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Temperature dependence of ionic and acid–base regulation in boreal and arctic *Crangon crangon* and *Pandalus borealis*.

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Abstract

The effects of temperature on intracellular pH were investigated in the abdominal muscle tissue of two latitudinally separated populations of the euryhaline and eurythermic common sand shrimp *Crangon crangon* and in the stenohaline and stenothermic deep water shrimp *Pandalus borealis*. The contribution of passive mechanisms (due to the physico-chemical responses of intracellular buffers) and active mechanisms (due to ion exchange) to the pH change was quantified at different temperatures. In addition the extracellular ion composition was measured at the same temperatures. Acclimation in full strength sea water at various temperatures had relatively minor and inconsistent effects on haemolymph ion concentrations. The changes in pHi due to temperature were not reflected by alterations of haemolymph ion concentrations.

The pHi/T-relationship after 4 h of incubation at various temperatures differs between the two populations of *C. crangon*, with ΔpHi/Δ°C values of -0.008 in North Sea *C. crangon* and -0.018 in *C. crangon* from the White Sea while the absolute pHi values in White Sea *C. crangon* were lower by about 0.15 pH units than in North Sea *C. crangon* or in *P. borealis* at the same temperature. In *P. borealis* as in White Sea *C. crangon*, 4 h of incubation were sufficient to regulate ΔpHi/Δ°C at a level (-0.016) close to that predicted by the alphastat hypothesis (ΔpHi/Δ°C = -0.018, Reeves, 1972). For the two populations of *C. crangon* the contribution of active mechanisms to alphastat control was about 50% compared to only 15% in *P. borealis*. In winter and summer animals of North Sea *C. crangon* the passive fraction was -0.009 ΔpHi/Δ°C and thus similar to those observed after 4 h of incubation (-0.008 ΔpHi/Δ°C), indicating the absence of active mechanisms during this period. The subsequent increase in ΔpHi/Δ°C after 6 days of incubation (-0.017 units · °C^{-1}) demonstrated that the active adjustment of constant alpha imidazole was slower in the boreal North Sea *C. crangon* compared to Arctic *P. borealis*, while in the sub-Arctic White Sea *C. crangon* time-dependent changes in the pHi/T relationship resulted in constant alpha imidazole after 12 h of incubation with a value only slightly larger than the one seen after 4 h. © 1997 Elsevier Science B.V.

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1. Introduction

As a unifying hypothesis, a constant relative alkalinity is postulated to be maintained during temperature changes in body fluids (Howell et al., 1973) resulting in a nearly constant dissociation of the imidazole group of proteins (alphastat hypothesis, Reeves, 1972, 1977). Typically an average pH change of $-0.017/°C$ is interpreted to meet this requirement. The temperature induced pH shift supporting extra- and intracellular alphastat control of pH may consist of a passive component, meaning that the pH shift during temperature change may be caused by the pk shift of body buffers, or by an active component meaning that changing rates of proton equivalent ion transfer are responsible for the pH shift. The number of studies addressing these relationships is scarce. In the crayfish *Austropotamobius pallipes* passive contributions to the temperature induced pH shift in the haemolymph were determined under closed system conditions by warming haemolymph samples anaerobically. As a result of passive changes in physicochemical conditions the $\Delta pH/\Delta T$ value followed constant relative alkalinity (Whiteley et al., 1995) indicating that the contribution of active mechanisms to the extracellular alphastat control was minor. Nonetheless, shifting set points of pH regulation are required to maintain pH at those values defined by alphastat regulation. In crustaceans, as in other water breathers, shift and maintenance of steady state acid–base parameters is not performed by altering gill ventilation but, due to low oxygen solubility in water, ventilation control is dominated by the need to obtain oxygen and not to adjust carbon dioxide tension as observed in air breathers (for review see Taylor et al., 1991). For the maintenance of acid–base homeostasis water-breathers must therefore actively move significant amounts of ions (Na^+ in exchange for H^+ and Cl^- in exchange for HCO_3^-) through the gill epithelium ion transport mechanisms are not only relevant to extracellular acid–base regulation but also play an important role in intracellular acid–base homeostasis during temperature changes (Malan, 1985). Considering the high concentrations of Na^+ and Cl^- in marine crustaceans incubated in full strength sea water (≈ 450 mmol·l^{-1}) no changes in the levels of these major ions are likely to be detected due to temperature dependent pH regulation. However, since ambient temperature differentially affects various ion transport mechanisms, changes in body temperature may disturb cellular ion homeostasis (Cossins et al., 1995). If ion regulation in stenothermic/-haline animals is impaired by changing temperature, this might influence acid–base regulation and may thus prevent maintenance of constant extra- and/or intracellular imidazole dissociation during temperature stress.

