HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 42, 99–111 (1988)

Osmotic and ionic regulation in shore crabs *Carcinus maenas* inhabiting a tidal estuary*

A. Winkler[1], D. Siebers[1] & W. Becker[2]

[1] *Biologische Anstalt Helgoland (Zentrale); Notkestr. 31, D-2000 Hamburg 52, FRG*
[2] *Zoologisches Institut und Museum der Universität Hamburg; Martin-Luther-King-Platz 3, D-2000 Hamburg 13, FRG*

ABSTRACT: Shore crabs *Carcinus maenas* were exposed to salinities fluctuating according to the natural tidal rhythm. To this end they were maintained in net cages positioned in the estuarine waters of the river Elbe. The cages were lifted every hour, and between 8–12 specimens were analyzed for hemolymph concentrations of Na, K, Ca, Mg, and osmolality. The results obtained were compared with the respective data measured in external brackish water. In addition, the specific activity of Na-K-ATPase in a posterior gill was determined. Hemolymph Na and Mg as well as branchial Na-K-ATPase were also determined in crabs collected in the North Sea and the Baltic. The results show that in *C. maenas* living in salinities fluctuating with the tides by approx. 15 ‰ S, Na, K and Ca were hyperregulated, and Mg was effectively hyporegulated. The concentrations of all hemolymph ions and the activity of the Na-K-ATPase were kept constant over the whole tidal cycle. In Baltic crabs, Na was effectively hyperregulated and gill Na-K-ATPase was significantly elevated by a factor of ca 2 when compared with North Sea crabs. It is suggested that long-term hyperregulation of Na in constant salinities results from an increased number of Na-K-ATPase molecules which may change by synthesis or degradation following salinity stress. Constant hemolymph levels of hyperregulated Na in crabs inhabiting fluctuating brackish water are accomplished by activation of existing Na-K-ATPase by low Na and inhibition by higher ambient concentrations.

INTRODUCTION

In northern European waters, the shore crab *Carcinus maenas* inhabits a large area between the Azores and the Baltic. The area is characterized by salinity levels between 9 and 35 ‰ S, and comprises regions, such as tidal estuaries, where the salinity fluctuates rhythmically with the tide. Under relatively constant salinity conditions, the extracellular fluids (ecf) of the crab are isosmotic to the external medium when this is seawater (Duval, 1925; Krogh, 1939). In shore crabs living in brackish water, the extracellular fluid is effectively hyperregulated. According to Zanders (1980a), *C. maenas* may be regarded as a transitional species between the truly marine decapods (e.g. *Cancer, Maia*) and those successfully thriving in freshwater or semi-terrestrial habitats (e.g. *Eriocheir, Potamon, Ocypode, Uca*). The euryhalinity of *Carcinus*, stemming from its capacity of hyperosmotic regulation, separates it distinctly from stenohaline marine species; shore crabs, however,

* This work is part of the first author's doctoral thesis submitted to the Department of Biology at the University of Hamburg.

cannot live in very dilute water or freshwater due to their limited capacity of osmotic and ionic regulation, and are thus clearly separated from limnic species.

To the authors' knowledge, the salinity and ionic relations between ambient medium and ecf have not been investigated in an osmoregulating species exposed to a natural salinity regime fluctuating twice daily with the tide. It is not known to what degree internal salinity is influenced by ambient salinity changes in terms of hemolymph osmolalities and concentrations of the major ionic blood constituents such as Na, K, Ca and Mg.

We therefore measured hemolymph ions and osmolalities every hour in shore crabs *C. maenas*, kept in net cages in the estuary of the river Elbe in the harbour of Cuxhaven and exposed to salinities that fluctuated with the tides. The results obtained were related to the brackish-water samples taken simultaneously.

In addition, specific activities of the Na-K-ATPase of the gills were determined according to the same time schedule in order to find out potential changes in this enzyme that is known to play a central role in osmotic regulation (Towle, 1981). In the crab *Callinectes sapidus* transferred from 30 to 5 ‰ S, gill Na-K-ATPase activity increased within 2½ h (Towle et al., 1976), but other studies (Neufeld et al., 1980) showed that the enzyme activity in the same species significantly increased after 3–4 days reaching equilibrium levels 12 to 18 days after abrupt transfer from 1000 to 200 mOsm kg^{-1}. The short-term changes observed by Towle et al. indicate rapid transformation of preexisting latent enzyme molecules into an active form. The long-term changes noted by Neufeld et al. (see also Siebers et al., 1982) indicate the time-consuming process of a de-novo enzyme synthesis. In order to obtain information on the mode of regulation of branchial sodium pump activity, it is of particular importance to monitor the specific activities of Na-K-ATPase in the gills of *C. maenas* exposed to the fast salinity fluctuations occurring in its natural habitat.

