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MECHANISM OF THE NET UPTAKE OF WATER IN MOULTING BLUE CRABS (*CALLINECTES SAPIDUS*) ACCLIMATED TO HIGH AND LOW SALINITIES

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Summary

Blue crabs (*Callinectes sapidus* Rathbun) acclimated to a salinity of 2‰ approximately doubled in wet mass (excluding carapace) during the period from 10 h before moult to 2 h after moult. Both in blue crabs acclimated to 2‰ salinity and in crabs acclimated to 28‰ salinity, the drinking rate increased from approximately 0.4 ml $100\,g^{-1}\,h^{-1}$ at 1 day prior to moult to approximately 8 ml $100\,g^{-1}\,h^{-1}$ during the first hour after moult. The drinking rate had decreased 1 day after moult in both salinities, but was significantly higher in crabs acclimated to high salinity (1.84±0.16 ml $100\,g^{-1}\,h^{-1}$) than in crabs acclimated to low salinity (0.26±0.04 ml $100\,g^{-1}\,h^{-1}$). Drinking accounted for two-thirds of the weight gain during the first hour after moult at both acclimation salinities, indicating that water enters the body at moult primarily through the gut rather than through the gills. [^{14}C]polyethylene glycol, added as a tracer in the bath water, was concentrated in the midgut gland rather than in the stomach, implicating the midgut gland as the primary site of water absorption. The rate of water efflux was significantly greater in crabs acclimated to 30‰ salinity (66.4±9.0 ml $100\,g^{-1}\,h^{-1}$) than in crabs acclimated to 2‰ salinity (34.0±4.7 ml $100\,g^{-1}\,h^{-1}$). The osmotic uptake of water is equal at both salinities as a result of the decreased water permeability at low salinity. The rate of urine formation was estimated to be between 0.5 and 1 ml $100\,g^{-1}\,h^{-1}$ during the first hour after moult in crabs acclimated to both low and high salinities, suggesting that the antennal gland plays a relatively small role in water regulation during this period.

Introduction

There is a large net influx of water during the moult of crustaceans that causes a large increase in hydrostatic pressure in the blood space (deFur *et al.* 1985). This water uptake provides the force necessary to break open the old exoskeleton and expand the body, and also provides a rigid structure for muscular movements (deFur *et al.* 1985; Cheng and Chang, 1991). Both the gills and the gut have been suggested as the major organs

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responsible for the changes in water fluxes that occur at moult. The gills are considered to be the major pathway for fluxes of ions (Mantel and Farmer, 1983) and water (Capen, 1972) between the external environment and the blood, and it has been suggested that the uptake of ions by the gills at moult creates an osmotic gradient that provides the force necessary for water influx (Travis, 1954; Dandrifosse, 1966; Lockwood and Andrews, 1969; Towle and Mangum, 1985). However, increased drinking rates in moulting lobsters (Dall and Smith, 1978; Mykles, 1980) suggest that the gut can be a major route of water influx. Water uptake by drinking might be expected in marine crustaceans, in which there is no overall osmotic gradient across the body, but there are no measurements of drinking rates for crustaceans moulting in low salinity where a large osmotic gradient would favour the osmotic influx of water. The other organ involved in water regulation, the antennal gland, produces little urine at moult in lobsters (Cheng and Chang, 1991), but there is no information on its role in the water balance of a crustacean moulting in low salinity.

In low salinities, the euryhaline blue crab maintains its blood at a much higher osmotic pressure than the external medium (Ballard and Abbott, 1969). Blue crabs are able to moult in fresh water (deFur *et al.* 1988) and often appear to prefer moulting in areas with lower salinities (Hines *et al.* 1987). Although movement into low salinities may have less to do with selection of salinity than other factors, such as predation (Shirley *et al.* 1990; Ryer *et al.* 1990), it is possible that the osmotic difference between the blood and water at low salinities aids in the uptake of water at moult and allows a greater increase in size (Hines *et al.* 1987; deFur *et al.* 1988). It is also possible that the mechanism of water uptake is different in low salinity, where there is a large osmotic gradient, than in crabs in high salinity, where no osmotic gradient is maintained at moult (Wheatly, 1985). We compared the weight gain at moult with the routes and magnitudes of water fluxes in blue crabs acclimated to both high and low salinities in order to determine how a euryhaline crustacean regulates water content when it moults in water with different osmotic strengths.

Materials and methods

We collected blue crabs and acclimated them to either high or low salinity as described by Neufeld and Cameron (1992). High-salinity water was taken from the Port Aransas ship channel and varied according to tide and season but was 28 ‰ salinity or greater in all cases. Premoult wet mass was estimated by subtracting the mass of the exuvium from the total mass at premoult. We were careful to blot the exuvium completely dry before weighing to avoid errors due to residual water. Percentage mass gain is expressed as the change in wet mass during the moult period divided by the premoult wet mass (total mass minus exuvium mass). We measured the water content of whole crabs by measuring the mass loss after oven-drying (60 ˚C) to a constant mass.

