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POSTMOULT UPTAKE OF CALCIUM BY THE BLUE CRAB (*CALLINECTES SAPIDUS*) IN WATER OF LOW SALINITY

BY DOUGLAS S. NEUFELD AND JAMES N. CAMERON

The University of Texas at Austin, Marine Science Institute, Port Aransas, TX 78373, USA

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Summary

After moulting, blue crabs (*Callinectes sapidus*) acclimated to a salinity of 2‰ were able to calcify as rapidly and accumulate as much calcium as crabs in sea water. Immediately after moult, the total masses of calcium, magnesium and strontium present in the whole body were 4.6, 15.6 and 3.0%, respectively, of their intermoult values. During the time of most rapid calcification, calcium uptake was 5.4±1.4 mmol l^{-1}, which is comparable to the maximum rate found in seawater-acclimated crabs. The concentrations of bound and free calcium in the blood changed very little with acclimation salinity, remaining at approximately 3 and 8 mmol l^{-1}, respectively, both during intermoult and 1 day postmoult. Free calcium changed relatively little through the moult cycle, varying between 6.9 and 8.1 mmol l^{-1}, but bound calcium rose to a peak of 6.4 mmol l^{-1} prior to moult then dropped to 2.6 mmol l^{-1} after moult, concurrent with a decrease of approximately 80% in the protein concentration of the blood. The concentration of total magnesium in the blood increased from a premoult low of 9.0 mmol l^{-1} to a postmoult high of 11.7 mmol l^{-1} and remained elevated throughout the period of rapid mineralization. During the postmoult period of rapid calcium uptake, the internal-to-external concentration ratio for total calcium was 6.6 to 1. The activity ratio, however, was only 2.5 to 1 because 28% of the calcium in the blood was bound to protein, and because the lower ionic strength of the medium resulted in a 2.5-fold higher activity coefficient for the water compared to blood. The transepithelial potential at postmoult (−5.4±0.7 mV) was significantly more negative than at intermoult (−3.1±0.6 mV). In artificial 2‰ sea water, the transepithelial potential (−9.3±0.7 mV) was higher than the equilibrium potential for calcium (−12.0±0.5 mV), implicating active transport in the uptake of calcium.

Introduction

The body of a crustacean is soft after moulting, affording almost no protection against predators and no structural support other than that produced by hydrostatic pressure. No doubt there is great selective pressure for these animals to harden the new carapace rapidly, primarily through the deposition of calcium

Key words: moulting, calcification, crab, *Callinectes sapidus*.

carbonate into the organic matrix. Crustaceans that mineralize their exoskeleton must be physiologically equipped to move large amounts of the requisite ions to the new exoskeleton in a relatively short period. This is less problematic in sea water, which contains a relatively high concentration of calcium ions, than in environments where calcium is far less available. There are a number of possible strategies which facilitate hardening of the exoskeleton in low-calcium environments and which seem to be more prevalent in freshwater than in marine crustaceans. (1) Storage: in some species, the storage of calcium from the old exoskeleton in the blood, gut or midgut gland (summarized by Greenaway, 1985) provides some of the calcium needed after moult for hardening of the new exoskeleton. (2) Reduced mineral content: in comparing two species of crayfish, Mills and Lake (1976) noted that the total amount of calcium accumulated was less in the species inhabiting an environment with a lower calcium concentration, suggesting that the total amount of calcium accumulated can be dependent on the availability of calcium in the environment. (3) Reduced uptake rate: in calcium-limited environments the time needed for calcification may increase without a reduction in final content. The rate of calcium accumulation in *Gammarus pulex*, for instance, is slower in water with less calcium (Vincent, 1969). (4) Reduced activity of calcium in the blood: although the total concentration of calcium in the blood of crustaceans in fresh water is greater than the total concentration of calcium in the external medium, it is the calcium activity that determines the electrochemical gradient. The relationship of activity to total calcium content is a function of the ionic strength of the solution and the proportion of calcium in ionized form. Anything that lowers the calcium activity in the blood will reduce or eliminate the unfavourable electrochemical gradient correspondingly. Although there are few data on the proportion of total calcium that is bound in the blood, there may be a tendency for freshwater species to have lower calcium activities while maintaining total concentrations equal to those of seawater species (Table 3 in Greenaway, 1985). (5) Active transport: in some freshwater species, net calcium accumulation occurs in waters with extremely low calcium concentrations (Vincent, 1969; Malley, 1980) where the electrochemical gradient is certainly directed outwards. Under these conditions crustaceans must rely on active transport since a net passive influx is not possible against the existing electrochemical gradient.

The blue crab, *Callinectes sapidus*, is notable for its ability to survive in salinities ranging from fresh to hypersaline water. It cannot reproduce at low salinities and has the high water and salt permeabilities (Cameron, 1978) characteristic of a marine species, but it can successfully moult in fresh water. Calcium regulation over the postmoult period in seawater-acclimated blue crabs may involve only passive mechanisms (Cameron, 1989), but the large difference in calcium concentrations of blood and water at low salinities suggests some other mechanism of regulation when crabs acclimate to fresh water. We investigated the pattern of calcium metabolism in moulting blue crabs acclimated to 2‰ salinity as an example of the method used by a euryhaline species at a very low salinity. In particular, we looked for the methods of calcium accumulation more frequently

found in freshwater species and measured the electrochemical gradient as an indicator of whether uptake was by active transport.