To compare the metabolic responses resulting from fluctuating and constant salinities, some of the measurements performed at Cuxhaven were repeated with crabs collected in situ under the comparatively constant and low salinity conditions of the Baltic and the constantly high salinities of the North Sea.

MATERIALS AND METHODS

Shore crabs

Shore crabs *Carcinus maenas* to be exposed to tidal salinity were obtained from the by-catch of prawn-fishermen at Cuxhaven, at the mouth of the river Elbe. Shore crabs inhabiting brackish water and seawater of relatively constant salinities were collected in shallow waters to the North of the island of Sylt (North Sea), or were obtained from a fisherman at Kiel-Heikendorf (Baltic Sea). Samples of hemolymph and gill tissue were taken at a close-by laboratory in Cuxhaven harbour, at the Biologische Anstalt Helgoland in List/Sylt (North Sea) or after a 1 h transfer of crabs from Kiel to our laboratory at Hamburg. During this transfer, the crabs were kept partially submersed in aerated original habitat water of approx. 3 cm depth.

Exposure of crabs to the tidal rhythm

Crabs were kept in net cages (approx. 0.5 m^3) covered by nets (0.5 cm mesh) pulled over wooden frames. By means of weights and long lines the enclosures were positioned at the bottom of the river Elbe, close to the pier, "Alte Liebe", in the harbour of Cuxhaven. The net cages were lifted every hour, and between 8 and 12 crabs were taken out in order to analyze hemolymph and gill tissues. This procedure enabled us to plot the osmolalities and cation concentrations in the hemolymph together with the specific activities of the Na-K-ATPase of a posterior gill over a complete tidal cycle.

Determination of salinities, osmolalities and cation concentrations

The salinities of brackish water and seawater were determined aerometrically using the salinity tables of Gillbricht (1959), based on measured gravities and temperatures.

Hemolymph was obtained by puncturing the membrane of a walking leg of *C. maena* with a hypodermic needle. After centrifugation at 2000 g for ca 8 min at room temperature, the supernatant serum was deep-frozen until analysis. Seawater, brackish water and serum samples were analyzed for osmolality by means of an osmometer designed for small sample volumes (Knauer, Berlin). Sodium concentrations were determined with a sodium selective electrode in combination with a sensitive ion analyzer (SelectIon 5000, Beckman) according to Winkler et al. (1982).

In order to compare the measurements of salinity, osmolality and sodium concentration in brackish-water and seawater samples, we used the following interrelation published by Winkler et al. (1982):

$$1\ ‰\ S = 29.4\ mOsm\ kg^{-1} = 13.2\ mM\ Na$$

Concentrations of K, Ca and Mg in brackish water, seawater and serum samples were determined by means of a flame atomic absorption spectrophotometer (AAS 300, Perkin Elmer) using commercial cation standards (Titrisol, Merck) and a reference solution for serum analysis (Merck). Internal standard was added to every sample.

Activity of Na-K-ATPase

A posterior gill (No. 8) was freed of adhering water with soft paper towels and homogenized (1 mg gill fresh weight + 0.029 ml buffer) for 45 secs with a teflon-glass Potter Elvehjem homogenizer at 0 °C, using Tris-EDTA buffer, pH 7.6 (250 mM sucrose, 20 mM Tris, 2 mM EDTA-Na_2, 0.1 % Na-deoxycholate). Na-K-ATPase activity was measured spectrophotometrically by monitoring the absorbance at 366 nm (25 °C) in a coupled enzyme assay (Allan & Schwartz, 1969).

The final test volume was 2.25 ml, pH 7.25, and composed of 0.1 M imidazol/HCl, 75 mM NaCl, 100 mM NH_4Cl, 5 mM $MgCl_2$, 5 mM ATP, 2.5 mM PEP, 0.5 mM NADH, 20 U ml^{-1} LDH, 5 U ml^{-1} PK. Assays were performed at 25 °C in the absence and in the presence of $5 \cdot 10^{-4}$ M ouabain, which completely inhibits Na-K-ATPase activity. Therefore, the reduction of total ATPase activity due to the presence of ouabain represents Na-K-ATPase activity. For calculations of activity we used the linear changes in absorbance between 5 and 15 min after starting the assays by addition of 0.1 ml

homogenate. Specific activities were calculated on the basis of protein concentrations determined with the Folin phenol reagent according to Lowry et al. (1951).

