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Temperature-dependent pH regulation in eurythermal and stenothermal marine fish: an interspecies comparison using ^{31}P-NMR

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Abstract

Temperature-induced pH changes in white muscle tissue of three eelpout populations with different levels of eurythermy (the cold stenothermal Antarctic species *Pachycara brachycephalum* and the temperate eelpout *Zoarces viviparus* from the North Sea and the Baltic Sea) were monitored online by use of in vivo ^{31}P-NMR in unrestrained, unanaesthetized fish. An intracellular pH (pH_i) change of around −0.015 pH units/°C was observed in all eelpout populations in accordance with the α-stat hypothesis. The pH change was completed earlier (within 4 h) in the stenothermal Antarctic eelpout than in the Baltic population (within 8 h) and latest (not within 12 h) in the eurythermal North Sea population. These findings confirm the hypothesis that the kinetics of temperature-dependent pH_i regulation is reflected by the relative contribution of active and passive processes to a temperature-induced pH change. The extent of passively induced pH_i changes is in line with the general hypothesis that the temperature-dependent adjustment of pH_i occurs mostly by active mechanisms in eurythermal animals, whereas in stenothermal animals pH changes are largely elicited by passive processes. Temperature changes had no influence on high-energy phosphates like phosphocreatine and ATP or on the Gibbs free energy change of ATP hydrolysis ($\Delta G/\Delta\xi$).

Keywords: In vivo MRI; Temperature physiology; α-stat pH regulation; Marine fish

1. Introduction

Generally, pH plays an important role in the maintenance of protein function during temperature change. Preservation of the structural integrity of proteins, especially enzymes, is a prerequisite for the maintenance of cellular function. Maintenance of the dissociation state (α) of histidine residues within proteins, especially in activity sites of enzymes, is seen as a key factor in this process (α-stat hypothesis). A pH change of around −0.018 pH units/°C is interpreted to maintain protein function when body temperature changes in ectothermic animals (α-stat pattern; Reeves, 1972). Even though literature is not uniform concerning the validity of the α-stat hypothesis recently the work of Ultsch and Jackson, 1996 and our own work (Pörtner et al., 1998) indicates that at least intracellular pH (pH_i) is generally regulated according to α-stat, especially in the normal temperature range of species and in between critical temperatures (Sommer et al., 1997). In other words, if animals deviate from α-stat there likely is a reason like hibernation or metabolic depression behind. Some experimental data obtained in marine ectotherms suggest that the adjustment of α-stat is significantly slower in eurythermal than in stenothermal animals (Sartoris and Pörtner, 1997; Pörtner and Sartoris, 1999). The pH change will be induced by both passive and active mechanisms. The change in dissociation equilibria

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(p*K*-values) of intra- or extracellular buffers with temperature accounts for the passive contribution to α-stat pH regulation. In water breathers observed changes in intra- or extracellular pH are in general supported by active ion exchange. Interspecies comparisons suggest that the temperature-induced adjustment of pH_i mostly occurs by active mechanisms in eurythermal animals, whereas in cold stenothermal animals pH changes mostly owing to passive processes (Sartoris and Pörtner, 1997; Pörtner and Sartoris, 1999). The active process involves the transcellular movement of ions, which may be the time-limiting process and involves enhanced energy requirements of acid–base regulation (Pörtner et al., 2000). This implies that the overall kinetics of α-stat pH regulation is controlled by the relative contribution of active and passive processes, with a largely delayed response in eurytherms. With a large delay, an α-stat pattern of pH regulation may not be maintained in animals living in a variable environment like in shallow coastal waters or the intertidal zone where temperature may change drastically during the tidal cycle. In these species α-stat regulation may only be important on a seasonal time scale. In contrast, cold stenothermal animals will never face large temperature changes and may not have evolved or may have secondarily reduced the capability for active temperature-dependent pH regulation. The capability for active temperature-dependent pH regulation appears as a prerequisite for the colonization of thermally instable environments, not as a means to support rapid pH shifts but as a tool to maintain new steady-state pH changes and to compensate for the minimized passive contribution to the pH shift.

