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Oxygen limited thermal tolerance in fish? Answers obtained by nuclear magnetic resonance techniques

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Abstract

In various phyla of marine invertebrates limited capacities of both ventilatory and circulatory performance were found to set the borders of the thermal tolerance window with limitations in aerobic scope and onset of hypoxia as a first line of sensitivity to both cold and warm temperature extremes. The hypothesis of oxygen limited thermal tolerance has recently been investigated in fish using a combination of non-invasive nuclear magnetic resonance (NMR) methodology with invasive techniques. In contrast to observations in marine invertebrates arterial oxygen tensions in fish were independent of temperature, while venous oxygen tensions displayed a thermal optimum. As the fish heart relies on venous oxygen supply, limited cardio-circulatory capacity is concluded to set the first level of thermal intolerance in fish. Nonetheless, maximized ventilatory capacity is seen to support circulation in maintaining the width of thermal tolerance windows. The interdependent setting of low and high tolerance limits is interpreted to result from trade-offs between optimized tissue functional capacity and baseline oxygen demand and energy turnover co-determined by the adjustment of mitochondrial densities and functional properties to a species-specific temperature range. At temperature extremes, systemic hypoxia will elicit metabolic depression, thereby widening the thermal window transiently sustained especially in those species preadapted to hypoxic environments.

Keywords: Fish; Teleost; Hypoxia; Thermal tolerance; Metabolism; Cold adaptation; Temperature; Thermal tolerance; Hypoxia

1. Introduction: a role for hypoxia in thermal limitation?

The physiological mechanisms setting thermal tolerance and defining thermal sensitivity have recently come into focus due to rising interest in the effects of climate change on organisms and ecosystems. In this context, the question has regained interest whether limitations in oxygen availability or supply are involved in thermal limitation. Early evidence collected in marine invertebrates (annelids and sipunculids) demonstrated a transition to anaerobic metabolism (including mitochondrial anaerobiosis) at both cold and warm temperature extremes (Zielinski and Pörtner, 1996; Sommer et al., 1997), later on confirmed in crustaceans (Frederich and Pörtner, 2000) and molluscs, i.e. bivalves, gastropods and cephalopods (Pörtner and Zielinski, 1998; Pörtner et al., 1999; Peck et al., 2002; Sokolova and Pörtner, 2003). Studies in a sipunculid (*Sipunculus nudus*,

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Zielinski and Pörtner, 1996) and the spider crab (*Maja squinado*, Frederich and Pörtner, 2000) investigated the pattern of coelomic fluid/haemolymph oxygen tensions in relation to warming and/or cooling and demonstrated development of hypoxia which preceded the onset of anaerobic metabolism towards both cold and warm temperature extremes. A temperature dependent optimization of oxygen supply capacity was visible in the form of maximized arterial P_{O_2} within a limited temperature range, equivalent to the window of mean temperatures in the natural environment (Frederich and Pörtner, 2000). Limitation of both ventilatory and circulatory capacities towards thermal extremes was found to explain these patterns of body fluid oxygenation (Zielinski and Pörtner, 1996; Frederich and Pörtner, 2000).

Such a restriction of optimized oxygen supply to a limited thermal window demonstrated that temperature extremes are suitable to induce hypoxia in the organism despite ample oxygen supply from the environment. Work on temperate invertebrates and their populations in a latitudinal cline demonstrated a shift of oxygen dependent thermal thresholds depending on the ambient climate regime and winter or summer seasons (Sommer et al., 1997; Sommer and Pörtner, 1999, 2002). Inclusion of Antarctic marine invertebrates in this picture revealed very narrow windows of thermal tolerance in these organisms, in a temperature range just above freezing. An early transition to "heat" induced anaerobiosis between 2 and 6 °C seen in bivalves reflected the permanently low temperatures of Antarctic seas (Pörtner et al., 1999; Peck et al., 2002).

The work of Zielinski and Pörtner (1996), Sommer et al. (1997), Pörtner et al. (1999) and Frederich and Pörtner (2000) in marine invertebrates led to the concept of oxygen-limited thermal tolerance, which suggests that towards cold or warm extremes progressively inadequate oxygen supply and thus, decreasing body fluid oxygen levels finally lead to temperature induced anaerobiosis. Terminology was applied by adopting the one used in Shelford's law of tolerance (Shelford, 1931). In the spider crab *Maja squinado*, early limits of thermal tolerance during both heating and cooling were indicated by a set of low and high *peius* temperatures (T_p), which denote the beginning of insufficient oxygen supply to an organism, or in other words, the onset of mildly hypoxic conditions associated with a progressive loss in aerobic scope. This transition occurs in fully oxygenated environments. The point of transition where increasing internal hypoxia at more extreme temperatures finally leads into anaerobiosis, was termed *critical* temperature, T_c (Frederich and Pörtner, 2000).

Evidently, adaptations to ambient temperature and oxygen levels were found closely related in the marine invertebrate species studied. The question arose early on whether the concept of an oxygen limitation of thermal tolerance is applicable to (aquatic) vertebrates, especially marine teleost fish. Initial evidence demonstrated that the indicator of mitochondrial anaerobiosis, succinate, accumulated in liver of North Sea eelpout, *Zoarces viviparus*, during heat stress (Van Dijk et al., 1999). Particularly in fish, however, further study of temperature dependent oxygen limitation (or vice versa, oxygen limited thermal tolerance) proved difficult, firstly due to more limited hypoxia tolerance and stress resistance of most fish compared to invertebrates and secondly due to limited accumulation of anaerobic mitochondrial end products like succinate, especially in bulk tissues like white muscle. To monitor the onset of temperature induced hypoxia, particularly the early stages of transition from normoxic to hypoxic conditions, and in order to overcome these constraints, non-invasive whole animal experiments or, alternatively, a combination of non-invasive and invasive techniques proved useful. Obtaining the respective evidence in fish was supported by recent developments in the non-invasive techniques of nuclear magnetic resonance imaging and spectroscopy (MRI and MRS) and their applicability to unrestrained, non-anaesthetized aquatic animals. The present study is intended to review these accomplishments and the available information on thermal limitation in (marine) fishes. From a wider perspective, it also examines to what extent hypoxia induced hypometabolism may support survival at thermal extremes. The respective findings are in line with the results obtained in invertebrates and suggest that thermal limitations in oxygen supply occur in fish, however, with an emphasis on a limiting role for circulation rather than ventilation. These comparative analyses have thus supported and are in line with a unifying conceptual framework of the physiological principles setting thermal tolerance windows and of the key mechanisms of thermal adaptation and limitation (Pörtner, 2001, 2002a,b).

2. Methodological developments

Over the last 2 decades magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI) techniques have extensively been used to study hypoxia with its immediate and drastic effects on cellular energy metabolism and acid-base regulation. In vivo experiments were carried out in invertebrates like lugworms (Kamp et al., 1995), mussels (Tjeerdema et al., 1991; Shofer et al., 1998, Shofer and Tjeerdema, 1998) or prawns (Raffin et al., 1988; Thébault and Raffin, 1991) and fish. However, the latter were anaesthetized and restrained during early studies. Various review articles about NMR applications in comparative physiology already exist in the literature (e.g. Ellington and Wiseman, 1989; Van den Thillart and Van Waarde, 1996). Our emphasis in this review is on the contribution of MRS and MRI combined with invasive techniques to studies of temperature hypoxia interactions, in the context of testing the concept of oxygen limited thermal tolerance in fish.