RESULTS

Salinity, osmolality, and cation concentrations

In the estuary of the river Elbe, salinities fluctuated by approx. 10–15 ‰ S within a tidal cycle, with pronounced seasonal differences. In spring, due to the large freshwater load of the river, salinities ranged between ca 5 and 20 ‰, and in late summer between ca 13 and 24 % S. In terms of osmolalities, the mean value of Elbe water at Cuxhaven, measured in July 1982, was 556 ± 83 mOsm kg^{-1} (N = 31), ranging between 418 and 670 mOsm kg^{-1}. Under these conditions, serum osmolalities of *Carcinus maenas* were hyperregulated and not influenced by fluctuations of external osmolality (Fig. 1).

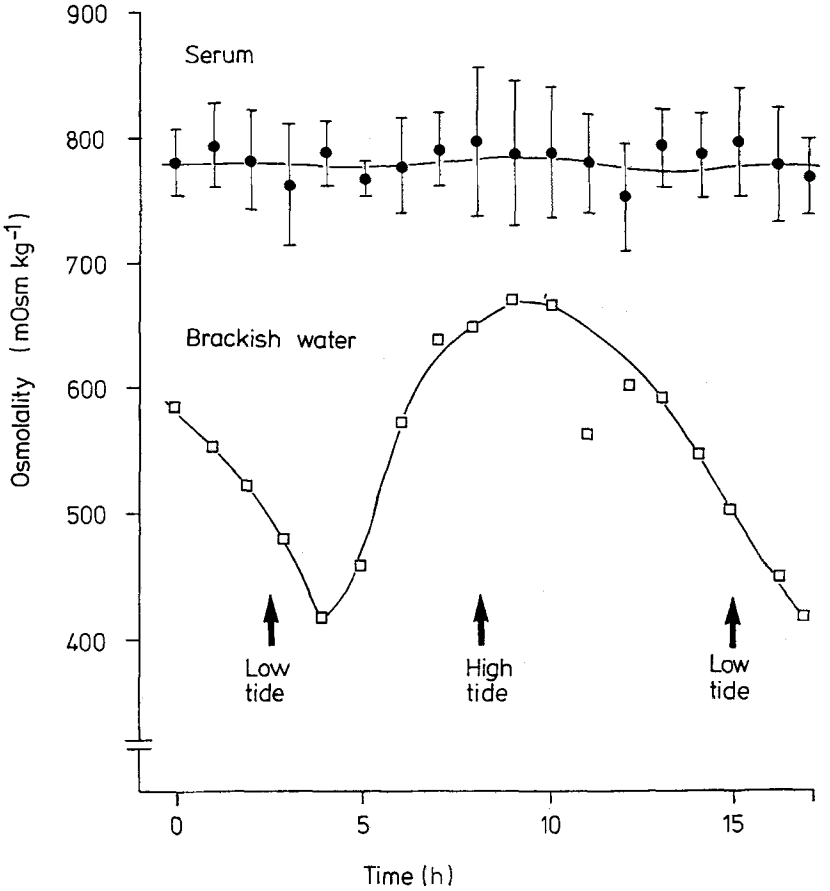

Fig. 1. Serum osmolalities of shore crabs *Carcinus maenas* in relation to brackish-water osmolalities measured in the Elbe estuary at Cuxhaven during a tidal cycle in July, 1982. Data represent means ± s. d. obtained from 8–12 crabs and 2–4 water samples per hour