Materials and methods

Specimens

We obtained crabs (*Callinectes sapidus* Rathbun) from several sources and a variety of salinities. Those obtained from higher-salinity areas were acclimated to 2‰ for 3 weeks, sufficient time for the physiological changes that occur on acclimation to a low salinity (R. Henry, personal communication). Crabs caught or obtained from bayous (0–0.5‰) near Tivoli, Texas, or from the lower Mission or Aransas Rivers (0–15‰) were sometimes acclimated for a shorter time, but not for less than 1.5 weeks. Crabs were kept in recirculation tanks until entering the premoult stage, when they were isolated in darkened containers connected to the same recirculation system. Dechlorinated Port Aransas tap water was mixed with sea water to a salinity of 2‰; the resulting mixture had proportions of ions similar to low salinities found in the field. Calcium concentrations in the tanks ranged from 1.61 to 2.13 mmol l^{-1}, and magnesium concentrations ranged from 1.80 to 2.94 mmol l^{-1}. Tank temperatures varied with the ambient room temperature, from 20.5 to 25.0°C; the crabs were fed *ad libitum* with frozen fish and shrimp. All experiments were performed with crabs in tank water unless otherwise noted. Data for crabs acclimated to sea water were from crabs held in a flow-through system containing water pumped from the Port Aransas ship channel. Seawater salinity was 30‰ with calcium concentrations ranging from 7.8 to 10.3 mmol l^{-1}. Intermoult data are from crabs that did not enter the premoult stage for several weeks after sampling.

Ion analyses

Crabs to be analyzed for total mineral contents were stored frozen at −10°C. The carcasses were dried at 60°C to a constant weight, then broken into small pieces and dissolved in 500 ml of 2 mol l^{-1} HCl to extract the mineral portion. We strained the solution to remove the remaining pieces of undissolved chitin, and analyzed the liquid for total calcium, magnesium and strontium. All samples measured for total concentrations of divalent cations were diluted in a solution of 1% lanthanum oxide and 5% HCl for analysis by atomic absorption spectrophotometry (Perkin-Elmer model 360).

We glued a small neoprene disk on a thinned portion of the carapace over the pericardial cavity for blood sampling. Depending on the number of variables to be measured, 0.5–1 ml of haemolymph was drawn from each individual, with sampling of individual crabs not more than once a day. We estimated the protein concentration by comparing the refractive index (American Optical Corp. refractometer) of the sample with a protein calibration curve.

The free calcium concentration was measured with a calcium-selective electrode (Radiometer, Inc. model F2112) and a Ag–AgCl microreference electrode

(Microelectrodes, Inc. model MI-402). Standards were mixed from a commercial standard consisting solely of calcium chloride in water (Radiometer, Inc.), with magnesium, sodium and potassium added as chlorides to approximate the average concentration of blood samples; the pH was adjusted with NaOH to the physiological range. Samples and standards were both measured at room temperature and the electrode was frequently checked against the standards, usually after several sample readings. To obtain the activity coefficients for calcium in water and blood, we made solutions with known calcium concentrations and ionic strengths equivalent to 2 ‰ salinity and blood. The electrode response to these solutions was then compared with calcium chloride solutions of various concentrations whose corresponding activities are defined by the National Bureau of Standards (NBS). The lack of a pH effect on electrode response was verified in two ways: we observed no significant changes in electrode response when a large volume of calcium standard solution was adjusted with small volumes of NaOH from pH 6.8 to 8.1; and we changed the pH of a blood sample by equilibrating it with air containing 0.033, 1, 3 and 5 % CO_2 without detecting a change in electrode response. The ionic strength of blood samples did not vary enough from that of the standard to make a large difference in the activity coefficient. Rather than dilute the blood samples to control for small differences in electrode response caused by variation in pH and ionic strengths, and thus to risk changing the proportion of total calcium in the free state, we immersed the electrodes directly in the blood samples. Since blood generally clotted rapidly after sampling from the crab, the clot was centrifuged and the liquid decanted for measurements. Owing to a chloride interference component on the reference electrode, we calculated the concentration of free calcium from the Nernst equation modified to include the response of the reference electrode to chloride [$E=E_o+k\log(C_{Ca}+k_{Ca,Cl}C_{Cl}^2)$; Covington, 1979]. The selectivity constant for chloride, $k_{Ca,Cl}$, was determined by observing the change in electrode response on addition of NaCl to a standard solution; E_o, the 'standard' potential, and k, the slope of the electrode response, were determined using standard solutions. The chloride concentration in blood (C_{Cl}) was measured by amperometric titration (Buchler-Cotlove).

Ion-specific electrodes respond to the ion activity in the aqueous space, which can be significantly less than the total blood volume because of exclusion by proteins and lipids. As an approximate correction for this effect, we used the formula for human blood, $W_s=99.1-1.03[\text{lipid}]-0.73[\text{protein}]$, where W_s is aqueous space (Waugh, 1969), to calculate the free calcium in the total blood volume of the crabs. The concentration of lipids in crustacean blood is relatively small (Allen, 1972) and was therefore ignored. Free calcium concentrations calculated for the total blood space were 2.5–7.5 % lower than those for the aqueous space.