Drinking rate

We calculated drinking rates by adding [^{14}C]polyethylene glycol ([^{14}C]PEG, relative molecular mass 4000) as a tracer in the water bath and measuring its appearance in the

gut. We compared the activity of tissue samples with the specific activity of the water bath to determine the equivalent volume of water collected by the tissue. In trials of crabs at moult, 0.19 MBq of [^{14}C]PEG was added to 2 l of water taken from the appropriate holding tank. The bath was stirred well before introducing the crab and the water was aerated throughout the experiments. We took crabs that had just moulted and placed them in the experimental bath for 1 h, recording the mass of the crab at the start and finish of the trial. After the trials, crabs were rinsed for several minutes in two baths containing 2 l of unlabelled tank water to wash [^{14}C]PEG from the gill chambers and the external surface of the cuticle. After immobilizing the crab by cooling it on ice for approximately 10 min, a blood sample was collected to check for the appearance of [^{14}C]PEG in the blood. The foregut, midgut and hindgut were dissected separately. Although the foregut was greatly distended with water after moult, it was possible to remove it without visible loss of contents. As much of the midgut gland was removed as possible, both within the main portion of the body and over the branchial chambers. We used a syringe to remove a sample of foregut fluid from some crabs to determine whether [^{14}C]PEG was concentrated in the foregut relative to the water bath. In some cases, we collected a sample of the residual fluid in the body chamber after dissection to check for leakage of [^{14}C]PEG out of the organs during dissection; this fluid had low radioactivity in all instances that we tested. For experiments on crabs at 1 day before and after moult, we performed experiments in an identical fashion except that 0.19 MBq of [^{14}C]PEG was added to 1 l of water and the trial period was 30 min.

We burned all tissue and water samples in a Tri-Carb sample oxidizer (Packard Instrument Company, Inc.) prior to scintillation counting. The recovery of activity after burning, estimated by comparing a burned quantity of water with the same quantity of water added to a blank burn, averaged 85 %. Tissue was burned in samples of 1 g or less for 3–4 min and may have averaged a lower recovery rate because a small amount of residue was often left after burning. Carry-over of activity from one sample to the next was tested at the beginning of this series of experiments and found to be negligible (<0.1 %).

Water permeability

We estimated the water permeability of crabs at moult by injecting tritiated water (THO) into the blood and monitoring its appearance in the water bath. Consecutive blood sampling at moult proved fatal to crabs in preliminary tests, so we based our calculations on the appearance of THO in the water bath. Crabs that had just withdrawn from the old exoskeleton were injected with 0.19 MBq of THO through a foam pad glued over the cardiac region. We held crabs out of the bath water and covered them with moist paper towels for approximately 30 min after the injection to allow mixture of THO. Crabs were held in 1 l of water from the appropriate holding tank for 45–60 min and water samples were taken at approximately 10 min intervals. A blood sample was taken after the completion of the experiment and the weight gain was recorded.

The rate of water efflux was calculated for the final sample time from the equation $J_{out}=(dQ_{in}/dt+J_{net}C_{out})/(C_{in}-C_{out})$, where J_{out} is the rate of water efflux, dQ_{in}/dt is the rate of change of total activity in the blood (calculated as described in the Appendix), J_{net}

is the rate of net water flux (equal to the rate of mass gain), and C_{out} and C_{in} are the specific activities of the bath and blood respectively. This equation was derived in a manner analogous to the equation for tracer influx described by Kirschner (1970).

Urine production

The rate of urine formation was measured using [^{14}C]inulin as a clearance marker. We injected 0.19 MBq of [^{14}C]inulin through a foam pad glued over the cardiac region of crabs that had just moulted. We held the crabs out of bath water and kept them covered with moist paper towels for 30 min prior to the start of the experiment in order to allow mixing of the marker throughout the blood space. Calculations were based on blood and water samples taken at the start and finish of a 1 h trial period. The first water sample was taken several minutes after the start of the experiment in order to allow mixing into the water bath of any [^{14}C]inulin that may have been present on the surface of the pad. Crab masses were also measured before and after the experiment. The clearance rate was calculated for the first sample point from the equation $[(dQ_{in}/dt)/C_{in}]\times[C_{urine}/C_{blood}]$, where C_{urine} and C_{blood} are the specific activities of the urine and blood. dQ_{in}/dt was estimated as described in the Appendix. The urine/blood ratio of inulin (U/B) in blue crabs is approximately 1 in other studies performed over a longer period (Cameron and Batterton, 1978; Robinson, 1982), but was probably somewhat less over the period of our experiments. Cannulation of the antennal glands was unsuccessful in the soft crabs; estimation of U/B in this study was therefore based on collection of urine with glass micropipettes. After carefully drying the area around the operculum, we gently inserted the glass micropipette at the opening of the antennal gland and were able to collect liquid samples of approximately 20 μl from several crabs at the end of the trial period.

Differences in all experiments were tested by the Mann–Whitney U-test unless otherwise noted. All values are expressed as means ±1 S.E.M. Since crabs at moult are not of a constant mass, we standardized all rates on the basis of the mass at the end of experiments.