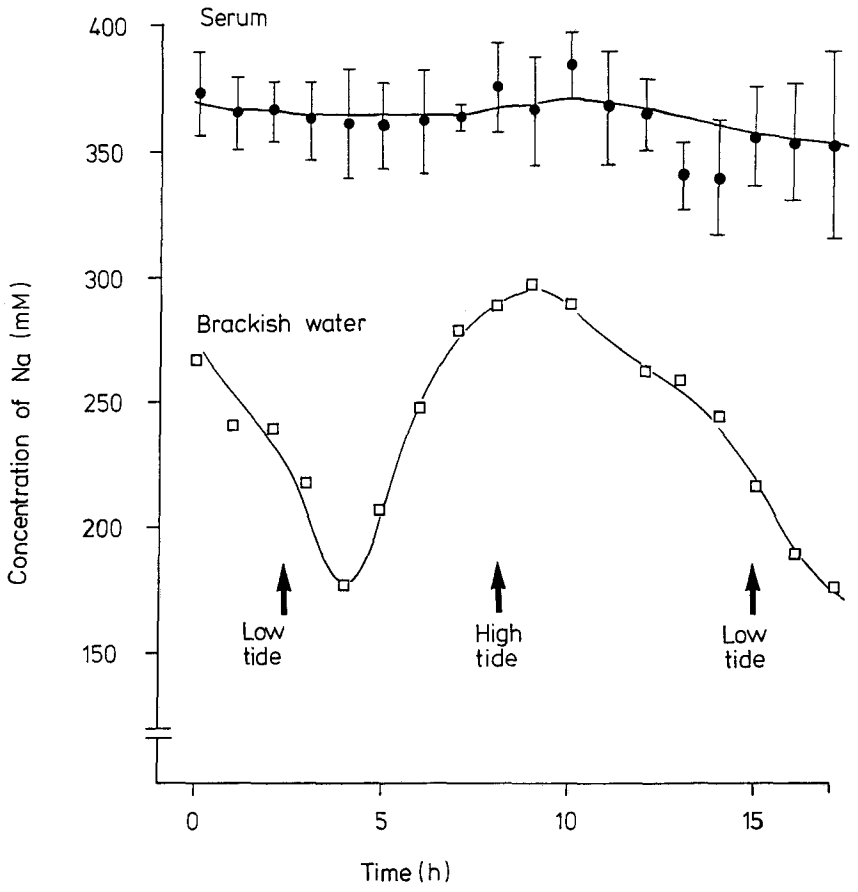

Fig. 2. Concentrations of sodium in samples of serum taken from shore crabs *C. maenas* and external brackish water measured in the Elbe estuary at Cuxhaven during a tidal cycle in July, 1982. Data represent means ± s. d. obtained from 8–12 crabs and 2–4 water samples per hour

Similarly, hemolymph sodium concentrations remained constant over the tidal cycle (Fig. 2). Approximately 1½ h following low tide, a sodium minimum of 179 mM was measured in the water; 1 h after high tide a maximum of 289 mM was measured. While the mean concentration of Na in the waters of the Elbe over the tidal cycle was 245 mM, the mean hemolymph level was 368 ± 20mM (N = 145).

The mean concentration of potassium in the river water was 7 mM, ranging between a minimum of 5 and a maximum of 10 mM. In hemolymph, potassium levels were constant over the whole period of investigation, amounting to 10.7 ± 1.2 mM (N = 158) (Fig. 3). Also hemolymph calcium concentrations were hyperregulated and remained constant during the whole tidal cycle (Fig. 4) at a mean of 12.3 ± 1.7 mM (N = 154). Calcium levels in the ambient water averaged at 7.8 mM with a minimum of 5.8 and a maximum of 9.6 mM.

Magnesium levels during the tidal cycle varied between 16.5 and 32.9 mM.

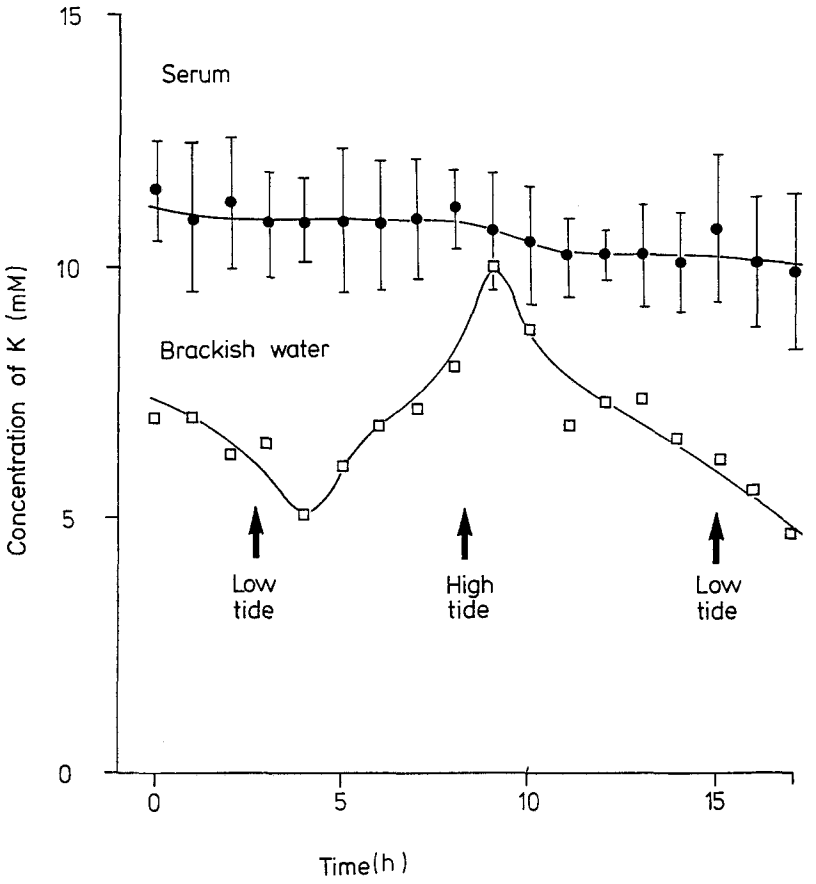

Fig. 3. Concentrations of potassium in samples of serum taken from shore crabs *C. maenas* and external brackish water measured in the Elbe estuary at Cuxhaven during a tidal cycle in July, 1982. Data represent means ± s. d. obtained from 8–12 crabs and 2–4 water samples per hour

Hemolymph Mg concentrations were strongly hyporegulated to a constant value of 11.2 ± 1.4 mM (N = 137) (Fig. 5). This concentration of magnesium was close to the levels determined in the hemolymph of crabs collected in the Baltic and the North Sea (Fig. 6).