Blood was stored in syringes at 4°C until analysed for total calcium and/or magnesium. The calcium concentrations of samples did not change over a period of at least 5 weeks when stored in this manner. After pushing the sample through the syringe needle to break any clot which had formed, we centrifuged the sample

and used the liquid portion for the remainder of the procedure. This portion was diluted with 0.1 mol l^{-1} HCl, centrifuged and diluted appropriately for analysis by atomic absorption spectrophotometry.

Ion fluxes

We measured calcium and apparent hydrogen ion fluxes by observing calcium concentration and pH changes in a closed system regulated at 20.0±0.5 °C, with a small volume circulating through three CO_2 equilibration chambers followed by a chamber containing a pH electrode (Heisler, 1984). The system was calibrated for measurements of apparent hydrogen ion fluxes by observing the change in pH upon addition of a known volume of 1 mol l^{-1} HCl.

Transepithelial potential

Polyethylene tubing was filled with agar and 3 mol l^{-1} KCl for use as catheter electrodes, and an annular leak electrode (HNU Systems Inc.) was used as a reference. We first measured the potential with the catheter electrode bridging from a calomel well electrode directly to the water to ensure stability and to allow correction for tip asymmetry. The electrode was then inserted through a thinned portion of the carapace into the pericardial space and secured with epoxy. The crab was immersed entirely in the water and a measurement was taken after the potential had stabilized, usually within 5 min. Finally, the electrode was cut from the crab close to the carapace and a second measurement was taken with the electrode in the water to check for drift. Virtually all crabs survived after this procedure; we discarded data from the few crabs which subsequently died.

Electrochemical gradient

In assessing the electrochemical gradient at the time of calcium uptake, we performed experiments with crabs placed in the acidification apparatus at approximately 1 day postmoult to confirm a high rate of calcium uptake. Artificial 2‰ water was used with the major ions at the approximate concentrations of natural 2‰ water (in mmol l^{-1}: Na^+, 26; K^+, 0.8; Ca^{2+}, 1.7; Mg^{2+}, 3.6; Cl^-, 33.2; HCO_3^-, 1.7; SO_4^{2-}, 2.6). After obtaining a stable transepithelial potential measurement, we took water and blood samples to measure the calcium activity. Since the electrode response at the low ionic strength of 2‰ sea water was often unstable, we used atomic absorption spectrophotometry for water samples and estimated the ionic calcium to be 85 % of the total concentration (Thompson and Ross, 1966). The activity coefficients of 2‰ water and blood were estimated to be 0.50 and 0.19, respectively, based on the comparison of solutions to standards with NBS-defined calcium activities. The minimum, or thermodynamic, work (W_i) required for transport of an ion was calculated using the equation:

$$W_i = [Z_i F E_m + \boldsymbol{R}T\ln(C_i/C_o)](M_i),$$

where Z_i is the charge on the ion, E_m is transepithelial potential, C_i is the internal concentration of the ion i, C_o is the external concentration of the ion, M_i is the flux

rate of the ion, and F, R and T have their usual values (Woodbury, 1965). All values are expressed as mean±1 S.E. unless otherwise stated.

Results

Mineral content of the whole body

Relative to the intermoult period, there was very little calcium, magnesium or strontium in the whole body immediately after moult (Fig. 1), indicating that there was no significant storage of the divalent cations that form the mineral portion of the exoskeleton. Calcium, magnesium and strontium contents of crabs immediately after moult were 1.63±0.16, 0.29±0.01, and 0.008±0.003 g kg^{-1} wet mass (N=5 for each) respectively. The increases in concentrations of all three cations occurred in concert, levelling off several weeks after moult. As a proportion of the 'soft-shell' content, calcium and strontium increased more than magnesium.

Calcium and apparent hydrogen ion fluxes

There was a strong correlation between the inward flux of calcium and the outward apparent flux of hydrogen ions, with a slope not significantly different (P>0.1) from 2 (Fig. 2). This relationship appeared to hold throughout the 5 days after moult.

There were no significant calcium or hydrogen ion fluxes during the 48 h period prior to moulting when the means were compared to zero with t-tests (Table 1; P>0.1 for all periods). Following the moult, there was a lag period of several

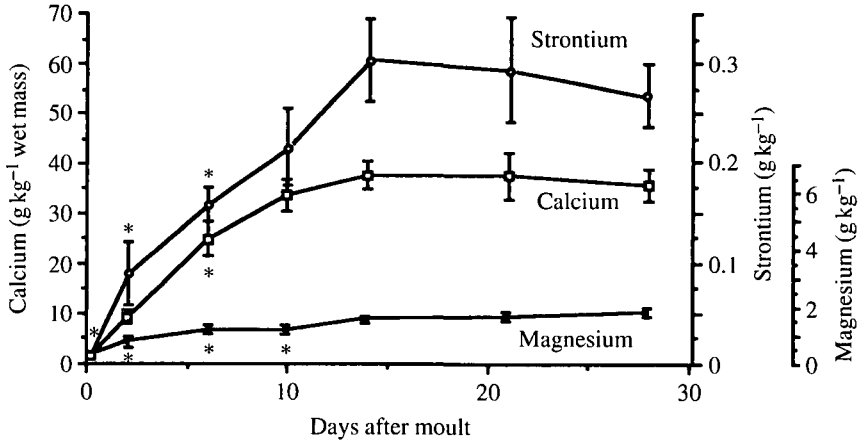

Fig. 1. The total amounts of the three major divalent cations in whole bodies of blue crabs, scaled to emphasize the increase relative to the total amount immediately after the moult (N=5–7; mean mass 137±6 g). Asterisks indicate values that are significantly different (P<0.05) from intermoult (day 28) values (Dunnett's test for comparing a control mean to each other group mean). Values are mean±S.E.

