

A Contribution of Acid-base Regulation to Metabolic Depression in Marine Ectotherms

HANS OTTO PÖRTNER¹ AND CHRISTIAN BOCK¹

Abstract. Adaptation to permanent cold in marine ectotherms has been discussed to be associated with mitochondrial proliferation as well as a more or less non-compensated decrease in metabolic rate. This process must involve the respective tradeoffs in energy consuming processes. Only recently it was demonstrated that acid-base regulation is associated with a considerable cost. Metabolic depression is accompanied by a decrease in the rate of cellular net proton excretion leading to a decrease in the setpoint of intracellular pH regulation. Also a shift is observed to more economic mechanisms of proton equivalent ion exchange. In this chapter the physiological relevance of acid-base parameters in various thermal environments is reevaluated based on these findings. During temperature change a wide range of tissues in ectothermal species displays pH changes in accordance with an alphastat mode of acid-base regulation. Exceptions from this pattern described in the literature are discussed in the light of cold induced mitochondrial proliferation, of metabolic depression during (seasonal) cold and of differences between the various methods of pH_i analysis applied. In animals adapted to various temperature regimes it is possible to distinguish between changes in pH_i elicited by physico-chemical buffering and those caused by a readjustment of the setpoints of proton equivalent ion exchange. The thermal flexibility and higher metabolic rate of eurythermal animals is reflected in the predominant use of more costly active mechanisms of pH_i regulation during temperature induced pH_i changes, whereas acid-base regulation appears to be less flexible and less costly in cold stenotherms and displays largely passive temperature dependent pH_i changes.

Temperature and the physiological relevance of acid-base regulation

Temperature is considered to be one of the most important abiotic factors due to its major impact on all biological processes. In marine ecosystems, low or high temperature extremes often define the limits of geographical distribution of many species, and global change has already caused a change in the distribution of species (Southward et al. 1995). From an evolutionary point of view, life evolved in the warm and adaptation to permanent cold started from life forms that evolved in warm waters (e.g. Arntz et al. 1994, Thiel et al. 1996). Adaptation to cold temperatures then becomes a special physiological feature rather than a basic ability of all life forms. Survival in either seasonally or permanently cold (or warm) ocean environments has required evolutionary adaptations which either

¹ Ökophysiologie und Ökotoxikologie, Alfred-Wegener-Institut für Polar- und Meeresforschung, D-27515 Bremerhaven, F.R.G.

permit short term shifts between different temperature regimes or adjustment of all physiological processes to a narrow range of either low (polar) or high (tropical) temperatures.

This chapter investigates the role and specific feature of acid-base regulation in the cold and especially, to what extent the modulation of acid-base regulation may contribute to metabolic depression. To this end, the physiological relevance of acid-base parameters, especially pH will be briefly reviewed, and the question will be investigated whether there is an energy demand associated with acid-base regulation.

pH maintenance and control of pH within a narrow window appears relevant for functional maintenance and the turnover rates of many cellular and physiological processes. Changes in pH_i alter the ionization states of certain key low molecular weight metabolites with pK 's in the physiological range. pH affects the dissociation equilibria of substrates or products of enzymatic reactions, and if several species of substrate exist in the range of physiological pH, which are just different in the level of protonation or dissociation, enzymes may react with or produce only one of those and will thereby exhibit specific, but apparent pH optima with respect to the total concentration of substrate. As a corollary, alterations in pH_i may change the relative proportions of reactants and, hence, may influence fluxes through key cellular reactions (see Pörtner and Ellington 2000, for a recent review). Furthermore, protons are direct participants in key reactions of cellular energy metabolism (ATPases, guanidino kinases) as well as the major component of proton motive force driving mitochondrial ATP synthesis.

The Gibb's free energy change of ATP hydrolysis $dG/d\xi_{ATP}$ quantifies the actual energy status of the cell *in vivo* (Kammermeyer 1987). Control values are usually well above critical $dG/d\xi_{ATP}$ values at which functional deficits should occur (Kammermeyer et al. 1982, Kammermeyer 1987, 1993). At constant adenine nucleotide and total Mg^{2+} concentrations, a reduction in pH of 0.5 pH units in the physiological range results in a modest 2 to 3 kJ mol^{-1} reduction in ATP free energy (Pörtner 1993). Some comparative data are available for muscle tissue of ectotherms *in vivo* and *in vitro*. The comparison of data evaluated for aerobic control conditions reveals that resting values of the Gibb's free energy change fluctuate with aerobic metabolic rate, values ranging from -58 kJ mol^{-1} in resting (ventilating) squid mantle to -66 kJ mol^{-1} in isolated (non-active) body wall musculature of a marine worm (see Pörtner and Ellington 2000 for review). The aerobic metabolic rate and the energy status of a tissue appear to be inversely coupled with large factorial changes in the level of free ADP, which reaches high values of $0.1 \mu\text{mol g}^{-1}$ wet wt. in squid mantle under control conditions (Pörtner et al. 1996). pH_i is only one of several factors contributing to the setting of ATP free energy change levels.

Reeves (1972, 1985) introduced the imidazole α -stat (=alphastat) hypothesis stating that poikilotherms regulate pH such that the degree of protonation (α) of imidazole groups is maintained despite changes in body temperature. The importance of this concept is emphasized by the observation that the average pK of histidyl residues exposed to the solvent falls in the vicinity of pH_i values of

typical cells (Somero 1986). Thus, relatively small changes in pH_i could produce significant changes in protein ionization. Alaphastat pH regulation is also beneficial for energy status. A pH rise with falling temperature ensures that the ATP free energy is maintained at a high level (Pörtner *et al.* 1998 a). Cameron (1989) proposed a "Z-stat" model which emphasizes that protein net charge Z is maintained rather than α in diverse histidine groups. This treatment may be more adequate since $\Delta pK \cdot C^{-1}$ depends upon local charge configurations in the environment of the imidazole group as well as on ionic strength and, therefore, varies between -0.016 and $-0.024 \text{ } ^\circ C^{-1}$ for histidine and free imidazole compounds and range between -0.0010 and $-0.051 \text{ } ^\circ C^{-1}$ for histidine residues in proteins (Heisler 1986). $\Delta pK \text{ } ^\circ C^{-1}$ of histidine residues will also depend on a temperature induced change in the protonation of neighbouring groups. However, the picture is not clear enough to decide whether maintenance of Z-stat or α -stat of special histidine groups is the more appropriate view. For the time being these concepts represent two sides of the same coin since Z-stat is a consequence of the maintenance of mean α for any protein mixture.