Na-K-ATPase

Specific activities of Na-K-ATPase in homogenates of posterior gills (No. 8) in relation to external salinities are shown in Figure 7. The overall picture indicates that Na-K-ATPase activity was not modified during the course of the tidal cycle which was characterized by salinity fluctuations between 15 and 24 ‰ S. Mean specific activity of Na-K-ATPase amounted to 8.0 ± 2.4 μ mole P_i mg protein^{-1} h^{-1} (N = 88). Na-K-ATPase activities seemed to oscillate around average values determined by the mean ambient salinity. This assumption is evident from comparison with data of experiments performed

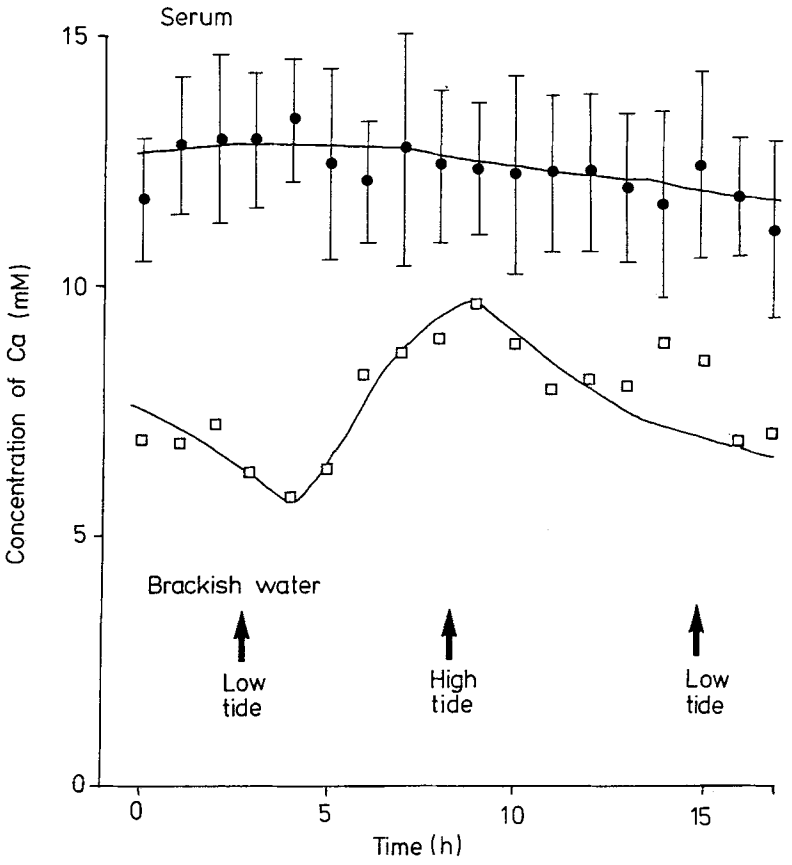

Fig. 4. Concentrations of calcium in samples of serum taken from shore crabs *C. maenas* and external brackish water measured in the Elbe estuary at Cuxhaven during a tidal cycle in July, 1982. Data represent means ± s. d. obtained from 8–12 crabs and 2–4 water samples per hour

in autumn 1981, when salinities varied between 10 and 15 ‰ S and Na-K-ATPase activity in gill 8 was 9.7 ± 1.9 μ moles P_i mg protein^{-1} h^{-1} (N = 36). In conclusion, the results obtained indicate that the specific activity of Na-K-ATPase did not change in response to the fluctuating salinities of the tidal estuary.

It is, however, obvious that hyperregulation of Na, observed at the comparably constant salinities of the Baltic Sea (Fig. 8), was related to adaptive changes in specific activities of Na-K-ATPase present in homogenates of posterior gills (Fig. 9). Specific activities of branchial Na-K-ATPase were 4.6 ± 2.2 μ moles P_i mg protein^{-1} h^{-1} (N = 35) in crabs collected in the North Sea and 8.8 ± 1.6 μ moles P_i mg protein^{-1} h^{-1} (N = 24) in Baltic crabs.