The contribution of active and passive mechanisms to the intracellular pHi/T shift has not been measured to date. Deviation from intracellular alphastat control was observed in many species and tissues (Walsh and Moon, 1982; Whiteley and Taylor, 1993; Whiteley et al., 1995). The question of whether these deviations are due to passive or active mechanisms remained also unanswered. A previously developed homogenate

technique (Pörtner et al., 1990) allows the rapid measurement of pHi in tissue samples collected from animals exposed to various temperatures. Since the influence of cell metabolism is excluded in the measurement procedure, pH can also be determined in tissue samples collected at one temperature but analyzed at various temperatures. Since in this closed system no exchange of ions or CO_2 occurred, slight changes in P_{CO_2} with changing temperature could be expected. In a companion study, this effect has been demonstrated to contribute by only 10% to the observed passive temperature response in the white muscle of the marine teleost *Zoarces viviparus* (Van Dijk et al., 1996).

Populations of a species often show differences in physiological responses to temperature. The goal of this study was the comparative investigation of two eurythermal populations of *Crangon crangon* living under different temperature regimes with a stenothermal species (*Pandalus borealis*) adapted to low environmental temperatures. Therefore we measured the extracellular ionic composition and the pHi in the abdominal muscle during temperature change to investigate whether temperature-induced impairment of ion regulation influences intracellular alphastat regulation. In addition we investigated the passive component of the pHi change with temperature in order to decide to what extent the adjustment of constant alpha imidazole is passive and depends on the season or species.

2. Material and methods

2.1. Experimental animals

Sand shrimps *Crangon crangon* were collected in February (Winter animals) and July (Summer animals) 1994 in shallow water (1.5–6 m) of the Wadden Sea of Lower Saxony near Neuharlingersiel. In the laboratory the shrimps were kept for several weeks in large plastic aquaria with a 1–2 cm layer of sand in natural sea water ($S=32‰$) at a temperature of $T=4\pm1°C$ (W.) or at $12\pm1°C$ (S.).

C. crangon from the White Sea was sampled with a hand-dredge in shallow waters (0.3–1.5 m) near the "White-Sea Biological Station of the Russian Academy of Science" (66°21′N 33°40′E) in Karelia (Russia). The animals were held in aquaria containing sand from the site of collection in natural sea water (30‰ salinity) at $15\pm1°C$.

The shrimps (North Sea and White Sea) were allowed to acclimate for at least one week prior to incubation. The water was replaced every second day and the animals were fed twice a week with small pieces of *Arenicola* body wall and fish. Incubations at different temperatures were carried out in aerated aquaria with filtered sea water at -1.5, 4, 15 and 20°C (White-Sea animals) or at 0, 5, 10, 15, 20 and 25°C (North Sea shrimps) for different time periods. We only used adult shrimps between 50 and 70 mm, reflecting the size class of animals caught by commercial fishing. Animals of this size have fresh weights between 2.5 and 4.5 g.

The deep-water shrimp *Pandalus borealis* was obtained from local fishermen trawling near Kongsfjord on Svalbard (79°N 11°E). The shrimps were kept in a flow-through system with natural sea water in the Laboratories of the Norsk Polar Institute in Ny Ålesund (Svalbard)(4±1.5°C, 28 to 30‰.) The measurement of haemolymph ions and intracellular parameters during temperature incubations did not reveal any effect of these minor fluctuations in water temperature and salinity.

All animals were fed pieces of fish on a regular basis and were allowed to acclimate for at least 3 days prior to experimentation. The temperature incubations were performed in large aerated plastic aquaria for 4 h at −1.5, 8, 11 and 14°C.

At the end of the incubation period the animals were carefully captured to avoid escape swimming and dried by use of a paper tissue. Animals which showed vigorous tail-flipping were rejected. Blood samples for the determination of extracellular ion concentrations were obtained with a Hamilton syringe inserted into the pericardium. After blood sampling, the tail of the animal was cut off, the muscle separated from the exoskeleton, divided into two parts, freeze clamped and stored in liquid nitrogen.

2.2. Analyses

Ion concentrations in the haemolymph were determined by ion chromatography (DIONEX BioLC 4500i) at 28°C using a conductivity cell and the DIONEX suppressor technology to reduce background conductivity. Anions were separated on an AS-4A column using an eluent containing Na_2CO_3 (1.8 mmol·l^{-1}) and $NaHCO_3$ (1.7 mmol·l^{-1}) at 2 ml·min^{-1}. H_2SO_4 (12.5 mmol·l^{-1}) was used as a regenerent for the AMMS-1 supressor. Mono- and divalent cations were measured using the CS-3 column and the CMMS-1 supressor. Separation of Na^+ and K^+ was performed using HCl (30 mmol·l^{-1}) as an eluent at 1.7 ml·min^{-1} and 100 mmol·l^{-1} KOH as a regenerent. For the determination of Ca^{2+} and Mg^{2+} we used HCl (30 mmol·l^{-1}) with DAPHCI (6 mmol·l^{-1}) at 1.7 ml·min^{-1} as an eluent and TBAOH (100 mmol·l^{-1}) as a regenerent.

Intracellular pH was measured in abdominal muscle tissues following the homogenate technique described by Pörtner et al. (1990). In vitro experiments were performed by measuring the tissue extract at various temperatures, which allows us to distinguish between passive and active processes (see Section 1).

