

OSMOREGULATION, IONIC EXCHANGE, BLOOD CHEMISTRY, AND  
NITROGENOUS WASTE EXCRETION IN THE LAND CRAB  
*CARDISOMA CARNIFEX*: A FIELD AND LABORATORY STUDY

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ABSTRACT

*Cardisoma carnifex* in Moorea, French Polynesia, were sampled in the field and after exposure in the laboratory to either fresh- or seawater under conditions which allowed the crabs to flush their branchial chambers with the medium but not to ventilate it. Relative to field data, ionic and osmotic status of the hemolymph was virtually unchanged by exposure to freshwater, but markedly disturbed by seawater. The crabs were capable of net  $\text{Na}^+$  and  $\text{Cl}^-$  uptake from freshwater. Water sampled from natural crab burrows was essentially freshwater. It is concluded that the population was "in equilibrium" with freshwater in the wild.

Net  $\text{H}^+$  uptake (= base excretion) occurred in both fresh- and seawater; in freshwater there was a 1:1 relationship between net  $\text{H}^+$  flux and strong cation minus anion flux (*i.e.*,  $\text{Na}^+ + \text{Mg}^{++} + \text{Ca}^{++} + \text{K}^+ - \text{Cl}^-$ ). Unidirectional  $\text{Na}^+$  and  $\text{Cl}^-$  exchanges, measured radioisotopically, were typical of euryhaline crabs in freshwater, but influxes were unusual in showing no increase in seawater. Mild dehydration caused complex alterations in these exchanges in both media, associated with small and quickly reversed changes in hemolymph composition in freshwater, but larger effects in seawater which were not reversed. High levels of ammonia in hemolymph occurred in the field but declined in the laboratory, while the level of urea was low in both situations. Both wastes were excreted into the water. Neither uric acid nor gaseous ammonia excretion were detected, and uric acid was generally not found in hemolymph. The results are discussed in relation to the ecology of this unusual animal.

INTRODUCTION

Water and electrolyte balance has now been studied extensively in the land crabs (for reviews, see Bliss, 1968, 1979; Edney, 1977; Mantel, 1979; Mantel and Farmer, 1983; Powers and Bliss, 1983). In gecarcinids, the general picture includes an ability to iono- and osmo-regulate, though far from perfectly, over a range of external salinities from 0 to >100% seawater, combined with an ability to exist for prolonged periods in the absence of free environmental water. Adaptations include reduced integumental permeability to evaporative water loss relative to aquatic crabs (*e.g.*, Herreid, 1969), tolerance of wide variations in hemolymph ionic strength and body water content (*e.g.*, Gross, 1963, 1964), and an ability to take up interstitial water from soil (*e.g.*,

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Wolcott, 1984). While the gut may have a role (Mantel, 1968), the gills are thought, though not yet proven, to be the major site of ionoregulation, as is true in aquatic crabs (Kirschner, 1979). Certainly, the branchial epithelium is rich in cells and enzymes thought to be associated with salt transport (Quinn and Lane, 1966; Copeland, 1968; Towle, 1981; Henry, 1984). The renal system (antennal gland and bladder) has been more thoroughly investigated (Gross, 1963, 1964; de Leersnyder and Hoestlandt, 1963, 1964; Gross *et al.*, 1966; Harris, 1977; Harris and Kormanik, 1981; Kormanik and Harris, 1981). It appears to be of major importance in volume regulation, minor importance in ionoregulation, and it makes a negligible contribution to osmoregulation.

Despite this intensive study, there exist only very limited data on the hydro-mineral status of gecarcinids under natural conditions in the wild (Skinner *et al.*, 1965; Gross *et al.*, 1966) and on normal rates of ionic exchange with the environment (Kormanik and Harris, 1981). The relative importance of various nitrogenous end products (ammonia, urea, uric acid) appears to be in conflict (Gifford, 1968; Horne, 1968; Henry and Cameron, 1981). The present study addresses these questions in a natural population of *Cardisoma carnifex*. We have assessed chemical conditions in the burrows, the blood chemistry of animals sampled in the field, and the changes which occurred in the latter, together with the flux rates of ions and nitrogenous wastes, when the same crabs were subsequently held in the laboratory under controlled conditions. *C. carnifex* was especially suitable for such study, since the water normally carried in its branchial chambers is intermittently changed over when an external pool is provided (Wood and Randall, 1981a). Exchange rates of substances between the whole organism and its environment could therefore be determined by periodic analysis of a closed external pool, an approach supplemented by the use of radiotracers to measure unidirectional  $\text{Na}^+$  and  $\text{Cl}^-$  fluxes.

## MATERIALS AND METHODS

### *Experimental animals*

Land crabs [*Cardisoma carnifex*;  $181.6 \pm 11.4$  g ( $\bar{x} \pm 1$  S.E.M.);  $n = 39$ ] were collected between 19:30 and 01:00 h (air temperature = 20–24°C) within 0.5 km of the shore on the island of Moorea, French Polynesia, during July and August. The crabs were actively foraging on fallen coconuts, fruits, and leaves at this time, and were generally caught after a brief chase. Within 5 min of capture, the crabs were dried and dusted with a towel as far as practical, then weighed to an accuracy of 0.2 g. No effort was made to remove water from the branchial chambers. If required, a venous hemolymph sample (0.8 ml) was immediately withdrawn from the arthrodistal membrane at the base of a walking leg. The crabs were transported back to the laboratory in a closed container filled with plant material (to prevent aggression) and set up in the flux experiments within 4 h. Four groups of animals were examined. The first ( $n = 12$ ) were carried through the flux protocol in a 1% seawater:99% Moorea tapwater mixture ("freshwater"; *cf.*, Table I) with hemolymph sampling at appropriate times. The second ( $n = 12$ ) were treated identically in 100% seawater. The third ( $n = 7$ ) were treated as the first but without hemolymph sampling, to check on possible disturbances due to this procedure. The fourth ( $n = 9$ ) were simply held in freshwater for 10 days prior to a 12 h flux measurement in the standard fashion followed by a single hemolymph sample. This series, which also served as a control in a gas exchange study (Wood, Boutilier, and Randall, in prep.) thereby assessed the effects of longer term exposure to the freshwater condition in the laboratory.

### *Experimental protocols*

In the laboratory (temperature = 24–26°C), the crabs were washed free of remaining superficial dirt by repeated brief immersion in the appropriate water (freshwater or seawater) and then placed in polyethylene buckets (22 cm diameter × 25 cm depth) fitted with lids pierced with small airholes. A water volume of 2 l·kg<sup>-1</sup> was added, providing a depth of approximately 1 cm, which allowed the animals to draw the medium into the branchial chamber through the posterior margin of the branchios-tegite, as described by Wood and Randall (1981a), but not to ventilate it. Thus the animal could only ventilate air, though some equilibration of O<sub>2</sub> and CO<sub>2</sub> between air and water phases in the branchial chambers undoubtedly occurred (Wood and Randall, 1981a; Boutilier and Wood, in prep.). As the branchial water is changed over every few minutes when an external pool is available, water samples were taken after allowing 15 min for initial flushing of the branchial chamber and again after 12 h. Both the crab and the bucket were then washed again (to remove fecal material), clean water was introduced, and the procedure was repeated so that by 48 h after capture four consecutive 12 h flux measurements had been completed. Crabs were not fed during the experiment. Fluxes determined in this manner represent movements of substances across the total body surface (*i.e.*, gills, gut, renal system, etc.). In a few instances, fresh fecal material was extracted in water and analyzed. These tests indicated that feces may have made a small contribution to measured K<sup>+</sup>, Mg<sup>++</sup>, and Cl<sup>-</sup> losses, but were of negligible influence for other substances.

After 48 h in fresh- or seawater, the animal was then towel dried, weighed, and another hemolymph sample (0.8 ml) was taken (if required). The crab was then placed in a dry, covered bucket for an additional 48 h to evaluate the effects of mild dehydration. At the end of this period, the animal was reweighed, blood sampled (0.8 ml), and then returned to its original water for a 12 h flux determination during rehydration, followed by final weighing and blood sampling.

Unidirectional flux rates of Na<sup>+</sup> and Cl<sup>-</sup> were determined during the 12 h periods immediately prior to and following dehydration by adding radiotracers (<sup>22</sup>Na, <sup>36</sup>Cl; New England Nuclear; 1.0 µCi/l) to the external water pool. Additional water samples were taken throughout these periods to monitor the disappearance of radioisotope from the water into the animal. <sup>22</sup>Na and <sup>36</sup>Cl exchanges were measured separately in half (*i.e.*, n = 5–6) of groups 1 and 2; in the third (no blood sampling) only <sup>22</sup>Na fluxes were determined. Net fluxes of acidic equivalents were also measured during these two periods in all crabs. This was feasible because defecation, which previously confounded titration alkalinity determinations by altering water buffer capacity, had ceased by these times in all animals. Changes in titration alkalinity are an important component in the calculation of acidic equivalent flux (see below).

### *Burrow measurements*

Conditions in the burrows of an active colony in a low lying region (elevation <3 m) within 50 m of the shore were investigated during the hottest part of a sunny day (14:00–17:00 h), a period when most of the burrows were likely to be occupied. Burrows were probed with a flexible stick to which was attached a temperature sensor and a cannula for sampling water or air at known depths. Samples were analyzed for PO<sub>2</sub> and PCO<sub>2</sub> within either 10 min (air) or 60 min (water) of collection. A time series control for two sets of burrow samples showed that changes due to permeability of the syringe or endogenous metabolism by micro-organisms in the water were negligible over these periods. Water samples were centrifuged to remove silt prior to storage.