The alaphastat concept implies that changes in pH_i fully or partly offset temperature-induced changes in protein ionization thereby maintaining relative constancy of protein structure and function (Hochachka and Somero 1984). Since the pK of imidazole groups may (on average) change at -0.018 units $\cdot C^{-1}$, a shift in intra- and extracellular pH with body temperature by $\Delta pH/\Delta T \sim -0.018 \cdot C^{-1}$ is seen to keep α constant. In a comprehensive paper Ultsch and Jackson (1996) recently reviewed the literature on the relationship between intracellular pH (pH_i) and temperature in ectothermic vertebrates concluding that the data in general support the concept of alaphastat regulation. With some exemptions, the alaphastat pattern of intracellular pH regulation could also be confirmed for marine ectotherms (invertebrates and fish) exposed to various temperatures both depending on the season or in a latitudinal cline (review by Pörtner *et al.* 1998a). However, a rather large variability found in the data requires explanation. Values range from $-0.003 \cdot C^{-1}$ in crayfish cheliped muscle (Whiteley *et al.* 1995) to $-0.033 \cdot C^{-1}$ in fish red muscle (Cameron 1984).

On the one hand, use of different methods of pH analysis may have contributed to some of this variability. The various methods yield different mean values of pH_i . The homogenate technique provides an estimate of mean intracellular pH emphasizing cytosolic pH in accordance with the distribution of buffers between various compartments (Pörtner *et al.* 1990; Pörtner and Sartoris 1999). A low non-bicarbonate buffer value appears typical for mitochondria, a conclusion supported by the observation that the mitochondrial influence on mean pH_i derived from homogenate analyses is small (Pörtner and Sartoris 1999).

The DMO technique not only provides a volume weighted mean pH_i but also largely weighs alkaline compartments, i.e. mitochondria and, therefore, yields a high estimate of mean pH_i . Interestingly, mitochondrial proliferation occurs in the cold, further increasing the discrepancy between, e.g. DMO and homogenate techniques. The localization of pH values measured by ^{31}P -MRS is not yet satisfactorily explained, but mostly assumed to reflect cytosolic pH (Gadian *et al.* 1982).

On the other hand, temperature induced changes in metabolic regulation may be related to deviations from the alphastat pattern. Animals acclimatised to low temperatures during the winter season frequently exhibit relatively low pH values. At temperatures below 10° C in winter, metabolic depression in the shrimp *Palaemon elegans* is reflected by a drop in intracellular pH (Thebault and Raffin 1991). Low pH_i values were also reported by Whiteley *et al.* (1995a) for the crayfish *Austrapotamobius pallipes* in winter. Only tissues like abdominal muscle, which remained operative, followed α -stat while less active tissues like claw muscle and hepatopancreas did not. Based on these results Whiteley and colleagues speculated that the relative acidosis observed in the haemolymph of the isopod *Glyptonotus antarcticus* and *A. pallipes* may be characteristic of crustaceans living at low temperatures when rates of protein synthesis and possibly catabolism are low (Whiteley *et al.* 1995b).

A downregulation of ion exchange would lead to a passive reduction of pH_i since pH would then approach a lower equilibrium value expected from passive proton distribution according to the membrane potential. In support of these conclusions, a reduction in metabolic activity was not observed in the sand shrimp *C. crangon* originating from the North Sea in winter and, as a consequence, both winter and summer animals followed the α -stat pattern (Sartoris and Pörtner 1997a,b).

Last but not least, a progressive rise in mitochondrial content as it occurs during cold adaptation (cf. Pörtner *et al.* 1998a) may counteract the upregulation of intracellular pH according to the α -stat pattern. There is some circumstantial evidence that cytosolic pH and, as a consequence, mean intracellular pH is lower in tissues containing high fractions of mitochondria (Pörtner and Sartoris 1999). Mitochondrial proliferation or metabolic downregulation in the cold appear as alternative explanations why, in interspecies comparisons, pH changes may not fully follow the alphastat slope (Ultsch and Jackson 1996) or why the alphastat pattern is not evident in some comparisons of eurytherms and cold stenotherms (e.g. Moerland and Egginton 1998, Bock *et al.* 2000).

Nonetheless, polar species could act as model organisms to demonstrate α -stat regulation in animals with a "normal" level of activity at very low temperatures. Measurements in the deep water shrimp *Pandalus borealis* (Spitzbergen), the Antarctic eelpout *Lycodichthys dearborni* or *Pachycara brachycephalum* under control conditions indicate that, in a comparison of species from different latitudes, some cold-stenothermal animals display control values in accordance with an α -stat pattern of intracellular pH (Pörtner *et al.* 1998a). More importantly, it could be demonstrated that pH_i changes monitored by ³¹P-NMR in individual Antarctic eelpout, *Pachycara brachycephalum*, followed the α -stat pattern during warming (Bock *et al.*, 2000). These findings are in agreement with invasive studies temperature dependent pH_i changes in this species (van Dijk *et al.* 1999). However, a uniform picture is again not evident for Antarctic species. For example, control pH_i values in the Antarctic bivalve, *Limopsis marionensis* do not reflect α -stat regulation if compared to other species and do not follow the alphastat pattern during temperature change (Pörtner *et al.* 1998a, 1999).

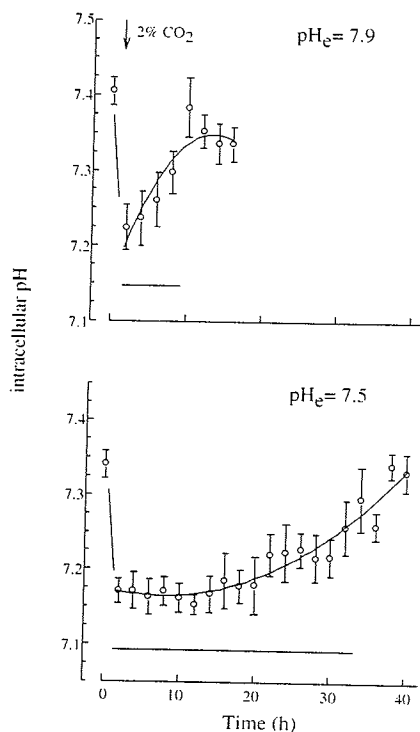


Fig. 1. Time course of pH_i recovery during acute hypercapnia under 2% CO_2 at pH_e 7.9 and 7.5 in isolated body wall musculature of *Sipunculus nudus*, studied by ^{31}P -NMR. Note the delay in the onset of pH recovery and the fourfold longer time course at pH_e 7.5. Horizontal lines indicate onset and maintenance of a significant effect by the previous treatment (modified after Pörtner et al., 2000).