The homogenate technique is the only method which allows such a differentiation between active and passive components in intracellular pH regulation. The medium used to inhibit metabolism contained 160 mmol·l^{-1} of potassium fluoride and 1 mmol·l^{-1} nitrilotriacetic acid; pH 6.9.

Lactate concentrations were determined enzymatically after Noll (1974) in neutralized perchloric acid extracts (PCA) of abdominal muscle tissue (Beis and Newsholme, 1975).

First order regressions were calculated for the changes in pHi with temperature (Figs. 1–3)(Sigmaplot, Jandel Scientific). Temperature induced changes in lactate (Fig. 5) and ion concentrations (Figs. 6 and 7) were tested for significance at the 5% level by use of one-way analysis of variance followed by the Bonferroni/Dunn post hoc test (Super-Anova, Abacus Concept Inc. Berkeley).

3. Results

3.1. Intracellular pH

Fig. 1 shows the temperature dependence of abdominal muscle pHi after 4 h of incubation at various temperatures in *Pandalus borealis* and in both populations of *Crangon crangon* as well as the pHi values obtained after 6 days in North Sea *C. crangon*. It is clearly demonstrated that pHi increased in a linear fashion with decreasing temperature. After 4 h of incubation this slope (−0.008) was distinctly lower in *C. crangon* from the North Sea compared to that predicted by the alphastat hypothesis (≈ −0.018), while in *P. borealis* (ΔpHi/°C = −0.016) and in White Sea *C. crangon* (ΔpHi/°C = −0.018) the pHi/*T* slope was in good agreement with alphastat. To test the hypothesis that part of this pHi change was caused by active regulation and thus ought to be time dependent, the pHi/*T* slope was measured for North Sea *C. crangon* again after

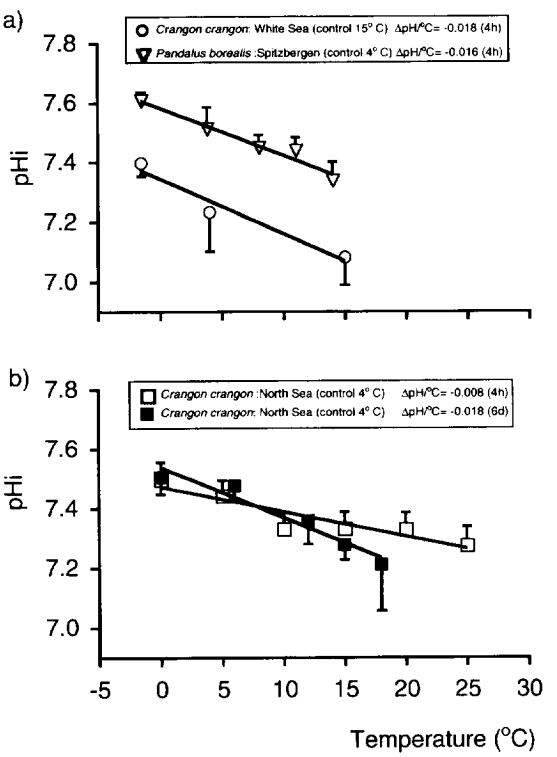

Fig. 1. Temperature dependence of intracellular pH in abdominal muscle in boreal and Arctic populations of *C. crangon* and *P. borealis*. Intracellular pH was analyzed after 4 h (*P. borealis* and *C. crangon* from the White Sea) (a) and after 4 h and 6 days (North Sea *C. crangon*) (b) of incubation at various temperatures. Note the increase in slope depending on the length of incubation in *C. crangon*. All values are means±SD of at least 5 measurements.

6 days of exposure to the same temperatures. The slope changed from −0.008 after 4 h to −0.017 after 6 days of incubation, a ratio which is similar to the one obtained from *P. borealis* and White Sea *C. crangon* already after 4 h.

To determine the contribution of passive mechanisms to the pH change we analyzed tissues obtained from control animals at various temperatures. The results are shown in Fig. 2. The slope of the passive pH/temperature relationship (determined in vitro) was −0.009 in North Sea *C. crangon* and thus the same as the value seen after 4 hours of exposure in vivo indicating that no temperature related contribution of active pH regulation to alphastat occurs during this time period. In *C. crangon* from the White Sea passive contribution to alphastat was similarly low with a ΔpHi/°C of about −0.008, while in the cold stenothermal *P. borealis* the passive component predominates with a contribution of about 85% to the pH change in vivo (% value calculated as the % fraction of the in vitro pH change in the pH change determined in vivo). The achievement of constant $_{alpha}$Im is a result of both active and passive mechanisms (each contributing by about 50% in the eurythermic *C. crangon*) and dependent upon incubation time. To answer the question whether the passive changes in pHi also depend upon the season and, thus, long term acclimation to temperature, we measured the pHi/temperature relationship in animals from the North Sea caught in summer. From Fig. 3 it is obvious that buffer characteristics are independent of the season since in vitro slopes are similar in tissues from summer and winter animals. In addition to the studies of North Sea *C. crangon* we investigated the time dependence of pHi manifestation in animals from the White Sea (Fig. 4) at 4°C where the final value is reached after 12 h and remained constant thereafter. If we consider this final value in calculating $\Delta pH/\Delta T$ the slope rises from −0.018 after 4 h to −0.020 after 12 h.