### Analytical techniques

Hemolymph and water samples were frozen at  $-20^{\circ}\text{C}$  for all later analyses, except for water pH and titration alkalinity measurements, which were performed shortly after collection. Hemolymph was routinely analyzed for osmolality,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , total protein, total ammonia, urea, uric acid, and glucose. Freshwater samples were assayed for  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , ammonia, urea, and uric acid in order to determine the flux rates of these substances between the crabs and their environmental water. Seawater samples were routinely assayed only for ammonia, urea, and uric acid, because changes in electrolytes over 12 h periods proved too small to reliably measure against their very high background levels in seawater. Selected samples of both media were analyzed for titration alkalinity and osmolality. Burrow samples were assayed for  $\text{PO}_2$  and  $\text{PCO}_2$  using Radiometer electrode methodology as described by Wood and Randall (1981a). Osmolality was measured by vapor pressure osmometry (Wescor 5100B),  $\text{Cl}^-$  by coulometric titration (Radiometer CMT10),  $\text{Na}^+$  and  $\text{K}^+$  by flame photometry (Eel Mk. II or Radiometer FLM3),  $\text{Mg}^{++}$  by atomic absorption spectrophotometry (Varian 1275AA), and  $\text{Ca}^{++}$  by either atomic absorption or colorimetric reaction with 0-cresolphthalein complexone using Sigma (1981a) reagents. The former was used for all water samples and a few hemolymph samples, the latter for most hemolymph samples; the two techniques were cross-validated. Micro-modifications of commercial diagnostic kits were used for the spectrophotometric assay of glucose (0-toluidine method; Sigma, 1980), urea (diacetyl monoxime method; Sigma, 1981b), uric acid (uricase/phosphotungstate method; Sigma, 1981c), hemolymph total protein (Lowry method; Sigma, 1982b), and hemolymph total ammonia, (1-glutamic dehydrogenase/NAD method; Sigma 1982a). Total ammonia in water was determined by a micro-modification of the salicylate-hypochlorite method of Verdouw *et al.* (1978). Different ammonia assays were used for water and hemolymph as the simpler salicylate-hypochlorite method gave spurious values for hemolymph; the two assays were cross-validated on water standards. Titratable alkalinity was determined by titration of air-equilibrated water samples (10 ml) to  $\text{pH} = 4.00$  with  $0.02\text{ N HCl}$  using a Gilmont micrometer burette as described by McDonald and Wood (1981).  $^{22}\text{Na}$  and  $^{36}\text{Cl}$  radioactivities were determined by counting 5-ml water samples or 0.2-ml hemolymph samples (in 4.8 ml water) in 10 ml ACS fluor (Amersham) on a Beckman LS 250 scintillation counter operated with a maximum window. At the 33% water content, the ACS fluor gives a very high and uniform counting efficiency ( $\sim 94\%$ ) for both of these highly energetic radioisotopes. Quenching is a constant, as determined by addition/recovery tests, and therefore cpm (after correction for background), rather than dpm, could be used directly in radiotracer calculations (see below).

### Calculations

Net flux rates ( $J_{\text{net}}$ ) of each substance (*e.g.*, X) were calculated as:

$$J_{\text{net}} = \frac{([X]_i - [X]_f) \times V}{t \times W} \quad (1)$$

where *i* and *f* refer to initial and final concentrations in  $\mu\text{equiv} \cdot \text{ml}^{-1}$  or  $\mu\text{mol} \cdot \text{ml}^{-1}$ , *V* the volume of the external water pool in ml, *t* the elapsed time in h, and *W* the body weight in kg. Thus net losses by the animal have a negative sign, and net gains a positive sign. By reversing the *i* and *f* terms, the net titratable acidity flux was calculated from the titratable alkalinities.

The net flux of acidic equivalents ( $J_{\text{net}}^{\text{H}^+}$ ) was calculated as the sum of the titratable acidity and ammonia fluxes, signs considered, which derives from the original principles

outlined by Maetz (1973). In brief, an increase in titratable acidity of the water results from either an addition of acidic equivalents and/or a removal of basic equivalents by the animal. A decrease in titratable acidity results from the opposite processes. Ammonia is produced metabolically as  $\text{NH}_3$ . If ammonia is excreted as  $\text{NH}_3$ , it will trap protons in the medium (as  $\text{NH}_4^+$ ) which will escape titration in the alkalinity measurement. Alternately, if ammonia is excreted as  $\text{NH}_4^+$ , then the protons carried out on  $\text{NH}_4^+$  represent an addition of acidic equivalents to the water which will again escape titration. Thus the sum of the titratable acidity and ammonia fluxes yields the net acidic equivalent flux. It should be pointed out that this procedure does not distinguish between  $\text{NH}_3$  and  $\text{NH}_4^+$  fluxes, nor between a net excretion of acidic equivalents and a net uptake of basic equivalents, or *vice versa*. Fortunately neither matters in terms of the net acid-base status of the animal.

Unidirectional influxes ( $J_{\text{in}}$ ) of  $\text{Na}^+$  and  $\text{Cl}^-$  were calculated from the disappearance of radioactivity from the external water using a logarithmic model:

$$J_{\text{in}} = \frac{(\ln Q_{\text{out}i}^* - \ln Q_{\text{out}f}^*) \cdot Q_{\text{out}}}{t \cdot W} \quad (2)$$

where  $Q_{\text{out}}^*$  (in cpm) was the total amount of radioactivity in the external pool at each time and  $Q_{\text{out}}$  (in  $\mu\text{equiv}$ ) was the average amount of the ion in the external pool, with appropriate correction for backflux when internal specific activity exceeded 5% of external activity (*cf.*, Kirschner, 1970). Because of the very large size of  $Q_{\text{out}}$  in seawater, changes in  $Q_{\text{out}}^*$  over time were very small, and thus measurements of  $J_{\text{in}}$  were less accurate than in freshwater. Therefore, a second, independent estimate of  $J_{\text{in}}$  in seawater was obtained from the appearance of radioactivity in the hemolymph over 12 h, using an equation analogous to (2) and assuming that the distribution volumes [radiospaces, see equation (4) below] for  $^{22}\text{Na}$  and  $^{36}\text{Cl}$  in seawater animals were the same as in freshwater animals. In all treatments, values of  $J_{\text{in}}$  determined by the two techniques were not significantly different, and well correlated in individual determinations ( $r = 0.92$ ,  $P < 0.01$ ,  $n = 16$ ); mean values are presented in Results.

Unidirectional outflux rates ( $J_{\text{out}}$ ) could then be calculated by the conservation equation (in freshwater only):

$$J_{\text{net}} = J_{\text{in}} - J_{\text{out}} \quad (3)$$

Internal distribution volumes ( $V_{\text{int}}$ ) for  $^{22}\text{Na}$  and  $^{36}\text{Cl}$  were calculated from the equation of Mayer and Nibelle (1969):

$$V_{\text{int}} = \frac{Q_{\text{out}i}^* - Q_{\text{out}f}^*}{\Delta C \times W} \quad (4)$$

where  $\Delta C$  represented the increase in radioactivity of hemolymph ( $\text{cpm} \cdot \text{ml}^{-1}$ ) between the start and end of the flux period. The total exchangeable internal pools (*i.e.*,  $\text{Na}_{\text{int}}^+$ ,  $\text{Cl}_{\text{int}}^-$ ) could then be estimated. For example:

$$\text{Na}_{\text{int}}^+ = V_{\text{int}}^{\text{Na}^+} \times [\text{Na}^+]_{\text{h}} \quad (5)$$

where  $[\text{Na}^+]_{\text{h}}$  was the concentration of sodium in hemolymph in  $\mu\text{equiv} \cdot \text{ml}^{-1}$ .

Data have been expressed as means  $\pm 1$  S.E.M. ( $n$ ) unless otherwise stated. The significance ( $P < 0.05$ ) of differences between means was assessed with Student's two tailed  $t$ -test, using either a paired (within group comparisons) or unpaired design (between group comparisons) as appropriate; data were transformed (logarithmic function) when necessary to match variance ratios.

## RESULTS

*Field observations*

Crab colonies were common in low lying swampy or grassy areas, such as disused coconut plantations, within  $\sim 0.5$  km of the shore. The animals exhibited mainly crepuscular or nocturnal activity, retreating to their burrows during the heat of the day, though daylight foraging increased greatly after rainfall. The burrows were simple, largely vertical holes, with one or occasionally two changes in angle (Fig. 1) and a diameter just large enough to allow the crab to enter sideways. The elaborate "castles" described for this species in the Andaman Islands (Silas and Sankarankutty, 1960) were not seen. A typical burrow was about 1 m in depth, and the bottom 15–25% was invariably filled with muddy water (Fig. 1). The air at the base of the burrows was significantly reduced in  $PO_2$  and elevated in  $PCO_2$  relative to the surface, presumably reflecting the presence of the crab and lack of mixing in these long, narrow blind-ended tubes. The water was markedly hypoxic and hypercapnic, even relative to burrow air (Fig. 1), and contained substantial levels of ammonia, and low levels of both urea and uric acid (Table I), suggesting that the water was used by the crab to flush the branchial chambers, as a ventilatory medium, and/or as a depository for urine. With one exception (burrow #7), burrow water was essentially freshwater (Table I, *cf.* Table II), despite the fact that all samples were taken within 50 m of the shore. Even in burrow #7, which was less than 10 m from shore, groundwater was less than 15% seawater. Ionic ratios in individual samples were highly variable, and did not reflect those in seawater (*cf.* Table II). Again this may have reflected the influence of the crab. For example,  $Mg^{++}$ , which was relatively high in burrow waters, is reported to be the one cation which is concentrated above hemolymph levels in *Cardisoma* urine (de Leersnyder and Hoestlandt, 1964).