Goal of this study was a comparative investigation of temperature-dependent pH regulation in ectothermal animals living under different temperature regimes at different levels of eurythermy. We wanted to investigate whether the larger contribution of passive processes causes the pH change to occur faster in stenothermal than in eurythermal animals. We chose two species of the cosmopolitan fish family Zoarcidae for this study, the circum-Antarctic cold stenothermal species *Pachycara brachycephalum* and the temperate eelpout *Zoarces viviparus* with populations in the German Wadden Sea and the Baltic Sea. Since the Wadden Sea displays larger temperature fluctuations than the Baltic Sea, the two populations may differ with respect to the level of eurythermy. Invasive studies in specimens from the North Sea Population as well as in the stenothermal Antarctic eelpout *P. brachycephalum* had demonstrated that pH regulation follows the α-stat pattern in both species in vivo (Van Dijk et al. 1997, 1999). With the advent of ^{31}P-NMR techniques for whole animal studies (Van den Thillart and van Waarde, 1996; Wasser et al., 1996; GrØttum et al., 1998; Borger et al., 1998; Moerland and Eggington, 1998; Bock et al., 2001, 2002) the change in pH_i can be monitored online in vivo thereby allowing to closely follow the time course of temperature-dependent pH_i regulation. By use of a horizontal rather than vertical magnet the analysis no longer requires anaesthesia or immobilization of the fish for these analysis.

2. Material and methods

2.1. Experimental animals

North Sea eelpout (*Z. viviparus*) were caught in trawls in shallow water (1.5–6 m) of the Wadden sea of Lower Saxony near Neuharlingersiel in the summer of 1998. *Z. viviparus* from the Baltic Sea was obtained from local fishermen fishing with traps in the Kiel Bay at 20 m depth in the summer months of 1998. Specimens from both populations were kept in aquaria at 12°C in water with the appropriate salinity, 30‰ for North Sea eelpout and 15‰ for Baltic Sea eelpout. Antarctic eelpout (*P. brachycephalum*) were caught in baited traps at a depth of 400 m in Admiralty Bay, Antarctica during the ANT XV/III Cruise (1998) and kept in well-aerated seawater at 0 ± 0.5°C. The fish were allowed to acclimate for at least 3 weeks prior to temperature incubation, at a daily light period of 12 h. The animals were fed twice a week ad libitum with shrimp. Feeding was terminated 3 days before experimentation. Pregnant females could be identified with MR imaging and were not used in this study.

2.2. Temperature incubations

The fish were transferred to a flow through chamber with a constant flow of recirculating aerated seawater ($1.5 l/min^{-1}$). Temperature was measured directly inside the chamber using a fluoroptic thermometer (Luxtron 504, Polytec, Waldheim). Temperature control (stability ± 0.3°C in the range between 0°C and 30°C) was achieved by a cryostat connected to the water reservoir. The experimental chambers were equipped with variable slide barriers to centre the animal in the chamber. Otherwise, the fish were not anaesthetized, unrestrained and free to move inside the chamber during the whole experimental time. Since the design of the chambers requires a certain length range of the fish only fish were used with a maximum length of about 40 cm and an average weight of 60 g. During an acclimation period of at least 24 h prior to temperature incubations stress-free conditions were certified by constant in vivo ^{31}P-NMR spectra. Temperature was increased from 0°C to 6°C for Antarctic eelpout, from 12°C to 18°C for Baltic and from 12°C to 21°C for North Sea eelpout, respectively, in steps of 1°C within 3 h. When a new steady state was reached (3°C and 6°C, Antarctic eelpout; 15°C and

18°C, Baltic and 15°C, 18°C and 21°C, North Sea eelpout) the temperature was kept constant for at least 12 h. Temperature was decreased to control levels again at the end of the experiment and the animal was put back into the aquarium. As long as the temperature was kept below the high critical temperature all animals survived the temperature increase and no mortality could be observed afterwards.

2.3. *In vivo* ^{31}P-NMR spectroscopy

The determination of pH_i and the high-energy phosphates phosphocreatine (PCr) and ATP followed the protocol outlined by Bock et al. (2001). All experiments were carried out using a 4.7 T magnet with actively shielded gradient coils (Bruker Biospec 47/40 DBX System). A 5 cm surface coil was placed directly onto the chamber wall and positioned close to tail of the animal for ^{31}P-NMR spectroscopy.

Pilot scans were collected right before temperature variation and directly after reaching of the desired temperature to control the position of the animal. For anatomical studies multi-slice RARE images were performed in coronal and transversal directions. In vivo ^{31}P-NMR spectra were acquired continuously over 1200 scans resulting in a measurement time of 10 min. All spectra were processed automatically using an user program (Bock et al., 2001). The spectra were calibrated using PCr as an internal standard. Signal integration and chemical shifts were calculated from an automatic fit routine (mdcon, Bruker Analytical, Rheinstetten) and compared with results determined by automatic peak picking. A calibration curve for the calculation of pH values from the chemical shift of the inorganic phosphate (P_i) signal was obtained from standard solutions with a simulated intracellular ion content at different pH and temperatures of 0°C and 20°C. The temperature-dependent p*K*s for P_i and temperature correction factors were obtained from Kost (1990).