The early NMR investigations of the effects of environmental hypoxia on fish muscle energy metabolism started by Van den Thillart et al. (1989a). They developed a flow through probe for in vivo ^{31}P-NMR spectroscopy in a 9.4 T vertical NMR spectrometer (Van den Thillart et al., 1989b). The set-up allowed long term online recordings of energy metabolism in muscle of carp, tilapia and goldfish during anoxia and hypoxia by means of ^{31}P-NMR spectroscopy. Data recordings at 10 min intervals were characterized by high signal to noise ratios. Control values indicated by a high phosphocreatine to inorganic phosphate ratio (PCr/Pi) were reached after 2 h and did not change significantly over an experimental period of 8 h. Hypoxia induced a rapid decline in the PCr/Pi ratio accompanied by a drop in intracellular pH (Van den Thillart et al., 1989a). Return to normoxic control conditions occurred within 3 h in carp as well as in goldfish. Besides the determination of high energy phosphate concentrations, of inorganic phosphate and of intracellular pH, free ADP concentrations were calculated from the equilibrium of creatine kinase (Van Waarde et al., 1990) and provided insight into the functional coupling of phosphocreatine utilisation and glycolysis in these three species in vivo. Nevertheless, the animals had to be anaesthetized prior to experimentation and were fixed in a vertical position inside the probe, limiting experiments to more robust fish species like carp or eel.

Blackband and Stoskopf (1990) reported the first combined MR imaging and spectroscopy studies in marine, albeit anaesthetized, fish. They focused mainly on the feasibility of NMR experiments with marine animals and did not describe any dynamic observations or metabolic patterns. Eight years later, Borger et al. (1998) reported in vivo ^{31}P-NMR experiments with common carp using a similar approach as the one described by van den Thillart. However, this time the fish was placed in a horizontal MR scanner at a magnetic field strength of 7 T. These experiments investigated the combined effects of temperature (acclimation as well as rapid change) and hypoxia on fish energy metabolism over several hours with a temporal resolution of minutes. These in vivo ^{31}P-NMR observations confirmed a negative correlation between temperature and intracellular pH in fish muscle in accordance with the alphastat pH regulation hypothesis developed by Reeves (1972). However, these experiments again involved the shortcomings of pre-experimental anaesthesia and fixation of the animal. Such experiments preclude long term analyses (for days or even weeks) especially of delicate organisms like polar animals or of animals displaying some of their normal physiological activities under resting conditions.

The shortcomings involved in studies of anaesthetized or immobilized animals were finally overcome when MRI and MRS experiments were successfully carried out in non-anaesthetized, unrestrained marine teleosts like benthic zoarcids (eelpout) or demersal gadids like Atlantic cod (Bock et al., 2001; Mark et al., 2002; Sartoris et al., 2003a,b). The experimental set-up (Fig. 1) allowed long term MRS and MRI experiments for more than 8 days. Polar organisms like Antarctic eelpout (*Pachycara brachycephalum*) were studied under controlled and stable conditions allowing extensive physiological monitoring with high localized and temporal resolutions. Animals were not even anaesthetized prior to experimentation, thereby excluding possible long-term effects of narcotics (Iwama et al., 1989). The fish usually recovered from handling stress within 1 h. Excellent resting conditions were reflected by extremely high and constant PCr/Pi ratios and stable intracellular pH values. The fish was free to move inside the chamber, but were imperturbable even during

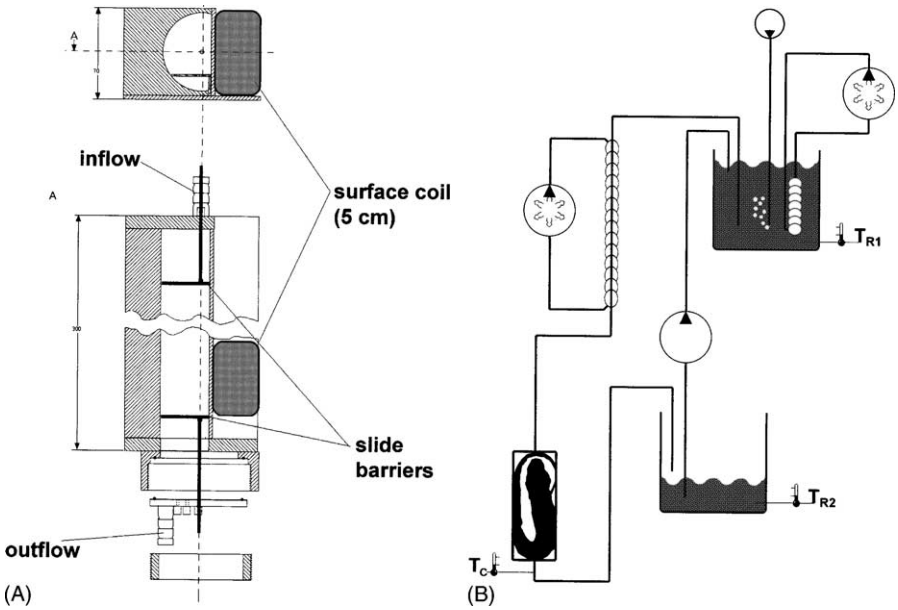


Fig. 1. (A) Experimental set-up for in vivo NMR and MR studies of un-anaesthetized and un-restrained fish (adapted from Bock et al., 2001). The animal is positioned inside the chamber with slide barriers parallel to a NMR surface coil, ensuring the mobility of the fish. (B) Water was continuously supplied from a temperature controlled water reservoir (minimum 50 l, T_{R1}) by hydrostatic pressure. Temperature stability in the chamber and reservoirs was ±0.5 °C as confirmed by continuous T-measurements (T_C, T_{R1}, T_{R2}).

periods of MR scanner sounds; therefore MR images were obtained with high anatomical resolution allowing for localized MR spectroscopy of different organs (Bock et al., 2001, 2002a). For example, Fig. 2 depicts perfectly localized in vivo ^{1}H-NMR spectra obtained in embryos of the North Sea eelpout *Z. viviparus*.

This methodology proved applicable to demersal, more mobile fish. Fig. 3 presents a stack plot of in vivo ^{31}P-NMR spectra from North Sea cod *Gadus morhua* during hypoxia and recovery. The time interval was 5 min between each spectrum. Under control conditions, only NMR signals from the high-energy phosphates PCr and ATP could be detected, inorganic phosphate signals did not even reach noise levels, indicating minimal activity levels and undisturbed resting conditions of the fish. Onset of hypoxia resulted in an immediate increase of inorganic phosphate levels at the expense of phosphocreatine. Values returned to control levels within 15 min of post-hypoxic recovery (Fig. 3). In the meanwhile, this technology has been developed even further to allow online study of tissue energetics of unrestrained swimming cod in swim tunnels fed through the NMR system (Pörtner et al., 2002; Bock et al., 2002b).