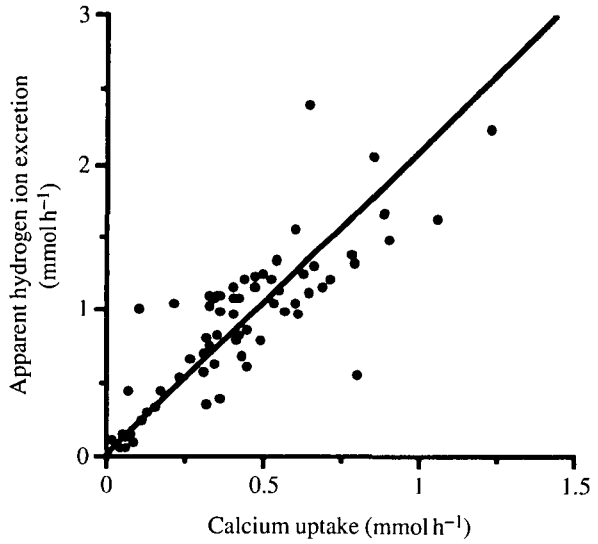

Fig. 2. Correlation between apparent hydrogen ion excretion and calcium uptake in 30 postmoult crabs. The equation of the line fitted by geometric mean regression is $y=2.08x+0.02$ ($r^2=0.67$).

Table 1. *Calcium and apparent hydrogen fluxes over four periods during the 48 h prior to moulting*

Hours prior to moult (approximate)	Stage	Apparent hydrogen efflux (mmol kg^{-1} h^{-1})	Calcium influx (mmol kg^{-1} h^{-1})	*N*
36	'Red line', sutures not yet weakened	1.07±0.79 (NS)	−0.26±0.29 (NS)	9
16	Sutures weak, no swelling of body	0.36±0.39 (NS)	0.29±0.29 (NS)	9
6	Swelling begun, sutures cracked open	0.63±0.53 (NS)	−0.08±0.16 (NS)	11
2	'Busting', period up to ecdysis	0.28±0.36 (NS)	−0.08±0.60 (NS)	9

NS, not significant ($P>0.1$, Dunnett's test).

hours before acidification began, after which the rate rose rapidly to a maximum (Fig. 3). Acidification continued for at least 10 days after moult, although the rate dropped slowly after the first several days and was only one-tenth of the maximum rate by day 10.

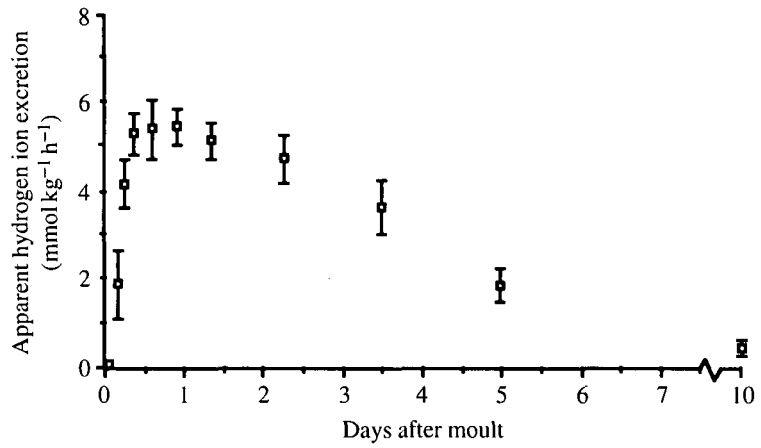

Fig. 3. Rate of acidification over the postmoult period in blue crabs acclimated to 2‰ sea water. Sample sizes range from 3 to 11. All masses are adjusted to the final mass of the crab (mean mass 129±5 g). Values are mean±S.E.

Blood constituents

The blood protein concentration during the early premoult stage was substantially greater than during the intermoult stage (Fig. 4). The concentration gradually decreased until moult, when there was an abrupt drop correlated with the uptake of water and resulting dilution of the blood. Protein content remained low for several weeks after moult.