Results

Mass gain and water content

The mass gain of blue crabs acclimated to 2 ‰ salinity was 94±3 % (N=19). The mass gain began more than 10 h before moult, when the cuticular sutures began to split, and gradually became more rapid as the moult approached (Fig. 1). Approximately half of the mass gain was achieved prior to moult. Mass continued to increase rapidly in the first hour after moult, but stabilized within 2 h after moult. The water content of crabs was significantly higher (P<0.05) at moult (85.6±1.9 %; N=5) than at intermoult (75.4±1.7 %; N=6).

Drinking rate

In both high and low salinities, the drinking rate was much greater during the first hour after moult than either 1 day before or after moult (Table 1). Drinking during the first hour after moult was responsible for 71 % of the mass gain in crabs acclimated to low

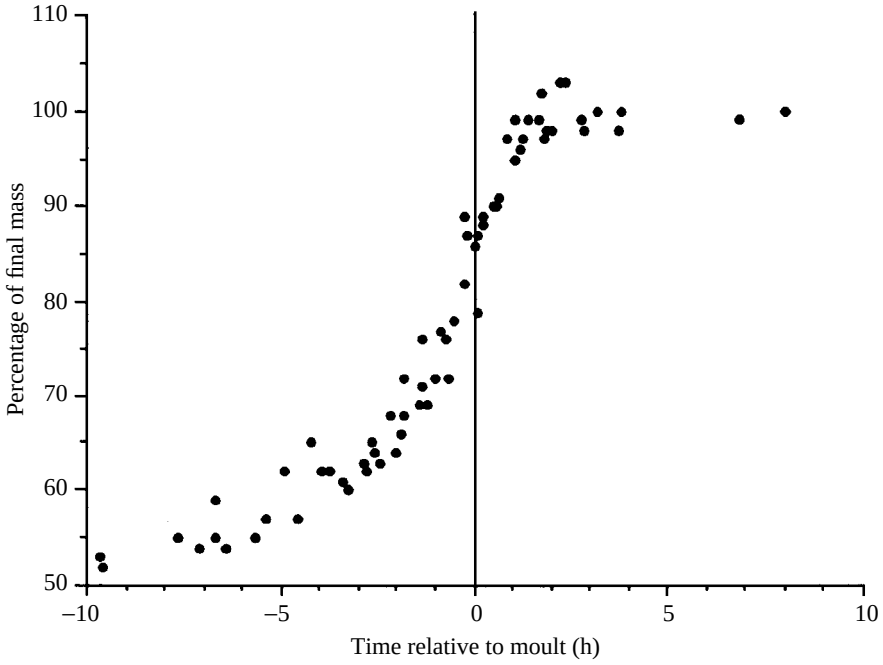

Fig. 1. Time course of mass gain at moult in five crabs acclimated to low salinity.

salinity and 58 % of the mass gain in crabs acclimated to high salinity. The drinking rates were equal at both salinities in crabs at 1 day before moult and at moult, but at 1 day after moult the rate was greater (P<0.05) in crabs acclimated to high salinity. [^{14}C]PEG did not appear in the blood over the period of our experiments. In all cases, the amount of activity found in the midgut or hindgut was equivalent to the amount of activity in 0.01 ml or less of bath water. Approximately 2–3 ml of fluid was present in the foregut of crabs at moult. Compared with the concentration of tracer in the bath water, tracer was concentrated in the foregut of low- and high-salinity crabs by factors of 1.34±0.26 and 1.44±0.33, respectively; neither value is significantly different from 1 (P>0.1; Wilcoxon signed-rank test). More tracer was found in the midgut gland than in the stomach at moult (Table 1). Samples of midgut gland taken from areas close to the foregut had activities that were equal to 1.42±0.22 ml bath-water g^{-1} tissue (N=3) and 1.75±0.29 ml bath-water g^{-1} tissue (N=3) in crabs acclimated to low and high salinity, respectively. Midgut glands sampled from over the branchial chamber, located away from the foregut, had activities equal to 0.66 ml bath-water g^{-1} tissue (N=2) and 0.90±0.26 ml bath-water g^{-1} tissue (N=3) in low and high salinity, respectively.

Water permeability

The rate of water efflux was significantly higher (P<0.05) in crabs acclimated to high salinity than in crabs acclimated to low salinity (Table 2).

Table 1. *Drinking rate of blue crabs acclimated to low and high salinity during the premoult stage, at 1 h after moult and during the postmoult stage*

	1 day before moulting		At moulting		1 day after moulting	
	2 ‰	28 ‰	2 ‰	28 ‰	2 ‰	28 ‰
Sample size	3	3	5	5	3	3
Mass gain ($g\,100\,g^{-1}$ final mass h^{-1})	–	–	12±2	16±2	–	–
Drinking rate ($ml\,100\,g^{-1}\,h^{-1}$)						
Midgut gland	0.26±0.08	0.12±0.04	5.48±1.76	5.83±1.14	0.10±0.02	0.74±0.28
Foregut	0.14±0.04	0.20±0.16	3.05±0.23	3.48±0.63	0.16±0.02	1.08±0.26
Total	0.42±0.10	0.32±0.22	8.53±1.84	9.31±1.11	0.26±0.04	1.84±0.16

Crab masses range from 47 to 178 g.