3. Evidence for temperature induced hypoxia in fish

In fish, heart rate and both ventilation frequency and amplitude have frequently been reported to increase in association with a temperature-induced rise in oxygen consumption in order to compensate for elevated oxygen demand by progressively enhanced oxygen supply (Barron et al., 1987; Graham and Farrell, 1989; Mark et al., 2002). According to the concept of oxygen limited thermal tolerance (see Section 1) onset of thermal limitation should be elicited by limited capacity of oxygen supply mechanisms to match oxygen demand beyond low or high peius temperatures (T_p, see above), thereby eliciting a drop in aerobic scope. In NMR experiments monitoring of blood flow changes by a flow weighted MR imaging sequence was combined with localized ^{1}H-NMR spectroscopy in the North Sea eelpout *Z. viviparus* at different tem-

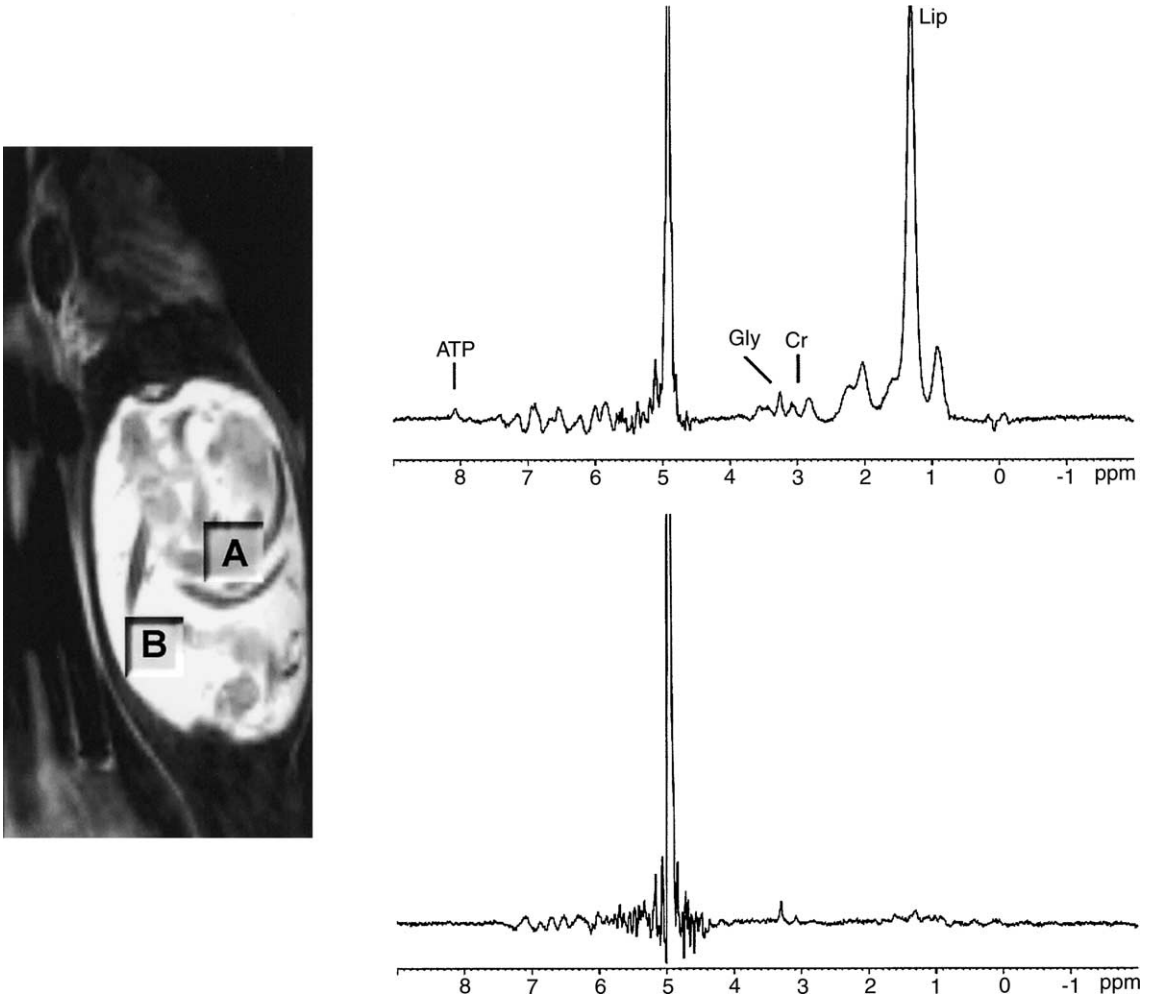


Fig. 2. Anatomical MR image of a pregnant specimen of the North Sea eelpout *Zoarces viviparus*. Localized in vivo ^{1}H-NMR spectra obtained in two voxels are presented on the right. Metabolites in spectrum (A) originated from embryonic fish, whereas almost no signal (except for residual water) arose in spectrum (B) where no embryo was present (after Bock et al., 2002a).

peratures. Progressive warming resulted in increased arterial and venous blood flow until it remained unchanged despite a continued rise in oxygen demand (Van Dijk et al., 1999; Zakhartsev et al., 2003). Above a critical temperature invasive work reported succinate accumulation in liver (Van Dijk et al., 1999). In the MR studies a sudden drop in blood flow was observed and lactate accumulation in the white muscle of the fish was detected in localized ^{1}H-NMR spectra (Fig. 4). Lactate as an anaerobic end product is a more indirect marker for cellular hypoxia than succinate. It nonetheless indicates that critical metabolic conditions were reached at this temperature; consequently, the animal died.

These findings already indicated closely coordinated adaptation to ambient temperature and oxygen levels as derived for the marine invertebrates. In consequence, Zakhartsev et al. (2003) studied the temperature dependence of the critical oxygen tension (P_c) in eelpout, *Z. viviparus*. The P_c was determined as the oxygen tension below which the rate of oxygen consumption fell below the regulated value when the animal was exposed to progressive hypoxia. Zakhartsev et al. reported that the P_c rose linearly depending