3. Results

The absence of movement artefacts enabled good resolution of the MR images and allowed to control the orientation of the fish in the flow through chamber (Fig. 1). This indicates low stress conditions which are also reflected by low P_i signals, resulting in a high PCr/P_i ratio and stable steady-state pH_i values under control conditions (Fig. 2). As previously reported by Bock et al. (2001) high amounts of phosphomono- and diesters could be detected in *P. brachycephalum* which were not present in the two eurythermal populations of the common eelpout, *Z. viviparus*. However, pH_i estimation is not affected by the phosphodiester signal since the

Fig. 1. Anatomical picture from unanaesthetized and unrestricted Antarctic eelpout in the flow trough chamber. Note the excellent anatomical resolution which allows the identification of different tissues.

position of this signal is not in the region of the P_i signal. The spectra obtained in North Sea eelpout were different from those of the other two populations in a way that two P_i signals were visible. Within a typical line width of 10–15 Hz two distinguishable P_i signals could be detected since even in the worst case the distance between the two signals is at least 19 Hz. Both signals showed the same temperature dependence which allows the clear differentiation of pH_i1 and pH_i2 at each temperature. According to the pH_i calculated from the signals one signal could be identified as white muscle P_i reflecting a value of 7.25 at 12°C (pH_i1), very similar to values determined by the homogenate technique in the same species at 12°C (Van Dijk et al., 1999). pH_i2 calculated from the second P_i signal was about 0.25 pH units higher compared to pH_i1. Both pH values were obtained from the tail of the fish where white muscle tissue represents the dominating tissue fraction. Although the origin of the pH_i2 signal remains unclear both pH values displayed the same changes with temperature. We have performed preliminary tests with different positions of the coil. This led to changes in pH_i2. Since we used a 5 cm coil we cannot exclude the contribution of different tissues according to partial volume effects.

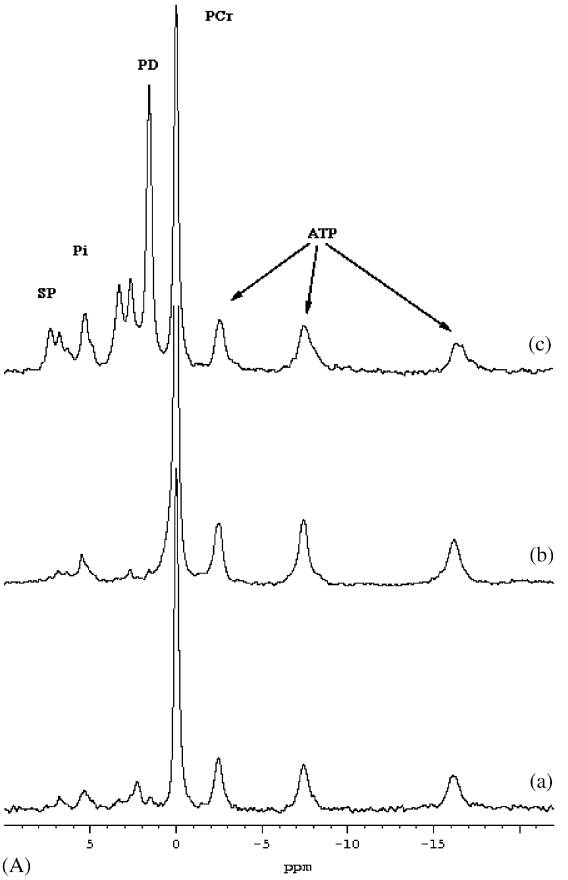

Fig. 2. (A)In vivo ^{31}P-NMR spectra at control temperatures of (a) *Z. viviparus* (North Sea, 12°C), (b) *Z. viviparus* (Baltic Sea, 12°C) and (c) *P. brachycephalum* (0°C). The spectrum of Antarctic eelpout showed additional signals in the phosphodiester region. (B) In vivo ^{31}P-NMR spectra at control temperatures of North Sea eelpout. The P_i signal regularly splits into two distinguishable P_i signals.