Table 2. *Rate of water efflux in crabs acclimated to low and high salinity during the first hour after moult*

	Acclimation salinity	
	2 ‰	30 ‰
Sample size	5	4
Mass gain ($g\,100\,g^{-1}$ final mass h^{-1})	8±4	8±3
Rate of water efflux ($ml\,100\,g^{-1}\,h^{-1}$)	34.0±4.7	66.4±9.0
Estimated extrarenal efflux (total efflux – urine production, Table 3)	33.2	65.8

Crab masses ranged from 53 to 146 g.

Urine production

The *U*/*B* ratio of [^{14}C]inulin was 0.65 for seawater-acclimated crabs (N=3) after the 1 h trial period. We were unsuccessful at collecting urine at the beginning of the trial period. Blood space at ecdysis (after the 30 min mixing period; Table 3) calculated from inulin distribution was reasonably close to blood space expected on the basis of other studies (Cameron and Batterton, 1978; Robinson, 1982), suggesting that blood circulation during the period of air exposure was adequate for mixing of tracers. The rates of urine formation during moult were equal in crabs at high and low salinities (Table 3).

Discussion

The mass of blue crabs acclimated to 2 ‰ salinity approximately doubles at moult, as it

Table 3. *Approximate rates of urine production in blue crabs acclimated to low and high salinity during the first hour after moult*

	Acclimation salinity	
	2‰	30‰
Sample size	3	4
Mass gain ($g\,100\,g^{-1}$ final mass h^{-1})	13±7	6±5
Blood space at ecdysis ($ml\,100\,g^{-1}$)	40.2±4.2	34.3±6.6
Rate of urine production using *U*/*B* ratio of 1 ($ml\,100\,g^{-1}\,h^{-1}$)	0.48±0.11	0.38±0.05
Rate corrected for *U*/*B* ratio after 1 h (0.65) ($ml\,100\,g^{-1}\,h^{-1}$)	0.8	0.6

Crab masses ranged from 95 to 126 g.

does in blue crabs collected from the same area and acclimated to sea water (Cameron, 1989). Our results are in agreement with the study of Haefner and Shuster (1964), who found that the increase in carapace width was equal in blue crabs acclimated to 13, 19 and 28‰ salinity. Mass gains were likewise equal in the amphipod *Gammarus duebeni* moulting in 100, 50 and 2 % sea water (Lockwood and Inman, 1973). deFur *et al.* (1988), however, reported that the percentage increase in mass at moult in blue crabs was dependent on salinity. The rate of water uptake is independent of salinity because the time course of mass gain in blue crabs acclimated to high salinity (Cameron, 1989) was equivalent to the time course we report for crabs in low salinity. Although blue crabs sometimes move into lower salinities to moult (Hines *et al.* 1987), our findings do not support the hypothesis that this provides a physiological advantage in allowing a greater uptake of water and resulting expansion of the body (deFur *et al.* 1988).

The reliance on drinking for water uptake in blue crabs is consistent with the role of the gut in water absorption during moulting in the lobsters *Homarus americanus* (Mykles, 1980) and *Panulirus longipes* (Dall and Smith, 1978). The volume of water in the stomach and midgut gland is also greater at moult in *Carcinus maenas* (Robertson, 1960), suggesting that drinking is the mechanism of mass gain in most marine crustaceans. Drinking appears to have a function in volume regulation during intermoult, since the drinking rate decreases when crustaceans are moved to lower salinities (Bolt *et al.* 1980; Greco *et al.* 1986). Cheng and Chang (1991) found that drinking was necessary for lobsters to withdraw their claws in 100 % sea water, but lobsters in 75 % sea water could withdraw their claws from the exuvium without drinking. These results suggested that drinking may not be necessary at moult in low salinities, where there is an osmotic gradient across the body wall; however, we found the contribution of drinking to be nearly the same in low and high salinities in moulting blue crabs. Water and ion permeabilities are sometimes correlated (Shaw, 1959;

Subramanian, 1975), and water uptake in low salinity by drinking rather than by the maintenance of a high enough permeability for the osmotic influx of water may prevent excessive losses of ions.