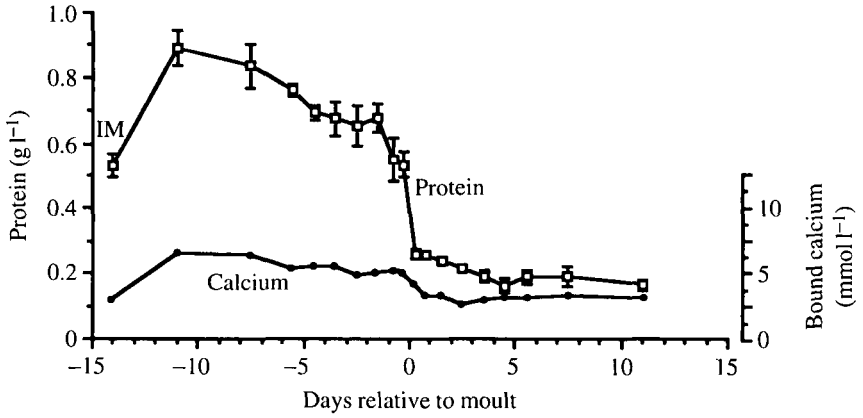

Fig. 4. Concentrations of protein and bound calcium in the blood through the moult cycle. Bound calcium is calculated as mean total calcium minus mean free calcium (see Fig. 5). Protein sample sizes range from 4 to 25. IM, intermoult value. Values are mean±S.E.

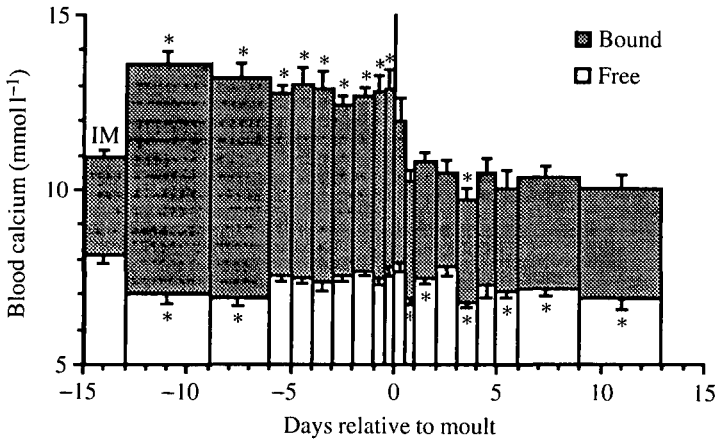

Fig. 5. Changes in the concentration of total and free calcium in the blood over the moult cycle (N=3–11). Top error bars are for the total calcium measurements; lower error bars are for free calcium measurements; bound calcium was obtained by difference. Asterisks indicate values significantly different from the intermoult value (IM). Note that the y-axis does not start at zero.

The concentration of calcium in the bound state correlated with the changes in protein concentrations through the moult period (Fig. 4). Thus, the substantial increase in the concentration of total calcium (Fig. 5) by the 'white-line' stage (when formation of the organic matrix for the new cuticle has begun) several weeks before moult was the result of an increase in bound calcium, which remained elevated until after moult. Likewise, the decrease in the concentration of total calcium after moult was primarily the result of a drop in the bound component. There was also a smaller, statistically significant, decrease in the concentration of free calcium (Fig. 5) correlated with the start of calcium uptake.

The pattern of regulation of total blood magnesium was approximately opposite to that of free calcium. Magnesium concentration did not change significantly from intermoult through the moult until 12 h after moult, when there was an increase which lasted about 1 week (Fig. 6). The delay in the rise in magnesium concentrations correlated with the small decrease in free calcium.

Effect of acclimation salinity on blood calcium distribution

Crabs acclimated to 2‰ salinity had a distribution of blood calcium that was comparable to that of crabs acclimated to 30‰ salinity, both at intermoult and 1 day postmoult (Fig. 7). A bivariate two-way analysis of variance (ANOVA) indicated a significant effect of moult stage on free calcium concentration (P<0.001) and an interaction effect of moult stage and acclimation salinity on bound calcium concentration (P<0.05). The differences in concentrations due to acclimation salinity, however, were small compared to absolute concentrations and were probably of minor physiological significance.

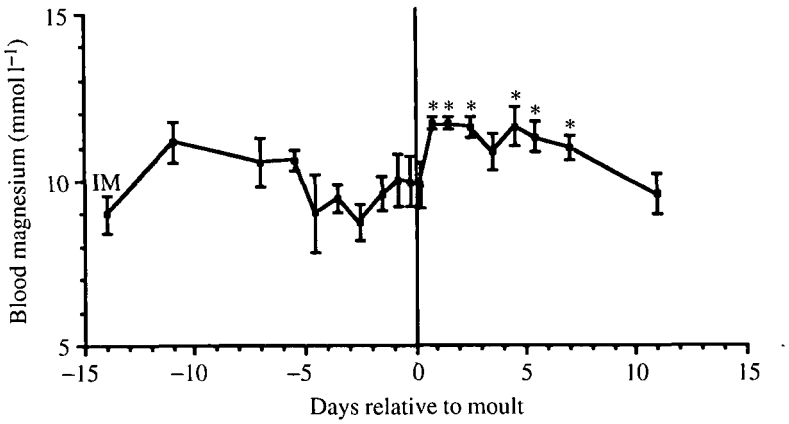

Fig. 6. Concentrations of total magnesium in the blood over the moult cycle (N=3–10). Asterisks indicate values significantly different from those for the intermoult period (IM). Values are mean±S.E.

