{mmol NH}_4^+ \text{l}^{-1}$ , which is less than in the blood (Table 1). However, reprocessed 'P' contains up to

$10.8 \text{mmol l}^{-1}$  (Greenaway and Nakamura, 1991), and this is sufficient to excrete up to 68% of the total nitrogenous output because 'P' production was  $\sim 450 \mu\text{l kg}^{-1} \text{h}^{-1}$ . This flow rate is much greater than the  $3 \mu\text{l kg}^{-1} \text{h}^{-1}$  in *Discoplax hirtipes*, which is unable to sustain ammonia excretion in air (Dela-Cruz and Morris, 1997). The rate of 'P' production in *Gecarcinus lateralis* was  $>900 \mu\text{l kg}^{-1} \text{h}^{-1}$ , which facilitated an excretion rate of  $20 \mu\text{mol kg}^{-1} \text{h}^{-1}$  (Wolcott, 1991), compared with the  $25 \mu\text{mol kg}^{-1} \text{h}^{-1}$  rate in *Gecarcoidea natalis* (Greenaway and Nakamura, 1991). These authors (Greenaway and Nakamura, 1991; Wolcott, 1991) concluded that acid trapping in the 'P' was not involved and, thus, the outward gradient across the gill epithelia is not favorable for gaseous  $\text{NH}_3$  diffusion. In addition, Wolcott (1991) measured the urine pH of *Gecarcinus lateralis* and *Cardisoma guanhumi*, and in both crabs found it to be greater than that of the hemolymph. However, branchial  $\text{Na}^+/\text{H}^+$  exchange would assist in  $\text{NH}_3$  diffusion through reciprocal pH changes of intracellular and extra-corporeal fluids. The gills of *Gecarcoidea natalis* are highly active in  $\text{Na}^+$  transport and  $\text{NH}_4^+$  might easily substitute for  $\text{K}^+$  in the basal  $\text{Na}^+/\text{K}^+$ -ATPase (Morris, 2001; Morris and Ahern, 2003) but the necessary active transport of  $\text{NH}_4^+$  across the apical membrane into the 'P' remains unresolved. A net exchange of  $\text{Na}^+$  for  $\text{NH}_4^+$  (e.g. Pressley et al., 1981) would facilitate salt reclamation and nitrogen excretion, but would be hampered as the external  $\text{Na}^+$  declined. The possibility of exocytotic mechanisms and/or involvement of a Rhesus-related ammonia transporter needs to be evaluated in the excretory processes of these species.

While this branchial system allows routine excretion of ammonia to air, it also seems to make  $\text{NH}_4^+$  excretion dependent on the urinary flow rate, as well as on the extent of ion re-absorption. For example, in *Gecarcoidea natalis*, urine and 'P' flow can decline to zero under dry season conditions (Morris and Ahern, 2003) and so this mode of  $\text{NH}_4^+$  clearance becomes inoperable. In *Cardisoma guanhumi* the fluid retained within the abdominal flap ( $\sim 13.5 \text{mmol l}^{-1}$ ) is contiguous with that in the branchial chamber ( $\sim 6.5 \text{mmol l}^{-1}$ ) and further  $\text{NH}_4^+$  excretion may occur *via* unknown mechanisms (Wolcott, 1991) but, even so, this would be unavailable to land crabs in the dry season. Purine is stored in large amounts in connective tissue cells throughout the bodies of some land crabs (Linton and Greenaway, 1997b). In *Gecarcoidea natalis* this stored purine is normally synthesized *de novo*, from excess dietary nitrogen (Linton and Greenaway, 1997a). Recent data, including enzyme activities and nitrogen utilization (Linton and Greenaway, 1998, 2000) have led to the suggestion of a storage-excretion function for the urate accumulated by *G. natalis* (Greenaway, 2003). However, this seems likely to be infrequently called upon (Linton and Greenaway, 1997a) and evidence is required to show that waste amino N is incorporated as well as dietary N.

Other lineages of terrestrial crabs have not been investigated to the same extent as the gecarcinids, but at least one air-breathing ocy podid, *Ocypode quadrata*, has been shown to sustain ammonia excretion (DeVries and Wolcott, 1993).