The foregut was notably distended during the period of water uptake, but the amount of liquid in the foregut was less than the total amount taken up, indicating that most of the water rapidly passes through this organ. [^{14}C]PEG used as a tracer in the bath water may have been slightly concentrated in the foregut, but it is evident that most of the water passes into another organ before being absorbed. Water passing through the foregut can move either to the midgut and hindgut, or into the midgut gland which arises at the juncture of the foregut and midgut (Johnson, 1980). The amount of activity found in dissected midgut and hindgut was negligible, indicating that very little bath water was passing into these organs. The activity in 1 g of midgut gland sampled close to the foregut, however, was greater than the activity in 1 ml of bath water. Since this ratio is based on the entire tissue sample, the ratio of activity in the luminal fluid of the midgut gland to activity in the bath water would have been even greater. Obviously [^{14}C]PEG was concentrated in the midgut gland and water moves through this organ into the internal compartments of the crab. More [^{14}C]PEG was present in the portion of the midgut gland close to the foregut than in the portion far away from the foregut, as would be expected if water were moving rapidly from the foregut to the midgut gland and thence into internal compartments of the crab. Our results are in agreement with histological observations on tissues in the gut (Johnson, 1980). The morphology of midgut, midgut caeca and hindgut epithelia change somewhat at moult, but the greatest changes occur in the midgut gland during the short period when water is taken up.

The midgut gland is evidently involved in water fluxes at moult in several other species of crustaceans. Robertson (1960) found that tracer was concentrated in the midgut gland rather than the foregut of *Carcinus maenas* at moult. Bliss (1968) found a large amount of water in the midgut gland at moult in the land crab *Gecarcinus lateralis*, although drinking was not observed. Dall and Smith (1978) assumed that water passed into the midgut gland of *Panulirus longipes* since it was not absorbed in the foregut, although uptake could also have occurred in the midgut, as it does in another species of lobster (Mykles, 1980). The role of the midgut gland in the regulation of water and ions is not well understood and it is not known whether water moves from the midgut gland into the internal compartments of the body as the result of hydrostatic pressure created by gut musculature or by an osmotic gradient.

We measured water fluxes during the first hour after moult only, and it is possible that the relative importance of drinking may be different during the period prior to ecdysis, when water uptake not only expands the body but also provides the force to break open the old exoskeleton. The low drinking rate at 1 day after moult, when calcium uptake is at a maximum in both low (Neufeld and Cameron, 1992) and high salinities (Cameron, 1989), confirms that the gut is an insignificant route for calcium uptake during the postmoult period. At the recorded drinking rates and external concentrations of calcium, accumulation of calcium through the gut could only reach a maximum rate of $0.3\,\mu mol\,100\,g^{-1}\,h^{-1}$ in crabs acclimated to 2‰ and $18\,\mu mol\,100\,g^{-1}\,h^{-1}$ in crabs acclimated to 30‰ salinity, whereas the net uptake of calcium during this period is

approximately 500 μmol 100 g^{-1} h^{-1} in crabs acclimated to both salinities (Cameron, 1989; Neufeld and Cameron, 1992). We are uncertain why the drinking rate after moult is higher in crabs acclimated to high salinity than in crabs acclimated to low salinity. The cuticle is still somewhat pliable at this stage and a continued water uptake may be necessary to maintain a rigid body in the absence of an osmotic gradient, although internal pressure decreases to intermoult levels by 1 day postmoult (stage A2; deFur *et al.* 1985).

The remaining one-third of water uptake not due to drinking presumably enters *via* the gills and therefore must occur by osmotic uptake. The lower rate of water efflux in low salinity, despite an osmolarity gradient between the bath and blood that is certainly higher than in high salinity, indicates a lower permeability to water when crabs are acclimated to low salinity. Although an osmotic gradient is present in moulting crabs in low salinity, there is no overall osmotic gradient between the blood and water during this period in high salinity (Wheatly, 1985), suggesting that local osmotic gradients may be responsible for the uptake (Towle and Mangum, 1985). The increased number of mitochondria at moult in the gill epithelium of blue crabs acclimated to low salinity (D. S. Neufeld and J. N. Cameron, in preparation) suggests an increased transport of ions, although an uptake of ions need not be for the purpose of maintaining an osmotic gradient for water uptake. In crabs maintained at high salinity, transport enzyme activities are reported to be greater at moult and during the postmoult period (Towle and Mangum, 1985), but changes in enzyme activity appear to occur over a longer period than changes in rates of water uptake. In addition, these measurements are standardized to a quantity of protein and do not account for changes in cell size. In low-salinity crabs, the size of epithelial cells is greater at premoult than at postmoult or intermoult (D. S. Neufeld and J. N. Cameron, in preparation), so that the total amount of enzyme activity may not be any greater at moult than at premoult or postmoult.

In the presence of differing osmotic gradients, a lower apparent permeability in crabs acclimated to low salinity allows the proportion of water uptake from osmotic fluxes to be equal in both salinities. A reduction in apparent permeability to ions and water is a widespread method of acclimation to reduced salinities (e.g. Smith, 1967, 1970; Capen, 1972; Lockwood *et al.* 1973; Shaner *et al.* 1985) and is also found in blue crabs at intermoult (Robinson, 1982). In seawater-acclimated blue crabs, the rate of water efflux at moult (Table 2) is approximately double the rate of water efflux at intermoult (31.3±5.3 ml 100 g^{-1} h^{-1}; Robinson, 1982), although this is probably due in part to a lower acclimation temperature (15 °C) for the intermoult crabs. Ventilation decreases greatly at moult and circulation slows somewhat at early postmoult (deFur *et al.* 1985), so the greater permeability at moult is not a result of ventilatory or cardiovascular changes. Crabs frequently get stuck in their old shells at moult, presumably because of water uptake that is too rapid, and it is likely that moulting would not be successful at low salinities if the water permeability were as high as that of seawater crabs unless the drinking rate were reduced or urine production were increased.