In winter animals of North Sea *C. crangon* lactate levels were determined to decide whether critical temperatures characterised by an onset of anaerobic metabolism (Zielinski and Pörtner, 1996; Sommer et al., 1996) interfere with alphastat and ion regulation. Compared to control values lactate concentrations were significantly increased after 4 h at 0°C but not after 6 days, and were also elevated after prolonged

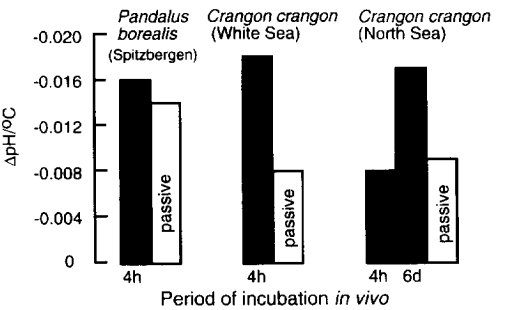

Fig. 2. Temperature dependence ($\Delta pHi/\Delta T$) of abdominal muscle intracellular pH in boreal and Arctic populations of *C. crangon* and *P. borealis*, including the contribution of passive mechanisms. Intracellular pH was analyzed after 4 h (*P. borealis* and *C. crangon* from the White Sea) and after 4 h and 6 days (North Sea *C. crangon*) of incubation at various temperatures. Note the delayed contribution of active mechanisms to pH regulation in North Sea compared to White Sea animals.

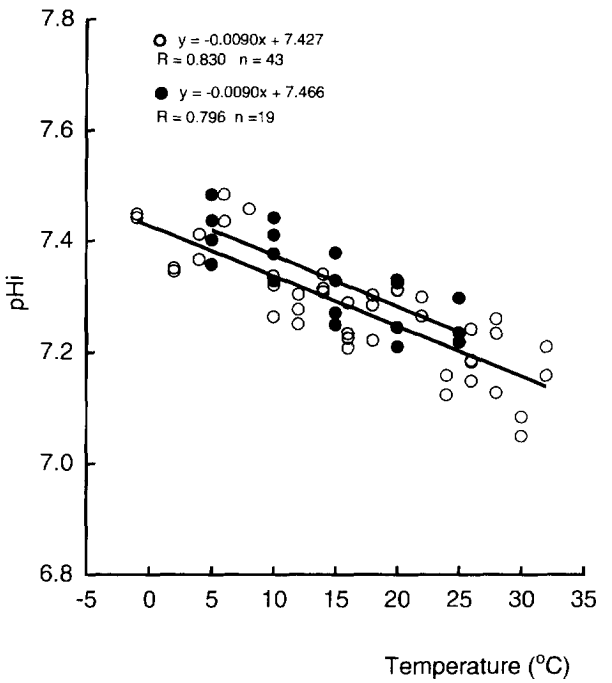

Fig. 3. Temperature dependence of intracellular pH measured in tissue extracts (in vitro closed system) prepared from tail muscle of North Sea *C. crangon*. (○) tissues from animals adapted to 12°C (Summer animals); (●) tissues from animals adapted to 4°C (Winter animals). The passive change in pHi with temperature results as −0.009 pH units/°C.

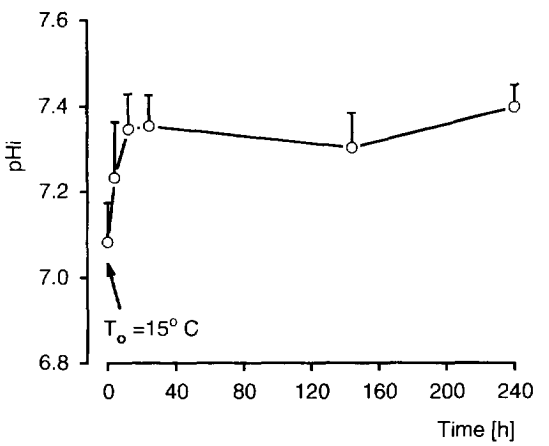

Fig. 4. The time dependent adjustment of pHi in White Sea *C. crangon*. The pH at $t=0$ is obtained from control animals adapted to 15°C. Open circles represent subsequent incubations at 4°C.