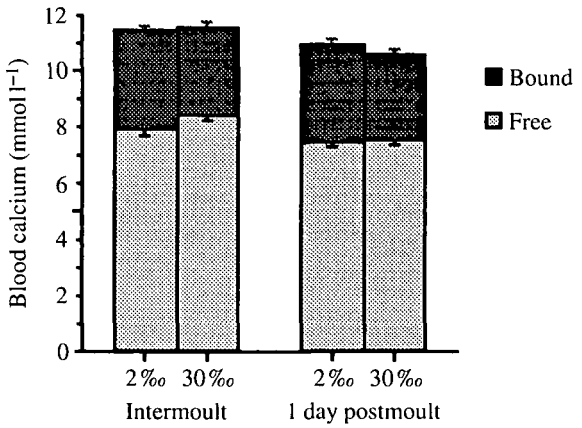

Fig. 7. Distribution of blood calcium with the moult stage and acclimation salinity (N=8–10). Top error bars are for the total calcium measurements; lower error bars for the free calcium measurements.

Transepithelial potential and electrochemical gradient

The transepithelial potential in natural 2‰ water was significantly more negative (t-test, P<0.025) at postmoult (−5.4±0.7 mV, N=8) than at intermoult (−3.1±0.6 mV, N=9). Postmoult crabs in artificial 2‰ water had a more negative potential (−9.3±0.7 mV, N=9) than those held in natural water, probably because of the strong dependence of the transepithelial potential on external calcium concentrations (Cameron, 1978), which were slightly lower in the artificial 2‰ water. The calcium equilibrium potential at 1 day postmoult, calculated using

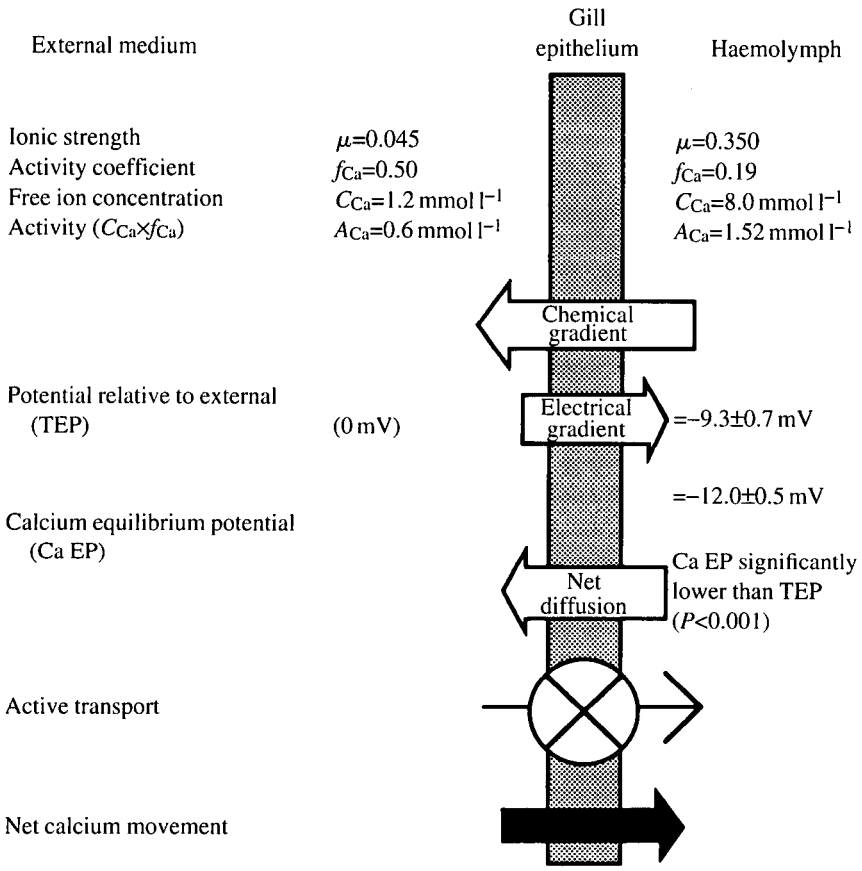

Fig. 8. Diagram of calcium distribution and fluxes across the gill epithelium at 1 day postmoult with 2‰ sea water as the external medium.

the Nernst equation, was significantly lower than the transepithelial potential (*t*-test with paired data, $P<0.001$), indicating an outward-directed electrochemical gradient and a tendency for calcium to diffuse out of the organism (Fig. 8).

Discussion

The external solution provides virtually all the necessary divalent cations for hardening of the exoskeleton when blue crabs are acclimated to 2‰ salinity, as it does in seawater-acclimated blue crabs (Cameron and Wood, 1985). Since the total content of calcium in the blood, assuming a blood space of 25 ml for a 100 g crab (Gleeson and Zubkoff, 1977), accounted for only about 6% of the calcium content in whole bodies after moult, small stores of this ion probably existed in compartments other than the blood. Some of this may be accounted for by small calcium reserves in the midgut gland (Becker *et al.* 1974), which may be important for maintaining blood concentrations or rapidly mineralizing critical parts of the

exoskeleton. The whole-body content of calcium in intermoult crabs at 2‰ salinity (35.5±3.1 g kg^{-1} wet mass, N=6) was comparable to that found in blue crabs that moulted in sea water (36.4±3.0, Cameron and Wood, 1985). There was therefore no reduction in the total amount of calcium accumulated in the postmoult period attributable to the lower calcium content of the water. The magnesium and strontium contents of whole bodies of intermoult crabs were very small in comparison to the calcium content, as they are in the exoskeletons of crabs that moult in sea water (Cameron and Wood, 1985). The importance of magnesium and strontium in hardening the exoskeleton is therefore equal in crabs acclimated to low-salinity water and sea water.

