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15 Levels of Metabolic Cold Adaptation: Tradeoffs in Eurythermal and Stenothermal Ectotherms

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ABSTRACT

A hypothesis is developed which links the phenomenon of metabolic cold adaptation in marine ectotherms with the level of temperature tolerance from an energetic point of view. Once temperature drops below a low or exceeds a high critical threshold (Tc) aerobic energy production becomes limiting indicated by low oxygen levels in the body fluids, the transition to an anaerobic mode of mitochondrial metabolism and progressive insufficiency of cellular energy levels. Both low and high Tcs are set by the failure of ventilatory mechanisms and/or the insufficiency of circulation in oxygen uptake and distribution. The transition to mitochondrial anaerobiosis is most pronounced in facultatively anaerobic marine invertebrates but is still visible in vertebrate tissues characterized by high mitochondrial densities and a high steady state energy demand.

Critical temperatures shift within, and differ between, populations depending on seasonal temperature acclimatisation and latitude. We propose that, among other processes, the adjustment of mitochondrial densities and, thus, aerobic capacity is critical in determining thermal tolerance. An increase of aerobic capacity during cold adaptation and, conversely, a decrease during warm adaptation will, therefore, lead to a shift of both critical temperatures to lower or higher values. In addition, the Tcs and the width of the tolerance window are likely to be set by the adjustment of kinetic properties of mitochondria, e.g. maximum respiratory as well as proton leakage rates and their temperature dependence. Potential mechanisms of proton leakage are discussed.

Adaptation to cold in general and especially to permanent cold may lead to an increasing temperature dependence of mitochondrial oxygen demand, especially proton leakage, and of flux limiting enzymes in metabolism. A hypothesis is presented which explains the maximization of Arrhenius activation energies (Ea) in some enzymes and the minimization of this term in others. The comparison of eurythermal species (from marine intertidal zones and shallow waters) in a latitudinal gradient with stenothermal animals (from polar environments including the Antarctic) suggests that the level of metabolic cold adaptation may depend upon the extent of diurnal and seasonal temperature fluctuations leading to higher costs of maintenance in eurythermal than in stenothermal animals. In stenothermal animals aerobic capacity and energy expenditure is minimized as far as possible according to environmental and lifestyle requirements. A model is developed which takes these considerations into account.

Key Words: Arrhenius activation energy, cold adaptation, critical temperatures, flux limiting enzymes, mitochondria, proton leakage, standard metabolic rate, transhydrogenase.

INTRODUCTION

Oxygen limitations at critical temperatures

Critical temperature thresholds (Tc) have been defined for various marine invertebrate species and most recently for fish as being due to the transition to an anaerobic mode of mitochondrial metabolism, once temperature reaches low or high extremes (Fig. 1, Zielinski and Pörtner 1996; Sommer *et al.* 1997; van Dijk *et al.* 1999; for review see Pörtner *et al.* 1998a). The transition to anaerobiosis occurs close to the lower and upper lethal temperature and indicates strict limits of tolerance. Critical temperatures differ between species and populations. They shift depending on latitude or seasonal temperature acclimatisation and are related to geographical distribution. For example, a within species comparison of *Arenicola marina* populations in a latitudinal gradient revealed that both low and high Tcs were lower in cold adapted, sub-Arctic animals from the Russian White Sea than in boreal, North Sea specimens (Sommer *et al.* 1997).