*O. quadrata* also recycles the urine to produce 'P', which can be as little as 10% of the osmotic strength of the primary urine (Wolcott and Wolcott, 1985). However, the mechanism of  $\text{NH}_4^+$  excretion is quite different from that of gecarcinids because the concentration in the primary urine of the ghost crab is extraordinarily high. For example, in *O. quadrata* (DeVries and Wolcott, 1993; DeVries et al., 1994) this reaches 116–212  $\text{mmol l}^{-1}$ , and in *O. ceratophthalma* and *O. cordimanus* (under field conditions) >40 and 27  $\text{mmol l}^{-1}$ , respectively (S. Morris, unpublished). The primary urine of *Ocypode quadrata* is unusually acidic ( $\text{pH } 5.36 \pm 0.21$ ), providing an 'acid-trap' for  $\text{NH}_4^+$ . On passage over the gills, the pH is increased ( $\text{pH } 7.01 \pm 0.24$ ) and  $\text{Cl}^-$  (but not  $\text{Na}^+$ ) is reclaimed, such that the alkalization of the fluid promotes significant  $\text{NH}_3$  volatilization ( $\sim 71 \mu\text{l kg}^{-1} \text{h}^{-1}$  in control crabs). While pH 7 is not alkaline, the increase in pH is quite effective. For example, at an ammonia concentration of 116  $\text{mmol l}^{-1}$  and pH 5.4 for primary urine (DeVries & Wolcott, 1993), if the pH is increased to pH 7 it is possible to estimate  $P_{\text{NH}_3}$  using the pK and solubility for  $\text{NH}_3$  provided by Kormanik and Cameron (1981) as used by Varley and Greenaway (1994). In the primary urine  $P_{\text{NH}_3} = 11.6 \text{ Pa}$  whereas at pH 7 the  $P_{\text{NH}_3} = 460 \text{ Pa}$ , which is a 40-fold increase in potential diffusive gradient. In view of the concomitant increase in the fluid  $\text{CO}_2$  concentration and the uptake of  $\text{Cl}^-$ , the most obvious candidate for the net base excretion is transport by an apical  $\text{HCO}_3^-/\text{Cl}^-$  exchanger (DeVries & Wolcott, 1993). Reclamation of urinary  $\text{Na}^+$  appears to be accomplished within the antennal gland (DeVries et al., 1994). These authors (DeVries et al., 1994) report high activity of  $\text{Na}^+/\text{K}^+$ ATPase in the antennal gland of *O. quadrata* for which  $\text{NH}_4^+$  may substitute for  $\text{K}^+$  in the basal membrane exchange. Furthermore, apical  $\text{Na}^+/\text{H}^+$  antiporters in the antennal gland may sustain both  $\text{Na}^+$  reclamation and acidification of urine to promote  $\text{NH}_4^+$ -trapping (Fig. 8).

Study of the more-terrestrial grapsid, *Geograpsus grayi*, has revealed further modifications of the  $\text{NH}_3/\text{NH}_4^+$  excretory system (Greenaway and Nakamura, 1991; Varley and Greenaway, 1994). *G. grayi* is a highly active carnivorous land crab and also reprocesses the urine to reclaim salts via branchial uptake (Greenaway and Nakamura, 1991) but, unlike *Ocypode* sp., does not employ ion reclamation within the antennal gland (Varley and Greenaway, 1994). Clearly *Ocypode* and *Geograpsus* represent separate radiations in to the terrestrial habitat, but with superficially analogous ammonia excretion physiology. *G. grayi* volatilizes  $\text{NH}_3$  from the limited volume of 'P' within the branchial chamber and, thereby, increases the effective  $\text{NH}_4^+$  capacity of the fluid, which may achieve concentrations in excess of 80  $\text{mmol l}^{-1}$  compared with <1  $\text{mmol l}^{-1}$  in the urine (Varley and Greenaway, 1994). However, *G. grayi* manages a rate of ammonia excretion comparable to that of aquatic crabs in water (107–220  $\mu\text{mol kg}^{-1} \text{h}^{-1}$ ; Greenaway and Nakamura, 1991; Varley and Greenaway, 1994). Gaseous ammonia contributes  $\sim 78\%$  of this total excretion in a discontinuous process over 3 h to 3 days (Varley and Greenaway, 1994) although urine

flow is apparently limited, restricting fluid available for 'P' formation. The pH of this fluid (pH 8.07) is higher than that of the hemolymph (pH 7.66–7.59) and at the same time the  $\text{CO}_2$  content (36  $\text{mmol l}^{-1}$ ) is considerably greater than that of the hemolymph (13.7–17.2  $\text{mmol l}^{-1}$ ). Amiloride reduced  $\text{NH}_4^+$  efflux by 83% in this system and reduced unidirectional  $\text{Na}^+$  uptake. Thus,  $\text{NH}_3$  volatilization is achieved by raising the fluid pH towards the pK, such that gaseous  $\text{NH}_3$  becomes 8% of the total ammonia, creating a diffusive gradient ( $P_{\text{NH}_3} \sim 3.3 \text{ Pa}$ ) into the convective air stream. Varley and Greenaway (1994) discussed the difficulties in transporting  $\text{NH}_3/\text{NH}_4^+$  outward into this fluid in the absence of 'acid-trapping' and proposed a net excretion reaction  $\text{NH}_4^+ + \text{HCO}_3^- \rightarrow \text{H}_2\text{O} + \text{CO}_2 \uparrow + \text{NH}_3 \uparrow$ . The volatilization of  $\text{NH}_3$  and  $\text{CO}_2$ , together with formation of water, all contribute to lowered ionic strength in the extrabranchial fluid. Again, apical  $\text{NH}_4^+$  transport, possibly mediated by a putative Rhesus-related ammonia transporter protein, needs to be investigated in further studies. This mechanism in *G. grayi* effectively increases the functional volume of the 'P' offering some escape from the dependency on regular and significant urine flow rate. Thus, there are significant increases in the amount of ammonia excreted per unit volume of 'P', while retaining the advantages of ammonia excretion, thereby allowing a more terrestrial habit (Fig. 9). At the same time, the system remains potentially limited by the supply of  $\text{Na}^+$  and  $\text{Cl}^-$  in a lowered supply of urine.