We did not rigorously test for the contribution of diet as a source of calcium. It is unlikely that this was a major source of calcium since the uptake of calcium from the water rose to a maximum rate before feeding resumed after moult and the apparent rate of hydrogen ion excretion was still closely coupled to the rate of calcium removal from the water until the fifth day postmoult (Fig. 2), by which time crabs were feeding. It is also not necessary for blue crabs to ingest their old carapaces after the moult for use as a source of calcium, as some species do (summarized in Greenaway, 1985). Postmoult crabs ingested very little, if any, of the exuviae in those cases when it was left in the chamber for several days. The normal diet and shed exuviae may, however, play a greater role in the calcium balance of crabs under more natural conditions.

The freshwater crayfish *Procambarus clarkii* (Wheatly and Ignaszewski, 1991) and *Austropotamobius pallipes* (Greenaway, 1974*a*) lose calcium to the water before moult, presumably because of the partial dissolution of the mineral portion of the old exoskeleton. Resorption also occurs in blue crabs as the sutures weaken, but we found no evidence of substantial premoult fluxes of either hydrogen or calcium ions. Either the resorbed calcium remained in the body or the fluxes were too small to be detected by the methods we used.

After the moult, the experiments concerning both acidification rates and whole crab mineral contents showed that there was no decrease in the overall rate or degree of calcification as a response to the lower calcium availability. Although acidification seems to begin slightly later in lower salinities, the basic pattern is the same and the maximum rate attained is comparable to that of blue crabs in sea water (Cameron and Wood, 1985). The peak uptake rate was greater in this species than in other freshwater species (Greenaway, 1974*b*; Malley, 1980; Sparkes and Greenaway, 1984; Wheatly and Ignaszewski, 1991), although interspecific comparisons are difficult because of differences in experimental temperatures and size.

Ammonia excretion in blue crabs is much slower than the observed rates of apparent hydrogen ion excretion (Mangum *et al.* 1976) and was probably only responsible for a small portion of the observed pH change. The apparent hydrogen ion excretion is a result of the need for carbonate ions in calcium carbonate formation, in which a bicarbonate ion is imported and the hydrogen ion released during carbonate formation is excreted (Cameron, 1989).

The protein concentration increases in the blood of other crustaceans before moult (e.g. McWhinnie, 1962; Djangmah, 1970) as it does in blue crabs acclimated to 2‰ salinity (present study). This is unlike blue crabs acclimated to sea water, whose blood protein concentrations at premoult are equivalent to those at intermoult (DeFur *et al.* 1985). It is possible that material from the breakdown of the proteins in muscle and integument during this period is a source of protein (Travis, 1955). The large dilution of blood which occurs in all aquatic crustaceans undergoing moult causes the postmoult halving in protein concentration found in all crustaceans studied (e.g. Travis, 1955; Glynn, 1968). The reason for the lack of a rapid postmoult return to intermoult concentrations, also reported by Glynn (1968) and DeFur *et al.* (1985), is less obvious. The haemocyanin concentration in the blood increases in less than 24 h in response to salinity stress (Boone and Schoffeniels, 1979), suggesting that the time required to synthesize haemocyanin, the major protein in the blood, was not preventing the return to intermoult concentrations. Protein synthesis in the carapace and muscle tissues, or the strong effect of diet and health (Djangmah, 1970; Mangum *et al.* 1985), may be responsible for the lack of a rapid recovery.

The concentration of total calcium in the blood of seawater-acclimated blue crabs (Cameron, 1989) and other species of crustaceans (Table 3 in Greenaway, 1985) rises before moult and declines after moult, as it did in our study of blue crabs acclimated to 2‰ salinity. There are fewer data for changes in free calcium, the more pertinent variable with respect to thermodynamic processes such as transport. Free calcium in freshwater blue crabs changes little throughout the moult cycle, a pattern of stasis also found in the freshwater crayfish *Austropotamobius pallipes* (Greenaway, 1974*a*,*b*). Unlike Towle and Mangum (1985), we did not find a substantially lower postmoult concentration of free calcium in seawater-acclimated blue crabs.

The regulation of blood calcium concentrations primarily involves the interaction of the blood space with three other major compartments: the surrounding water, the mineralized carapace and, to a lesser extent, the midgut gland. Changes in the size of the blood space and fluxes between blood and the other compartments are therefore responsible for the observed changes. The partial dissolution of the old carapace probably serves as a source for the extra calcium needed to increase the total amount of calcium in the blood during premoult. Interestingly, the concentration of free calcium did not drop with blood dilution at the time of water uptake. Clearly these crabs are able to adjust quickly to the increased blood space and maintain free calcium within a relatively narrow range of concentrations. The small decrease in the concentration of free calcium after the first 12 h and the subsequent slight increase after 1 day probably represent differences in the rate of calcium uptake into the blood compared with calcium movement from blood to carapace (Towle and Mangum, 1985). As in seawater-acclimated blue crabs, the lower calcium concentration in postmoult crabs would aid the uptake of calcium by creating a more favourable chemical gradient (Cameron, 1989). Although this may be the reason for a greater proportion of

bound calcium in some freshwater species, blue crabs do not lower their calcium activity by binding more of the ion when acclimating to low-salinity water (Fig. 7).