Table II compares the composition of hemolymph sampled from freshly captured crabs in the wild to that of the freshwater and seawater used in flux experiments in the laboratory. Note that with the exception of  $Mg^{++}$  and nitrogenous wastes, the freshwater used was similar to burrow water (*cf.* Table I). In the field, *Cardisoma* hemolymph was osmotically equivalent to about 70% seawater, with major, approximately equal, contributions from  $Na^+$  and  $Cl^-$ . The total measured concentration of

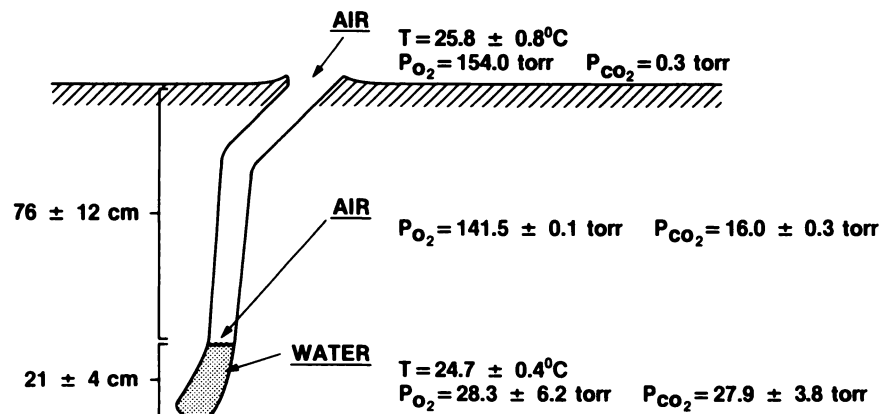


FIGURE 1. Dimensions and conditions in the burrows of *Cardisoma carnifex*. Means  $\pm 1$  S.E.M.  $n = 7$ –9 different burrows for all measurements except air  $PO_2$  and  $PCO_2$ , where  $n = 2$ .

TABLE I

*The composition of water sampled from the burrows of Cardisoma carnifex*

Burrow	Osmolality (mOsm · kg <sup>-1</sup> )	Na <sup>+</sup> (mequiv · l <sup>-1</sup> )	Cl <sup>-</sup> (mequiv · l <sup>-1</sup> )	K <sup>+</sup> (mequiv · l <sup>-1</sup> )	Ca <sup>++</sup> (mequiv · l <sup>-1</sup> )	Mg <sup>++</sup> (mequiv · l <sup>-1</sup> )	Ammonia (μmol · l <sup>-1</sup> )	Urea (μmol · l <sup>-1</sup> )	Uric acid (μmol · l <sup>-1</sup> )	pH
1	18	2.35	3.40	0.85	1.48	10.02	172	10.5	8.6	7.07
2	21	3.33	10.40	0.80	1.79	3.31	253	12.3	6.1	6.94
3	26	1.94	2.90	0.74	1.01	8.81	141	12.9	4.0	6.99
4	41	5.98	7.00	0.32	5.39	2.48	38	13.1	5.3	7.10
5	17	4.03	4.90	0.39	1.16	6.17	290	21.5	0.7	7.01
6	16	1.77	2.58	0.17	1.44	6.31	57	14.8	1.1	6.91
7	(-)	(38.58)	(72.00)	(1.36)	(8.95)	(35.92)	(193)	(44.9)	(12.9)	(7.02)
$\bar{x}$ <sup>1</sup>	23	3.23	5.20	0.55	2.05	6.18	159	14.2	4.3	7.00
S.E.M. <sup>1</sup>	4	0.65	1.24	0.11	0.68	1.21	41	1.6	1.2	0.03

<sup>1</sup> Excluding the data of burrow 7.

TABLE II

*A comparison of the composition of hemolymph of Cardisoma carnifex sampled in the field with that of freshwater and seawater used in flux experiments in the laboratory*

	Hemolymph	Freshwater <sup>1</sup>	Seawater <sup>1</sup>
Osmolality (mOsm · kg <sup>-1</sup> )	733 ± 6 (24)	18 ± 1 (10)	1055 ± 3 (11)
Na <sup>+</sup> (mequiv · l <sup>-1</sup> )	351.3 ± 2.1 (24)	6.59 ± 0.04 (93)	496.7 ± 7.2 (38)
Cl <sup>-</sup> (mequiv · l <sup>-1</sup> )	359.4 ± 3.6 (24)	6.36 ± 0.12 (91)	567.6 ± 1.9 (38)
K <sup>+</sup> (mequiv · l <sup>-1</sup> )	8.3 ± 0.1 (24)	0.20 ± 0.01 (91)	10.6 ± 0.3 (38)
Ca <sup>++</sup> (mequiv · l <sup>-1</sup> )	22.0 ± 0.6 (24)	0.70 ± 0.04 (92)	20.9 ± 0.3 (37)
Mg <sup>++</sup> (mequiv · l <sup>-1</sup> )	18.8 ± 0.8 (24)	1.24 ± 0.04 (92)	106.6 ± 1.2 (37)
Ammonia (μmol · l <sup>-1</sup> )	2673 ± 424 (24)	41 ± 5 (92)	59 ± 6 (68)
Urea (μmol · l <sup>-1</sup> )	226.8 ± 30.0 (24)	5.9 ± 0.4 (92)	5.6 ± 0.5 (68)
Uric acid (μmol · l <sup>-1</sup> )	<3.0 (24)	<0.5 (93)	<0.5 (38)
Glucose (μmol · l <sup>-1</sup> )	160 ± 38 (24)	—	—
Protein (g · 100 ml <sup>-1</sup> )	4.98 ± 0.54 (24)	—	—
Titration alkalinity (μequiv · l <sup>-1</sup> )	—	971 ± 31 (42)	2527 ± 31 (24)
pH	—	7.03 ± 0.12 (42)	8.01 ± 0.17 (24)

<sup>1</sup> Initial measurements at start of flux periods.  
Means ± 1 S.E.M. (n).

osmolytes in the hemolymph was 742 mmol · l<sup>-1</sup>, in comparison to a measured osmolality of 733 mOsm · kg<sup>-1</sup> (Table I). As one major osmolyte (SO<sub>4</sub><sup>2-</sup>) was unmeasured, while ion pairing and protein binding undoubtedly decreases osmotic activity in hemolymph, agreement between the two values seems quite reasonable. Hemolymph Mg<sup>++</sup> was maintained at less than 20% seawater levels, while the Ca<sup>++</sup> concentration was equal to that in full strength seawater. All major electrolytes were 15 to 60× freshwater levels. Ammonia was by far the most abundant of the measured nitrogenous wastes in blood, reaching very high concentrations (9.42 mmol · l<sup>-1</sup> in one crab). Urea was less than one tenth the ammonia level, but was detected in all animals. The detection limit for uric acid in hemolymph was ~3 μmol · l<sup>-1</sup>; in only 2 of 24 crabs were levels above this value (5, 6 μmol · l<sup>-1</sup>) observed.



### Laboratory observations

Blood was taken from two groups in the field prior to the flux protocol in the laboratory in fresh- and seawater respectively, a third was put through the flux protocol in freshwater without blood sampling, and the fourth was examined after 10 days in freshwater in the laboratory without prior sampling. There were no significant differences in any measured parameters in the hemolymph samples taken in the field from the freshwater and seawater flux groups (Figs. 2, 3, 4, 5).

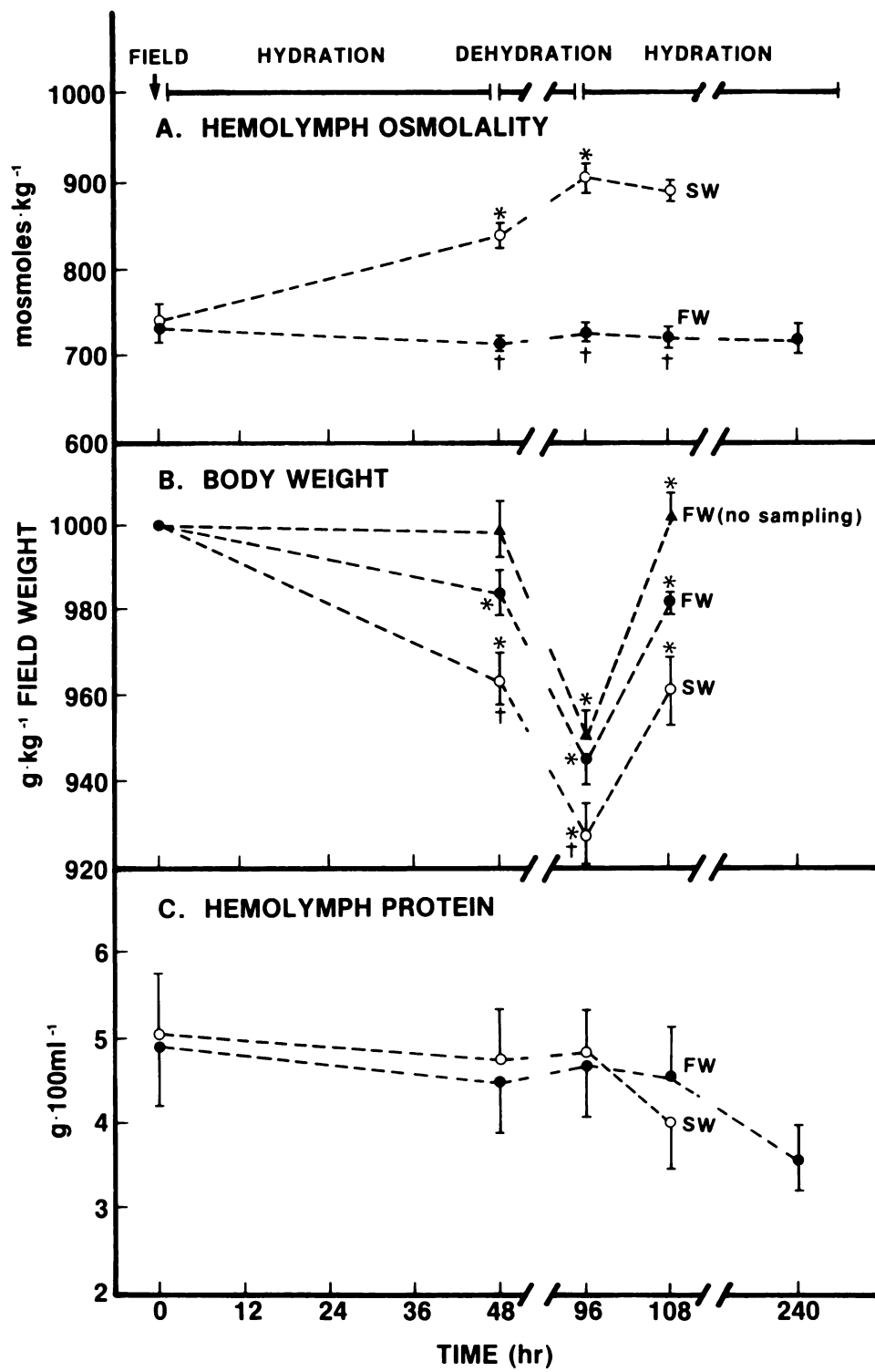
*Ionic and osmotic status of hemolymph.* Exposure to freshwater for 48 h after capture caused minimal net gain or loss of water, as witnessed by the constancy of hemolymph osmolality, total protein (mainly hemocyanin), and body weight, the latter in non-sampled animals only (Fig. 2). Inasmuch as the crabs defecated, some weight loss was to be expected, so a small net uptake of water may have occurred. (The crabs from which blood samples were taken lost about  $15 \text{ g} \cdot \text{kg}^{-1}$  over the same period, only 40% of which could be directly attributed to the weight of the removed sample, so there may have been some stress resulting in loss of more weight.) Similarly, the crabs maintained unchanged levels of  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{Ca}^{++}$ , and  $\text{Mg}^{++}$  in hemolymph (Fig. 3A–D). The only electrolyte to change significantly after 48 h in freshwater was  $\text{K}^+$ , which decreased by 28% (Fig. 3E). After 10 days in freshwater, hemolymph osmolality (Fig. 2A), protein (Fig. 2B), and  $\text{Cl}^-$  (Fig. 3B) still remained at levels seen in the field, while  $\text{Na}^+$  had decreased by only 5% (Fig. 3A). However,  $\text{Ca}^{++}$  had fallen by ~25% (Fig. 3C) and both  $\text{K}^+$  and  $\text{Mg}^{++}$  by ~50% (Fig. 3D, E) by this time.