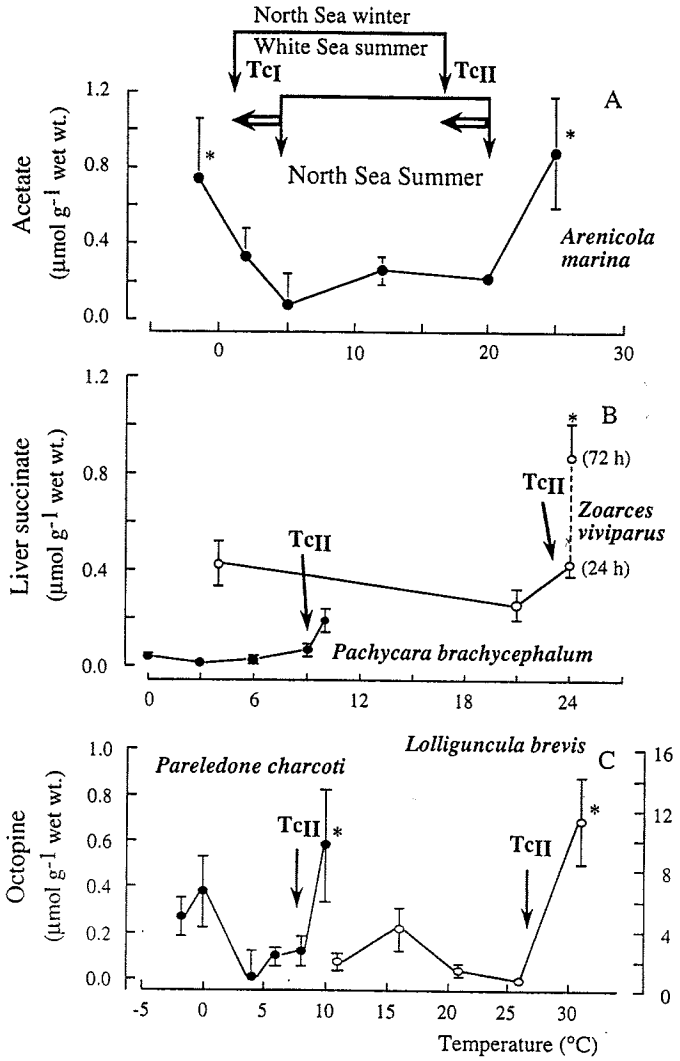

Fig 1. In ecothermal animals, low (Tc_I) and and high (Tc_{II}) critical temperatures can be characterized by the accumulation of anaerobic endproducts. A) Increased concentrations of acetate in the body wall tissue of the lugworm *Arenicola marina* at critical temperatures. Both Tc_I and Tc_{II} shift to lower values during cold adaptation. Lugworms collected from higher latitudes (White Sea) display lower values for Tc_I and Tc_{II} than animals from the North Sea (modified after Sommer *et al.*, 1997). B) Increased concentrations of succinate in the liver of North Sea eelpout, *Zoarces viviparus*, and Antarctic eelpout, *Pachycara brachycephalum* characterize transition to mitochondrial anaerobiosis at largely different values of Tc_{II} (after van Dijk *et al.*, 1999). C) Accumulation of the anaerobic end product octopine in the subtropical cephalopod *Lolliguncula brevis* and in the Antarctic cephalopod *Pareledone charcoti* may reflect failure of the ventilating tissue, again at different upper critical temperatures (cf Psrtner and Zielinki, 1999). *, significantly different from controls. Note the slightly different temperature scales.

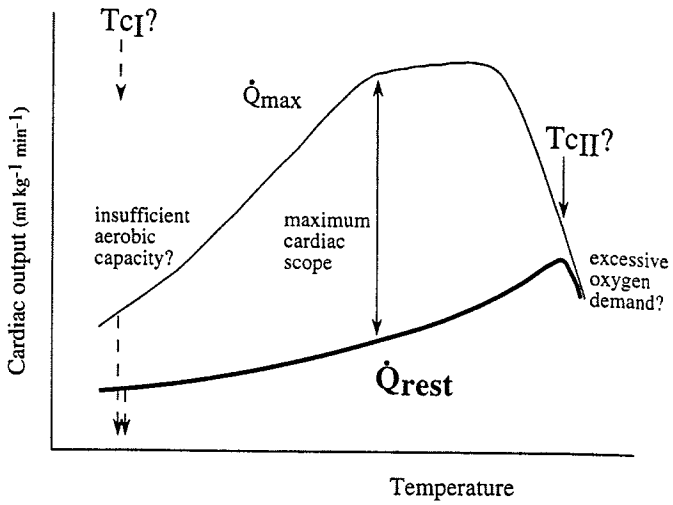

Fig 2. Effect of temperature on routine cardiac output (Q_{rest}) and maximum cardiac output (Q_{max}) in rainbow trout (Farrell, 1997). Critical temperatures may relate to insufficient Q_{rest} and the failure of the heart to perform adequately beyond a low and a high critical temperature.

In fish the onset of mitochondrial anaerobic metabolism at a high Tc was difficult to demonstrate since, as in other facultatively anaerobic vertebrates, mitochondrial metabolism does not contribute large amounts of energy to long term anaerobiosis (Grieshaber *et al.* 1994). Onset of mitochondrial anaerobiosis is most visible in organs with a high mitochondrial density and a large, usually aerobic energy (oxygen) demand such as liver (Fig. 1). These organs are the first to be affected by developing hypoxia (cf. Pörtner *et al.* 1991). Liver mitochondria are capable of anaerobic succinate formation by fumarate reduction (Hoberman and Prosky 1967). The factorial changes in succinate levels occurring during heat stress in Antarctic and temperate fish are similar to those found during anaerobiosis in other fish species (e.g. Johnston 1975; Smith and Heath 1980, van Waarde *et al.* 1983).