The most successful terrestrial animals have abandoned  $\text{NH}_3$  as an N-excretory vehicle in favor of urea or purines. The anomuran *Birgus latro* is the only identified purinotelic crab (Greenaway and Morris, 1989) but is sympatric with several species of gecarcinids that retain  $\text{NH}_3$  excretion. Excreting purines allows greater flexibility of urinary water flow independently of urine reprocessing and salt reclamation, but significant energetic advantages may accompany ammonotelic.

#### Implications

Decapod crustaceans exhibit a wide variety of ammonia excretion mechanisms and consequently provide good models for general investigation of nitrogen excretion. The debate as to whether ammonia is lost via  $\text{NH}_3$  diffusion or by  $\text{NH}_4^+$  transport remains active, but the answer may be both or either depending on circumstance and species. The ability to move ammonia against its gradient is obviously essential. There is a clear continuum of increased terrestriality accompanied by managed and active excretion with lowered water loss. This continuum represents multiple transitions onto land and is underpinned by phylogenetic differences. The increased application of molecular and post-genomic methodologies to the question will reveal, for example, the role of Rhesus-related proteins and vesicular transport systems in the physical extrusion of ammonia.

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## References

- Adamczewska, A. M., van Aardt, W. J. and Morris, S. (1997). The role of lungs and gills in an African freshwater crab *Potamonautes warreni* (Calman), (Decapoda, Potamoidea) in gas exchange with water, with air and during exercise. *J. Crust. Biol.* **17**, 596-608.
- Atanassov, C. L., Muller, C. D., Sarhan, S., Knodgen, B., Rebel, G. and Seiler, N. (1994). Effect of ammonia on endocytosis, cytokine production and lysosomal enzyme activity of a microglial cell line. *Res. Immunol.* **145**, 277-288.
- Atanassov, C. L., Muller, C. D., Dumont, S., Rebel, G., Poindron, P. and Seiler, N. (1995). Effect of ammonia on endocytosis and cytokine production by immortalized human microglia and astroglia cells. *Neurochem. Int.* **27**, 417-424.
- Avenet, P. and Lignon, J. M. (1985). Ionic permeabilities of gill lamina cuticle of the crayfish *Astacus leptodactylus* (E). *J. Physiol.* **363**, 377-401.
- Bakouh, N., Benjelloun, F., Hulin, P., Brouillard, F., Edelman, A., Cherif-Zahar, B. and Planelles, G. (2004). NH<sub>3</sub> is involved in the NH<sub>4</sub><sup>+</sup> transport induced by the functional expression of the Human Rh C glycoprotein. *J. Biol. Chem.* **279**, 15975-15983.
- Binstock, L. and Lecar, H. (1969). Ammonium ion currents in the squid giant axon. *J. Gen. Physiol.* **53**, 342-361.
- Böttcher, K. and Siebers, D. (1993). Biochemistry, localization, and physiology of carbonic anhydrase in the gills of euryhaline crabs. *J. Exp. Zool.* **265**, 398-409.
- Burckhardt, B.-C. and Frömter, E. (1992). Pathways of NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> permeation across *Xenopus laevis* oocyte cell membrane. *Pflügers Arch.* **420**, 83-86.
- Burnett, L. E. and McMahon, B. R. (1985). Facilitation of CO<sub>2</sub> excretion by carbonic anhydrase located on the surface of the basal membrane of crab gill epithelium. *Respir. Physiol.* **62**, 341-348.
- Burnett, L. E. and Towle, D. W. (1990). Sodium ion uptake by perfused gills of the blue crab *Callinectes sapidus*: Effect of ouabain and amiloride. *J. Exp. Biol.* **149**, 293-305.
- Butterworth, R. F. (2002). Pathophysiology of hepatic encephalopathy: a new look at ammonia. *Metab. Brain Dis.* **17**, 221-227.
- Cameron, J. N. and Batterton, C. V. (1978). Antennal gland function in the freshwater crab *Callinectes sapidus*: Water, electrolyte, acid-base, and ammonia excretion. *J. Comp. Physiol.* **123**, 143-148.
- Cameron, J. N. and Heisler, N. (1983). Studies of ammonia in the rainbow trout: Physicochemical parameters, acid-base behaviour and respiratory clearance. *J. Exp. Biol.* **105**, 107-125.
- Chen, J. C. and Lin, C. Y. (1992). Lethal effects of ammonia on *Penaeus chinensis* Osbeck juveniles at different salinity levels. *J. Exp. Mar. Biol. Ecol.* **156**, 139-148.
- Claybrook, D. L. (1983). The biology of crustacea. In *Internal Anatomy and Physiological Regulation*, vol. 5 (ed. L. H. Mantel), pp. 163-213. London: Academic Press.