Exposure to seawater after capture produced a very different pattern. After 48 h, body weight had declined by over twice as much ( $\sim 36 \text{ g} \cdot \text{kg}^{-1}$ ), suggesting a net loss of water (Fig. 2B). Hemolymph osmolality (Fig. 2A),  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{Ca}^{++}$  (Fig. 3A–C) increased by 14–20%, and  $\text{Mg}^{++}$  by ~40% (Fig. 3D). However  $\text{K}^+$  was well regulated (Fig. 3E), although it should be noted that levels of  $\text{K}^+$  in seawater were only slightly higher than those in hemolymph (Table II).

Dehydration for 48 h decreased body weight by  $\sim 40 \text{ ml} \cdot \text{kg}^{-1}$  in both freshwater groups, an effect which was fully reversed during 12 h of rehydration (Fig. 2B). This may have reflected changes in branchial chamber water, true body water, or both. Greatly increased flushing of the branchial chambers occurred during the rehydration period. While hemolymph osmolality, protein (Fig. 2A, C),  $\text{Mg}^{++}$ , and  $\text{K}^+$  (Fig. 3D, E) were unaffected, very small but significant increases (2–3%) and subsequent decreases in  $\text{Na}^+$  and  $\text{Cl}^-$  (Fig. 3A, B) were seen. Interestingly,  $\text{Ca}^{++}$  rose by about 8%, but only during the rehydration period (Fig. 3C).

The weight changes during dehydration and rehydration in seawater were similar (Fig. 2B), but disturbances in hemolymph composition more pronounced than in freshwater. Osmolality (Fig. 2A),  $\text{Na}^+$ , and  $\text{Cl}^-$  levels (Fig. 3A, B) rose by 6–9% and did not fall again during rehydration. Other osmolytes were not significantly affected.

*Ionic exchange rates and radiotracer studies.* During the first 12 h after capture, crabs maintained significant net uptakes ( $\sim +400 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) of both  $\text{Na}^+$  and  $\text{Cl}^-$  (Fig. 4A, B) from freshwater. These rates subsequently declined, becoming significantly negative at 36–48 h. Throughout this same period, the crabs exhibited net losses of  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , and  $\text{K}^+$  (Fig. 4C–E), though these fluxes were much smaller and showed no significant trends over time. After 48 h dehydration,  $J_{\text{net}}^{\text{Na}^+}$  and  $J_{\text{net}}^{\text{Cl}^-}$  returned to significantly positive values similar to those immediately after capture (Fig. 4A, B), while  $\text{Mg}^{++}$  and  $\text{K}^+$  losses were significantly reduced (Fig. 4D, E);  $\text{Ca}^{++}$  balance was unaffected (Fig. 4C). These responses were generally similar regardless of whether blood samples had been taken, suggesting that they were not due to ionic depletion



as a result of hemolymph withdrawal. After 10 days in freshwater, the crabs were in positive balance for  $\text{Na}^+$  and  $\text{Cl}^-$  (Fig. 4A, B), while  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , and  $\text{K}^+$  fluxes, although still negative, were significantly lower than during the first 48 h.

Net flux rates for these ions could not be directly measured in seawater. However the hemolymph data suggest net uptakes of all ions except  $\text{K}^+$  over the experimental period (Fig. 3).

Determinations of  $J_{\text{net}}^{\text{H}^+}$ , calculated as the sum of the titratable acidity and ammonia fluxes (Fig. 8A), signs considered, are summarized in Table III. After 36–48 h “hydration” in the laboratory, all groups exhibited a net acidic equivalent uptake (= base excretion). After 10 days in freshwater,  $J_{\text{net}}^{\text{H}^+}$  was significantly reduced but still positive. Dehydration for 48 h had no effect in freshwater, but reduced  $J_{\text{net}}^{\text{H}^+}$  to approximately zero in seawater. Figure 5 illustrates that over a wide range of flux rates, there was an approximate 1:1 relationship between  $J_{\text{net}}^{\text{H}^+}$  and the measured strong cation minus anion fluxes (i.e.,  $\text{Na}^+ + \text{K}^+ + \text{Ca}^{++} + \text{Mg}^{++} - \text{Cl}^-$ ) in *Cardisoma*.

Unidirectional flux determinations with  $^{22}\text{Na}$  and  $^{36}\text{Cl}$  yielded almost identical values for sampled and non-sampled groups, so the data have been combined in Figure 6. During the 36–48 h post-capture period (“hydration” in Fig. 6),  $J_{\text{in}}^{\text{Cl}^-}$  ( $\sim +1500 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) was approximately twice as large as  $J_{\text{in}}^{\text{Na}^+}$  in freshwater. For both ions,  $J_{\text{out}}$  exceeded  $J_{\text{in}}$ , resulting in negative net balance. After 48 h dehydration, this balance became positive during rehydration, but whereas  $J_{\text{out}}^{\text{Na}^+}$  was reduced without significant change of  $J_{\text{in}}^{\text{Na}^+}$ ,  $J_{\text{in}}^{\text{Cl}^-}$  was stimulated without significant alteration of  $J_{\text{out}}^{\text{Cl}^-}$ .

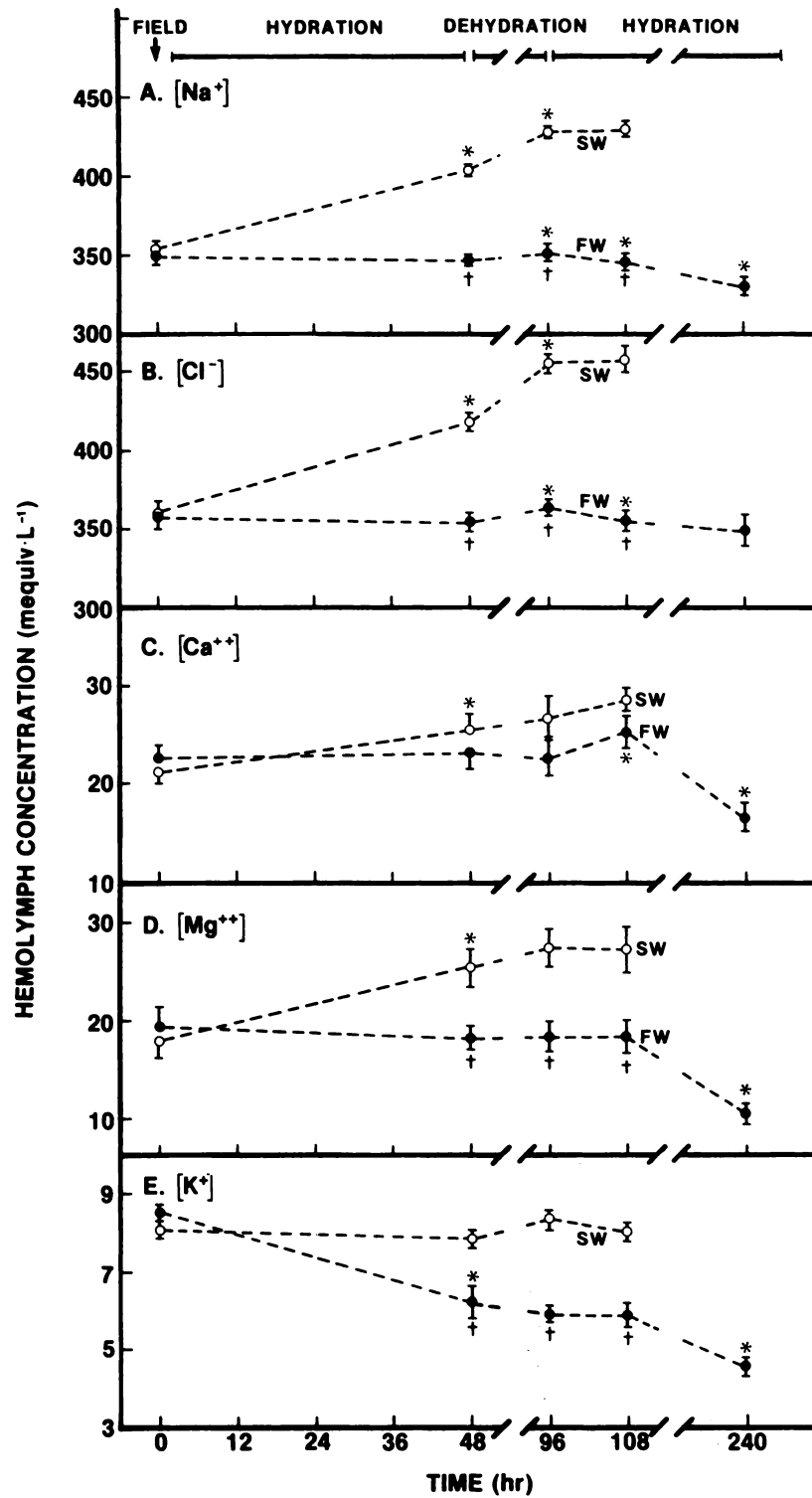
Interestingly, despite the very different gradients in the two situations, unidirectional  $\text{Na}^+$  and  $\text{Cl}^-$  influx rates in seawater were similar to those in freshwater during “hydration.” However in contrast to the freshwater picture, both  $J_{\text{in}}^{\text{Na}^+}$  and  $J_{\text{in}}^{\text{Cl}^-}$  approximately tripled after 48 h dehydration in seawater (Fig. 6).