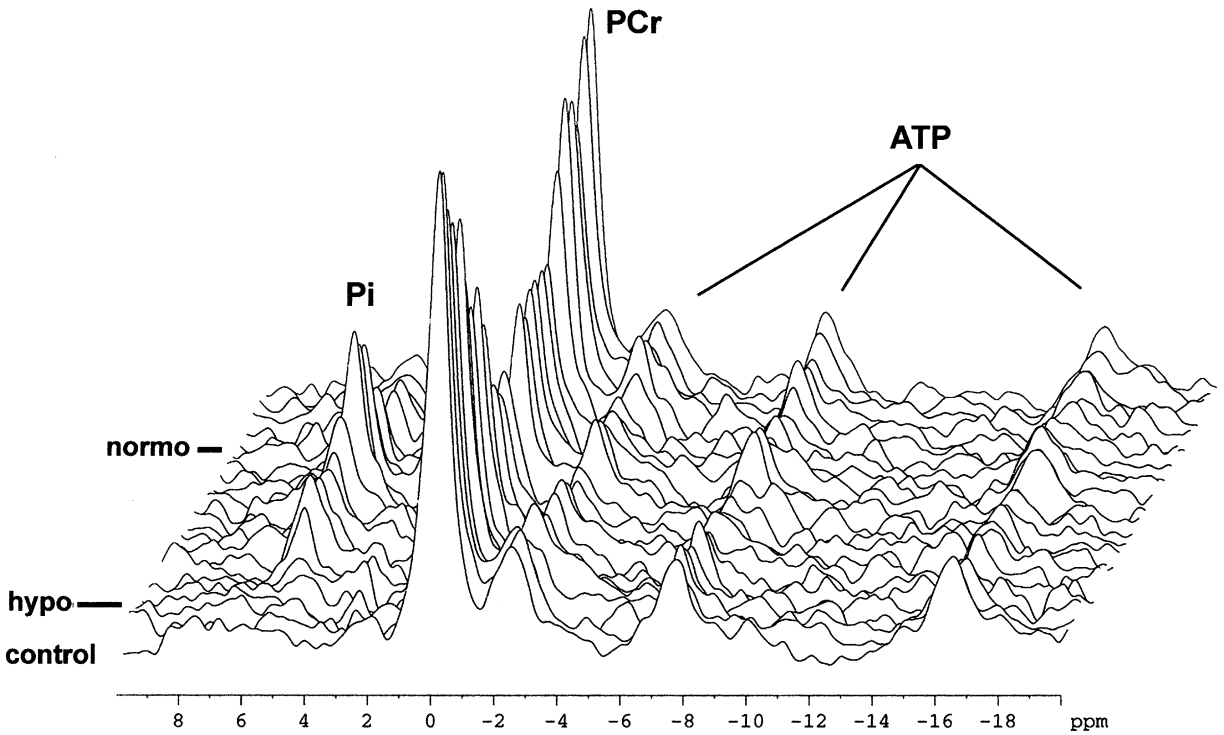
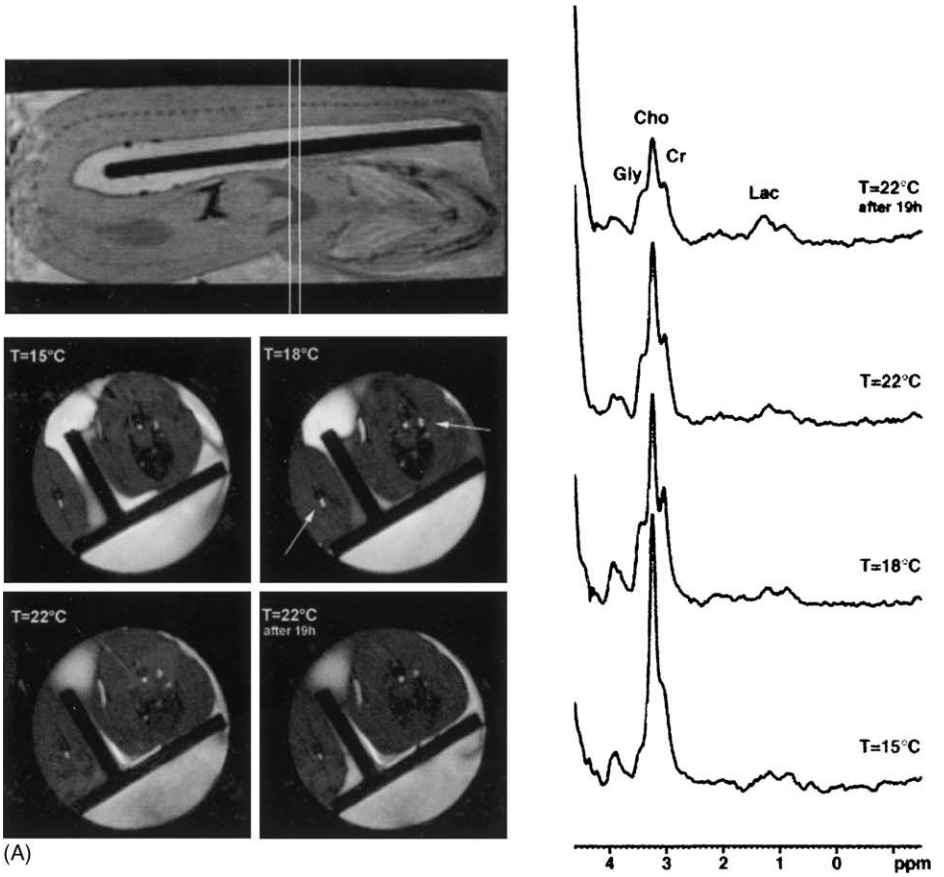


Fig. 3. Stack plot of in vivo ^{31}P-NMR spectra collected in Atlantic cod *G. morhua* during normoxia (front), hypoxia and post-hypoxic normoxia (after Bock et al., 2002a). Time resolution between spectra was 5 min. Note the drastic decrease of phosphocreatine and increase of inorganic phosphate under hypoxia. Switching to normoxia caused values to return to control levels within 15 min.

on water temperature and would reach normoxic levels at those temperatures where Van Dijk et al. (1999) had found the critical temperature and onset of mitochondrial anaerobiosis.

Mark et al. (2002) tested whether temperature induced hypoxia was alleviated by ambient hyperoxia in the Antarctic eelpout *P. brachycephalum*. They monitored the effects of temperature on oxygen demand, ventilatory effort and blood flow at normoxic and hyperoxic oxygen levels (Fig. 5). Under normoxia arterial blood flow rose distinctly between 0 and 7 °C. It reached a plateau above 7 °C, possibly due to a limitation of heart and/or vascular capacity. In contrast, experiments carried out under hyperoxia ($P_{O_2} = 45$ kPa, ~2-fold normal O_2 tension) did not cause a significant rise in blood flow in the *Aorta dorsalis*, in line with an alleviation of temperature induced oxygen shortage by hyperoxia. At elevated temperatures this effect was also clear from significantly lower oxygen consumption rates under hyperoxia than seen under normoxia. In fact, hyperoxia alleviated the "typical" exponential increase in oxygen consumption with temperature,