- Cooper, A. J. and Plum, F. (1987). Biochemistry and physiology of brain ammonia. *Physiol. Rev.* **67**, 440-519.
- Copeland, D. E. and Fitzjarrell, A. T. (1968). The salt absorbing cells in the gills of the blue crab (*Callinectes sapidus*, Rathbun) with notes on modified mitochondria. *Z. Zellforsch.* **92**, 1-22.
- Corotto, F. S. and Holliday, C. W. (1996). Branchial Na,K-ATPase and osmoregulation in the purple shore crab, *Hemigrapsus nudus* (Dana). *Comp. Biochem. Physiol. A* **113**, 361-368.
- Corpet, F. (1988). Multiple sequence alignment with hierarchical clustering. *Nucl. Acids Res.* **16**, 10881-10890.
- D'Orazio, S. E. and Holliday, C. W. (1985). Gill NaK-ATPase and osmoregulation in the sand fiddler crab *Uca pugnator*. *Physiol. Zool.* **58**, 364-373.
- Dagg, M. J. (1976). Complete carbon and nitrogen budgets for the carnivorous amphipod *Calliopius laevisculus* (Kroyer). *Int. Rev. Ges. Hydrobiol.* **61**, 297-357.
- Davie, P. J. F. (2002). Crustacea: Malacostraca: Eucarida (Part 2): Decapoda-Anomura, Brachyura. In *Zoological Catalogue of Australia*, vol. 19.3B (ed. A. Wells and W. W. K. Houston). Melbourne, Australia: CSIRO Publishing
- deFur, P. L. and McMahon, B. R. (1984). Physiological compensation to short-term air exposure in red rock crabs, *Cancer productus* Randall, from littoral and sublittoral habitats. 2. Acid-base-balance. *Physiol. Zool.* **57**, 151-160.
- Dela-Cruz, J. and Morris, S. (1997). Water and ion balance and nitrogenous excretion as limitations to terrestrial excursion in the Christmas Island Blue Crab, *Cardisoma hirtipes* (Dana). *J. Exp. Zool.* **279**, 537-548.
- DeVries, M. C. and Wolcott, D. L. (1993). Gaseous ammonia evolution is coupled to reprocessing of urine at the gills of ghost crabs. *J. Exp. Zool.* **267**, 97-103.
- DeVries, M. C., Wolcott, D. L. and Holliday, C. W. (1994). High ammonia and low pH in the urine of the ghost crab, *Ocypode quadrata*. *Biol. Bull.* **186**, 342-348.
- Durand, F. and Regnault, M. (1998). Nitrogen metabolism of two portunid crabs, *Carcinus maenas* and *Necora puber*, during prolonged air exposure and subsequent recovery: A comparative study. *J. Exp. Biol.* **201**, 2515-2528.
- Durand, F., Devillers, N., Lallier, F. H. and Regnault, M. (2000). Nitrogen excretion and changes in blood components during emersion of the subtidal spider crab *Maia squinado* (L.). *Comp. Biochem. Physiol. A* **127**, 259-271.
- Eladari, D., Cheval, L., Quentin, F., Bertrand, O., Mouro, I., Cherif-Zahar, B., Cartron, J.-P., Paillard, M., Doucet, A. and Chambrey, R. (2002). Expression of RhCG, a new putative NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> transporter, along the rat nephron. *J. Am. Soc. Nephrol.* **13**, 1999-2008.
- Enoksson, V. and Samuelsson, M.-O. (1987). Nitrification and dissimilatory ammonium production and the effects on nitrogen flux over the sediment-water interface in bioturbated coastal sediments. *Mar. Ecol. Prog. Ser.* **36**, 181-189.
- Furriel, R. P. M., Masui, D. C., McNamara, J. C. and Leone, F. A. (2004). Modulation of gill Na<sup>+</sup>, K<sup>+</sup>-ATPase activity by ammonium ions: putative coupling of nitrogen excretion and ion uptake in the freshwater shrimp *Macrobrachium olfersii*. *J. Exp. Zool.* **301A**, 63-74.
- Garvin, J. L., Burg, M. B. and Knepper, M. A. (1987). NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup> transport by rabbit renal proximal straight tubules. *Am. J. Physiol.* **252**, F232-F239.
- Goodman, S. H. and Cavey, M. J. (1990). Organization of a phyllobranchiate gill from the green shore crab *Carcinus maenas* (Crustacea, Decapoda). *Cell Tissue Res.* **260**, 495-505.
- Greenaway, P. (1988). Ion and water balance. In *The Biology of Land Crabs* (ed. W. W. Burggren and B. R. McMahon), pp. 211-248. New York: Cambridge University Press.
- Greenaway, P. (1991). Nitrogenous excretion in aquatic and terrestrial Crustacea. *Mem. Qld. Mus.* **31**, 215-227.
- Greenaway, P. (1999). Physiological diversity and the colonisation of land. In *Proceedings of the Fourth International Crustacean Congress*, pp. 823-842 (ed. F. R. Schram and J. C. von Vaupel Klein), Amsterdam, The Netherlands, July 20-24, 1998, vol. I. Leiden: Brill Academic Publishers.