Another important point to be considered in such analyses is that α -stat pH regulation only occurs in the temperature range between low and high critical temperatures beyond which anaerobic metabolism becomes involved in energy production. Temperature dependent pH changes becomes unpredictable beyond these thresholds (Sommer et al. 1997, Zielinski and Pörtner 1996). In polar and possibly also in tropical (stenothermal) species the temperature window for α -stat regulation is expected to be much smaller than in temperate zone (eurythermal) species.

An energy cost of acid-base regulation?

Transmembrane or transepithelial pump and leak processes are differentially affected by temperature changes. This also appears to be the case for ion exchange mechanisms involved in acid-base regulation. While ion transport mechanisms like Na^+/K^+ -ATPase display a Q_{10} of 2 - 4, leak processes are

relatively temperature insensitive with Q_{10} values close to unity (Ellory and Hall 1987, Gibbs 1985, Raynard and Cossins 1991). This differential effect of temperature on ion movements will lead to an excess of dissipative fluxes over active ion pumping during cold exposure unless the organism is able to compensate for these differences.

Acid-base regulation should be an energy dependent process since acid-base equivalents (protons or bicarbonate) are transported by H^+ -ATPases or by secondary active processes, e.g. via the Na^+/H^+ -exchanger, which depends upon the Na -gradient established by Na^+/K^+ -ATPase. Most animal cells maintain a disequilibrium of proton distribution between intracellular and extracellular compartments - pH_i is more alkaline than the equilibrium value calculated from membrane potential and extracellular pH (Roos and Boron 1981, Aickin 1984). Passive proton distribution over the cell membrane is determined by the membrane potential and would lead to a ΔpH of about one pH unit between intra- and extracellular compartment (Thomas 1984). In this context the following key questions arise: Which mechanisms determine the setpoints of acid-base regulation and what are the costs of acid-base regulation? It has recently been demonstrated that certain species are capable of modulating the energy (i.e. ATP) cost of acid-base regulation as a means of adjusting metabolic rate to environmental requirements (Reipschläger and Pörtner 1996; Pörtner et al. 2000a).

In those studies the effect of different ion transport inhibitors on the rate of pH recovery during hypercapnia, on energy turnover and on steady state acid-base parameters was analysed in isolated body wall musculature of the marine worm *Sipunculus nudus* under control conditions and during steady state extracellular acidosis using *in vivo* ^{31}P -NMR and oxygen consumption analyses (Reipschläger and Pörtner 1996, Pörtner et al. 2000). This study identified a role for amiloride sensitive Na^+/H^+ exchange, DIDS sensitive Na^+ -dependent Cl^-/HCO_3^- exchange, and a bafilomycin sensitive H^+ -ATPase in pH_i regulation.

During hypercapnia oxygen consumption fell and pH_i recovery was delayed during extracellular acidosis (Figure 1). Effects of ouabain, DIDS and DMA on metabolic rate were reduced at low pH_e thereby confirming that acidosis caused the ATP demand of Na^+/K^+ -ATPase to fall. This drop likely occurred via an inhibiting effect on Na^+/H^+ - and Na^+ -dependent Cl^-/HCO_3^- (i.e. $Na^+/H^+/Cl^-/HCO_3^-$) exchange. The latter may become predominant during acidosis indicated by its capacity to compensate for the effect of DMA at both low and high pH_e . Na^+ dependent Cl^-/HCO_3^- -exchanger transports 2 acid-base equivalents per Na^+ instead of 1 (in the case of the Na^+/H^+ exchanger), such that the Na^+/K^+ -ATPase, which provides the driving force for both systems, should be used at a lower rate when Na^+ dependent Cl^-/HCO_3^- exchange predominates. Falling rates of H^+ equivalent ion exchange and the potential shift from Na^+/H^+ to $Na^+/H^+/Cl^-/HCO_3^-$ exchange under conditions of extracellular acidosis together with a reduced rate of oxygen consumption (Figure 2) provide evidence that the ATP demand of acid-base regulation comprises a significant fraction of metabolic rate and that this fraction falls during metabolic depression (Pörtner et al. 2000 a).

Metabolic depression occurred even at normal pH_i when extracellular pH was reduced (Pörtner et al. 1998b).

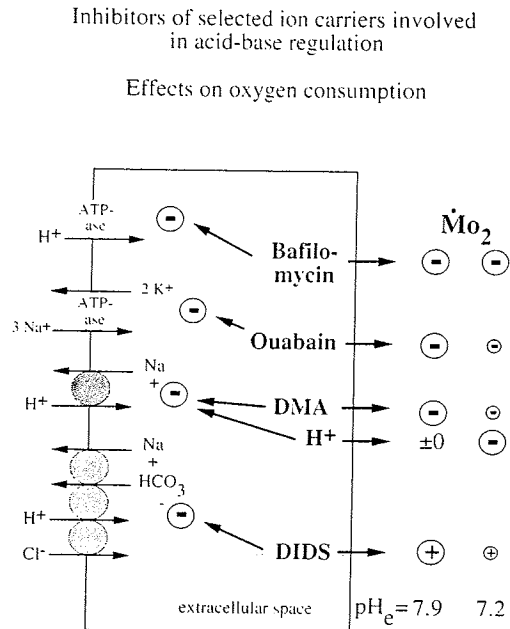


Fig. 2. Mechanisms involved in metabolic depression at low extracellular pH. The model is supported by changes in oxygen consumption elicited by the specific effects of changes in intra- and extracellular acid-base parameters (Reipschläger and Pörtner, 1996) and by the effect of inhibitors (DMA, 5-(*N,N*-dimethyl)-amiloride; DIDS, 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid) on metabolic rate and pH_i recovery at various values of extracellular pH (Reipschläger and Pörtner 1996, Pörtner et al. 2000). In addition to an overall inhibition of the rate of proton equivalent ion exchange by low pH_e (Figure 1) the specific inhibition of the Na^+/H^+ exchanger by a decrease in extracellular pH (pH_e) may lead to an increased contribution of the $Na^+/H^+/Cl^-/HCO_3^-$ (or equivalent) exchanger to acid-base regulation. Since the $Na^+/H^+/Cl^-/HCO_3^-$ -exchanger transports 2 acid-base equivalents per Na^+ (instead of 1) Na^+/K^+ -ATPase turnover rate falls. (The activity of bafilomycin A1 sensitive H^+ transport remains unaffected by pH. Inhibition of the $Na^+/H^+/Cl^-/HCO_3^-$ exchanger by DIDS causes oxygen consumption to rise, very likely owing to an increased contribution of the Na^+/H^+ exchanger.)