The small but significant increase in the concentration of total magnesium in blood 12 h after moult correlates with the start of calcium uptake and the small drop in free calcium. Magnesium concentration remained elevated through the first 5 days after moult, when mineralization occurred most rapidly. This contrasts with seawater-acclimated blue crabs in which total (Wheatly, 1985) and free (Towle and Mangum, 1985) magnesium concentrations increase at the time of water uptake and drop after 1 day, at the peak of the mineralization process. The reasons for these changes are unknown. Blood magnesium concentration is inversely related to the transepithelial potential when external calcium is varied during the postmoult period (D. S. Neufeld and J. N. Cameron, unpublished results).

Acclimation to low salinity evidently entails a negative shift in the transepithelial potential, since our measurements were more negative than those reported by Cameron (1989) for seawater-acclimated crabs. This inward electrical gradient for calcium increased further after moult, as indicated by the more negative transepithelial potential, but was still offset by the stronger chemical gradient resulting from a higher internal activity of calcium (Fig. 8). The sum of electrical and chemical forces therefore favoured net efflux. Nevertheless, net calcium uptake occurred, indicating the presence of some active transport component. The identification of active transport by this means does not necessarily imply the action of an ATPase directly on calcium. In this study, although there must have been some source of the energy needed to move calcium against its electrochemical gradient from the water to blood, this could have been through coupling to the movement of another ion such as sodium (Ahearn, 1978) or hydrogen (Ahearn and Franco, 1990).

Although the great difference in calcium concentrations between blood and water in some freshwater species makes it very likely that transport occurs by an active mechanism (Vincent, 1969; Malley, 1980; Sparkes and Greenaway, 1984), the freshwater crayfish *Austropotamobius pallipes* is the only other species for which measurements of activities have been used to demonstrate the presence of active transport during the postmoult period (Greenaway, 1974*b*). In seawater-acclimated blue crabs the chemical gradient for calcium appears to be directed inwards, based on total concentrations (Cameron, 1989). This conclusion would undoubtedly be strengthened by the use of activities rather than total concentrations, since the percentage of calcium in the free state is less in blood than in water. Assuming 72 % free calcium in the blood (present study) and about 85 % free calcium in sea water (Thompson and Ross, 1966), the external:internal activity ratio would be around 0.76 and the equilibrium potential would be +3.5 mV, greater than the published transepithelial potential (+2.1 mV) and thus favouring influx. Since the use of the Nernst equation in the evaluation of active transport assumes steady-state conditions (Kirschner, 1970), there may still be an active component if the inward electrochemical gradient is not strong enough to maintain the observed rate of uptake.

Table 2. *Comparison of flux rates and thermodynamic work for postmoult Ca^{2+} uptake and intermoult Na^+ and Cl^- regulation in a 100 g crab*

Ion	Stage	External solution	Flux rate ($mmol\,h^{-1}$)	Work ($J\,h^{-1}$)	Reference
Ca^{2+}	1 day postmoult	2‰ sea water	545	0.25	Present study
Na^+	Intermoult	Tap water	472	5.18	Cameron (1978)
Cl^-	Intermoult	Tap water	531	6.16	Cameron (1978)

The uptake rate for calcium ions during the period of rapid calcium uptake is similar to those of both sodium and chloride at intermoult in blue crabs acclimated to fresh water (Table 2). We calculated the minimum energy required to move calcium ions against the electrochemical gradient to be much less than that reported for sodium or chloride, although this may not provide an accurate comparison of the relative metabolic costs of regulating calcium as opposed to sodium and chloride. The metabolic cost of ion transport across an epithelium is dependent on the mechanism of transport, which is unknown for this epithelium, and may be independent of the electrical and chemical gradient across the epithelium as a whole. For instance, if calcium moves transcellularly and is actively transported across the basolateral membrane, transport would occur against a much larger electrochemical gradient than is suggested by the present data for the epithelium as a whole. In addition, the flux rate used may not include the work associated with the normal maintenance of blood calcium level. The portion of uptake required to counteract calcium efflux is probably substantially less than the flux associated with cuticle hardening since the uptake rate for calcium at intermoult is $0.014\,mmol\,kg^{-1}\,h^{-1}$ in a freshwater crayfish (Greenaway, 1972) and $0.133\,mmol\,kg^{-1}\,h^{-1}$ in *Carcinus maenas* in 35 % sea water (Greenaway, 1976). Finally, the transport of the hydrogen and bicarbonate needed for mineralization may also require energy.

Acclimation for postmoult calcification at low salinities in blue crabs involves a lowering of the transepithelial potential and a reliance on calcium uptake through active transport. Although many freshwater crustaceans also rely on active transport of calcium from the external medium for calcification of the exoskeleton, other strategies that are found primarily in crustaceans inhabiting fresh water are not adopted by blue crabs in low salinity. These results are consistent with other studies of the regulation of salts and water by blue crabs (Cameron, 1978) showing that, when in environments requiring ionic or osmotic regulation, blue crabs rely more on transport processes than on physiological changes that would allow the animal to avoid the need for increased metabolic expenditures directly on transport.

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