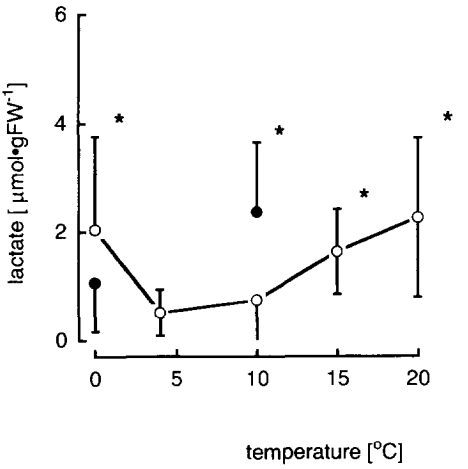

Fig. 5. Lactate concentrations in the muscle tissue at different temperatures and after periods of 4 h (○) and 6 days (●). Each value represents a mean of at least 5 animals±SD. (*) indicate significant differences ($P<0.05$) compared to control levels at 4°C.

incubation at 10°C and after 4 h at 15° and 20°C (Fig. 5), where prolonged incubation resulted in increasing mortality. Lactate production elicited by temperature stress was small enough to cause neither a deviation from linearity of the pH/T slope nor an impairment of ion regulation.

3.2. Ion regulation

The concentrations of inorganic anions and cations were measured in North Sea shrimps (winter animals), in White Sea shrimps and in the deep water shrimp *P. borealis* acclimated to various temperatures for different time periods. In shrimps from the North Sea a decrease in temperature had only a minor influence on haemolymph ion concentrations (Table 1). Four hours of incubation at 0°C resulted in significantly

Table 1
Concentrations of major ions in the haemolymph of North Sea *Crangon* in relation to incubation temperature and the length of the incubation period

	Inorganic ion concentration [mmol·l^{-1}]				
	Na^+	Cl^-	Mg^{2+}	Ca^{2+}	SO_4^{2-}
Control 4°C	509.3±65.1	551.6±76.2	9.97±6.66	14.05±3.65	2.89±1.51
4 h 0°C	524.3±13.2	469.2±81.3	16.40±14.63	18.57±2.37	6.68±6.75
6 days 0°C	563.9±41.0	513.9±40.0	8.40±6.95	11.51±4.87	3.84±2.75
4 h 10°C	489.6±28.7	473.9±48.0	18.65±17.25	18.29±2.92*	4.23±2.85
6 days 10°C	435.8±21.5*	409.0±19.9	5.55±1.55	11.59±3.10	3.20±0.96
4 h 15°C	509.3±29.5	486.1±35.0	21.62±8.19*	16.10±2.51	6.33±1.15*
4 h 20°C	481.8±34.1	484.2±48.1	8.75±5.60	13.26±2.57	2.90±0.96

All values are means±SD of at least 5 measurements.

increased Ca^{2+}-concentrations but haemolymph calcium returned to control levels after 6 days of exposure. Cl^--levels decreased significantly after 4 h and 6 days of incubation at 10°C. The changes were comparable to the alterations in Na^+, although Na^+-fluctuations were not significant after 4 h. Warmer temperatures resulted in an increase in $[Ca^{2+}]$ after 4 h at 10°C while SO_4^{2-}-concentrations only increased after 4 h at 15°C. The haemolymph levels of magnesium fluctuated and showed unexpectedly high interindividual variability. The implications of this response to temperature changes are discussed elsewhere (Sartoris and Pörtner, 1995, 1996).

In shrimps from the White Sea a time-dependent response to alterations in temperature was tested (Fig. 6). Na^+-concentrations decreased significantly after 24 h and 6 days of incubation at 20°C while 6 days of incubation at 4°C resulted in an increase in haemolymph sodium. Changes in Cl^--levels were slightly different since chloride concentrations increased significantly after 6 days at 20°C. Similar to alterations in haemolymph sodium, chloride values increased at 4°C although changes were already