Molecular studies of vertebrate systems suggest that intracellular pH is a major modulator of Na^+/H^+ exchange (reviewed by Demarex and Grinstein 1994, Bianchini and Pouyssegur 1994). In fact low pH_i most likely stimulates the Na^+/H^+ exchanger but low pH_e may limit the maximum rate of its activity, thus contributing to a drop in metabolic rate. Further evidence for a key role of extracellular pH in metabolic depression is provided by the observation that Ca^{2+} entry into cardiomyocytes is reduced (investigated in perfused ventricles from the land snail, *Helix lucorum*) with a consequent depression of heart rate (Michaelidis et al. 1999). In mammalian heart net proton extrusion from the

intracellular space was also reduced depending on the degree of extracellular acidosis (Vandenberg et al. 1994).

The rates of pH recovery and associated changes in oxygen consumption yield some insight into the contribution of membrane permeability to the cost of acid-base regulation (Pörtner et al. 2000a): At $pH_e = 7.9$ a total cost of ion regulation of about $1.8 \mu\text{mol ATP g}^{-1} \text{h}^{-1}$ was calculated from the sum of ouabain and bafilomycin A effects on oxygen consumption assuming glucose as a substrate. H^+ -ATPase and Na^+/H^+ -exchange would consume about $0.65 \mu\text{mol ATP g}^{-1} \text{h}^{-1}$ each in addition to a fraction smaller than the remaining $0.5 \mu\text{mol ATP g}^{-1} \text{h}^{-1}$ required by $Na^+/H^+/Cl^-/HCO_3^-$ -exchange, which shares this rate with other processes stimulating Na^+/K^+ -ATPase activity. Assuming an average ATP requirement of 0.33 ATP per proton extruded, net proton extrusion, quantified from the rate of pH recovery, would consume about $0.13 \mu\text{mol ATP g}^{-1} \text{h}^{-1}$, less than 10 % of the total energy allocated to acid-base regulation. It was concluded that futile cycling induced by membrane permeability contributes to the control of intracellular pH since more than 90 % of the energy used by acid-base regulation are consumed just for pH maintenance (no net H^+ extrusion, Pörtner et al. 2000).

The picture becomes even more complete when the example discussed here (hypercapnic acidosis) is combined with insight from the findings under hypoxia. A reduction in proton equivalent ion exchange occurs during anaerobiosis (Pörtner et al. 1991) accompanied by a reduction of pH_i at constant or reduced levels of extracellular bicarbonate. The drop in pH_i may reflect, in the first place, reduced ion exchange rates and reduced metabolism rather than a regulatory signal eliciting metabolic depression (Pörtner and Ellington 2000). At first sight metabolic depression might cause pH_i values to approach the equilibrium distribution of protons across membranes. However, extracellular pH values also fall and, as a consequence, the steady state transmembrane pH gradient is maintained (e.g. Pörtner et al. 1984) or even falls (Pörtner 1993, R. Oeschger and H.O. Pörtner, unpublished). Since energy turnover falls although a decrease in the pH gradient should cause a rise in the energy requirements of acid-base regulation, it needs to be investigated whether a drop in proton permeability may occur and support metabolic depression.

All of these findings have been obtained mostly in one example organism making more general conclusions impossible. However, other studies addressing the energy cost of acid-base regulation in invertebrate or vertebrate organs are not currently available. Work on the anoxic turtle heart suggests that similar principles may be operative. Acid-base regulation in this organ shifted to sodium and bicarbonate dependent ion exchange suggesting the use of an energy efficient mechanism of acid-base regulation during anaerobiosis (Shi et al. 1997). Further research should support a wider understanding of the evolution of acid-base regulation under the force of environmental factors and constraints.

In general, pH_i can be seen from this work as a dependent parameter that changes with the rate of ion exchange and depends on extracellular bicarbonate levels (Pörtner and Ellington 2000). In the same sense setpoints of intracellular pH are modified by hormonal influences. A steady state alkalization by 0.1 pH units was reported owing to the action of serotonin via cyclic AMP in *Mytilus*

edulis anterior byssus retractor muscle (ABRM) which activates the Na^+/H^+ exchanger (Zange et al. 1990 a,b). Other processes may also contribute to shifting setpoints. Studies on vertebrate cell preparations suggest that changes in ATP levels or in cell volume may cause a change in the pH sensitivity of the Na^+/H^+ exchanger, and, thereby, a change in the setpoints of pH_i regulation. It is unlikely that this change is mediated through a change in phosphorylation of the transporter (Demaurex and Grinstein 1994). Studies in anaerobic marine invertebrates appear highly interesting in this context since anaerobic changes in the setpoints of acid-base regulation mostly occur at largely unchanged ATP levels. These quantitative relationships and their regulatory background warrant further investigation.

All of these findings suggest that pH_i setpoints may change with metabolic rate although the observations of responses to hypercapnia discussed above indicate that the coupling between pH_i setpoints and level of metabolic rate is not tight but may fluctuate, e.g. depending on the levels of extracellular bicarbonate (Pörtner and Ellington 2000). Nonetheless, pH_i is likely to fall when metabolic depression occurs in accordance with a drop in the cost of acid-base regulation, e.g. during hypoxia or normocapnic extracellular acidosis. These relationships have not been investigated during other situations of hypometabolism like in the cold or during starvation or hibernation/aestivation. However, these considerations may help to understand some of the variability of control values found between tissues and organisms.