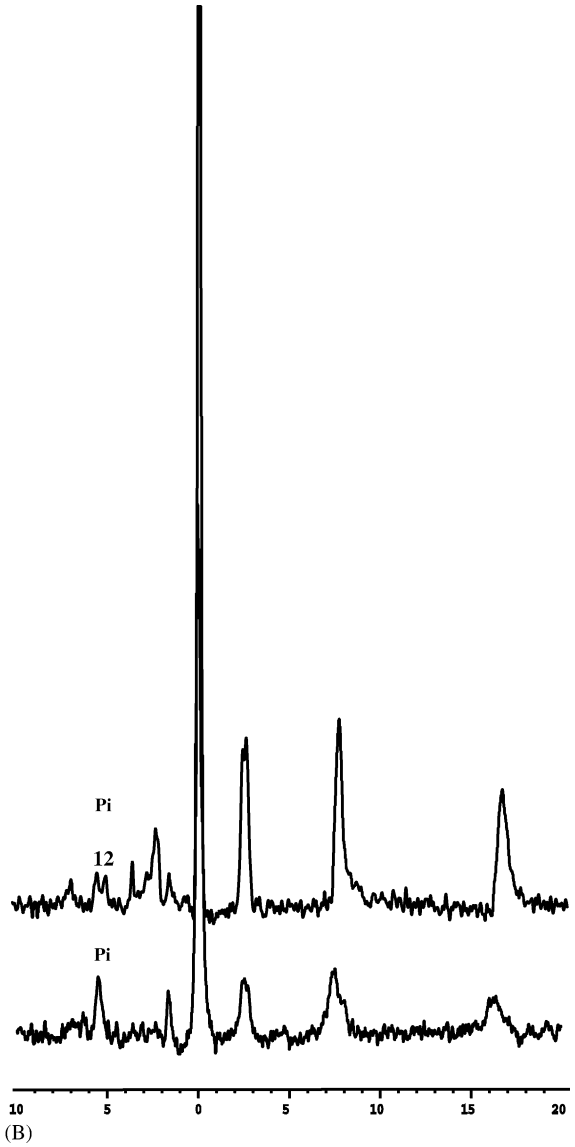

Fig. 2 (*continued*).

In earlier studies, it had been shown that beyond critical temperatures disruption of regulated physiological parameters like cellular energy levels and pH_i may lead to substantial changes in eelpout species (>6°C in Antarctic eelpout and >21°C in North Sea eelpout; Van Dijk et al., 1999). Preliminary studies were performed with Baltic Sea *Z.viviparus* showing that between 12°C and 18°C all parameters remained close to control levels. Therefore, we investigated only temperatures within this range in the present study. No mortality could be observed at temperatures below critical. In one trial with Baltic eelpout, carried out to evaluate the upper critical temperature, i.e. the temperature where aerobic scope is lost and transition to anaerobic metabolism occurs (for review see Pörtner (2001)), the fish lost balance during long time incubation at 18°C when PCr and ATP started to decrease followed by a drop in pH by 0.4 units (Figs. 3 and 4). The fish died within 1 h and even immediate cooling to control temperature (within 15 min) could not reverse this process.

Fig. 5 shows the typical time courses of pH changes in white muscle with increasing temperature. An acidification after temperature change could be observed in all populations. The stenothermal Antarctic eelpout with a high passive contribution to α-stat (Pörtner and Sartoris, 1999) was faster in adjusting pH_i (completed after 4 h) than the more eurythermal Baltic eelpout (pH_i adjustment completed after 8 h), while in the highly eurythermal North Sea eelpout steady-state values were not

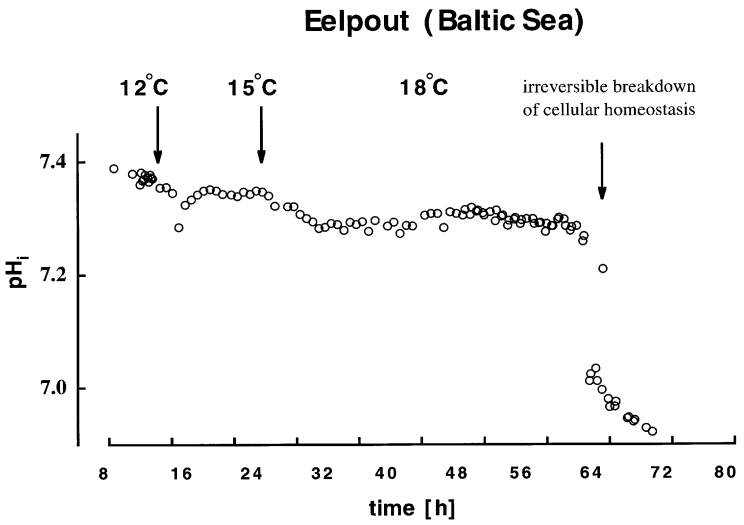

Fig. 3. Time course of changes in pH_i in the white muscle of Baltic eelpout. The pH change with temperature confirms α-stat regulation of pH_i for the individual fish. The abrupt decrease in pH_i after certain hours of incubation at 18°C was not preceded by changes in the energy status of the muscle. However, it was typical for all species although the "critical" temperature and the time course were different.