Internal distribution volumes ( $V_{\text{int}}$ ) and total exchangeable pools were similar for  $\text{Na}^+$  and  $\text{Cl}^-$  in freshwater ( $V_{\text{int}} \cong 35\%$  body weight; pool  $\cong 125 \text{ mequiv} \cdot \text{kg}^{-1}$ ) and were unchanged after the dehydration/rehydration treatment (Table IV). Volumes and pools could also be estimated at the immediate end of the 48 h dehydration period, assuming no radioisotope had been lost from the animal. These were only  $\sim 10\%$  higher than initial values (data not shown), suggesting that these treatments caused no marked alterations in internal distributions. Estimates in seawater (data not shown) were imprecise due to specific activity limitations (see Materials and Methods) but did not differ significantly from the freshwater data.

**Metabolites.** After 48 h in the laboratory in either fresh- or seawater, the glucose concentration of hemolymph increased approximately 5-fold from the very low levels measured in the field (Fig. 7C). Thereafter, glucose declined, irrespective of the dehydration/rehydration treatment; by 10 days in freshwater, the low levels seen in the field were restored.

Conversely, the ammonia concentration of hemolymph declined from the very high levels seen in the field (Fig. 7A). Ammonia excretion rates decreased over a similar time course (Fig. 8A). Sampled and non-sampled groups behaved similarly, so the data have been combined in Figure 8 for clarity. Hemolymph concentrations

FIGURE 2. Changes relative to field measurements in (A) hemolymph osmolality, (B) body weight, and (C) hemolymph protein concentration in *Cardisoma carnifex* exposed to either freshwater or seawater in the laboratory. See text for details of treatment regimes. Means  $\pm 1$  S.E.M.  $n = 6-12$  at each point.  $t$  = significant difference ( $P \leq 0.05$ ) between the freshwater and seawater groups at a comparable time. \* = significant difference ( $P \leq 0.05$ ) within each group; each mean is tested relative to the preceding one, except for the 10-day (240 h) freshwater hydration value, which is compared with the 48-h freshwater hydration value.



and flux rates after 10 days in freshwater were similar to those seen after 48 h. A comparable pattern of decline occurred in seawater, though net ammonia fluxes were only ~35% of those in freshwater. During the 12 h rehydration period immediately following 48 h dehydration, ammonia excretion rates increased to the levels seen immediately after capture in both groups (Fig. 8A), though this was not accompanied by significantly elevated hemolymph levels (Fig. 7A).

Levels of urea in hemolymph were much lower than those of ammonia, and unlike the latter, remained unchanged under all treatment conditions (Fig. 7B). Excretion rates of urea were generally less than 20% of the simultaneously measured ammonia fluxes and unaffected by experimental conditions or salinity (Fig. 8B). Uric acid could not be detected (detection limit  $\cong 3 \mu\text{mol} \cdot \text{l}^{-1}$ ) in the hemolymph under any laboratory condition, and there was no measurable uric acid excretion to either freshwater or seawater (detection limit in water  $\cong 0.5 \mu\text{mol} \cdot \text{l}^{-1}$ ).

To check the possibility that the animals might excrete ammonia gas by direct volatilization to the atmosphere, crabs were enclosed in jars (in the absence of water) through which air was pumped at  $\sim 1 \text{ l} \cdot \text{min}^{-1}$ . The air passed first through a 0.1 N HCl trap (for humidification and removal of any ambient ammonia), then to the crab jar, then to a second identical ammonia trap. In one 6-h and one 48-h experiment, no ammonia was detected in the outflow trap, despite considerable ambient ammonia accumulation in the inflow trap.

## DISCUSSION

The purpose of sampling crabs first shortly after capture in the wild and then subsequently after exposure to defined conditions in the laboratory was to assess their "natural" condition of water and electrolyte balance. The present population of *Cardisoma carnifex* in Moorea appeared to be essentially "in equilibrium" with freshwater when sampled on land in the field. Subsequent exposure to freshwater in the laboratory caused little change in most osmotic and ionic parameters, in contrast to seawater exposure. These physiological data are supported by the presence of freshwater in the burrows, where the animals spend at least half their time. It is likely that the crabs carry this burrow water with them in the branchial chambers when foraging above ground (*cf.*, Wood and Randall, 1981a). It is not clear whether this pattern can be extrapolated to the genus as a whole, for comparable information in the literature is fragmentary. Gross *et al.* (1966) sampled *C. carnifex* "in less than 12 h of capture" from a mangrove area in Madagascar and found blood osmolalities much higher than the present data and closer to those in animals "equilibrated" with seawater than with freshwater. Burrow water was not collected. Kormanik and Harris (1981) reported hemolymph osmolality identical to the present data in "freshly collected" *C. carnifex* from the Palau Archipelago; burrow waters ranged from 35 to 85% seawater, though it is not clear that these were *C. carnifex* burrows. Herreid and Gifford (1963) found that inland burrows of *C. guanhumi* in Florida contained freshwater but reported no blood data from crabs in the field.

The differences in hemolymph ionic and osmotic composition associated with fresh- or seawater exposure are in basic agreement with previous observations on *Cardisoma* sp. (Herreid and Gifford, 1963; de Leersnyder and Hoestlandt, 1963, 1964;

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FIGURE 3. Changes relative to field measurements in (A) sodium, (B) chloride, (C) calcium, (D) magnesium, and (E) potassium concentrations of hemolymph in *Cardisoma carnifex* exposed to either freshwater or seawater in the laboratory. Other details as in legend of Figure 2.

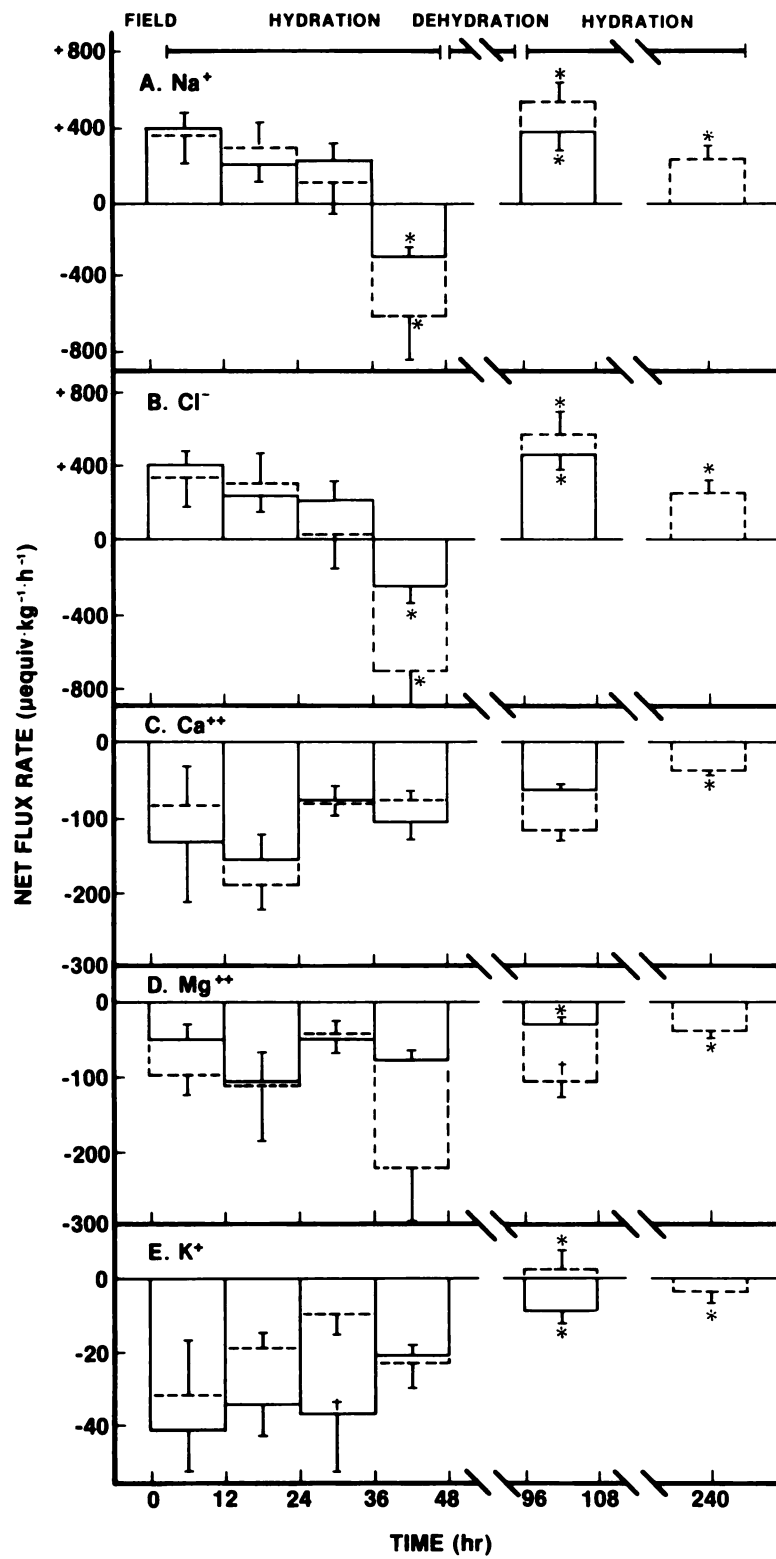


TABLE III

Net acidic equivalent fluxes ( $J_{\text{net}}^{\text{H}^+}$ ;  $\mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) in *Cardisoma carnifex* held in either freshwater or seawater under various conditions in the laboratory

	Hydration (36–48 h)	After 48 h dehydration (96–108 h)	Hydration (240 h)
Freshwater (no blood sampling)	+278.6 $\pm$ 73.4 (7)	+201.4 $\pm$ 37.3 (7)	+116.3 $\pm$ 23.0*
Freshwater (blood sampling)	+190.1 $\pm$ 26.1 (10)	+248.2 $\pm$ 31.8 (10)	–
Seawater (blood sampling)	+120.1 $\pm$ 30.1 (12)	+16.1 $\pm$ 32.3*† (12)	–

\* Significantly different ( $P \leq 0.05$ ) from 36–48 h hydration value.