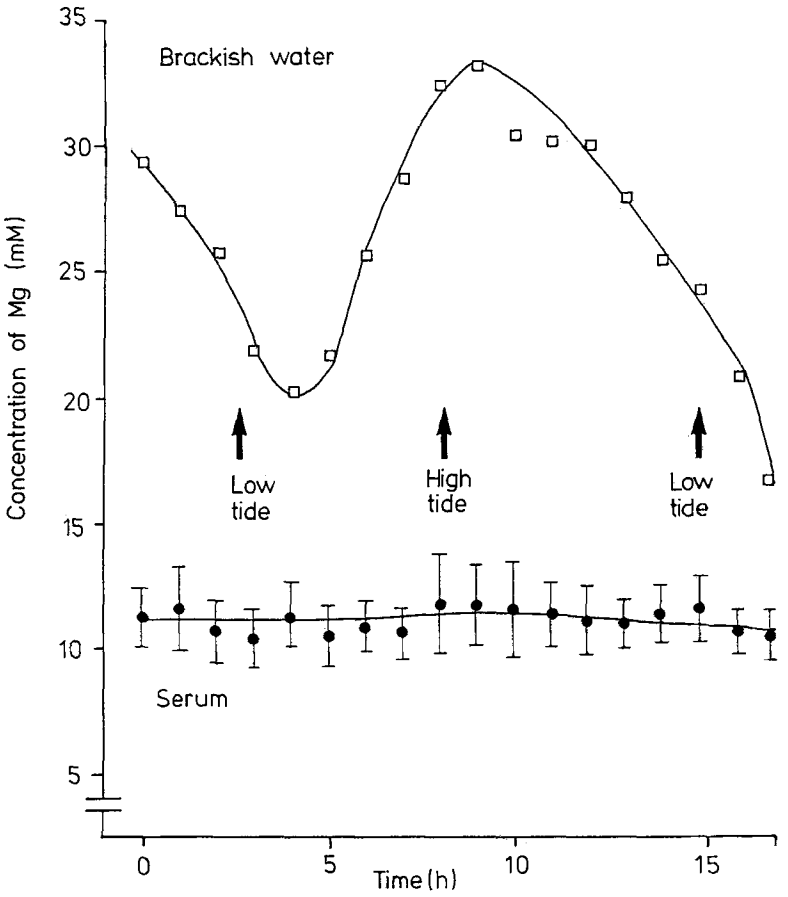

Fig. 5. Concentrations of magnesium in samples of serum taken from shore crabs *C. maenas* and external brackish water measured in the Elbe estuary at Cuxhaven during a tidal cycle in July, 1982. Data represent means ± s. d. obtained from 8–12 crabs and 2–4 water samples per hour

DISCUSSION

Hemolymph ion concentrations of shore crabs living in biotopes with constant and fluctuating salinities

Due to its capacities for effective osmotic and ionic regulation, the shore crab *Carcinus maenas* exhibits a high degree of euryhalinity enabling the species to thrive successfully in waters of highly variable salinity. In the tidal estuary of the river Elbe, hemolymph ion concentrations of Na, K, Ca and Mg were not influenced by salinity fluctuations of up to 15 ‰ S that occurred twice daily. These findings show that *Carcinus* is perfectly equipped with regulative properties allowing the metabolic functions to proceed in a practically constant ionic milieu of the body fluids when confronted with extensive and rhythmically occurring environmental changes.

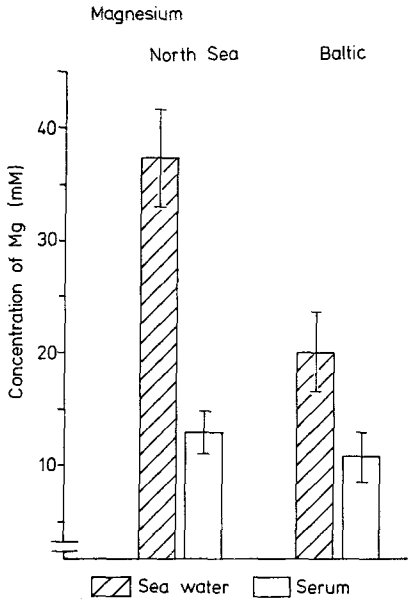

Fig. 6. Concentrations of magnesium in serum samples taken from shore crabs *C. maenas* in relation to Mg concentrations in ambient brackish water of the Baltic and seawater of the North Sea. Data represent means ± s. d. obtained from 29 (North Sea) and 37 (Baltic) serum samples and 12 (North Sea) and 11 (Baltic) water samples

In the Elbe estuary, Na, K, and Ca were hyperregulated, and Mg was hyporegulated. In the comparatively constant and low salinities of the Baltic the crabs also hyperregulated hemolymph sodium in contrast to specimens collected from the North Sea. Mg was effectively hyporegulated in all salinities whether high, low or fluctuating.