Fig. 4. (A) Axial views of flow weighted MR images of eelpout (*Zoarces viviparus*) from the North Sea at different temperatures. Blood flow in vessels, visible as bright spots, increased with temperature (see arrows). At a water temperature of 22 °C blood flow dropped abruptly after 19 h accompanied by an increase of lactate (Lac) in localized ^{1}H-NMR spectra from white muscle, indicating that the critical temperature was reached. (B) Development of arterial blood flow between 10 and 22 °C showed an early increment but no further rise despite increased oxygen demand. Similar to observations in Antarctic eelpout (Fig. 5) and in cod (Lannig et al., 2004) transition to saturated blood flow velocity is interpreted to reflect the peius temperature which indicates onset of a loss in aerobic scope. In *Z. viviparus*, T_p was found at 14 °C while the critical temperature was reached at 22 °C, in line with earlier observations of succinate accumulation (Van Dijk et al., 1999; modified after Bock et al., 2002a and unpublished).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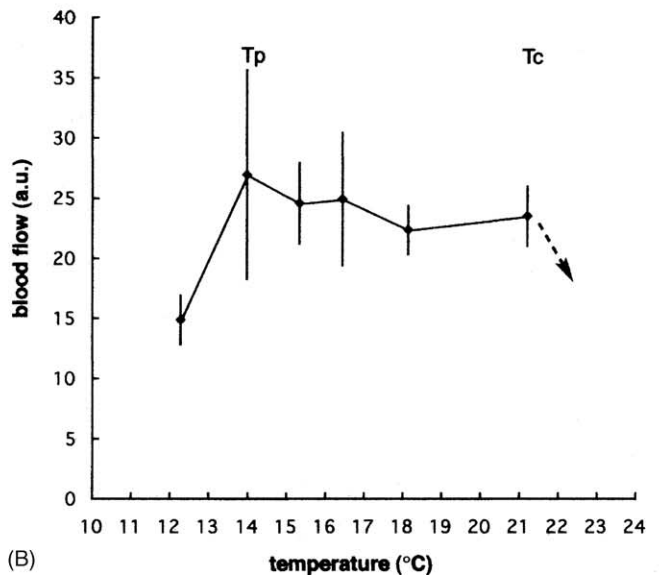
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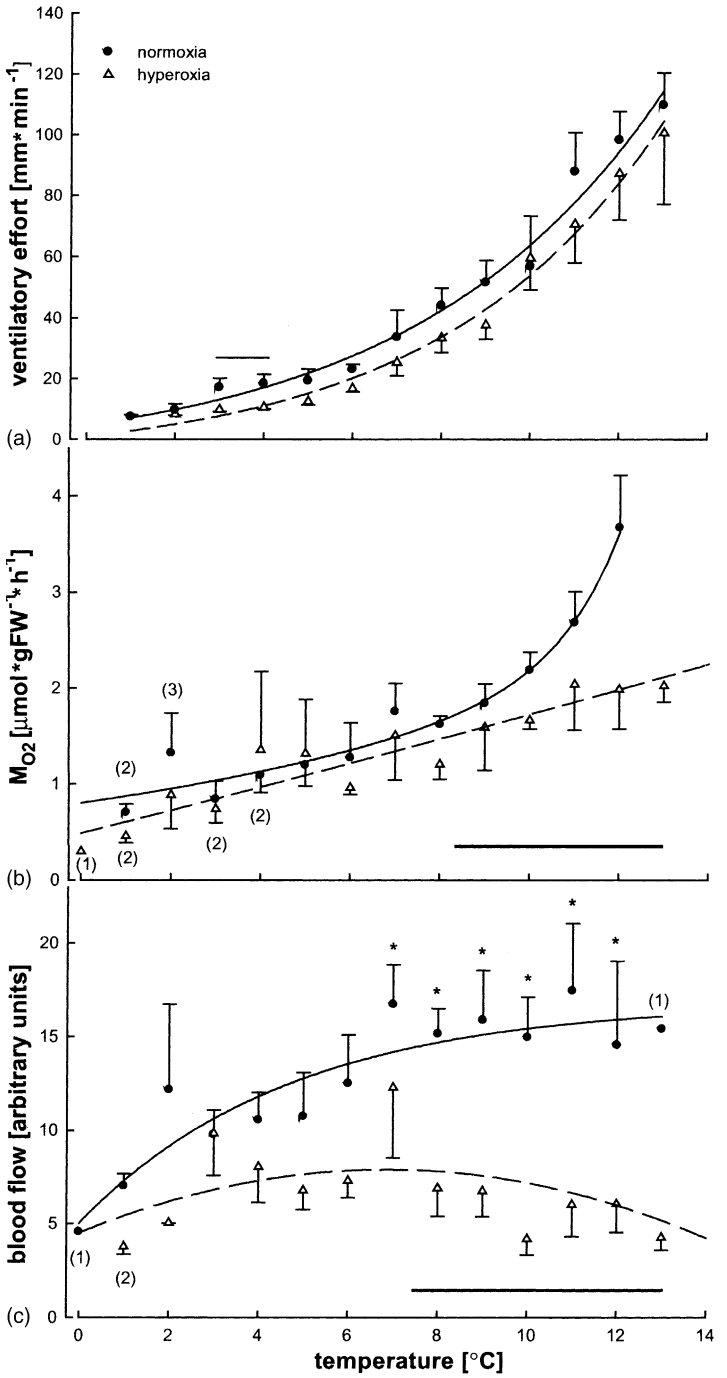

Fig. 5. Ventilatory effort (a), oxygen consumption (b) and blood flow (c) of Antarctic eelpout *P. brachycephalum* at different temperatures and external oxygen levels. In contrast to ventilation and oxygen consumption blood flow levelled off at higher temperatures. Interestingly, hyperoxia alleviated the effect of warming on systemic parameters (after Mark et al., 2002). The drop in oxygen consumption observed under hyperoxia at high temperatures indicates reduced cost of circulation due to ample oxygen supply. In contrast to *Z. viviparus*, T_p under normoxia was reached at about 7 °C in *P. brachycephalum*.

very likely due to energy savings in the cardiocirculatory system due to enhanced oxygen availability. Ventilatory effort under both treatments did not reveal a significant effect of hyperoxia as it followed the same exponential increment, regardless of ambient oxygen levels.

These findings indicated that the Antarctic eelpouts became hypoxic because of a temperature induced systemic limitation of oxygen supply at rising oxygen demand, which was alleviated by hyperoxia. At similar ventilation rates higher oxygen consumption and limited blood flow under normoxia on the one hand and reduced oxygen consumption and blood flow at elevated temperatures under hyperoxic conditions on the other hand suggested that the cardiovascular rather than the ventilatory system responds to changes in systemic oxygen availability and may thus play a key role in thermal tolerance in these Antarctic fish (Mark et al., 2002). Nonetheless, although hyperoxia likely widened the temperature range of available aerobic scope in the Antarctic eelpout, it did not cause large shifts of the limits of passive heat tolerance. A recent treatment of thermal limits in various systematic groups from prokaryotes via unicellular eukaryotes to metazoa suggested a systemic to molecular hierarchy of thermal limitation with the narrowest windows found at the highest levels of organisational complexity (Pörtner, 2002a). At the same time, the concept of symmorphosis indicates that the functional capacities of individual components contributing to the performance capacity of the higher unit, i.e. the organism, are usually not expressed in excess. With respect to the functional capacities setting thermal tolerance this would mean that, once the limits at the highest level are alleviated (in this case by hyperoxia), those at a lower (i.e. cellular or molecular) level of complexity may now predominate in limiting whole organism thermal tolerance at a slightly but not hugely widened window of thermal tolerance.

Studies carried out in Atlantic cod, *G. morhua* from the North Sea and in rainbow trout, *Oncorhynchus mykiss*, support a crucial role of the circulatory system in thermal limitation and temperature dependent aerobic scope and suggest that this may be a general pattern in fish. Early data by Heath and Hughes (1973) would also match this interpretation. They found that heart rate in rainbow trout decreased at temperatures above 24 °C, whereas ventilation remained virtually unchanged until death of the animals occurred. In cod implanted with micro-optodes in gill blood vessels, Sartoris et al. (2003b) demonstrated that arterial oxygen tensions (P_{a,O_2}) remained unaffected by progressive warming. However, venous oxygen tension (P_{v,O_2}) dropped progressively during warming, in line with limited cardiac rather than ventilatory performance. It was concluded that in resting cod at elevated temperatures, circulatory performance cannot fully compensate for excessive oxygen extraction from the blood (Pörtner et al., 2001; Sartoris et al., 2003b). Functionally, this pattern can seriously hamper myocardial oxygen supply, as most teleost fish lack or only possess a weak coronary circulation and hence almost exclusively rely on the venous oxygen reserve to provide the heart with oxygen (Farrell, 1993).

Farrell and Clutterham (2003) measured venous oxygen tension in the *ductus Cuvier* of rainbow trout, *O. mykiss*, during exercise at different acclimation temperatures. They discussed that a specific threshold P_{v,O_2} is required in fish in order to maintain sufficient oxygen supply to the myocardium and support cardiac output. Accordingly, a reduction in aerobic scope is likely to result when a temperature dependent decrease in venous oxygen tension (P_{v,O_2}) sets in. In line with these findings and with a limited temperature window of optimum oxygen supply, Farrell (2002) found maximum cardiac output of exercising salmonids within the optimal temperature range. Similarly, cod make use of their full aerobic capacity and use both the glycolytic and oxidative capacities of their musculature to support endurance swimming under 'normal' thermal conditions (Martinez et al., 2003).