An accurate measurement of the rate of urine production proved difficult and our measurements are meant to be rough estimates. Urine production has also been measured by cannulation (Cameron and Batterton, 1978; Cheng and Chang, 1991); however, the

cuticle was too soft after moulting for successful cannulation. We could not calculate urine production on the basis of tracer disappearance from the blood since the blood space increased by an unknown amount during water uptake, so we based our measurements on tracer appearance in the bath. Urine production based on the U/B ratio at the end of the trial period is probably an underestimate, because the U/B ratio was probably lower at the start of the trial period, but we believe our estimates were sufficiently accurate for comparing water loss *via* urine with other water fluxes.

The antennal gland of crustaceans is involved in ion and nutrient regulation, particularly in those crustaceans adapted to fresh water, but it is primarily an organ of volume regulation in the marine species (summarized by Mantel and Farmer, 1983). Cheng and Chang (1991) reported a cessation of urine production in *Homarus americanus* at moult, the only other crustacean for which urine rates have been measured at moult. A greater production of urine at moult might be expected if it were necessary to counteract an excessive osmotic uptake of water at moult in low salinity, as appears to be the case in intermoult blue crabs (Cameron and Batterton, 1978; Robinson, 1982). The permeability is evidently low enough to prevent large osmotic fluxes of water at moult. Consequently, the rate of water loss by urine production in moulting blue crabs is quite low compared with water fluxes *via* other routes (Fig. 2) and its role in volume regulation at moult appears to be minimal. The rate of urine production at moult in both salinities appeared to be similar to that of blue crabs in fresh water (Cameron and Batterton, 1978), but greater than that of blue crabs in 30 % and 100 % sea water (Robinson, 1982).

In summary, it is evident from this and other studies that drinking is the primary method of water uptake used by marine crustaceans at moult. This study shows that drinking is also important in a euryhaline animal moulting in a very low salinity. Uptake by drinking appears to be the most economical method of osmotic and ionic regulation at moult, since it allows crustaceans in fresh water to maintain a lower permeability that reduces ion loss by diffusive efflux and/or urine produced for volume regulation. Moulting in low salinities conveys no physiological advantage in terms of weight gain, since the lower permeability prevents a greater osmotic uptake of water.

Appendix

Since the tracer space (equivalent to the blood space in the urine production experiments and the water space in the water efflux experiments) is needed for the calculation of total activity in the crab (Q_{in}) and the tracer space changed by an unknown amount over the course of the experiment, we estimated dQ_{in}/dt as dQ_{out}/dt, where Q_{out} is the total activity of the external medium. Even though tracer space in the crab increased during the course of the experiment, the appearance of tracer in the bath conformed well ($r^2>0.9$) to the empirical relationship:

$$C_{out,t} = C_{out,eq}(1 - e^{-kt}) \quad (1)$$

(Motais and Isaia, 1972), where $C_{out,t}$ is the specific activity at time t, $C_{out,eq}$ is the specific activity at equilibrium and k is the turnover constant. The specific activity at equilibrium