The cost of acid-base regulation in the cold

The maintenance of acid-base homeostasis may also be affected by the difference in temperature sensitivities of pumps and leaks. A typical pH gradient of 0.4 - 0.6 pH units in most vertebrates and invertebrates is achieved by active proton extrusion from the cell through secondary active ion exchange, mostly via the Na^+/H^+ -exchanger. A reduction of pH gradients between intra- and extracellular spaces has been observed in the cold (Heisler 1986, Sommer et al. 1997) and suggests a larger deviation from passive proton distribution. If the membrane potential and the permeability for acid-base equivalents remain unchanged during cold exposure this may actually require enhanced rates of proton extrusion, possibly an upregulation of Na^+/H^+ -exchanger activity which was found to display a high Q_{10} of 7.9 in trout erythrocytes (Cossins and Kilbey 1990). These relationships warrant further investigation.

According to Reeves (1985), α -stat pH regulation is elicited by both active and passive components. The original hypothesis claimed that the observed changes in pH with temperature are only elicited by passive mechanisms, mostly proton binding or release from intra- and extracellular buffers owing to the change in dissociation equilibria (pK-values) of the buffer components. Later on, the alaphastat hypothesis was extended, integrating an active component. In air breathers active pH regulation is mainly due to the control of PCO_2 by means of

ventilatory adjustments while active ion exchange predominates in water breathers. The relative contributions of active and passive mechanisms to the adjustment of pH_i have been quantified by model calculations (Reeves and Malan 1976; Heisler 1984), however, this analysis is complicated by the variability of the $\Delta pK/\Delta T$ values (see above).

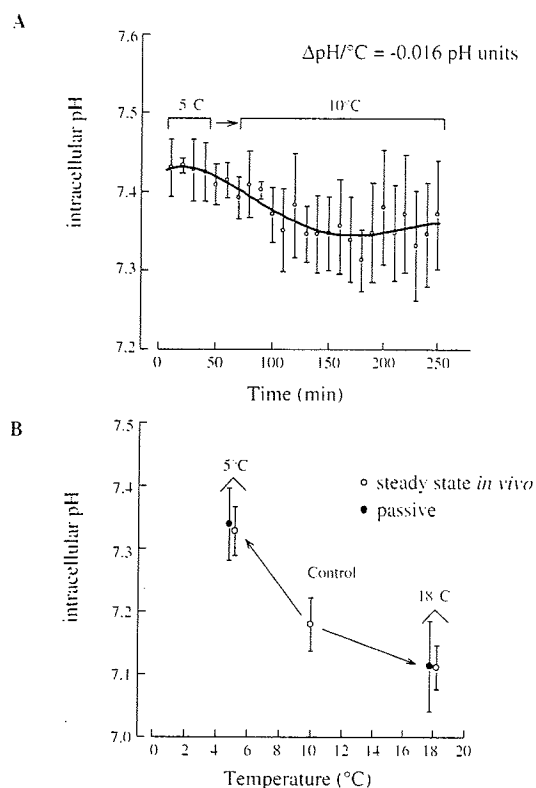


Fig. 3. A) Temperature dependent changes in intracellular pH during a temperature rise from 5 to 10 °C in *S. nudus in vivo*, investigated by ^{31}P -NMR. B) Temperature dependent pH shifts *in vivo* determined after a temperature change from control values to 5 or 18 °C, respectively. In this case steady state values *in vivo* were determined by use of the homogenate technique (data for A and B by C. Bock, P.L.M. van Dijk and H.O. Pörtner, unpublished).

The passive component depends upon the physicochemical composition of intra- and extracellular buffers and results from proton binding or release owing to the change in pK values of the buffer components with temperature. Active mechanisms are comprised of ventilatory and/or ion exchange adjustments. Ventilatory mechanisms of adjusting PCO_2 can only contribute to a limited extent in water breathers which are oxygen limited. Therefore, active ion exchange mechanisms predominantly contribute to the temperature-induced pH change. Previous model calculations of the relative contribution of various processes to pH_i adjustment exclusively relied on the $\Delta pK/\Delta T$ value of the imidazole group.