At constant levels of arterial oxygen tension (P_{a,O_2}) temperature dependent patterns of venous oxygen tension (P_{v,O_2}) should therefore delineate the window of thermal tolerance in fish in similar ways as previous recordings of arterial oxygen tensions in a crustacean (Frederich and Pörtner, 2000). In a combination of invasive oxygen analyses and measurements of blood flow by MRI, Lannig et al. (2004) found a decrease in venous P_{O_2} towards both sides of the thermal optimum. Resting heart rate in cod (*G. morhua*) rose exponentially upon warming from 10 to 16 °C. However, arterial and venous blood flow rose only slightly and did not compensate for the drop in venous P_{O_2} in the warm. Hence, loss of optimized oxygen sup-

ply to the heart and thus, decreased aerobic scope of the whole organism seems to be the first limiting factor in these fish. The maintenance of arterial P_{O_2} seen during warming in cod is in line with an excess capacity of the ventilatory system for oxygen uptake, if compared to oxygen distribution via circulation. However, arterial P_{O_2} fell drastically above 16 °C (Sartoris et al., 2003b), presumably indicating the temperature at which a minimum threshold P_{v,O_2} is reached in cod with the result of cardiac failure and organismic collapse.

As a corollary, the capacity of the teleost circulatory system likely becomes insufficient in the warm to match the rising oxygen demand. The question then arises how oxygen limitation develops in the cold. In the cold, oxygen provision appears facilitated due to high oxygen solubility in ambient water and body fluids. Moreover, oxygen diffusion should be facilitated in relation to oxygen demand as diffusion decreases less with temperature than baseline metabolic costs which are reflected in the level of standard metabolic rate (Q_{10} of ∼1.1 versus ∼2–3). The data obtained under hyperoxia in Antarctic eelpout (Mark et al., 2002) suggest that enhanced oxygen availability as in cold waters should allow for a reduction in the energy cost of circulation and ventilation and thereby support the reduction in metabolic rates typically seen in Antarctic stenotherms. Energy savings are also supported by the increasing importance of cutaneous uptake of O_2 in the cold, culminating in a 30% contribution of cutaneous oxygen uptake to standard metabolic rate (SMR) in the Antarctic icequab *Rigophila dearbornii* (Wells, 1986). Low metabolic rates at enhanced oxygen solubility in body fluids enable icefish (*Chaennichthyidae*) to survive without red blood cells that greatly contribute to blood viscosity (Davison et al., 1997)—an alternative way to cut cardiovascular costs at low temperatures.

Nonetheless, oxygen supply capacity becomes limiting in temperate water breathers exposed to cold temperatures, observed in cod (Lannig et al., 2004) in similar ways as seen in a temperate crustacean (Frederich and Pörtner, 2000) or in annelids and sipunculids (Zielinski and Pörtner, 1996; Sommer et al., 1997). The drop in venous P_{O_2} (in fish) or arterial P_{O_2} (in the crustacean) and the transition to anaerobic metabolism in the cold indicate a limited functional capacity of oxygen supply mechanisms likely elicited by cold induced slowing. Functional capacity of oxygen supply mechanisms and the muscular tissues involved falls below the one to cover metabolic requirements at low ambient temperatures, thereby setting the first limit to cold tolerance. The upregulation of mitochondrial densities in the cold discussed below indicates that loss in mitochondrial functional capacity contributes to the limited functional capacity of cells and organs and is therefore compensated for in the cold. Again, the symmorphosis concept would predict that cold induced limitations in oxygen supply are likely to be closely followed by limitations in the capacity of other systemic, cellular and molecular functions such a general functional collapse of the organism occurs beyond but close to the limits set by insufficient oxygen supply. These relationships warrant further investigation.

4. Trade-offs in thermal adaptation setting functional limits

The question arises which mechanisms cause animals and their oxygen supply systems to specialize on a limited range of thermal tolerance which matches the thermal range which a species usually experiences in its natural habitat. In particular, invertebrates and fish adapted to the stable temperatures of Antarctic waters rely on narrow thermal windows. As oxygen limitations set in at both sides of the temperature window it appears likely that mechanisms are involved which define oxygen demand in relation to the capacity of oxygen delivery by circulation and ventilation such that tissue functional capacities (esp. of the heart) are set to a level sufficient to match maximum oxygen demand between the average highs and lows of environmental temperatures. The responsible mechanisms should also characterize the trade-offs involved in thermal adaptation, i.e. they should explain why a downward shift of the oxygen limited cold tolerance threshold coincides with an increase in heat sensitivity and vice versa.

The mitochondrial metabolic background of setting both tissue and organismic functional capacity and oxygen demand at various temperatures has contributed to an understanding of the links between low and high thermal limits and thus, the trade-offs in thermal adaptation (Pörtner et al., 1998, 2000; Pörtner, 2002a). In ectothermic species, especially

fish a plethora of studies have identified mechanisms of seasonal and latitudinal cold versus warm adaptation which are suitable to modulate the capacity of aerobic metabolism. As outlined above, a rise in aerobic capacity permits maintenance of cell functions in the cold (for review Guderley, 1998; Pörtner et al., 1998, 2001). Associated processes are rising enzyme capacities (Crockett and Sidell, 1990; Guderley, 1990; Lannig et al., 2003), increased mitochondrial or capillary densities (Sisson and Sidell, 1987; Guderley and Blier, 1988; Ressel, 2001), changes in mitochondrial structure (Gaebel and Roots, 1989; St.-Pierre et al., 1998) and/or alterations in membrane composition (Miranda and Hazel, 1996; Logue et al., 2000). Mitochondrial densities are found esp. large in pelagic notothenioid fishes of the Antarctic, where densities in red muscle result beyond 50% (Dunn et al., 1989). Recent evidence indicates that the thermal adaptation of marine invertebrates follows similar principles (Sommer and Pörtner, 2002) which therefore appear unifying in thermal adaptation.

In the context of the concept of oxygen limited thermal tolerance, these mechanisms contribute to a unidirectional shift of both low and high peius and critical temperatures. Such a shift is associated with a change in mitochondrial density, which drops as temperature rises and increases as ambient temperature falls. The main advantage of an increase in mitochondrial density and activity in the cold is an increase in aerobic functional capacity. The associated increase in the network of intracellular membrane lipids constitutes a significant facilitation of intracellular oxygen diffusion in the cold (Sidell, 1998; cf. Pörtner, 2002b). In the cold, the improvement of aerobic energy production also supports the capacities of ventilation and circulation (Pörtner, 2001). As a consequence of elevated mitochondrial densities, however, baseline oxygen demand by mitochondria is enhanced, set by the level of mitochondrial proton leakage. This will cause earlier problems during warming, where associated with a rise in other baseline costs the overall increment in oxygen demand can no longer be met by the capacity of oxygen supply mechanisms. A trade-off results between the compensation of functional capacity in the cold and the resulting increase in baseline oxygen demand which contributes to lower the limits of heat tolerance. In this context, recent evidence indicates that these mechanisms of cold adaptation are likely modulated in Antarctic stenotherms in order to minimize the cost of cold adaptation below the one seen in cold adapted eurytherms (Pörtner et al., 2000). Especially in temperate to high latitudes of the Northern hemisphere several species are found eurythermal and, thus, experience high costs of cold adaptation (e.g. Sommer and Pörtner, 2002) associated with trade-offs in energy budget and their likely ecological consequences (Pörtner et al., 2000, 2001, 2004). Such differences will also have their bearing with respect to the sensitivity of animal species to climate and associated temperature change.