For crustaceans, comparable investigations on the interrelations between hemolymph ions and ambient salinity fluctuations in a tidal estuary have not been carried out. In a laboratory experiment, Spaargaren (1973) exposed *C. maenas* to abrupt salinity changes, and by employment of a mathematical model for sinus-shaped salinity changes he calculated that during a tidal cycle of 12 h hemolymph ionic changes might amount to approx. ⅓ of ambient fluctuations. These results could not be verified by our experiments.

Internal ionic constancy during tidal fluctuations is obviously a property of species capable of osmoregulation in constant salinities. In contrast to the findings presented for *C. maenas* the internal ions in various osmoconforming marine bivalves varied with in-situ tidal and artificially employed salinity fluctuations (Davenport et al., 1975; Shumway, 1977; Stickle & Denoux, 1976). Some of the mussel species, among them *Chlamys opercularis, Modiolus modiolus, Mytilus edulis* and *Crassostrea gigas* could avoid hemolymph ion changes by shell closure (Davenport et al., 1975).

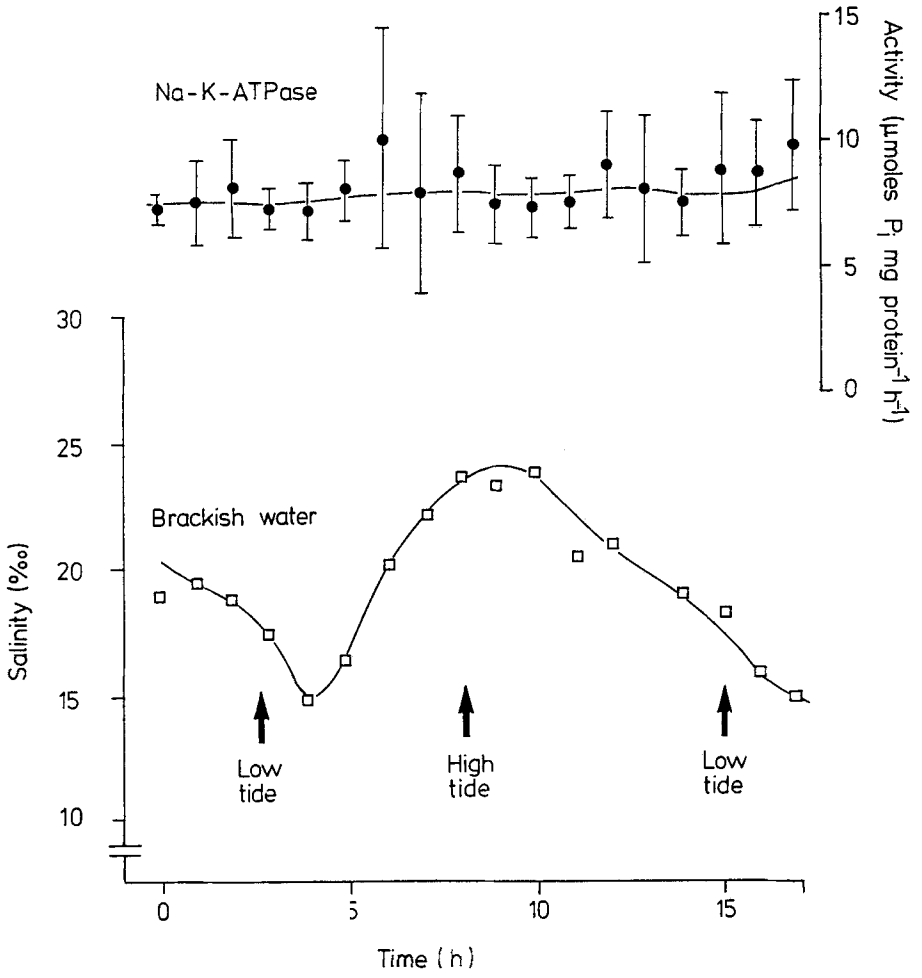

Fig. 7. Specific activities of Na-K-ATPase in gill 8 of shore crabs *C. maenas* in relation to ambient salinity of the Elbe estuary during a tidal cycle in July, 1982. Data represent means ± s. d. obtained from 6–8 crabs and 2–4 water samples taken every hour

Specific activity of Na-K-ATPase and its regulation

Hyperregulation of Na, K, Ca, and also Cl is considered to be the result of the balance between passive ion losses along the gradient through all permeable parts of the body surface and active uptake across the gills. In steady state conditions uptake equals loss.

Active uptake of Na, K and Cl in crustaceans is considered to be based on the activity of the branchial ouabain-sensitive Na-K-ATPase (Towle, 1984; for *Carcinus* see Lucu & Siebers, 1987; Siebers et al., 1982, 1983, 1985, 1986, 1987), which is located within the basolateral membranes of the epithelial cells (Towle & Kays, 1986). While hyperregulation of Ca presumably results from the activity of a still hypothetical branchial Ca pump, hyporegulation of Mg is effected by powerful renal excretory processes (Zanders, 1980b).