† Significantly different ( $P \leq 0.05$ ) from comparable freshwater values.

Means  $\pm$  1 S.E.M. (n).

Gross *et al.*, 1966; Kormanik and Harris, 1981). To our knowledge, however, the present data are the first to demonstrate a net uptake of  $\text{Na}^+$  and  $\text{Cl}^-$  from freshwater in land crabs. Renal  $\text{NaCl}$  loss rates in *Cardisoma* sp. in freshwater have been variously estimated as  $-400 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  (Kormanik and Harris, 1981) to  $-6500 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  (de Leersnyder and Hoestlandt, 1964). The former appears more reasonable, for our unidirectional estimates with radiotracers indicate whole body  $J_{\text{out}}$  values of only  $-1500 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , even in a period of negative  $J_{\text{net}}$  (Fig. 6), and some of this undoubtedly occurred at extra-renal sites. Thus positive  $J_{\text{net}}$  values of  $+100$  to  $+400 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  (Fig. 4) must reflect  $J_{\text{in}}$  values at least several-fold greater at the transport sites, a conclusion confirmed by the radiotracer  $J_{\text{in}}$  measurements ( $+700$  to  $+1500 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , Fig. 6). Although transepithelial potentials were not determined, active transport is almost certainly involved in view of the large chemical gradients between freshwater and hemolymph (Table II). The gills, and possibly the gut, are the likely sites of this active transport (see Introduction).

In contrast to the constancy of  $\text{Na}^+$  and  $\text{Cl}^-$  in hemolymph during freshwater exposure, there were decreases in  $\text{K}^+$  (after 48 h),  $\text{Mg}^{++}$  and  $\text{Ca}^{++}$  (after 10 days) (Fig. 3), and continuous net losses of these ions to the medium (Fig. 4). As the herbivorous diet of *Cardisoma* is rich in these electrolytes, the higher hemolymph levels in the field are probably maintained by dietary intake rather than uptake from water (*cf.*, Gross and Holland, 1960). However the latter source cannot be entirely discounted, for  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  levels were somewhat higher in burrow water than in the freshwater used in the laboratory experiments (Tables I, II).

While seawater exposure caused marked physiological disturbances, these were

FIGURE 4. Whole body net flux rates over 12 h periods of (A) sodium, (B) chloride, (C) calcium, (D) magnesium, and (E) potassium in *Cardisoma carnifex* exposed to freshwater in the laboratory. See text for details of treatment regimes. Positive values represent net uptakes, negative values net losses. Means  $\pm$  1 S.E.M. Data from hemolymph-sampled groups ( $n = 11$ – $12$ ; solid lines) and non-sampled groups ( $n = 6$ – $7$ ; dotted lines) are shown separately.  $t$  = significant difference ( $P \leq 0.05$ ) between the sampled and non-sampled groups at a comparable time. \* = significant difference ( $P \leq 0.05$ ) within each group for the following comparisons: the 36–48-h hydration value is tested against the 0–12-h hydration value; the 96–108-h rehydration value is tested against the 36–48-h hydration value; and the 10-day (240 h) hydration value is tested against the 36–48-h hydration value.

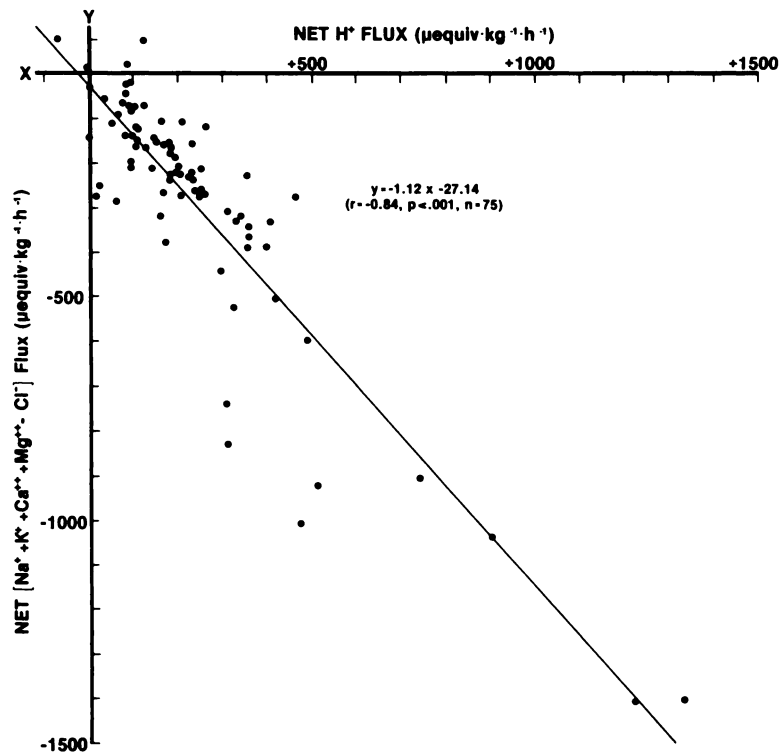


FIGURE 5. The regression relationship between the whole body net flux rate of acidic equivalents ( $J_{\text{net}}^{\text{H}^+}$ ; X) and the simultaneously measured difference (Y) between the whole body net flux rates of strong cations ( $\text{Na}^+ + \text{K}^+ + \text{Ca}^{++} + \text{Mg}^{++}$ ) and strong anions ( $\text{Cl}^-$ ) in *Cardisoma carnifex* exposed to freshwater in the laboratory. Positive values represent net uptakes; negative values net losses. Data from hemolymph-sampled and non-sampled groups during both hydration and rehydration have been combined. The slope of the regression relationship is not significantly different from 1.0, and the intercept is not significantly different from origin ( $P > 0.05$ ).

well within the tolerance of the animal. Indeed we observed that *C. carnifex* would readily enter seawater in the wild, and one group survived in the laboratory in 100% seawater for three weeks without mortality. Previous reports concur (Gifford, 1962; de Leersnyder and Hoestlandt, 1963, 1964; Gross *et al.*, 1966), and emphasize the role of the renal system in selective excretion of  $\text{Mg}^{++}$  and  $\text{SO}_4^-$ . Nevertheless, hemolymph levels of these ions do rise considerably in seawater (*e.g.*, Fig. 2, also de Leersnyder and Hoestlandt, 1964), and their neuro-muscular depressant action may explain the reduced activity commonly observed.

Forty-eight hours dehydration had minimal influence on the ionic composition of hemolymph in freshwater equilibrated crabs (Figs. 2, 3), despite a weight loss ( $\sim 40 \text{ g} \cdot \text{kg}^{-1}$ ) equivalent to about 20% of the extra-cellular fluid volume ( $\sim 195 \text{ ml} \cdot \text{kg}^{-1}$ ; Kormanik and Harris, 1981). This suggests that much of the loss was from branchial water, and that the crab used this pool to regulate the osmolality of hemolymph during dehydration, thereby emphasizing the value of this pool. While the weight loss was the same during dehydration in seawater equilibrated crabs, there were substantial further increases in hemolymph osmolality and ions, which were not corrected during



rehydration (Figs. 2, 3). This illustrates the disadvantage of seawater for *Cardisoma*. Other studies of dehydration in *C. carnifex* (Harris and Kormanik, 1981; Burggren and McMahon, 1981) employed much more severe, nearly terminal stress conditions (3–4 fold greater weight loss) and so are not directly comparable.

Unidirectional flux rates for  $\text{Na}^+$  and  $\text{Cl}^-$  for *C. carnifex* in freshwater (Fig. 6) were typical of euryhaline aquatic crabs, but the finding of unchanged  $J_{\text{in}}^{\text{Na}^+}$  and  $J_{\text{in}}^{\text{Cl}^-}$  in seawater was most unusual. Exchange rates one to two orders of magnitude higher normally occur, and are thought to at least partly reflect large exchange diffusion components (Kirschner, 1979). While most workers have measured efflux rates as an index of exchange in seawater because of the technical difficulty of influx determinations, we adopted the opposite approach for consistency with the freshwater measurements. We are confident of our results, as two independent methods (radiotracer disappearance from the water, appearance in the animal) yielded the same values ( $J_{\text{in}}^{\text{Na}^+} \cong +1000$ ;  $J_{\text{in}}^{\text{Cl}^-} \cong +1300 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ). From the hemolymph ion data (Fig. 3) and the space estimates (Table IV), net  $\text{Na}^+$  and  $\text{Cl}^-$  uptake rates of  $\sim +400 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  can be estimated over the first 48 h in seawater, indicating values of  $J_{\text{out}}^{\text{Na}^+} \cong -600$  and  $J_{\text{out}}^{\text{Cl}^-} \cong -1000 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ . Kormanik and Harris (1981) directly measured  $J_{\text{out}}^{\text{Na}^+}$  at  $-1600 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  in *C. carnifex* "immersed in seawater," so the two studies are in reasonable agreement. Thus  $\text{Na}^+$  and  $\text{Cl}^-$  exchange rates in *Cardisoma* are atypically low in seawater, perhaps reflecting an absence of exchange diffusion and/or simply reduced flushing of the branchial chambers. The tripling of exchange seen after dehydration (Fig. 6) could result from the greatly increased flushing noticeable during rehydration.