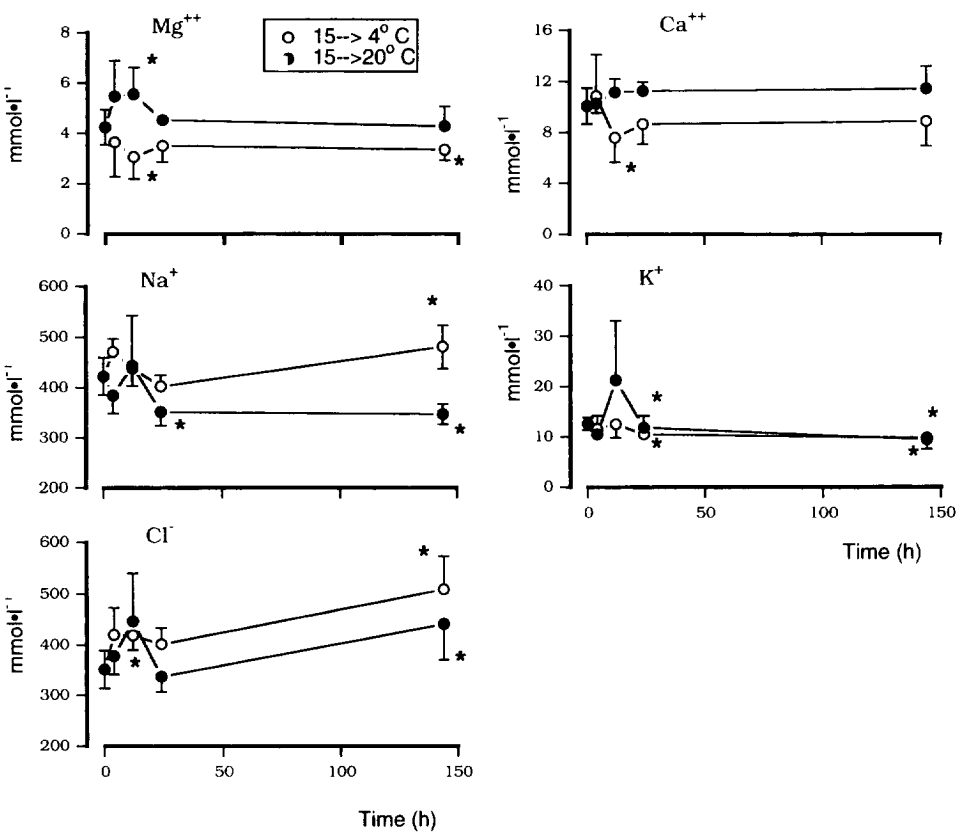

Fig. 6. Concentrations of some inorganic ions in the haemolymph of White Sea *C. crangon* in relation to the duration of temperature incubations at 4° (open symbols) and at 20°C (closed symbols). Ion concentrations at $t=0$ were obtained from animals adapted to 15°C. All values are means±SD of at least 5 measurements.

significant after 12 h. Haemolymph magnesium decreased significantly at 4°C where values were significantly lower after 12 h and 6 days of exposure. Incubation at 20°C increased magnesium significantly within 12 h followed by a return to control levels thereafter. Significant changes in Ca^{2+} were only seen after 12 h of exposure at 4°C. Haemolymph K^+ decreased at all temperatures tested resulting in significantly lower values after 36 h and 6 days at 4 and 8°C and after 4 h and 6 days at 20°C.

P. borealis showed no significant changes in haemolymph sodium or chloride during 4 h of temperature incubations, although Cl^- levels were higher than Na^+ levels at each temperature tested which is a rather unusual situation for crustaceans. Bivalent cation concentrations decreased with increasing temperature and were significantly lower at 8, 11 and 14°C (Ca^{2+}) and at 14°C (Mg^{2+}). Haemolymph K^+ levels remained constant after 4 h of incubation at increased temperatures but decreased significantly at −1.5°C (Fig. 7)

4. Discussion

For the analysis of temperature dependent changes in intracellular acid–base status the homogenate technique (Pörtner et al., 1990) has the major advantage that the contribution of active and passive mechanisms to changes in pHi can easily be quantified. Other methods do not permit such a clear distinction. Passive pHi adjustment does not depend upon the season, since no difference was detected in the passive $\Delta pHi/\Delta T$ relationship between summer and winter animals of North Sea *Crangon crangon* ($\Delta pHi/\Delta T = -0.009$ units·°C^{-1}), suggesting that no differences exist in the concentration and temperature characteristics of relevant intracellular buffer substances. However, such differences exist between the eurythermal *C. crangon* (pH adjustment by about 50% passive) and the stenothermal *Pandalus borealis*, where passive contribution to the pH change was about 85%. The major conclusion we have to draw from these results is that both passive and active mechanisms contribute to alphastat, while the fraction of (active) ion exchange mechanisms, many of which are energy dependent, is reduced in animals which are never exposed to temperature changes exceeding 3–5°C. The passive slopes of $\Delta pHi/\Delta T$ in *C. crangon* were similar to those observed in North Sea *C. crangon* after 4 h of incubation at various temperatures ($\Delta pHi/\Delta T = -0.008$ units·°C^{-1}), indicating the absence of active mechanisms contributing to pH regulation during this period. In contrast, 4 h of incubation were sufficient to reach $\Delta pHi/\Delta T$ slopes close to those predicted by the alphastat theory in *P. borealis* (−0.016 pH units·°C^{-1}) and in *C. crangon* from the White Sea (−0.018 units·°C^{-1}), where final values were only slightly higher and reached after 12 h with a $\Delta pHi/\Delta T$-ratio of −0.020 units·°C^{-1} (Fig. 4). The comparison of the two *C. crangon* populations suggests that the White Sea population was faster to adjust pHi to new steady state values after a temperature change. As a corollary, animals living at lower temperatures may, in general, be able to compensate acid–base disturbances faster since cold adaptation could increase the capacity of pH regulatory mechanisms.