- Greenaway, P. (2003). Terrestrial adaptations in the Anomura (Crustacea: Decapoda). *Mem. Mus. Victoria* **60**, 13-26.
- Greenaway, P. and MacMillen, R. E. (1978). Salt and water balance in the terrestrial phase of the inland crab *Holthuisana (Austrothelphusa) transversa* Martens (Parathelphusoidea: Sundathelphusidae). *Physiol. Zool.* **51**, 217-229.
- Greenaway, P. and Morris, S. (1989). Adaptations to a terrestrial existence by the robber crab, *Birgus latro* L. III. nitrogenous excretion. *J. Exp. Biol.* **143**, 333-346.
- Greenaway, P. and Nakamura, T. (1991). Nitrogenous excretion in 2 terrestrial crabs (*Gecarcoidea natalis* and *Geograpsus grayi*). *Physiol. Zool.* **64**, 767-786.
- Harris, R. R., Coley, S., Collins, S. and McCabe, R. (2001). Ammonia uptake and its effects on ionoregulation in the freshwater crayfish *Pacifastacus leniusculus* (Dana). *J. Comp. Physiol. B* **171**, 681-693.
- Hartenstein, R. (1970). Nitrogen metabolism in non-insect arthropods. In *Comparative Biochemistry of Nitrogen Metabolism*, vol. 1 (ed. J. W. Campbell), pp. 299-385. New York: Academic Press.
- Hazel, R. H., Burkhead, C. E. and Huggins, D. G. (1982). Development of water quality criteria for ammonia and total residual chlorine for the protection of aquatic life in Two Johnson County, Kansas Streams. In *Proceedings of 5th Annual Symposium Aquatic Toxicology* (ed. J. G. Pearson, R. B. Foster and W. E. Bishop), pp. 381-388. ASTM STP 766, Philadelphia.
- Henry, R. P. (1988). Subcellular distribution of carbonic anhydrase activity in the gills of the blue crab, *Callinectes sapidus*. *J. Exp. Zool.* **245**, 1-8.
- Henry, R. P. and Wheatly, M. G. (1992). Interaction of respiration, ion regulation, and acid-base balance in the everyday life of aquatic crustaceans. *Am. Zool.* **32**, 407-416.
- Henry, R. P., Weihrauch, D., Gehrich, S. and Towle, D. W. (2003). Salinity-mediated induction of carbonic anhydrase in the gills of the euryhaline green crab, *Carcinus maenas*. *Comp. Biochem. Physiol. A* **136**, 243-258.
- Hrnjez, B. J., Song, J. C., Prasad, M., Mayol, J. M. and Matthews, J. B.



- (1999). Ammonia blockade of intestinal epithelial  $K^+$  conductance. *Am. J. Physiol.* **277**, G521-G532.
- King, F. D., Cucci, T. L. and Bidigare, R. R.** (1985). A pathway of nitrogen metabolism in marine decapod crabs. *Comp. Biochem. Physiol. B* **80**, 401-403.
- Knepper, M. A., Packer, R. and Good, D. W.** (1989). Ammonium transport in the kidney. *Physiol. Rev.* **69**, 179-249.
- Kormanik, G. A. and Cameron, J. N.** (1981). Ammonia excretion in the seawater blue crab (*Callinectes sapidus*) occurs by diffusion, and not  $Na^+/NH_4^+$  exchange. *J. Comp. Physiol. B* **141**, 457-462.
- Koroleff, F.** (1983). Determination of ammonia. In *Methods of Seawater Analysis* (ed. K. Grasshoff, M. Ehrhart and K. Kremling), pp. 150-151. Weinheim: Verlag Chemie GmbH.
- Krishnamoorthy, R. V. and Srihari, K.** (1973). Changes in the patterns of the freshwater field crab *Paratellphusa hydrodromous* upon adaptation to higher salinities. *Mar. Biol.* **21**, 341-348.
- Laursen, H. and Diemer, N. H.** (1997). Morphometric studies of rat glial cell ultrastructure after urease-induced hyperammonaemia. *Neuropathol. Appl. Neurobiol.* **5**, 345-362.
- Lignon, J. M.** (1987). Ionic permeabilities of the isolated gill cuticle of the shore crab *Carcinus maenas*. *J. Exp. Biol.* **131**, 159-174.
- Lin, H. C., Su, Y. C. and Su, S. H.** (2002). A comparative study of osmoregulation in four fiddler crabs (Ocypodidae: Uca). *Zool. Sci.* **19**, 643-650.
- Linton, S. M. and Greenaway, P.** (1995). Nitrogenous excretion in the amphibious crab *Holthuisana transversa* under wet and dry conditions. *J. Crust. Biol.* **15**, 633-644.
- Linton, S. M. and Greenaway, P.** (1997a). Urate deposits in the gecarcinid land crab *Gecarcoidea natalis* are synthesised *de novo* from excess dietary nitrogen. *J. Exp. Biol.* **200**, 2347-2354.