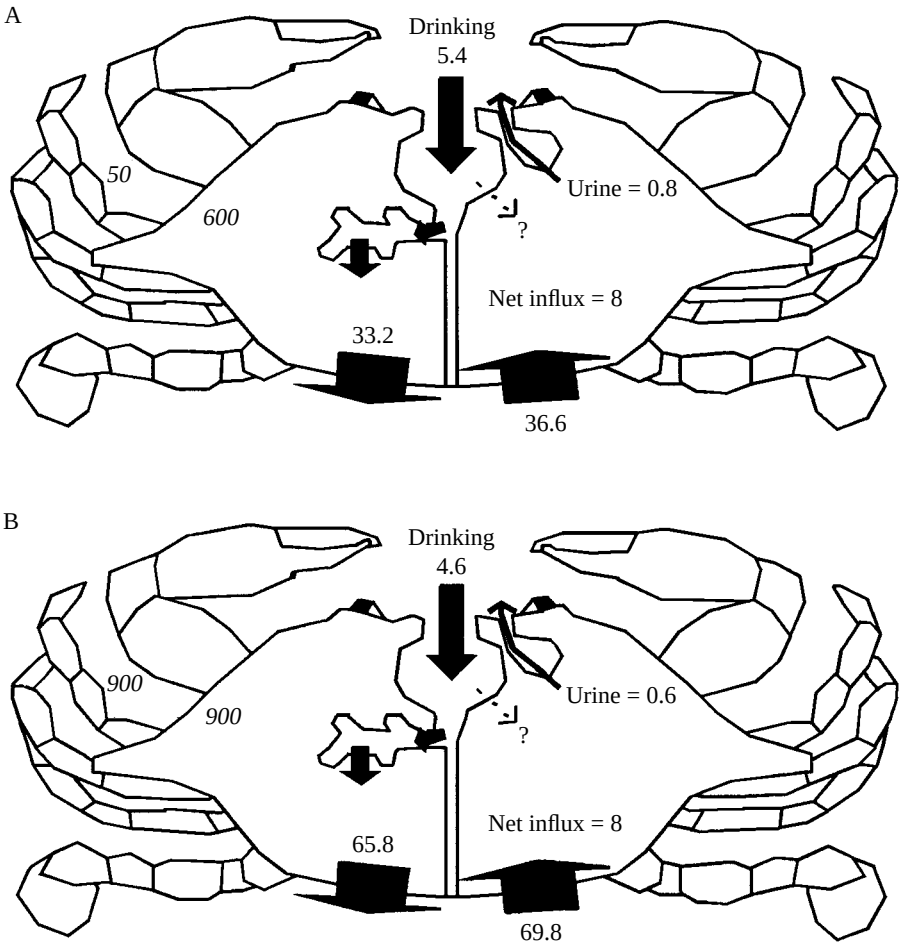

Fig. 2. Schematic diagram of routes and magnitudes of water fluxes during the first hour after moult in blue crabs acclimated to low (A) and high (B) salinity. All rates are in ml $100\,g^{-1}\,h^{-1}$. Osmotic strengths of blood and bath (given in italic type and measured in mosmol l^{-1}) are estimates based on unpublished data for low-salinity crabs or from Wheatly (1985) for high-salinity crabs.

was equal to $Q_{total}/(V_{out}+V_{in})$, where Q_{total} was the total activity injected and V_{out} was 1000 ml (bath volume). V_{in} was equal to V_{blood} (blood volume) for the urine production experiments and was calculated as $Q_{total}/X_{in,0}$ where $X_{in,0}$ is the specific activity in the blood after the mixing period and before the start of the experiment. For the water efflux experiments, V_{in} was equal to the water space and was estimated to be 85.6 % of the crab mass (see Results). The rate of change of tracer concentration in the bath is the derivative of equation 1:

$$dC_{out}/dt = kC_{out,eq}e^{-kt}. \quad (2)$$

Equation 2 was multiplied by V_{out} to calculate dQ_{out}/dt.

References

BALLARD, B. S. AND ABBOTT, W. (1969). Osmotic accommodation in *Callinectes sapidus* Rathbun. *Comp. Biochem. Physiol.* **29**, 671–687.

BLISS, D. E. (1968). Transition from water to land in decapod crustaceans. *Am. Zool.* **8**, 673–685.

BOLT, S. R. L., DAWSON, M. E., INMAN, C. B. E. AND LOCKWOOD, A. P. M. (1980). Variation of apparent permeability to water and sodium transport in *Gammarus duebeni* exposed to fluctuating salinities. *Comp. Biochem. Physiol.* **67**B, 465–473.

CAMERON, J. N. (1989). Post-moult calcification in the blue crab, *Callinectes sapidus*: timing and mechanism. *J. exp. Biol.* **143**, 285–304.

CAMERON, J. N. AND BATTERTON, C. V. (1978). Antennal gland function in the freshwater blue crab, *Callinectes sapidus*: water, electrolyte, acid–base and ammonia excretion. *J. comp. Physiol.* **123**, 143–148.

CAPEN, R. L. (1972). Studies of water uptake in the euryhaline crab, *Rhitropanopeus harrisi*. *J. exp. Zool.* **182**, 307–320.

CHENG, J. AND CHANG, E. S. (1991). Variations in urinary output of the lobster. *J. exp. Zool.* **260**, 288–294.

DALL, W. AND SMITH, D. M. (1978). Water uptake at ecdysis in the western rock lobster. *J. exp. mar. Biol. Ecol.* **35**, 165–176.

DANDRIFOSSE, G. (1966). Absorption d'eau au moment de la mue chez un Crustacé décapode: *Maia squinado* Herbst. *Archs Inst. Physiol. Biochim.* **74**, 329–331.

DEFUR, P. L., MANGUM, C. P. AND MCMAHON, B. R. (1985). Cardiovascular and ventilatory changes during ecdysis in the blue crab *Callinectes sapidus* Rathbun. *J. crust. Biol.* **5**, 207–215.

DEFUR, P. L., NUSBAUMER, D. AND LEWIS, R. J. (1988). Physiological aspects of moulting in blue crabs in tidal fresh water. *J. crust. Biol.* **8**, 12–19.

GRECO, T. M., ALDEN, M. B. AND HOLLIDAY, C. W. (1986). Control of hemolymph volume by adjustments in urinary and drinking rates in the crab, *Cancer borealis*. *Comp. Biochem. Physiol.* **84**, 695–701.

HAEFNER, P. A. AND SHUSTER, C. N. (1964). Length increments during terminal molt of the female blue crab, *Callinectes sapidus*, in different salinity environments. *Ches. Sci.* **5**, 114–118.