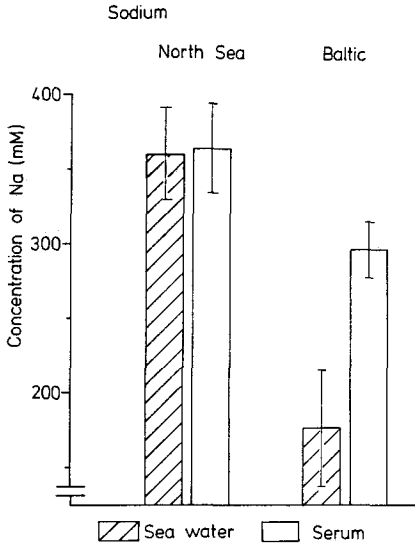

Fig. 8. Concentrations of sodium in serum samples taken from shore crabs *C. maenas* in relation to sodium concentrations in ambient brackish water of the Baltic and seawater of the North Sea. For sample size and s. d., see legend to Fig. 6

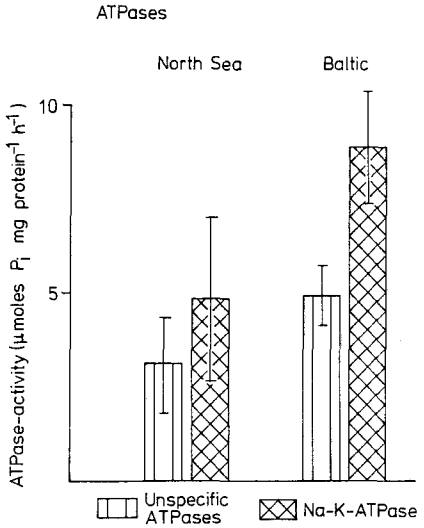

Fig. 9. Activities of unspecific (ouabain – insensitive) ATPases and Na-K-ATPase gill 8 of shore crabs *C. maenas* collected in the North Sea and the Baltic. Data represent means ± s. d. obtained from a sample size of 35 (North Sea) and 24 (Baltic)

Fluctuating salinities require the permanent regulation of active sodium uptake. Uptake has to increase at low tide and decrease at high tide. We did not, however, find changes in specific Na-K-ATPase activity during the tidal cycle. This corresponds to the view that the processes of enzyme synthesis and degradation do not proceed sufficiently to cope with tidal salinity changes. The question now is: How is the branchial Na-K-ATPase regulated in fluctuating salinities if not by fast changes in the number of Na-K-ATPase molecules?

We suggest that regulation of Na-K-ATPase in fluctuating salinities is achieved by an instantly operative activation/inhibition property based on the characteristic dependence of Na-K-ATPase on its ionic milieu, in particular the Na concentration. As published by Siebers et al. (1983), the specific activity of branchial Na-K-ATPase is highest at low Na concentrations of about 75 mM present in brackish water of ca 6 ‰ S. The higher the ambient levels of Na, the greater their inhibitory effect. At 400 mM Na present in 30 ‰ S, activities were completely inhibited. This type of inhibition/activation by its substrate Na is found also in the branchial Na-K-ATPase of the land crab *Cardisoma guanhumi* (Quinn & Lane, 1966), the freshwater crayfish *Procambarus clarki* (Horiuchi, 1977), the chinese crab *Eriocheir sinensis* (Pequeux et al., 1984), the fiddler crab *Uca minax* (Wanson et al., 1984) and various marine and limnic decapods (Winkler, 1986).

It must, however, be noted that in *C. maenas* intracellular concentrations of Na in the epithelial cells of the gill which supposedly control Na-K-ATPase activity are not known up-to-date. We have therefore to assume that intracellular levels of Na are coupled to and are thus representative of ambient concentrations, an assumption which would link external Na to Na pump activity.

Considering the result that branchial Na-K-ATPase in Baltic crabs, inhabiting brackish waters of relatively constant salinity, was significantly elevated compared to that in North Sea crabs, we assume the presence of 2 modes of Na pump regulation: a long-term regulation by synthesis and degradation of Na-K-ATPase molecules which is operative in constant salinities, and a short-term regulation of an unchanged number of enzyme molecules achieved by inhibition and activation by sodium. The latter mode of regulation is assumed to be operative in situations when salinities change abruptly or fluctuate with the tidal rhythm.

The finding in the Elbe estuary that mean Na-K-ATPase activity is influenced by mean salinities – no matter how strongly they vary according to each season – indicates that both mechanisms may also act in concert.

Acknowledgements. Financial support of this work by the Deutsche Forschungsgemeinschaft (Si 295/1–5) is gratefully accepted.

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