- Linton, S. M. and Greenaway, P.** (1997b). Intracellular purine deposits in the gecarcinid land crab *Gecarcoidea natalis*. *J. Morph.* **231**, 101-110.
- Linton, S. M. and Greenaway, P.** (1998). Enzymes of urate synthesis and catabolism in the gecarcinid land crab *Gecarcoidea natalis*. *Exp. Biol. Online* **3**:5.
- Linton, S. M. and Greenaway, P.** (2000). The nitrogen requirements and dietary nitrogen utilization for the gecarcinid land crab *Gecarcoidea natalis*. *Physiol. Biochem. Zool. Biochem.* **73**, 209-218.
- Lohse, L., Malschaert, J. F. P., Slomp, C. P., Helder, W. and Van Raaphorst, W.** (1993). Nitrogen cycling in North Sea sediments: Interaction of denitrification and nitrification in offshore and coastal areas. *Mar. Ecol. Prog. Ser.* **101**, 283-296.
- Lopez Mananes, A. A., Magnoni, L. J. and Goldemberg, A. L.** (2000). Branchial carbonic anhydrase (CA) of gills of *Chasmagnathus granulata* (Crustacea Decapoda). *Comp. Biochem. Physiol. B* **127**, 85-95.
- Lucu, C.** (1989). Evidence for  $Cl^-$  exchangers in perfused *Carcinus* gills. *Comp. Biochem. Physiol. A* **92**, 415-420.
- Lucu, C.** (1990). Ionic mechanisms in crustacean gill epithelia. *Comp. Biochem. Physiol. A* **97**, 297-306.
- Lucu, C., Devescovi, M. and Siebers, D.** (1989). Do amiloride and oubain affect ammonia fluxes in perfused *Carcinus* gill epithelia? *J. Exp. Zool.* **249**, 1-5.
- Luquet, C. M., Postel, U., Halperin, J., Urcola, M. R., Marques, R. and Siebers, D.** (2002). Transepithelial potential differences and  $Na^+$  flux in isolated perfused gills of the crab *Chasmagnathus granulatus* (Grapsidae) acclimated to hyper- and hypo-salinity. *J. Exp. Biol.* **205**, 71-77.
- Mantel, L. H. and Farmer, I. L.** (1983). Osmotic and ionic regulation. In *The biology of Crustacea*, vol. 5 (ed. L. H. Mantel), pp. 54-126. London: Academic Press.
- Marcaida, G., Felipo, V., Hermenegildo, C., Minana, M. D. and Grisolia, S.** (1992). Acute ammonia toxicity is mediated by NMDA type of glutamate receptors. *FEBS Lett.* **296**, 67-68.
- Marini, A. M., Matassi, G., Raynal, V., Andre, B., Cartron, J. P. and Cherif-Zahar, B.** (2000). The human Rhesus-associated RhAG protein and a kidney homologue promote ammonium transport in yeast. *Nat. Genet.* **26**, 341-344.
- Masui, D. C., Furriel, R. P., McNamara, J. C., Mantelatto, F. L. and Leone, F. A.** (2002). Modulation by ammonium ions of gill microsomal ( $Na^+, K^+$ )-ATPase in the swimming crab *Callinectes danae*: A possible mechanism for regulation of ammonia excretion. *Comp. Biochem. Physiol. C* **132**, 471-482.
- Morris, S.** (2001). Neuroendocrine regulation of osmoregulation and the evolution of air-breathing in decapod crustaceans. *J. Exp. Biol.* **204**, 979-989.
- Morris, S.** (2002). The ecophysiology of air-breathing in crabs with special reference to *Gecarcoidea natalis*. *Comp. Biochem. Physiol. B* **131**, 559-570.
- Morris, S. and Ahern, M. D.** (2003). 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. *J. Exp. Biol.* **206**, 2869-2881.
- Morris, S. and van Aardt, W. J.** (1998). Salt and water relations, and nitrogen excretion, in the amphibious African freshwater crab *Potamonautes warreni* in water and in air. *J. Exp. Biol.* **201**, 883-893.
- Morris, S., Greenaway, P., Adamczewska, A. M. and Ahern, M. D.** (2000). Adaptations to a terrestrial existence in the robber crab *Birgus latro*. IX. Hormonal control of post-renal urine reprocessing and salt balance in the branchial chamber. *J. Exp. Biol.* **203**, 389-396.
- O'Donnell M. J. and Wright J. C.** (1995). Nitrogen excretion in terrestrial crustaceans. In *Nitrogen Metabolism and Excretion* (ed. P. J. Walsh and P. Wright), pp. 105-118. Boca Raton, FL: CRC Press.
- Onken, H. and McNamara, J. C.** (2002). Hyperosmoregulation in the red freshwater crab *Dilocarcinus pagei* (Brachyura, Trichodactylidae): Structural and functional asymmetries of the posterior gills. *J. Exp. Biol.* **205**, 167-175.
- Onken, H. and Putzenlechner, M.** (1995). A V-ATPase drives active, electrogenic and  $Na^+$ -independent  $Cl^-$  absorption across the gills of *Eriocheir sinensis*. *J. Exp. Biol.* **198**, 767-774.