reached within 12 h. Clear distinction between the fast passive pH change and the slower active contribution was hampered by the variability of NMR derived pH_i values. However, after 4 h in *Z. viviparus* (North Sea), (when pH adjustment was already complete in Antarctic eelpout) a $\Delta pH/°C$ change of -0.006 ± -0.016 reflected exactly the passive value reported by Van Dijk et al. (1997). Similar results could not be obtained in Baltic eelpout since temperature increase in this species sometimes led to an immediate undershoot of pH which dominated the pH response during the first hours. The temperature-induced pH_i change did not lead to changes in high-energy metabolites. Decreasing the temperature to control levels at the end of the incubation period resulted in immediate (within 4 h) return to control levels of pH_i in all populations. Again this was not reflected in the energy status of white muscle.

The shift in pH_i with temperature in the white muscle of Antarctic ($\Delta pH/°C = -0.015$) and North Sea eelpout ($\Delta pH/°C = -0.017$) followed the α-stat pattern and was very similar to the values determined by invasive methods by Van Dijk et al. (1997, 1999) ($\Delta pH/°C = -0.015$ in *P. brachycephalum* and $\Delta pH/°C = -0.016$ in North Sea eelpout). In contrast to the findings of Van Dijk et al. (1999), no deviation from the α-stat slope could be observed between 3°C and 6°C in white muscle of Antarctic eelpout. α-stat pH regulation was found within each species. However, comparisons of Antarctic and North Sea and Baltic eelpout revealed large differences between the position of the slopes. In common eelpout the absolute pH_i values were about 0.15 units higher in animals from the Baltic than in those from the North Sea (Fig. 6). The extrapolated slope of pH_i in Antarctic eelpout is found between the two slopes of the common eelpout populations.

4. Discussion

The online monitoring of temperature-dependent changes in pH_i and energy status occurred in unanaesthetized and unrestrained marine fish under physiological conditions (Bock et al., 2001, 2002). Although the possibility for the fish to move inside the chambers could lead to movement artefacts, spectral quality and time resolution were comparable to investigations where the animal was either anaesthetized and/or vertically fixed (Van den Thillart et al., 1989a, b; Van Ginneken et al., 1995, 1996, Borger et al., 1998; Moerland and Eggington, 1998). The potential influence of anaesthetics on blood, tissue and acid–base parameters as reported for rainbow trout (Iwama et al. 1989) and in the Antarctic fish *Pagothenia borchgrevinki* (Ryan 1992) could be excluded. The eelpout was orientated towards the water inlet and, as a benthic species, did not move too much inside the chamber when stress-free conditions are maintained. This could be confirmed by control measurements where steady-state conditions were indicated by low P_i levels and physiological pH_i values and maintained for more than a week. In addition, all animals survived heat exposure as long as temperature was kept below the high critical level T_c (only reached in a single experiment with Baltic eelpout). This critical temperature threshold was about 18°C and