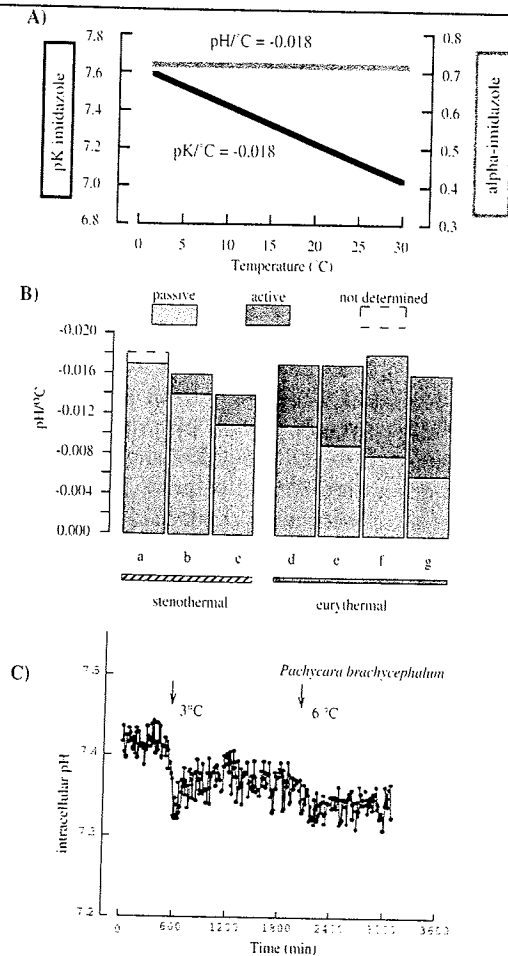


Fig. 4. Changes in the pH of body fluids with temperature (modified after Pörtner et al. 1998). A) Effects of temperature on the dissociation constant (pK) of the imidazole group in histidine, which is an important amino acid component of proteins. A parallel change in pK and cellular pH at $-0.018 \text{ } ^\circ\text{C}^{-1}$ is postulated to maintain the degree of dissociation, α -imidazole (alphastat hypothesis). Maintenance of alpha is interpreted as being essential for the maintenance of protein structure and function. B) Relative contributions of passive and active mechanisms to temperature induced changes in intracellular pH in stenothermal (a-c) marine ectotherms, the Antarctic amphipod *Tryphosella murrayi* (a), the deep water shrimp *Pandalus borealis* (Spitzbergen, b) and the Antarctic eelpout *Pachycara brachycephalum* (c), compared with eurythermal, more temperate zone species (d-g) like the lugworm *Arenicola marina* (d), the sand shrimp *Crangon crangon* from the North Sea (e) and the White Sea (f), and the eelpout *Zoarces viviparus* (g). In the polar species the temperature induced passive pH change is close to the pH change observed *in vivo* (passive slope = $\Delta pH_i/\Delta T = -0.017$ in *Tryphosella murrayi*, -0.013 in *Pandalus borealis*, -0.011 in *Pachycara brachycephalum*). In species a only the passive contribution was determined, the dashed lines reflect expected *in vivo* values according to the alphastat pattern. Data for species a,e and f are from Sartoris and Pörtner, 1997a, for g from van Dijk et al. 1997; for b,c and d are unpublished by F.J. Sartoris, C. Tesch and H.O. Pörtner (modified after Pörtner and Sartoris 1999). C) Temperature induced changes in intracellular pH monitored by ^{31}P -NMR in white muscle of the Antarctic eelpout *Pachycara brachycephalum* (after Bock et al. 2000).

With the reported range of $\Delta pK \cdot C^{-1}$ of histidine residues a large uncertainty arises about the accuracy of these model calculations. To overcome these limitations the contribution of passive and active mechanisms was quantified with the homogenate technique (Pörtner et al. 1990). pH changes resulting from passive (physicochemical) buffering were determined in tissues excised from animals exposed to control temperature. Measurements then occurred *in vitro* at different temperatures. In contrast, tissue samples collected from animals exposed to various temperatures yield the pH_i change as it results from the summed effects of active and passive processes.

Available data suggest that the contribution and velocity of active processes to the pH shift differs between species and populations of fish and invertebrates from different latitudes (Sartoris and Pörtner 1997, van Dijk et al. 1997, Pörtner and Sartoris 1999). The active component of pH regulation may be larger in eurythermal species reaching 50% of the pH change in the eurythermal shrimp *Crangon crangon* or more in eurythermal eelpout *Zoarces viviparus*, whereas it amounts to only 10% in stenothermal cold adapted species, the Arctic deep sea shrimp *Pandalus borealis*, Antarctic amphipods or Antarctic eelpout, *Pachycara brachycephalum*. These phenomena should rely on differences in the composition and physicochemical properties of buffers. Moreover, the velocity of the active component appears to increase as a consequence of metabolic cold adaptation (Pörtner et al. 1998a; Pörtner and Sartoris 1999). In general, the mechanisms behind these differences remain obscure and warrant further research. As a corollary, the hypothesis of alaphastat pH regulation is the only comprehensive hypothesis available that would logically explain these patterns although evidence for the general validity of this hypothesis is still circumstantial.

The homogenate technique (Pörtner et al. 1990) offers the opportunity to distinguish active and passive elements in temperature dependent pH regulation by direct analysis. For a quantification of passive mechanisms tissues collected from animals exposed to control temperatures are analysed *in vitro* at different temperatures, whereas *in vivo* $\bullet pH/T$ values are determined in animals exposed to various temperatures for long enough time periods to allow active mechanisms to achieve and maintain new steady state values of intracellular pH.

In contrast to the original hypothesis we found that the passive contribution to pH regulation was considerably below the alaphastat value in some of the animals investigated (van Dijk et al. 1997, Sartoris and Pörtner 1997a, Pörtner et al. 1998a, Pörtner and Sartoris, 1999). The passive contribution accounted for only 35% of the temperature induced pH shift in white muscle of the eelpout *Zoarces viviparus*. In contrast, passive pH changes in an isolated mixture of intracellular proteins revealed a slope not significantly different from the *in vivo* slope. This discrepancy could not be ascribed to low molecular weight components with low $\bullet pK/T$ values like bicarbonate ($\bullet pK/T = -0.006 \text{ } ^\circ C^{-1}$) or phosphate ($\bullet pK/T = -0.003 \text{ } ^\circ C^{-1}$, van Dijk et al. 1997). The nature of the buffers responsible for the passive contribution to alaphastat remains unexplained.

Comparative studies suggest that the relative contribution of active and passive processes to the pH shift can vary between animals. In *Sipunculus nudus* the pH shift is largely passive, possibly reflecting the low metabolic scope of this species

(Figure 3). Also the pattern observed may be largely influenced by the temperature regime of the animals' habitat (Figure 4). The temperature dependent adjustment of pH_i mostly occurs by active mechanisms in very eurythermal animals whereas in stenothermal animals pH adjustment is mostly achieved by passive processes. Lower passive pH shifts would, on the one hand, lead to more acidic pH values in the cold and leave a larger contribution to ion exchange mechanisms to accomplish alphastat pH regulation. On the other hand, a low passive slope allows flexible adjustments of pH according to metabolic requirements. Large passive slopes would require active pH regulation to compensate for their effect when more acidic pH values are to be maintained. Especially animals exposed to large seasonal temperature variations exhibit low pH values at low temperatures in the winter (Thebault and Raffin 1991, Spicer et al. 1994, see above). One might speculate that a capacity for metabolic depression in eurythermal animals is correlated with a reduced contribution of passive mechanisms to pH adjustment during temperature change and the down regulation of energy consuming ion exchange mechanisms otherwise responsible for alphastat pH regulation. A larger active than passive component of alphastat regulation may not only be a prerequisite to colonise shallow coastal waters but may also allow for a rapid and variable adjustment of metabolic activity on a daily or seasonal time scale. During active periods this occurs at the cost of a larger expense for acid-base regulation, thereby reflecting the higher baseline cost of metabolism in eurythermal animals (Pörtner et al. 2000b).

As a corollary, the patterns and changes observed in acid-base regulation appear to be correlated with the level of energy turnover and the changes in metabolic rate displayed by the animals under the effect of environmental stresses or temperature change. Intracellular pH appears as a dependent parameter fluctuating with metabolic rate in accordance with a down or up regulation of acid-base relevant ion exchange during metabolic depression or activation. Overall, it appears as a parameter set to a level required to support metabolic adjustments allowing for or restricting metabolic scope of a cell, rather than as a parameter that is required for metabolic fine tuning (Pörtner and Ellington, 2000).