At rest, *C. carnifex* has an unusually low respiratory quotient ( $\cong 0.58$ ), suggesting the fixation of some respiratory  $\text{CO}_2$ , perhaps as  $\text{CaCO}_3$  for carapace growth as earlier hypothesized (Wood and Randall, 1981a, b; Wood, Boutilier, and Randall, unpub.). Such a strategy would necessitate the excretion of two  $\text{H}^+$  ions for every  $\text{CO}_2$  stored as  $\text{CO}_3^{2-}$ . This situation has recently been documented in the immediately post-moult aquatic blue crab, *Callinectes sapidus*, during the period of rapid carapace mineralization (Cameron and Wood, 1985). In intermoult *C. carnifex* at normal resting metabolic rates ( $M_{\text{CO}_2} \cong 1400 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ;  $M_{\text{O}_2} \cong 2400 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ; Wood and Randall, 1981a), the difference between  $R:Q = 0.58$  and the theoretical minimum  $R:Q \cong 0.70$  (lipid metabolism) would imply the fixation of at least  $280 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  of respiratory  $\text{CO}_2$ , yielding a  $J_{\text{net}}^{\text{H}^+}$  of at least  $-560 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ . In contrast, the present measurements show a net  $\text{H}^+$  uptake ( $J_{\text{net}}^{\text{H}^+} = +100$  to  $+300 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ; Table III), suggesting either that the theory is wrong or that the predicted net  $\text{H}^+$  excretion in *C. carnifex* is swamped by metabolic base production. As most herbivorous diets are strongly base producing (Hills, 1973), this is quite possible; clearly further work is needed to settle the matter.

The only other measurements of acidic equivalent fluxes in crabs using comparable methodology are also in *Callinectes sapidus*, which is both carnivorous and euryhaline. Interestingly, this species in intermoult condition exhibits  $J_{\text{net}}^{\text{H}^+} \cong +380 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  (Cameron, 1979), declining to  $\cong 0 \mu\text{equiv} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  in seawater (Wood and Cameron, 1985), a comparable trend to that seen in *C. carnifex* (Table III).

According to the concept of strong ion difference (Stewart, 1978), solutions separated by membranes (e.g., the body fluids of the crab and the external water) can only interact in acid-base terms by processes which alter the values of their dependent variables, which are the difference in concentration between strong cations and strong anions, the  $\text{P}_{\text{CO}_2}$ , and the total weak acid present (mainly protein). If the latter two are constant, as was likely the case in the present flux experiments, a change in the

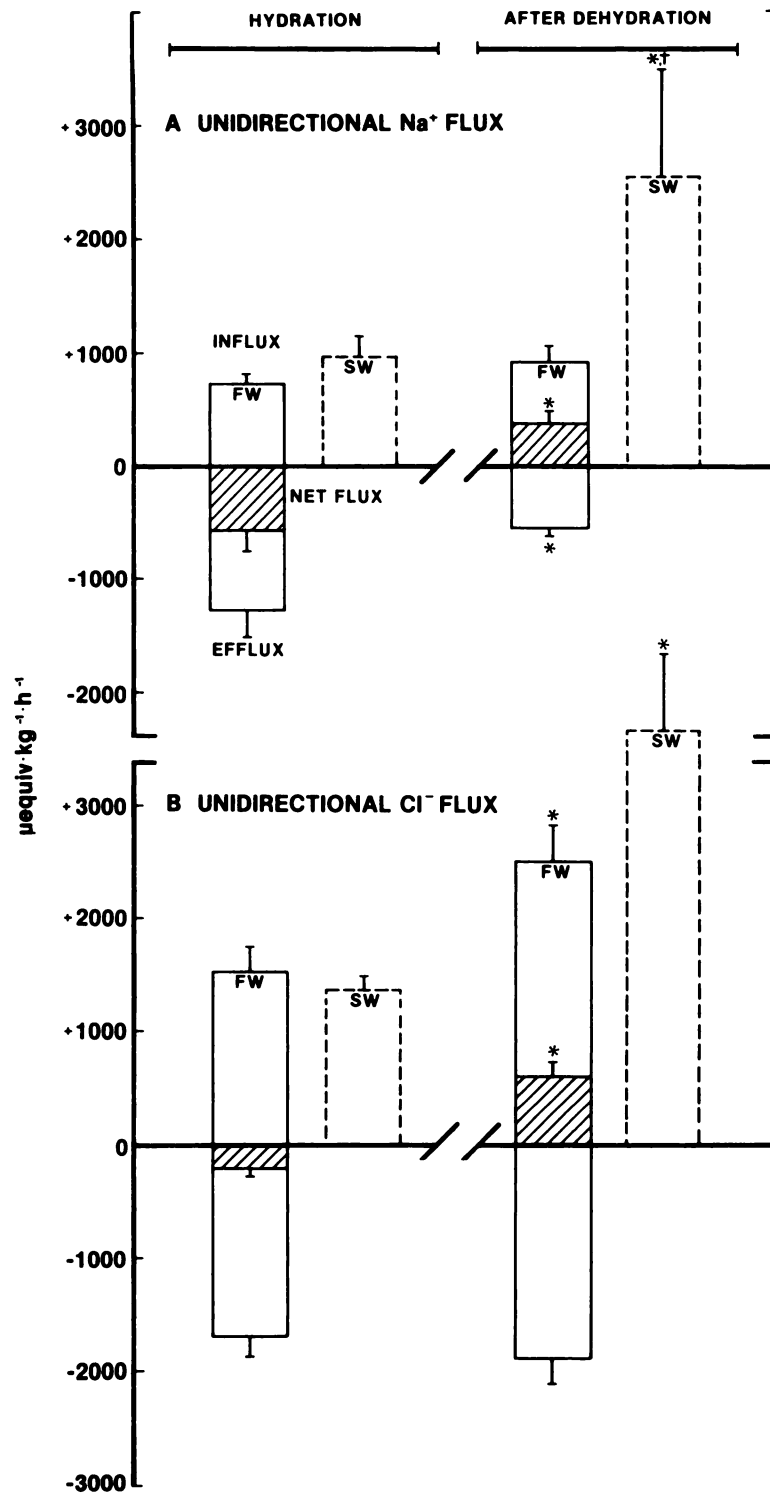


TABLE IV

Internal distribution volumes ( $V_{int}$ ) and total exchangeable internal pools ( $Na_{int}^+$ ,  $Cl_{int}^-$ ) as estimated with  $^{22}Na$  and  $^{36}Cl$  in *Cardisoma carnifex* held in freshwater

	Hydration (i.e., 48 h)	After 12 h rehydration (i.e., 108 h)
$V_{int}^{Na^+}$ (ml · kg <sup>-1</sup> )	363.8 ± 12.7 (5)	359.0 ± 6.8 (3)
$Na_{int}^+$ (mequiv · kg <sup>-1</sup> )	123.64 ± 4.15 (5)	120.64 ± 2.28 (3)
$V_{int}^{Cl^-}$ (ml · kg <sup>-1</sup> )	337.7 ± 21.6 (5)	352.6 ± 24.4 (4)
$Cl_{int}^-$ (mequiv · kg <sup>-1</sup> )	121.01 ± 8.59 (5)	127.82 ± 9.77 (4)

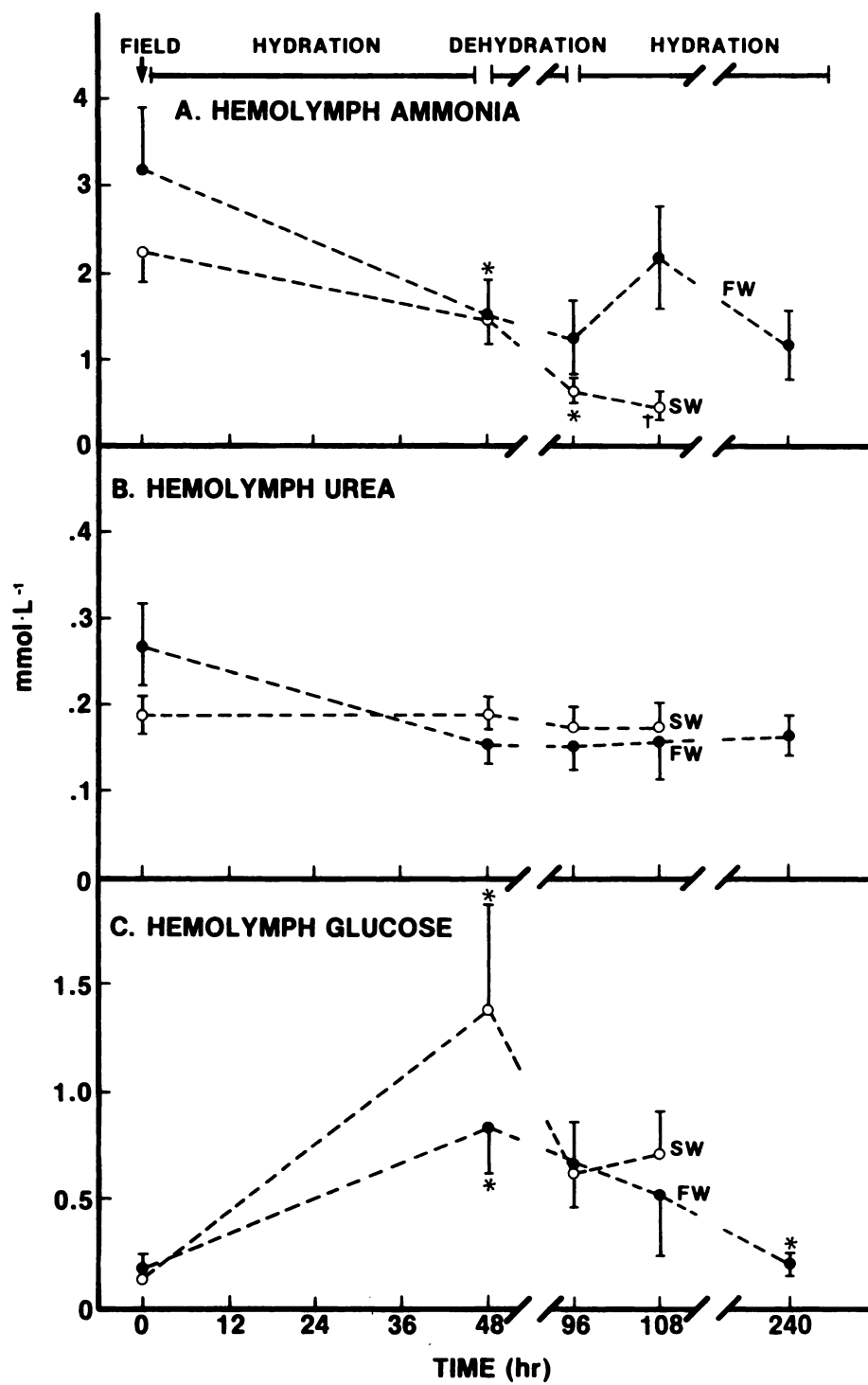
There were no significant differences between  $Na^+$  and  $Cl^-$  values at comparable times, or between comparable hydration and rehydration values.