The mechanistic and regulatory bases of the processes setting thermal tolerance and defining thermal adaptation as well as their integration into whole animal functioning are still incompletely understood. Thermal adaptation is linked to temperature dependent gene expression, for example of key aerobic enzymes, as seen during seasonal as well as latitudinal cold adaptation (e.g. Hardewig et al., 1999; Lucassen et al., 2003). Rearrangements of aerobic metabolism also occur with a shift to lipid accumulation and energy storage (cf. Pörtner, 2002b, for review). The fine tuning of these processes on a temperature scale or their functional consequences at the whole animal level remain to be quantified and the regulatory signals to be identified.

The mitochondrial trade-offs addressed above will relate to changes in functional capacity and oxygen demand of more or less all cells of the organism and these patterns transfer to the next hierarchical level, the functional capacity of tissues like the cardiovascular system and finally of the organism. Further trade-offs apply at the organismic level, like for the cardiovascular system of fish which supplies oxygen to tissues on the one hand but on the other hand relies on supply from residual oxygen in venous blood. Therefore, it is cost-effectively designed to consume rather small amounts of oxygen itself (Farrell and Clutterham, 2003). This constraint limits the development of functional capacity and may be the key reason why in fish the circulatory system appears more crucial in thermal limitation than the ventilatory system. However, compared to invertebrates and higher vertebrates, where cardiac supply is via arterial blood or haemolymph, the excess ventilatory capacity observed in fish may in fact be related to the unusual pattern of venous oxygen supply to the fish heart. This

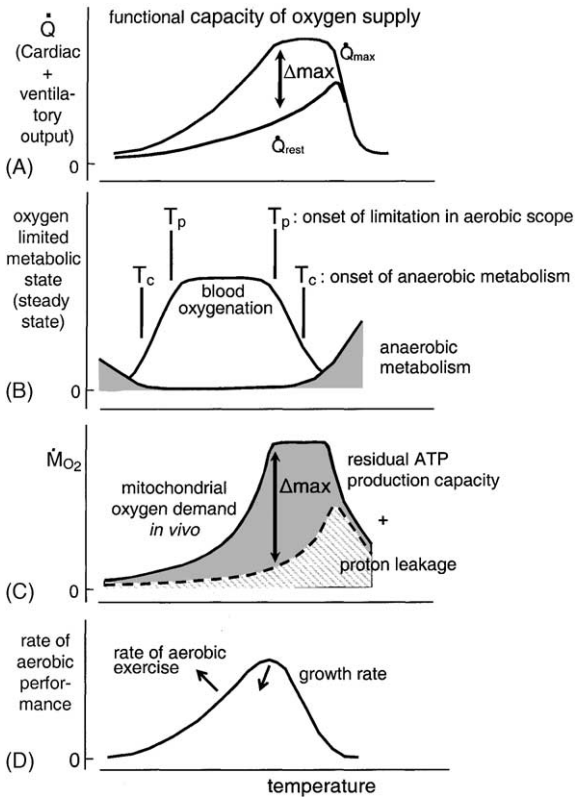

Fig. 6. Schematic model of oxygen limited thermal tolerance and performance capacity in fish and other metazoa, set by the capacity of oxygen supply mechanisms. (A) Functional reserves in oxygen supply result as combined ventilatory and cardiac output (Q) and are maximum at the upper pejus temperature T_p, before aerobic scope becomes thermally limited (B). Maximum scope (Δ_{max}) between resting and maximum output in oxygen supply is likely correlated with the one in mitochondrial ATP generation such that the functional capacity of the (ventilatory and circulatory) muscles is co-defined by the capacity of mitochondria to produce ATP which is limited by oxygen supply in vivo (C). Part of this limitation is elicited by the temperature dependent rise in oxygen demand by the cost of mitochondrial proton leakage which is no longer available to ATP formation. Low ATP formation capacity in the cold and high proton leakage in the warm contribute to insufficient oxygen supply, loss of aerobic scope and finally, transition to anaerobic metabolism (B). Maximum scope in ATP generation at the upper T_p not only supports maximum capacity of organismic oxygen supply by circulatory and ventilatory muscles, but also an asymmetric performance curve of the whole organism (D, after Angilletta et al., 2002) with optimal performance (e.g. growth, exercise) again expected at the upper peius temperature T_p. Here, functions are supported by both high temperatures and optimum oxygen supply in relation to baseline oxygen demand. As a trade-off in eurythermal cold adaptation (e.g. upper limits constant, lower limits shifting to colder temperatures), standard metabolism and, in consequence, aerobic exercise capacity may increase in the cold (cf. Pörtner, 2002b), while temperature specific growth performance is reduced likely due to enhanced mitochondrial proton leakage (Pörtner et al., 2001). These contrasting changes in exercise capacity vs. those in growth rate are indicated by arrows in (D) (for further explanations, see text).

excess ventilatory capacity likely supports wider tolerance windows. Maximized arterial oxygen supply regardless of temperature would help to prevent an earlier drop in venous P_{O_2} below critical values and thus support the cardiovascular system in counteracting thermal limitation. From this point of view, a coordinated thermal limitation by integrated ventilatory and circulatory capacities as observed in a crustacean (Frederich and Pörtner, 2000) exists in teleosts as well; however, limitation is first experienced by the circulatory system. The evolutionary constraint of venous oxygen supply to the heart in fish leads to specific patterns of temperature dependent oxygenation in arterial versus venous blood. In conclusion and in similar ways as in marine invertebrates, the integrated cardiovascular and ventilatory capacities of oxygen supply to tissues appear as the first thermally sensitive functional level that defines a fish's thermal limits of biogeography.

The principle trade-offs leading to thermal optimization of cellular and tissue function will not only be valid for the circulatory system but for many tissues and finally the intact organisms although details and trade-offs at the tissue and organism levels need to be investigated. These principle relationships between temperature dependent capacities and limits of organismal performance have been summarized in Fig. 6 (based on studies across phyla, mostly in aquatic ectotherms such as sipunculids, annelids, crustaceans, molluscs and fishes, cited above). Trade-offs as outlined above support optimized performance only within a limited temperature window. Minimum and maximum peius temperatures (T_p) delineate the first level of thermal limitation and indicate onset of a loss in aerobic scope, as the capacities of oxygen supply (integrated capacities of circulation and ventilation) become progressively unable to meet oxygen demand. Once aerobic scope is reduced towards thermal extremes, critical temperature thresholds (T_c) delineate the transition to an anaerobic mode of metabolism or

passive tolerance. Extended exposure of animals to temperatures above high or below low critical temperatures finally leads to death of the animal unless thermal acclimatization, i.e. a shift of T_c values occurs (Zielinski and Pörtner, 1996; Sommer et al., 1997). Limitation of survival is associated with a drop of the cellular Gibb's free energy change of ATP hydrolysis to a low, possibly critical value (Zielinski and Pörtner, 1996). In accordance with a hierarchy of thermal tolerance limits, a failure in oxygen delivery system at the whole-organism level occurs prior to a failure in mitochondrial and then molecular functions, thereby setting the ecologically relevant thermal tolerance thresholds of the intact organism (Pörtner, 2002a).