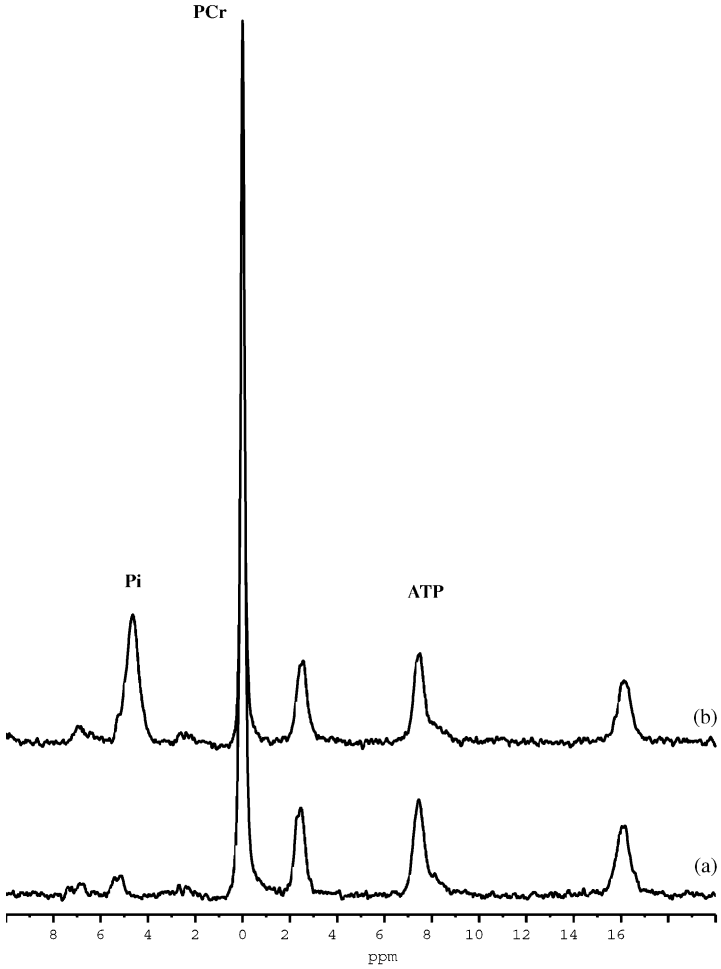

Fig. 4. In vivo ^{31}P-NMR spectra of white muscle of Baltic eelpout before and after the abrupt decrease in pH_i.

thus below the T_c reported by Van Dijk et al. (1999) for North Sea eelpout (between 21°C and 24°C). Since maximum summer water temperature are lower in the Baltic Sea than in the Wadden Sea the T_c in both population reflect the maximum temperature these populations are exposed to.

The tissue responsible for determining pH_i1 in North Sea *Z. viviparus* could be identified as white muscle by comparison with the data by Van Dijk et al. (1999). The origin of pH_i2 still remains unclear. The position of the coil was chosen such that mostly signals from white tail muscle tissue were monitored. Nevertheless, due to a coil diameter of 5 cm, red muscle, the liver and the blood might display a pH which is visible through in vivo ^{31}P-NMR and different from white muscle tissue pH. The differentiation of pH_i between white and red muscle is possible with in vivo ^{31}P-NMR as demonstrated by Van den Thillart et al. (1989a). Mitochondrial density is higher in red muscle and may thus be responsible for a more alkaline pH compared to white muscle. However, the difference between pH1 and pH2 in our study was above 0.2 pH units while the values measured by Van den Thillart et al. (1989a) were below 0.1 pH units. In liver tissue mitochondrial density is also elevated and might thus account for the high pH_i2 values. But again it is questionable whether the difference of 0.2 pH units can be explained since most mitochondrial phosphate is NMR invisible (for review of cellular compartmentalization of pH_i see Pörtner and Sartoris, 1999). The concentration of P_i in the blood of eelpout as well as the blood content is too low in the sensitive volume to account for pH_i2 (Mark et al., 2002). At the moment we cannot clearly identify the tissue in question.

The magnitude of temperature-induced pH changes measured by in vivo ^{31}P-NMR is similar in all eelpout populations investigated in this study (see also Bock et al., 2001) and confirms the results obtained by invasive studies for white muscle of Antarctic eelpout

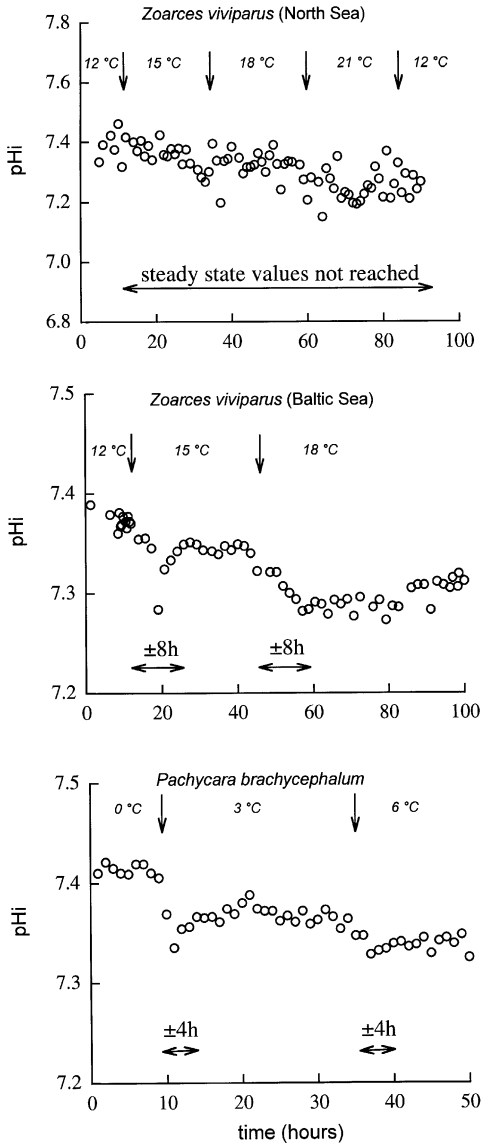

Fig. 5. Typical time course of pH_i changes with increasing temperatures obtained from in vivo spectra of white muscle of common eelpout *Z. vivparus* from North Sea (a), Baltic Sea (b) and Antarctic eelpout *P. brachycephalum* (c). Note the different time scales and the faster pH_i adjustment in the stenothermal Antarctic eelpout.