HINES, A. H., LIPCIUS, R. N. AND HADDON, A. M. (1987). Population dynamics and habitat partitioning by size, sex and molt stage of blue crabs *Callinectes sapidus* in a subestuary of central Chesapeake Bay. *Mar. Ecol. Prog. Ser.* **36**, 55–64.

JOHNSON, P. T. (1980). *Histology of the Blue Crab,* Callinectes sapidus. New York: Praeger.

KIRSCHNER, L. B. (1970). The study of NaCl transport in aquatic animals. *Am. Zool.* **10**, 365–376.

LOCKWOOD, A. P. M. AND ANDREWS, W. R. H. (1969). Active transport and sodium fluxes at molt in the amphipod *Gammarus duebeni*. *J. exp. Biol.* **51**, 591–605.

LOCKWOOD, A. P. M. AND INMAN, C. B. E. (1973). Changes in the apparent permeability to water at moult in the amphipod *Gammarus duebeni* and the isopod *Idotea linearis*. *Comp. Biochem. Physiol.* **44**A, 943–952.

LOCKWOOD, A. P. M., INMAN, C. B. E. AND COURTENAY, T. H. (1973). The influence of environmental salinity on the water fluxes of the amphipod crustacean *Gammarus duebeni*. *J. exp. Biol.* **58**, 137–148.

MANTEL, L. H. AND FARMER, L. L. (1983). Osmotic and ionic regulation. In *The Biology of Crustacea*, vol. 5 (ed. L. H. Mantel), pp. 54–162. New York: Academic Press.

MOTAIS, R. AND ISAIA, J. (1972). Temperature-dependence of permeability to water and to sodium of the gill epithelium of the eel *Anguilla anguilla*. *J. exp. Biol.* **56**, 587–600.

MYKLES, D. L. (1980). The mechanism of fluid absorption at ecdysis in the American lobster, *Homarus americanus*. *J. exp. Biol.* **84**, 89–101.

NEUFELD, D. S. AND CAMERON, J. N. (1992). Postmoult uptake of calcium by the blue crab (*Callinectes sapidus*) in water of low salinity. *J. exp. Biol.* **171**, 283–299.

ROBERTSON, J. D. (1960). Ionic regulation in the crab *Carcinus maenas* (L.) in relation to the moulting cycle. *Comp. Biochem. Physiol.* **1**, 183–212.

ROBINSON, G. D. (1982). Water fluxes and urine production in blue crabs (*Callinectes sapidus*) as a function of environmental salinity. *Comp. Biochem. Physiol.* **71**A, 407–412.

RYER, C. H., VAN MONTFRANS, J. AND ORTH, R. J. (1990). Utilization of a seagrass meadow and tidal marsh creek by blue crabs *Callinectes sapidus*. II. Spatial and temporal patterns of molting. *Bull. mar. Sci.* **46**, 95–104.

SHANER, S. W., CROWE, J. H. AND KNIGHT, A. W. (1985). Long-term adaptation to low salinities in the euryhaline shrimp *Crangon franciscorum* (Stimpson). *J. exp. Zool.* **235**, 315–323.

SHAW, J. (1959). Salt and water balance in the East African fresh-water crab, *Potamon niloticus* (M. Edw.). *J. exp. Biol.* **36**, 157–176.

SHIRLEY, M. A., HINES, A. H. AND WOLCOTT, T. G. (1990). Adaptive significance of habitat selection by molting adult blue crabs, *Callinectes sapidus* (Rathbun), within a subestuary of central Chesapeake Bay. *J. exp. mar. Biol. Ecol.* **140**, 107–119.

SMITH, R. I. (1967). Osmotic regulation and adaptive reduction of water-permeability in a brackish-water crab, *Rhithropanopeus harrisi* (Brachyura, Xanthidae). *Biol. Bull. mar. biol. Lab., Woods Hole* **133**, 643–658.

SMITH, R. I. (1970). The apparent water-permeability of *Carcinus maenas* (Crustacea, Brachyura, Portunidae) as a function of salinity. *Biol. Bull. mar. biol. Lab., Woods Hole* **139**, 351–362.

SUBRAMANIAN, A. (1975). Sodium and water permeabilities in selected crustaceans. *Physiol. Zool.* **48**, 398–403.

TOWLE, D. W. AND MANGUM, C. P. (1985). Ionic regulation and transport ATPase activities during the molt cycle in the blue crab *Callinectes sapidus. J. crust. Biol.* **5**, 216–222.

TRAVIS, D. F. (1954). The molting cycle of the spiny lobster, *Panulirus argus* Latreille. I. Molting and growth in laboratory-maintained individuals. *Biol. Bull. mar. biol. Lab., Woods Hole* **107**, 433–450.

WHEATLY, M. G. (1985). Free amino acid and inorganic ion regulation in the whole muscle and hemolymph of the blue crab *Callinectes sapidus* Rathbun in relation to the molting cycle. *J. crust. Biol.* **5**, 223–233.