Acknowledgements

A contribution to the ELOISE project: Effects of climate induced temperature change on marine coastal fishes (CLICOFI), funded by the European Union program "Climate and environment", contract No. ENV4-CT97-0596. An operating and equipment grant (NMR laboratory) by the German Minister of Education and Research is gratefully acknowledged. Alfred-Wegener-Institute Publication No.

References

- Aickin CC (1984) Direct measurement of intracellular pH and buffering power in smooth muscle cells of guinea-pig vas deferens. *J Physiol* 349: 571-585.

- Arntz WE, Brey T, Gallardo VA (1994) Antarctic Zoobenthos. *Ocean Mar Biol Ann Rev* 32: 241-304.
- Bianchini L, Pouysselgur J. (1994) Molecular structure and regulation of vertebrate Na^+/H^+ exchangers. *J Exp Biol* 196: 337-345.
- Bock C, Sartoris FJ, Wittig R-M, Pörtner HO. In vivo NMR studies of fish at different temperatures: observation of alaphastat pH regulation in stenothermal Antarctic and eurythermal temperate eelpout (Zoarcidae). *Pol Biol* in press
- Cameron JN (1984) Acid-base status of fish at different temperatures. *Am J Physiol* 246: R1452-1459.
- Cameron JN (1989) Acid-base homeostasis: past and present perspectives. *Physiol Zool* 62: 845-865.
- Cossins AR, Kilbey RV (1990) The temperature dependence of the adrenergic Na^+/H^+ exchanger of trout erythrocytes. *J Exp Biol* 148: 303-312.
- Demaurex N, Grinstein S (1994) Na^+/H^+ antiport: modulation by ATP and role in cell volume regulation. *J Exp Biol* 196: 389-404.
- Ellory JC, Hall AC (1987) Temperature effects on red cell membrane transport processes. In: Bowler K, Fuller BJ (eds) *Temperature and animal cells. Symposia of the Society for Experimental Biology* 21: 53-66.
- Gadian DG, Radda GK, Dawson MJ, Wilkie DR (1982) pH_i measurements of cardiac and skeletal muscle using ^{31}P -NMR. In: Nuccitelli R, Deamer DW (eds) *Intracellular pH: Its measurement, regulation and utilization in cellular functions*. Alan R. Liss Inc., New York, pp. 61-77.
- Gibbs A (1995) Temperature, pressure and the sodium pump: The role of homeoviscous adaptation. In: Hochachka PW, Mommsen TP (eds) *Biochemistry and molecular biology of fishes*. Vol. 5, Elsevier Science B.V., Amsterdam, pp. 197-212.
- Heisler N (1984) Role of ion transfer processes in acid-base regulation with temperature changes in fish. *Am J Physiol* 246: R441-R451.
- Heisler N (1986) Comparative aspects of acid-base regulation. In Heisler N. (ed) *Acid-base regulation in animals*, Elsevier North Holland, Amsterdam, pp. 397-450.
- Hochachka PW, Somero GN (1984) *Biochemical adaptation*. Princeton Univ. Press, 537 pp.
- Kammermeier H (1987) High energy phosphate of the myocardium: concentration versus free energy change. *Basic Res Cardiol* 82 (suppl. 2): 31-36.
- Kammermeier H (1993) Efficiency of energy conversion from metabolic substrates to ATP and mechanical and chemiosmotic energy. *Basic Res Cardiol* 88: 15-20.
- Kammermeier H, Schmidt P, Jungling E (1982) Free energy change of ATP-hydrolysis: a causal factor of early hypoxic failure of the myocardium? *J Mol Cell Cardiol* 14: 267-277.
- Michaelidis B, Rofalickou E, Grieshaber MK (1999) The effects of hypercapnia on force and rate of contraction and intracellular pH of perfused ventricles from the land snail *Helix lucorum* (L.). *J Exp Biol* 202: 2993-3001.
- Moerland TS, Egginton S (1998) Intracellular pH of muscle and temperature: insight from *in vivo* ^{31}P -NMR measurements in a stenothermal Antarctic teleost (*Harpagifer antarcticus*). *J therm Biol* 23: 275-282, 1998.
- Pörtner HO (1993) Multicompartmental analyses of acid-base and metabolic homeostasis during anaerobiosis: invertebrate and lower vertebrate examples. In: Hochachka PW, Lutz PL, Sick T, Rosenthal M, van den Thillart G (eds) *Surviving hypoxia: mechanisms of control and adaptation*. CRC Press Inc., Boca Raton FL, U.S.A. pp. 139-156.
- Pörtner HO, Sartoris FJ (1999) Invasive studies of intracellular acid-base parameters: environmental and functional aspects. In: Egginton S, Taylor EW, Raven JA (eds). *Regulation of tissue pH in plants and animals: A reappraisal of current techniques*. Cambridge University Press, pp. 69-98.
- Pörtner HO, Ellington WR (2000) Systemic and cellular acid-base regulation in marine invertebrates: Models for an evolutionary and ecological understanding. *Physiol Rev*