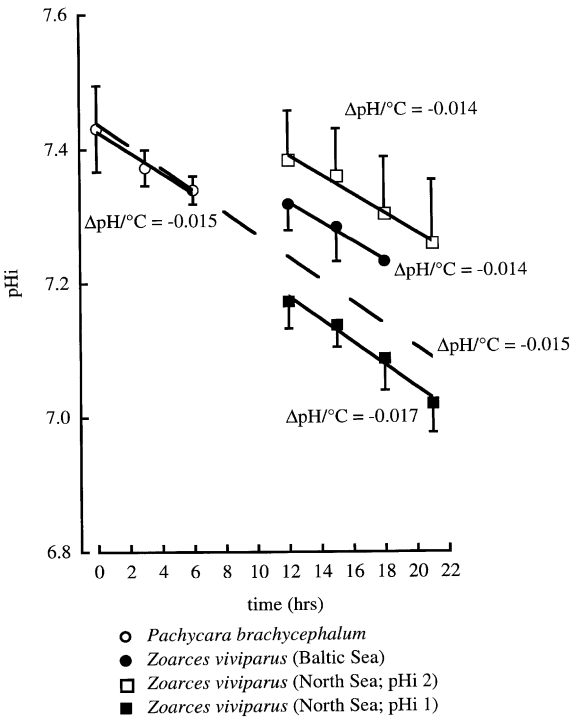

Fig. 6. Relationship between pH_i and ambient temperature in white muscle of Antarctic eelpout *P. brachycephalum* (open circles; $n = 6$ at 0°C, $n = 5$ at 3°C, $n = 3$ at 6°C), common eelpout *Z. viviparus* from Baltic Sea (filled circles; $n = 3$ at 12°C and 15°C, $n = 2$ at 18°C), common eelpout from the North Sea *Z. viviparus* (pH_i1, filled squares; $n = 4$ at all temperatures and pH_i2, open squares; $n = 4$ at all temperatures). α-stat pH regulation could be confirmed within each species. Note the differences in temperature specific pH_i values between species and populations.

(−0.015 pH/°C; Van Dijk et al., 1999) and North Sea eelpout (−0.016 pH/°C; Van Dijk et al., 1997). The incubation experiment was designed to assure that in vivo $\Delta pH/\Delta T$ values could be determined in animals exposed to increasing temperatures for time periods long enough to allow active mechanisms to reach and maintain new steady-state values of pH_i. This could be achieved in Baltic and in Antarctic eelpout, whereas in North Sea *Z. viviparus* active α-stat regulation was not evidently completed within the incubation period of 12 h at each temperature. However, even if the final steady-state value was not reached the exposure time was sufficient to confirm α-stat regulation as indicated by the slope of the ΔpH/°C relation which was –0.017 for pH_i1 and –0.014 for pH_i2, respectively.

Despite the excellent quality of the spectra a difference between an initial passive pH_i shift within few hours followed by an active pH_i decrease over a longer period could only be seen in Antarctic eelpout possibly due to the higher passive value in these population. In the other eelpout populations, the lower passive shift is very likely complemented by the active fraction as soon as temperature changes. In the NMR experiments, it is not possible to distinguish between the two mechanisms as it is with invasive methods. Here it is possible to block the active mechanisms and to follow the passive pH_i change with temperature. Using these techniques, the different time course of passive and active pH regulation have been demonstrated for crustaceans (Sartoris and