- Onken, H. and Riestenpatt, S.** (2002). Ion transport across posterior gills of hyperosmoregulating shore crabs (*Carcinus maenas*): Amiloride blocks the cuticular  $Na^+$  conductance and induces current-noise. *J. Exp. Biol.* **205**, 523-531.
- Ostrensky, A., Marchiori, M. A. and Poersch, L. H.** (1992). Aquatic toxicity of ammonia in the metamorphosis of post-larvae *Penaeus paulensis* Perez-Farfante, 1967. *An. Acad. Bras. Cienc.* **64**(4), 383-389.
- Péqueux, A. and Gilles, R.** (1981).  $Na^+$ -fluxes across isolated perfused gills of the Chinese crab *Eriocheir sinensis*. *J. Exp. Biol.* **92**, 173-186.
- Péqueux, A., Marchal, A., Wanson, S. and Gilles, R.** (1984). Kinetic characteristics and specific activity of ( $Na^+K^+$ )-ATPase in the euryhaline crab *Eriocheir sinensis* during acclimation. *Mar. Biol. Lett.* **5**, 35-45.
- Pressley, T. A., Graves, J. S. and Krall, A. R.** (1981). Amiloride-sensitive ammonium and sodium ion transport in the blue crab. *Am. J. Physiol.* **241**, R370-R378.
- Regnault, M.** (1987). Nitrogen excretion in marine and fresh-water crustacea. *Biol. Rev.* **62**, 1-24.
- Regnault, M.** (1992). Effect of air exposure on nitrogen-metabolism in the crab *Cancer pagurus*. *J. Exp. Zool.* **264**, 372-380.
- Regnault, M.** (1994). Effect of air exposure on ammonia excretion and ammonia content of branchial water of the crab *Cancer pagurus*. *J. Exp. Zool.* **268**, 208-217.
- Riestenpatt, S., Onken, H. and Siebers, D.** (1996). Active absorption of  $Na^+$  and  $Cl^-$  across the gill epithelium of the shore crab *Carcinus maenas*: Voltage-clamp and ion-flux studies. *J. Exp. Biol.* **199**, 1545-1554.
- Schleich, C. E., Goldemberg, L. A. and Lopez Mananes, A. A.** (2001). Salinity dependent  $Na^+K^+$ -ATPase activity in gills of the euryhaline crab *Chasmagnathus granulata*. *Gen. Physiol. Biophys.* **20**, 255-266.
- Schoffeniels, E. and Gilles, R.** (1970). Nitrogenous constituents and nitrogen metabolism in arthropods. In *Chemical Zoology*, vol. 5 (ed. M. Florkin and B. Sheer), pp. 199-227. New York: Academic Press.
- Siebers, D., Riestenpatt, S., Wehrauch, D. and Lucu, C.** (1995). Exkretion von Stickstoff über die Kiemen der Strandkrabbe *Carcinus maenas*. *Verh. Dtsch. Zool. Ges.* **88**, 134.
- Singh, S. K., Binder, H. J., Geibel, J. P. and Boron, W.** (1995). An apical permeability barrier to  $NH_3/NH_4^+$  in isolated, perfused colonic crypts. *Proc. Natl. Acad. Sci. USA* **92**, 11573-11577.
- Spaargaren, D. H.** (1990). The effect of environmental ammonia concentrations on the ion-exchange of shore crabs, *Carcinus maenas* (L.). *Comp. Biochem. Physiol. C* **97**, 87-91.
- Taylor, H. H. and Greenaway, P.** (2002). Osmoregulation in the terrestrial Christmas Island red crab *Gecarcoidea natalis* (Brachyura: Gecarcinidae): Modulation of branchial chloride uptake from the urine. *J. Exp. Biol.* **205**, 3251-3260.
- Towle, D. W.** (1981). Role of  $Na^+K^+$ -ATPase in ion regulation by marine and estuarine animals. *Mar. Biol. Lett.* **2**, 107-122.
- Towle, D. W. and Hølleland, T.** (1987). Ammonium ion substitutes for  $K^+$  in ATP-dependent  $Na^+$  transport by basolateral membrane vesicles. *Am. J. Physiol.* **252**, R479-R489.
- Towle, D. W. and Kays, W. T.** (1986). Basolateral localization of  $Na^+K^+$ -

- ATPase in gill epithelium of two osmoregulating crabs, *Callinectes sapidus* and *Carcinus maenas*. *J. Exp. Zool.* **239**, 311-318.
- Towle, D. W. and Weihrauch, D.** (2001). Osmoregulation by gills of euryhaline crabs: molecular analysis of transporters. *Am. Zool.* **41**, 770-780.
- Towle, D. W., Rushton, M. E., Heidysch, D., Magnani, J. J., Rose, M. J., Amstutz, A., Jordan, M. K., Shearer, D. W. and Wu, W. S.** (1997). Sodium/proton antiporter in the euryhaline crab *Carcinus maenas*: Molecular cloning, expression and tissue distribution. *J. Exp. Biol.* **200**, 1003-1014.