The increase in $\Delta pHi/\Delta T$ after 6 days of incubation in North Sea *C. crangon* (−0.018 units·°C^{-1}) emphasizes that the maintenance of alphastat is time dependent. Truchot

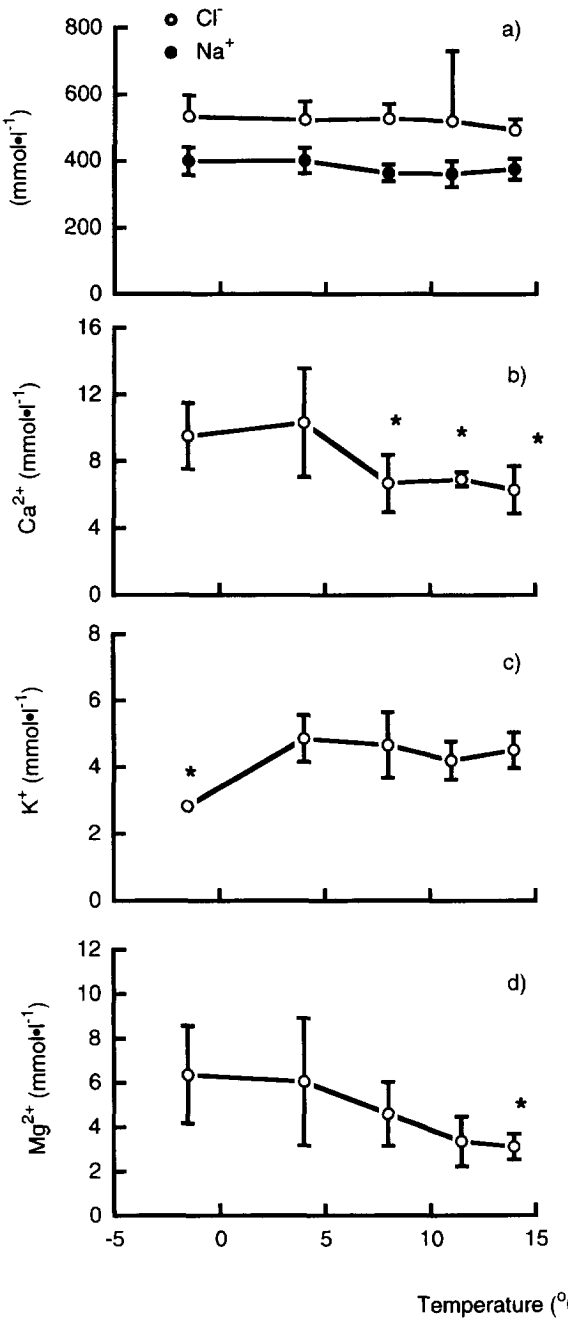

Fig. 7. Concentrations of some inorganic ions in the haemolymph of the deep water Arctic prawn *P. borealis* in relation to the incubation temperature. The incubation period was 4 h, temperature of controls was 4°C. All values are means±SD of at least 5 measurements.

(1978) found an immediate response in *Carcinus maenas* haemolymph pH and a slower phase lasting many hours. The time dependence of temperature related pHi regulation could be confirmed by a more complete time series in White sea *C. crangon* (Fig. 4), where final values were reached after approximately 12 h. The time dependence of alphastat regulation implies that during the tidal cycle, where in shallow coastal waters temperature may change drastically, alphastat regulation is not fast enough to keep alpha imidazole constant. The maintenance of alphastat may therefore be more beneficial and thus more important during seasonal changes in ambient temperature than during short term temperature fluctuations.

The values of pHi measured at 12°C in North Sea *C. crangon* correspond to the results reported by Thebault and Raffin (1991) for the tail muscle of *Palaemon elegans* in the summer (pHi = 7.3). In winter, *P. elegans* tends to be inactive at temperatures below 10°C, metabolic depression being reflected by a drop in pH and an increase in the levels of sugar phosphates. Acidic pHi values were also reported by Whiteley et al. (1995) for inactive winter tissues in crayfish. They explained this relative acidosis with low rates of protein synthesis and a lowered overall metabolism at low temperatures when crayfish are inactive. In order to save energy, animals may tolerate passive changes of pHi during periods of relative inactivity, since the active transport of ion equivalents depends on energy supply. A downregulation of ion exchange could lead to a reduction of pHi, since pH would then approach thermodynamic equilibrium according to the redistribution of H^+ following the membrane potential. This comparison emphasizes that the reduced velocity of alphastat regulation seen in North Sea *C. crangon* may help to reduce the energy requirements of acid–base regulation.