Pörtner, 1997), which is to our knowledge the only study available so far. The relative contribution of passive and active mechanisms to the temperature-dependent adjustment of pH_i in eurythermal and stenothermal animals is largely influenced by the width of the temperature window (Sartoris and Pörtner, 1997; Pörtner and Sartoris, 1999). In all species investigated so far the passive contribution to α-stat regulation was small in eurythermal animals, while in stenothermal species pH adjustment is mostly achieved by passive processes. Van Dijk et al. (1999) have shown that in *Z. viviparus* from the North Sea ventilatory regulation of CO_2 is not significantly involved in the active regulation of pH_i after temperature change. They calculated the net H^+-equivalent ion transfer with a temperature increase of 1°C as 0.52 mmol protons/kg cell water. A 10°C drop in temperature equals the proton transfer after strenuous exercise in dogfish white muscle (about 8 mmol H^+; Holeton and Heisler, 1983). The metabolic costs of pH_i adjustment after exercise and the velocity of recovery of intracellular acid–base homeostasis depend on the degree of exhaustion. Stating that the active ion transport mechanisms are similar both in pH_i recovery from exhaustion and adjustment of pH_i after temperature change (mainly through the Na^+/K^+-ATPase) the velocity of active α-stat regulation reflects the fractional contribution of active pH_i regulation. In consequence, the higher the active component the higher the energetic costs and the longer the period of pH_i readjustment. In fact, our online recording reveals different velocities of pH_i adjustments. In the Antarctic eelpout a pH shift with temperature is completed after 4 h, while the pH change in North Sea *Z. viviparus* is still approximate to the passive shift after this time. In Baltic eelpout temperature-dependent pH regulation was completed within 8 h. These results are in good agreement with our hypothesis that the kinetics of temperature-dependent pH regulation is linked to the fractional contribution of passive and active mechanisms. Furthermore, the immediate change in pH_i with temperature in Antarctic eelpout verifies that temperature-induced pH_i changes in stenotherms are largely elicited by passive mechanisms as reported by Pörtner and Sartoris (1999).

The decrease in the passive component of temperature-dependent pH regulation with increasing levels of eurythermy is compensated by active ion transport. Both active and passive processes act synergistically towards a pH shift according to the α-stat hypothesis. Stenothermal animals with a high passive slope might be energetically favoured during temperature fluctuations since the active transport of ion equivalents to accomplish α-stat pH depends on energy supply. This might be of superior importance in the cold where the higher thermal sensitivity of active transport in comparison to less sensitive leakage pathways (Hochachka (1986)) would increase the fractional cost of ion regulation in a decreasing metabolic rate. It remains to be shown whether warm adapted stenotherms also show higher levels of passive pH_i regulation or whether they can afford to display more active contribution as seen in eurythermal animals. In addition, the question arises what are the advantages of active α-stat pH regulation? At first sight, the higher cost as well as the decrease in velocity might appear as a disadvantage. On the other hand, a high active component allows a much more flexible response when temperature changes. In a previous paper (Sartoris and Pörtner, 1997), it was suggested that low passive slopes may support metabolic depression in winter which should comprise the down regulation of energy consuming ion exchange mechanisms otherwise responsible for α-stat pH regulation. The capacity for metabolic depression in eurythermal animals is correlated with a reduced contribution of passive mechanisms to pH adjustment during temperature change (Sartoris and Pörtner, 1997). It has been shown that ectothermal animals exposed to low temperatures in the winter (Thebault and Raffin, 1991; Spicer et al. 1994) exhibit tissue pH values below those expected from α-stat pattern. Metabolic depression in eurytherms, induced by a pH_i decrease to a level adjusted by passive components, would reduce the cost of active α-stat regulation. This might indicate that stenotherms would be faced with high costs of metabolic depression, since active pH regulation would be required to reduce pH below the values adjusted by the higher passive contribution to α-stat. However, seasonal metabolic depression in Antarctic stenotherms involves minor temperature changes, such that a reduction of the passive slope is not necessary.

Interestingly, we could not observe any significant changes in the levels of high-energy phosphates like PCr and ATP during temperature incubations, except during exposure to high critical temperatures (prolonged incubation at 18°C in Baltic eelpout) when the fish lost balance and PCr and ATP started to decrease. The fish died within 1 h and even immediately cooling could not reverse this process. In consequence, no deviation from α-stat regulation could be observed before this "point of no return" was reached. In conclusion, white muscle energy status appears to be very insensitive to thermal stress in all populations. Similar results have been obtained by Van Dijk et al. (1999) with invasive methods in Antarctic and North Sea eelpout.

A temperature change towards acclimation temperatures at the end of the experiments results in a fast return of pH_i values in all populations. No differences between stenothermal and eurythermal eelpouts could be seen in the velocity of this pH shift. This indicates that the eurythermal animals are flexible in the adjustment of the kinetics of pH adjustment. The benefit of this capacity is not clear, but it is possible that the capability to maintain metabolic depression is of great advantage in

an environment where temperature and other physical parameters may change drastically throughout the year.

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