- Towle, D. W., Paulsen, R. S., Weihrauch, D., Kordylewski, M., Salvador, C., Lignot, J. H. and Spanings-Pierrot, C.** (2001). Na<sup>+</sup>+K<sup>+</sup>-ATPase in gills of the blue crab *Callinectes sapidus*: cDNA sequencing and salinity-related expression of  $\alpha$ -subunit mRNA and protein. *J. Exp. Biol.* **204**, 4005-4012.
- Varley, D. G. and Greenaway, P.** (1994). Nitrogenous excretion in the terrestrial carnivorous crab *Geograpsus grayi* – site and mechanism of excretion. *J. Exp. Biol.* **190**, 179-193.
- Vitale, A. M., Monserrat, J. M., Castilho, P. and Rodriguez, E. M.** (1999). Inhibitory effects of cadmium on carbonic anhydrase activity and ionic regulation of the estuarine crab *Chasmagnathus granulata* (Decapoda, Grapsidae). *Comp. Biochem. Physiol.* **122C**, 121-129.
- Weihrauch, D. and Towle, D. W.** (2000). Na<sup>+</sup>/H<sup>+</sup>-exchanger and Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> cotransporter are expressed in the gills of the euryhaline Chinese crab *Eriocheir sinensis*. *Comp. Biochem. Physiol.* **126B, Suppl.**, S158.
- Weihrauch, D., Becker, W., Postel, U., Riestenpatt, S. and Siebers, D.** (1998). Active excretion of ammonia across the gills of the shore crab *Carcinus maenas* and its relation to osmoregulatory ion uptake. *J. Comp. Physiol. B* **168**, 364-376.
- Weihrauch, D., Becker, W., Postel, U., Luck-Kopp, S. and Siebers, D.** (1999). Potential of active excretion of ammonia in three different haline species of crabs. *J. Comp. Physiol. B* **169**, 25-37.
- Weihrauch, D., Ziegler, A., Siebers, D. and Towle, D. W.** (2001a). Molecular characterization of V-type H<sup>+</sup>-ATPase (B-subunit) in gills of euryhaline crabs and its physiological role in osmoregulatory ion uptake. *J. Exp. Biol.* **204**, 25-37.
- Weihrauch, D., Marini, A. M. and Towle, D. W.** (2001b). Cloning and expression of a putative Rh-like ammonium transporter from gills of the shore crab *Carcinus maenas*. *Am. Zool.* **41**, 1621.
- Weihrauch, D., Ziegler, A., Siebers, D. and Towle, D. W.** (2002). Active ammonia excretion across the gills of the green shore crab *Carcinus maenas*: Participation of Na<sup>+</sup>/K<sup>+</sup>-ATPase, V-type H<sup>+</sup>-ATPase and functional microtubules. *J. Exp. Biol.* **205**, 2765-2775.
- Wilkie, M. P.** (1997). Mechanisms of ammonia excretion across fish gills. *Comp. Biochem. Physiol. A* **118**, 39-50.
- Wilson, R., Wright, P., Munger, S. and Wood, C.** (1994). Ammonia excretion in freshwater rainbow trout (*Oncorhynchus mykiss*) and the importance of gill boundary layer acidification: Lack of evidence for Na<sup>+</sup>/NH<sub>4</sub><sup>+</sup> exchange. *J. Exp. Biol.* **191**, 37-58.
- Wolcott, D. L.** (1991). Nitrogen-excretion is enhanced during urine recycling in two species of terrestrial crab. *J. Exp. Zool.* **259**, 181-187.
- Wolcott, T. G.** (1992). Water and solute balance in the transition to land. *Am. Zool.* **32**, 428-437.
- Wolcott, T. G. and Wolcott, D. L.** (1985). Extrarenal modification of urine for ion conservation in ghost crabs, *Ocypode quadrata* (Fabricius). *J. Exp. Mar. Biol. Ecol.* **91**, 93-107.
- Wolcott, T. G. and Wolcott, D. L.** (1991). Ion conservation by reprocessing of urine in the land crab *Gecarcinus lateralis* (Freminville). *Physiol. Zool.* **64**, 344-361.
- Wood, C. M., Boutilier, R. G. and Randall, D. J.** (1986). The physiology of dehydration stress in the land crab, *Cardisoma carnifex*: respiration, ionoregulation, acid-base balance and nitrogenous waste excretion. *J. Exp. Biol.* **126**, 271-296.
- Wood, C. M., Matsuo, A. Y., Gonzalez, R. J., Wilson, R. W., Patrick, M. L. and Val, A. L.** (2002). Mechanisms of ion transport in *Potamotrygon*, a stenohaline freshwater elasmobranch native to the ion-poor blackwaters of the Rio Negro. *J. Exp. Biol.* **205**, 3039-3054.
- Young-Lai, W. W., Charmantier-Daures, M. and Charmantier, G.** (1991). Effect of ammonia on survival and osmoregulation in different life stages of the lobster *Homarus americanus*. *Mar. Biol.* **110**, 293-300.