In Winter animals of North Sea *C. crangon* no such reduction in activity could be observed. Assuming that a deviation from alphastat at low temperatures is a consequence of metabolic depression induced (or followed) by a pHi decrease, the maintenance of a normal activity pattern should correlate with pHi values according to alphastat as observed in *C. crangon*. Whiteley et al. (1995) determined $\Delta pHi/\Delta T$ values in hepatopancreas ($\Delta pHi/\Delta T = -0.006$ units·°C^{-1}) and claw muscle ($\Delta pHi/\Delta T = -0.003$ units·°C^{-1}) even lower than the passive component we demonstrated for *C. crangon*. During the actual temperature change these lower slopes may reflect a lower concentration of tissue buffers or the contribution of buffers with low $\Delta pHi/\Delta T$ values like phosphate ($\Delta pHi/\Delta T = -0.003$°C^{-1}) and bicarbonate ($\Delta pHi/\Delta T = -0.006$°C^{-1}). Longer term maintenance of these slopes may be due to the downregulation of ion exchange. The relatively acidic pH resulting at low temperatures in tissues, which do not regulate pH according to alphastat, may contribute to depress the activity of metabolic enzymes. In crayfish no lactate formation was observed during incubation at low temperature such that a contribution of anaerobic metabolism to the relative acidosis could be excluded (Whiteley et al., 1995). Our study and the study by Whiteley et al. (1995) would suggest that alphastat regulation is important in metabolically active tissues with active on top of passive mechanisms of pH control. This requires changing the set points of ion exchange mechanisms. These conclusions are also in line with the observation that cold water animals are faster in achieving alphastat control since downregulation by low temperature does not occur in these species.

If a change in the set points of acid–base regulation occurs, this is not reflected by

changes in the steady state levels of haemolymph ions. In general, short term incubations (4 h) at various temperatures had no major influence on ion concentrations either in *C. crangon* (both populations, (Fig. 6; Table 1) or in *P. borelis* (Fig. 7). Presumably, 4 h are not sufficient to impair ion regulation when temperature is the only stressor. In both populations of *C. crangon* increased fluctuations in haemolymph ions were observed, rather than a general trend in terms of a temperature induced disturbance of ion homeostasis, while in *P. borealis* only potassium showed significant alterations due to temperature change. The reduction in haemolymph potassium seen during the temperature decrease from 5°C to −1.5°C in *P. borealis* may be a result of fast temperature compensation to avoid a reduction in the ionic gradient for Na^+ and K^+. Two compensatory mechanisms are discussed in the literature, one involving enhanced pumping capacity, another one involving reduced passive transport by altering membrane viscosity (Cossins and Bowler, 1987; Cossins et al., 1989). Neither can explain the over-compensation seen at −1.5°C. However, no impact of temperature induced disturbance of ion regulation on active alphastat regulation could be observed in *P. borealis*.

In North Sea *C. crangon*, haemolymph [Cl^-] was always higher than [Na^+] at every temperature tested and even in control animals. So far, we have no explanation for the elevated extracellular Cl^--levels, since in crustaceans Cl^- concentrations are usually lower than [Na^+] due to the presence of bicarbonate and organic anions in the haemolymph. After 6 days of incubation at 10°C starting from a control value of 4°C, sodium and chloride decreased significantly while pHi decreased from 7.39±0.06 to 7.28±0.05. One of the processes involved in pHi regulation in crustaceans is the transfer of ions across the cell membrane, e.g. by one type of Na^+/H^+ exchanger in crayfish neurone (Moody, 1981) and muscle (Galler and Moser, 1986), or 2 Na^+/H^+ exchange mechanisms in hepatopancreas (Ahearn and Clay, 1989) and gill (Shetlar and Towle, 1989). One might speculate that a decrease in haemolymph sodium at high temperatures could reduce Na^+/H^+ exchange across the cell membrane and lead to an acidification of the cytoplasm. Nevertheless, this could not explain the simultaneous decrease in chloride which by itself might be due to extracellular osmoregulatory requirements.

The low haemolymph magnesium concentration observed in both shrimp species is typical for active crustaceans since high Mg^{2+} levels depress neuromuscular transmission in such a way that Mg^{2+} competes with Ca^{2+} for binding sites (Robertson, 1953). During exposure to low temperature of the sandhopper *Talitrus saltator* Spicer et al. (1994) observed the onset of inactivity, a marked increase in the concentration of magnesium and, although less pronounced, a rise in the concentrations of the other major ions in the haemolymph. Similar alterations in haemolymph ion concentrations or in the activity level at low temperatures could not be observed in either *P. borealis* or *C. crangon*.

5. Summary

In the abdominal muscle of shrimp the $\Delta pHi/\Delta T$ ratio followed the values predicted by the alphastat theory in *P. borealis* and *C. crangon* and was time-dependent in both

populations of *C. crangon*. Animals acclimated to lower seasonal temperatures like *P. borealis* and White Sea *C. crangon* exhibit a faster active contribution to alphastat regulation than the boreal species. At least in North Sea *C. crangon*, the time needed to achieve constant alpha imidazole exceeded the time period of temperature fluctuations during the rapid tidal cycle. Thus the benefit of alphastat control in the daily life of *C. crangon* is uncertain. Both active and passive mechanisms contribute to alphastat by about 50% in *C. crangon*, whereas the active component amounts to only 15% in *P. borealis*. The passive component of $\Delta pHi/\Delta T$ adjustment was quantified and proved to be independent of seasonal variations. Our findings suggest that a larger active than passive component of alphastat regulation may be a prerequisite to colonize shallow coastal waters, thereby increasing adaptational flexibility during temperature fluctuations. Concentrations of major inorganic haemolymph ions showed few changes upon temperature acclimation without substantial influence on acid–base balance.

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