Means ± 1 S.E.M. (n).

strong ion difference generated by a transmembrane flux will constrain an equal and opposite flux of acidic equivalents. The observed 1:1 relationship between  $J_{net}^{H^+}$  and the measured strong cation minus anion fluxes in *C. carnifex* in freshwater (Fig. 5) is in agreement with this strong ion difference concept (Stewart, 1978), and suggests that the fluxes of all quantitatively important strong electrolytes have been accounted for. (The only electrolyte of likely importance whose flux was not measured was  $SO_4^{2-}$ .) A similar relationship has been documented recently in the freshwater fish gill, taking only  $Na^+$  and  $Cl^-$  into account (Wood *et al.*, 1984). In *Cardisoma*, however, the relationship was rather weak unless other ions were included ( $K^+$ ,  $Ca^{++}$ ,  $Mg^{++}$ ), emphasizing the quantitatively greater importance of these exchanges in crabs.

In direct contrast to the current finding of high ammonia, low urea, and virtually no uric acid in hemolymph in *C. carnifex* in Moorea (Table II, Fig. 7), Henry and Cameron (1981) found high levels of uric acid, low levels of ammonia, and undetectable urea in the same species in the Palau Archipelago. We employed the same assays for urea and uric acid as Henry and Cameron (1981), and indeed confirmed that they accurately measured these substances in human body fluids, even when diluted with crab hemolymph, so this is not the source of the difference. Our own measurements of low ammonia levels in the Palau crabs (Wood and Randall, 1981b) were also in agreement with Henry and Cameron (1981), so the crabs appear fundamentally different in the two habitats. Interestingly, the present data are very similar to those of Horne (1968) on *C. guanhumi* (comparable high ammonia, low urea, and only very

FIGURE 6. Unidirectional measurements with radiotracers of (A) whole body sodium exchanges and (B) whole body chloride exchanges in *Cardisoma carnifex* exposed to either freshwater (influxes, effluxes, net fluxes, solid lines) or seawater (influxes only, dotted lines) in the laboratory. Means ± 1 S.E.M. Data did not differ significantly between hemolymph-sampled and non-sampled groups in freshwater and have been combined. n = 11 for sodium exchange in freshwater; n = 5 for chloride exchange in freshwater; n = 6 for sodium exchange in seawater; n = 6 for chloride exchange in seawater. "Hydration" measurements were taken at 36–48 h; "after dehydration" measurements at 96–108 h. t = significant difference ( $P \leq 0.05$ ) between freshwater and seawater groups under the same condition; \* = significant difference ( $P < 0.05$ ) within each group under different conditions.



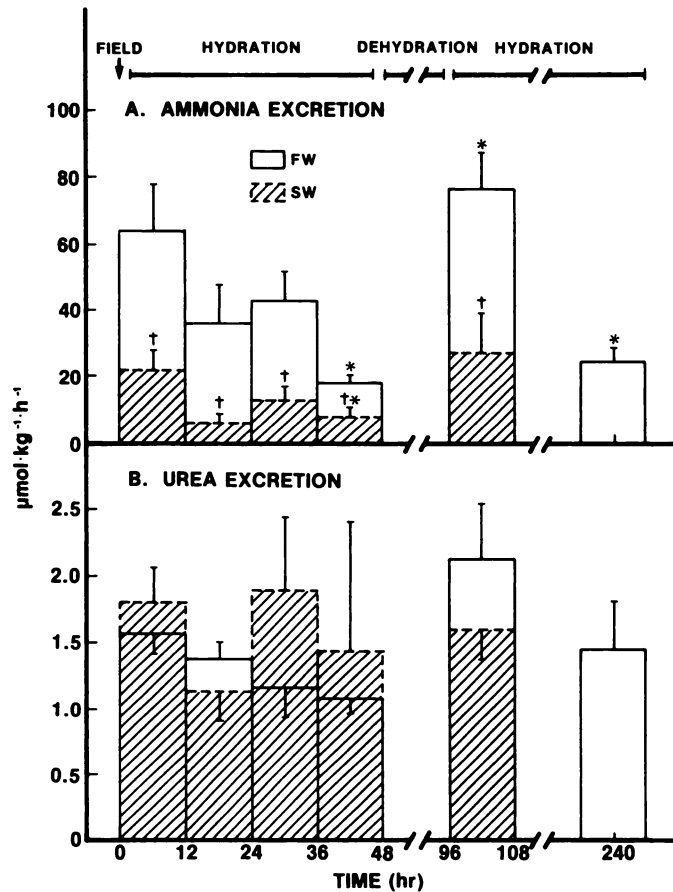


FIGURE 8. Whole body net excretion rates over 12 h periods of (A) ammonia and (B) urea in *Cardisoma carnifex* exposed to either freshwater (open bars, solid lines) or seawater (cross-hatched bars, dotted lines) in the laboratory. See text for details of treatment regimes. Means  $\pm$  1 S.E.M. Data did not differ significantly between hemolymph-sampled and non-sampled groups in freshwater and have been combined.  $n = 16-17$  for freshwater;  $n = 11-12$  for seawater.  $\dagger$  = significant difference ( $P \leq 0.05$ ) between freshwater and seawater groups at a comparable time. \* = significant difference ( $P \leq 0.05$ ) within each group for the following comparisons: the 36-48-h hydration value is tested against the 0-12-h hydration value; the 96-108-h re-hydration value is tested against the 36-48-h hydration value; and the 10-day (240 h) hydration value is tested against the 36-48-h hydration value.

low levels of uric acid) but not those of Gifford (1968) on *C. guanhumi* (high ammonia and high uric acid, urea not assayed). Gifford also noted copious white deposits of uric acid in the hemocoel. Similar white deposits were observed (without analysis) in the Palau specimens of *C. carnifex* (Henry and Cameron, 1981; Wood and Randall, 1981a) but did not occur in the Moorea animals. An anecdotal comment by Bright (1968) may offer some resolution of this dilemma. Bright noted that in *C. crassum*

FIGURE 7. Changes relative to field measurements in (A) hemolymph ammonia concentration, (B) hemolymph urea concentration, and (C) hemolymph glucose concentration in *Cardisoma carnifex* exposed to either freshwater or seawater in the laboratory. Other details as in legend to Figure 2.

the amount of uric acid varied with the habitat, and that none was present when the supply of groundwater was substantial, as was the case in the current study. Possibly nitrogen metabolism in *Cardisoma* sp. is very flexible, and biochemical variation can be brought on by seasonal changes or environmental pressures. Uric acid was found in burrow water in Moorea (Table I); this could reflect leaching of uric acid from cast carapaces from an earlier dry season, for crabs moult in the burrows (Bliss, 1968), or could simply originate from sources other than the crabs.

The very high blood levels of ammonia ( $1\text{--}10\text{ mmol}\cdot\text{l}^{-1}$ ) seen in *C. carnifex* in the field (Table I, Fig. 7A) and also in *C. guanhumi* (Gifford, 1968; Horne, 1968) are within the range considered toxic for most vertebrates (e.g., Warren, 1958; Hillaby and Randall, 1979). These levels fell and ammonia excretion occurred when the crabs were held in the presence of water, with subsequent increases after a period of dehydration (e.g., Fig. 8A). However from the work of Horne (1968) and our own more extensive studies on dehydration (Wood, Boutilier, and Randall, in prep.), it is clear that feeding history and metabolic rate are other important influences, and that excretion rates and hemolymph levels are not always closely related. Thus high ammonia levels and excretion rates (and low glucose levels) of field animals (Table II, Figs. 7, 8) probably reflected high metabolic rates (i.e., activity) associated with feeding. Changes in these parameters during holding in the laboratory may in turn have resulted from a combination of starvation and hydration effects. The much lower excretion rates of animals held in seawater (Fig. 8) may be indicative of a depression of metabolic rate. Alternately it could be associated with an enlargement of the free amino acid pool for tissue fluid balance (cf., Henry and Cameron, 1981).

The rates of total nitrogenous waste excretion recorded in *C. carnifex* in the present study ( $10\text{--}100\text{ }\mu\text{mol N}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) were similar to those reported for *C. guanhumi* (Horne, 1968; Gifford, 1968), but only 5–30% of those in a vertebrate of comparable size (e.g., Wright and Wood, 1985). While there is general agreement that nitrogen metabolism is reduced in land crabs, several authors have suggested that the animals might excrete undetected ammonia gas by direct volatilization to the atmosphere (Campbell *et al.*, 1972; Henry and Cameron, 1981). The tests for this in the present investigation, while limited, indicated that *C. carnifex* does not excrete ammonia by volatilization. In summary, the present and previous studies demonstrate that nitrogen metabolism in *Cardisoma* is unusual, complex, and labile. There is a clear need for a comprehensive investigation of the topic employing a thorough search for alternate end products and excretion routes, and careful control of feeding and hydration states.

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