Critical temperatures as discussed here border the temperature range that permits performance on top of baseline energy expenditure and are likely reached before the onset of spasms (Zakhartsev et al., 2003) which are traditionally used to define critical thermal maxima (Lutterschmidt and Hutchison, 1997a,b). Within the thermal tolerance window aerobic performance increases with temperature to a maximum and then decreases at higher temperatures yielding a species-specific asymmetric bell shaped curve, which shifts depending on thermal adaptation (Angilletta et al., 2002). Fig. 6 predicts that optimum performance occurs close to upper peius values linked to the maximum scope for ATP formation by mitochondria. The relationship between temperature dependent growth rates and aerobic scope may follow this pattern. Aerobic scope and growth rate were found related in a population of cod (Claireaux et al., 2000). Growth curves similarly shaped as in Fig. 6D were found in invertebrates (Mitchell and Lampert, 2000; Giebelhausen and Lampert, 2001) and in fish (Jobling, 1997). Moreover, protein synthesis rates will set the pace for organismic growth. Recent findings indicate that low blood oxygen tensions limit protein synthesis rates as seen in feeding crabs (Mente et al., 2003), thereby supporting the concept layed out in Fig. 6.

5. Temperature induced hypoxic hypometabolism?

The question arises whether temperature induced hypoxia and finally anaerobiosis at the edges of the thermal tolerance window have consequences other than reducing performance capacity of the organism. In principle, all processes will become involved that characterize survival strategies in hypoxia tolerant animals mainly through metabolic depression and associated passive tolerance of adverse environmental conditions as seen with respect to survival of turtles, frogs or fish during winter cold (Jackson, this volume). However, while transition to anaerobic metabolism has been clearly demonstrated in the turtles or goldfish other hibernating animals may succeed to use metabolic depression strategies while being fully aerobic. This is likely true for many hibernating amphibians and includes mammalian hibernators, where at least the brain remains fully aerobic, despite extremely cold body temperatures (Bock et al., 2002c). Passive hibernation is thus interpreted to be a strategy which allows the animal to survive at minimal cost and thereby escape from the costly mechanisms of eurythermal cold adaptation outlined above (Pörtner, 2004). The factors and mechanisms eliciting metabolic depression in excess of the one elicited by cold temperature itself are currently unknown.

Heat induced hypoxia will also elicit such responses which are likely beneficial to counteract the temperature induced acceleration of baseline metabolic costs. As the interdependence of thermal tolerance and aerobic scope have only recently been discussed as a unifying principle among animals (Pörtner, 2001) these relationships have not been systematically investigated. However, invertebrate examples from the intertidal zone where they may be exposed to midday sunshine and heat, would most adequately illustrate that extreme heat goes hand in hand with anaerobic metabolism and passive survival (Sokolova and Pörtner, 2003) and thus very likely involves a metabolic depression scenario which contributes to energy savings and thereby extends the period during which heat beyond the critical temperature can be tolerated.

6. Ecological perspectives

Temperature and global climate patterns have frequently been proposed as the most important factors governing marine zoogeography (Angel, 1991). Compared with terrestrial fauna, marine organisms cover larger ranges of geographical distribution and

exhibit distinct latitudinal zonation more frequently, especially in the near-shore environment where physical barriers prevent the migration of littoral species (Pielou, 1979; Rapoport, 1994). Along the same lines of thought identifying mechanisms of temperature adaptation (Johnston and Bennett, 1996) and their contribution to adjusting and limiting both cold and heat tolerance are considered important in the light of global warming (e.g. Wood and MacDonald, 1997; Pörtner et al., 2001) and the associated shifts in geographical distribution and/or physiological performance of ectothermic animals (Pörtner et al., 2001).

The ecological relevance of the physiological principles discussed in this chapter is emphasized by the observation that in the spider crab, *M. squinado*, upper and lower peius temperatures are more or less equivalent to the mean highs and lows of ambient temperature in the natural environment of this species (Frederich and Pörtner, 2000). This indicates that peius temperatures are prime candidates to relate to temperature dependent limits of geographical distribution. Due to the recent nature of the concept of oxygen limited thermal tolerance such patterns need to be investigated in more metazoan taxa and species including vertebrates and fish. The cellular and organismic principles of thermal limitation and adaptation outlined here may be influenced by overlying phylogenetic constraints of specific groups which may then contribute to modulate oxygen dependent thermal limits. For example, enhanced sensitivity to the anaesthetizing effect of magnesium reflects such a phylogenetic constraint in the special case of marine reptant decapod crustaceans (anurans and brachyurans). High magnesium levels in the haemolymph of this group likely limit its capability to adapt to extremely cold temperatures below 0 °C by the mechanisms outlined above and, thereby, excludes them from the respective temperature regimes in polar areas (Frederich et al., 2001). In contrast, marine ectothermic teleosts exist at all temperatures of the ocean. Although venous oxygen supply to the heart is an evolutionary constraint in teleost fish, it has not been found to limit cold adaptation capacity or biogeography of the whole group. Nonetheless, this constraint may limit the thermal range of individual species according to the trade-offs discussed above.

Some evidence indicates that adjustment to cold rather than warm temperatures is a more severe challenge for organismic physiology, in other words, small temperature changes on the cold side of the temperature spectrum may elicit larger effects than similar temperature changes in the warm. For example, warming by just 1 or 2 °C above ambient average temperatures will be fatal for extreme Antarctic stenotherms (Pörtner et al., 1999). The cost of adaptation to the same degree of cooling appears much larger at cold than at higher temperatures unless the width of thermal windows is minimized (Pörtner et al., 2000). This may be one reason for the impression that true stenotherms in the marine realm may only be found in polar esp. Antarctic cold (Pörtner, 2002a). Moreover, the decrease in biodiversity of extant marine macrofauna towards high, esp. Northern latitudes (Roy et al., 1998) may be due to the limited capacity of species to adapt to low but unstable temperatures. The picture is less clear for the southern hemisphere, where temperature oscillations are less expressed. However, a clear temperature dependent decrease in crustacean biodiversity has been shown by Astorga et al. (2003). The anaesthetizing effect of Mg^{2+} in decapod crustaceans is progressively increased during cooling and allows reptant decapods to live at 0 °C but no longer at −1 to −1.9 °C. In earth history decreasing winter temperatures by about 4 °C were associated with mass extinctions of marine invertebrates at the Eocene/Oligocene boundary despite constant summer temperatures (Ivany et al., 2000). During climate change scenarios alleviation of winter cold may, therefore, play a key role in changes of ecosystem structure and functioning, esp. in Northern temperate zones. As an example, in the North Sea, warmer water species immigrate including both invertebrates and fish (Hummel et al., 2001; Von Westernhagen and Schnack, 2001) while cold-water species like cod move further North (Fischer, 2002). Associated changes in biodiversity will have to be investigated.

Although the contribution of physiological constraints and capacities to these patterns remains to be clearly elaborated insight is only just emerging that the physiological basis for such ecological patterns may be associated with the width of the thermal tolerance window and its location on the temperature scale (Pörtner, 2002a,b). Temperature dependent shifts in geographical distribution may be related to the mismatch phenomena elaborated above and may be due to the limited phenotypic plasticity of the species involved, i.e. the limited capacity to shift the window of

oxygen limited thermal tolerance. A rising width of the thermal tolerance window, especially in the cold, was suggested to be associated with enhanced energy turnover due to the cost of cold adaptation. These relationships may elicit climate dependent trends in lifestyles and, last not least, biodiversity in ecosystems and will have to be considered in future analyses of such patterns.

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