- Pörtner HO, Grieshaber MK, Heisler N (1984) Anaerobiosis and acid-base status in marine invertebrates: effect of environmental hypoxia on extracellular and intracellular pH in *Sipunculus nudus* L. *J Comp Physiol* 155B: 13-20, 1984.
- Pörtner HO, Boutilier RG, Tang Y, Toews DP (1990) Determination of intracellular pH and P_{CO_2} after metabolic inhibition by fluoride and nitrilotriacetic acid. *Respir Physiol* 81: 255-274.
- Pörtner HO, Andersen NA, Heisler N (1991) Proton equivalent ion transfer in *Sipunculus nudus* as a function of ambient oxygen tension: relationships with energy metabolism. *J Exp Biol* 156: 21-39.
- Pörtner HO, Finke E, Lee PG (1996) Metabolic and energy correlates of intracellular pH in progressive fatigue of squid (*Lolliguncula brevis*) mantle muscle. *Am J Physiol* 271: R1403-1414.
- Pörtner HO, Hardewig I, Sartoris FJ, van Dijk P (1998a) Energetic aspects of cold adaptation: critical temperatures in metabolic, ionic and acid-base regulation? In: Pörtner HO, Playle R (eds.) *Cold Ocean Physiology*. Cambridge University Press, pp. 88-120.
- Pörtner HO, Reipschläger A, Heisler N (1998b) Metabolism and acid-base regulation in *Sipunculus nudus* as a function of ambient carbon dioxide. *J Exp Biol* 201: 43-55.
- Pörtner HO, Peck L, Zielinski S, Conway LZ (1999) Intracellular pH and energy metabolism in the highly stenothermal Antarctic bivalve *Limopsis marionensis* as a function of ambient temperature. *Pol Biol* 22: 17-30.
- Pörtner HO, Bock C, Reipschläger A (2000a) Modulation of the cost of pHi regulation during metabolic depression: a ^{31}P -NMR study in invertebrate (*Sipunculus nudus*) isolated muscle. in review
- Pörtner HO, van Dijk PLM, Hardewig I, Sommer A (2000b) Levels of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. In: *Antarctic Ecosystems: models for wider ecological understanding*, edited by Davison W, Howard Williams C: Caxton Press, Christchurch New Zealand, in press.
- Raynard RS, Cossins AR (1991) Homeoviscous adaptation and thermal compensation of sodium pump of trout erythrocytes. *Am J Physiol* 260: R916-R924.
- Reeves RB (1972) An imidazole alphastat hypothesis for vertebrate acid-base regulation: Tissue carbon dioxide content and body temperature in bullfrogs. *Respir Physiol* 14: 219-236.
- Reeves RB (1985) Alphastat regulation of intracellular acid-base state? In: Gilles R (ed.) *Circulation, respiration, and metabolism*. Springer-Verlag: Berlin Heidelberg 1985, pp. 414-423.
- Reeves RB, Malan A (1976) Model studies of intracellular acid-base temperature responses in ectotherms. *Respir Physiol* 28: 49-63
- Reipschläger A, Pörtner HO (1996) Metabolic depression during environmental stress: the role of extra- versus intracellular pH in *Sipunculus nudus*. *J Exp Biol* 199: 1801-1807.
- Roos A, Boron WF (1981) Intracellular pH. *Physiol Rev* 61: 296-434.
- Sartoris FJ, Pörtner HO (1997a) Temperature dependence of ionic and acid-base regulation in boreal and arctic *Crangon crangon* and *Pandalus borealis*. *J exp mar Biol Ecol* 211: 69-83.
- Sartoris FJ, Pörtner HO (1997b). Increased concentrations of haemolymph Mg^{2+} protect intracellular pH and ATP levels during temperature stress and anoxia in the common shrimp *Crangon crangon*. *J Exp Biol* 200: 785-792.
- Shi H, Hamm, PH, Meyers RS, Lawler RG and Jackson DC (1997) Mechanisms of pHi recovery from NH_4Cl -induced acidosis in anoxic turtle heart: a ^{31}P -NMR study. *Am J Physiol* 272: R6-R15, 1997.
- Somero GN (1986) Protons, osmolytes and the fitness of the internal milieu for protein function. *Am J Physiol* 251: R197-R213, 1986.
- Sommer A, Klein B, Pörtner HO (1997) Temperature induced anaerobiosis in two populations of the polychaete worm *Arenicola marina*. *J Comp Physiol* 167B: 25-35.

- Southward AJ, Hawkins SJ, Burrows MT (1995) Seventy years' observation of changes in distribution and abundance of zooplankton and intertidal organisms in the western English channel in relation to rising sea temperature. *J Therm Biol* 20: 127-155.
- Spicer, J I, Morrill, D., Taylor, AC (1994). Effect of low temperature on oxygen uptake and haemolymph ions in the sandhopper *Talitrus saltator* (Crustacea: Amphipoda). *J mar biol Ass U.K.* 74: 313-321.
- Thebault MT, Raffin JP (1991) Seasonal variations in *Palaemon serratus* abdominal muscle metabolism and performance during exercise, as studied by ^{31}P NMR. *Marine Ecology Progress Series* 74: 175-183.
- Thiel H, Pörtner HO, Arntz WE (1996) Marine life at low temperatures - a comparison of polar and deep-sea characteristics. In: Uiblein F, Ott J, Stachowitsch M. (eds) *Deep-sea and extreme shallow-water habitats: affinities and adaptations*. Vienna, Austrian Academy of Sciences, *Biosystematics and Ecology Series* 11: 183-219, 1996.
- Thomas RC (1984) Experimental displacement of intracellular pH and the mechanism of its subsequent recovery. *J Physiol* 354: 3P-22P.
- Ultsch GR, Jackson DC (1996) pH and temperature in ectothermic vertebrates. *Bull Alabama Mus Nat Hist* 18: 1-41.
- Vandenberg JJ, Metcalfe JC, Grace AA (1994) Intracellular pH recovery during respiratory acidosis in perfused hearts. *Am J Physiol* 266: C489-C497.
- van Dijk P, Hardewig I, Pörtner HO (1997) The adjustment of intracellular pH after temperature change in fish: relative contributions of passive and active processes. *Am J Physiol* 272: R84-R89.
- van Dijk PLM., Tesch C, Hardewig I, Pörtner HO (1999) Physiological disturbances at critically high temperatures. A comparison between stenothermal Antarctic, and eurythermal temperate eelpouts (Zoarcidae). *J Exp Biol* 202: 3611-3621.
- Whiteley NM, Naylor JK, Taylor EW (1995) Extracellular and intracellular acid-base status in the fresh water crayfish *Austropotamobius pallipes* between 1 and 12°C. *J Exp Biol* 198: 567-576.
- Zange J, Pörtner HO, Jans AWH, Grieshaber MK (1990). The intracellular pH of a molluscan smooth muscle during a contraction-catch-relaxation cycle as estimated by the distribution of ^{14}C -DMO and by ^{31}P -NMR spectroscopy. *J Exp Biology* 150: 81-93.
- Zange J, Grieshaber MK, Jan WH (1990b) The regulation of intracellular pH estimated by ^{31}P -NMR spectroscopy in the anterior byssus retractor muscle of *Mytilus edulis* L. *J Exp Biol* 150: 95-109.
- Zielinski S, Pörtner HO (1996) Energy metabolism and ATP free-energy change of the intertidal worm *Sipunculus nudus* below a critical temperature. *J Comp Physiol* 166B: 492-500.