Tissues like the body wall musculature of worms and the mantle musculature of cephalopods are special in that they not only represent a large fraction of the musculature but are also ventilatory organs actively involved in oxygen uptake. Anaerobic glycolysis is observed on top of the transition to mitochondrial anaerobic metabolism, and is thus a consequence of oxygen limitation (Sommer *et al.* 1997; Zielinski and Pörtner 1996; Pörtner and Zielinski 1998; cf. Fig. 1 C). The limitation of survival at extreme temperatures is not only characterized by transition to anaerobic metabolism but also by a drop of the cellular Gibbs free energy change of ATP hydrolysis to a low, possibly critical value as seen in cold exposed ventilating body wall musculature (Zielinski and Pörtner 1996). This indicates insuffient ATP production to meet energy requirements.

The overall conclusion from these data is that thermal adaptation is linked to the necessity to overcome the threat of temperature induced hypoxia. Oxygen is supplied to the tissues by ventilation and circulation. Ventilation was shown to fail in the cold in sipunculid worms linked to a drop in coelomic PO_2 (Zielinski and Pörtner 1996). Haemolyph PO_2 fell towards both a low and a high critical temperature in *Maja squinado* linked to a concomitant fall in ventilation and circulation (Frederich and Pörtner 2000). The upper critical temperature threshold in the notothenioid fish, *Lepidonotothen nudifrons* was correlated with a progressive deviation of ventilation frequency from that dictated by the normal Q_{10} relationship, also suggesting a potential capacity limitation of ventilation for oxygen uptake (Hardewig *et al.* 1999a). A decrease in oxygen uptake seen during long term recordings in heat stressed Antarctic clams, *Laternula elliptica* was accompanied by a concomitant decline in heart rate (L.S. Peck, H.O. Pörtner, I. Hardewig, unpublished). Studies summarized by Farrell (1997) suggest that in temperate salmonid fish the limits of aerobic scope in the heart may lead to insufficient blood circulation at extreme temperatures (Fig. 2). All of these examples strongly suggest that low and high critical temperatures are set by a mismatch of oxygen supply and demand

Species	(g tissue wet wt. per animal)	Mo_2 (µmol h^{-1} g^{-1} tissue wet wt. at 0°C)	Tc °C	Source
Invertebrates				
Lyothyrella uva (brachipod)	n.d.	Å0.07	4-5	Peck, 1989,1996
Limopsis marionensis (bivalve)	Å 0.5	0.7	2-3	Pörtner *et al.*, 1999b
Nacella concinna (gastropod)	Å 0.5	0.8	9	Houlihan and Allan, 1982, Peck 1989
Laternula elliptica (bivalve)	57	0.35	9	L.S. Peck, I. Hardewig, H.O. Pörtner, unpublished
Pareledone charcoti (octopod)	35.8	0.27	9	Zielinski *et al.*, 1999
Fish				
Lepidonotothen nudifrons (notothenioid)	24	?	9	Hardewig *et al.*, 1999a
Pachycara brachycephalum (zoarcid)	89	0.47	9	van Dijk *et al.*, 1999

Table 1. Critical temperatures in polar ectotherms in relation to body weight and metabolic rate.

(Pörtner *et al.* 1998). Insufficient capacity of ventilation or circulation would not only be the cause of the mismatch between oxygen supply and demand at extreme temperatures but vice versa, ventilatory or circulatory organs may also be the first to reflect oxygen deficiency, anaerobiosis and energetic failure (Fig. 1). Oxygen limitation and functional failure most likely go hand in hand since transition to anaerobiosis always indicates a time limited situation.

The width of the window between critical temperatures reflects the amplitude of temperature fluctuations in the habitat of a species. It will be small especially in polar areas and most explicitly in the Southern Ocean where the stenothermal marine fauna is constantly exposed to temperatures ranging between -1.9 and +1°C. Nonetheless, this window is not the same for all Antarctic species (Table 1). Upper lethal temperatures in Antarctic ectotherms range between 2 and 3°C in a bivalve from the Weddell Sea, *Limopsis marionensis*, and most commonly, 9 to 10°C in some Antarctic bivalves, octopods or fish (Table 1). A preliminary comparison of the lifestyles of these species suggests that exclusively sessile epifauna species like *Limopsis marionensis* and *Liothyrella uva* are characterized by lower critical temperatures than more mobile species. The capacity of ventilation and circulation is likely to be higher in active fish or octopods than in mussels. This may be related to the higher critical temperatures in more mobile compared to sessile Antarctic species.

The tolerance window is even larger in eurythermal species. The question arises to what extent the mechanisms used during cold adaptation are similar or different between stenotherms adapted to permanent cold and eurytherms adapted to tolerate short term (daily or seasonal) cold. This review adopts the view that life in warm waters is likely to reflect the original evolutionary situation (Arntz *et al.* 1994). It uses the available information for the construction of a comprehensive model of metabolic cold adaptation and thermal tolerance in polar stenotherms and temperate to subpolar eurytherms. Its goal is to identify some baseline constraints in cold adaptation that animals had to cope with on the way from stenothermal warm via eurythermal to stenothermal cold. The model considers cellular and mitochondrial energetics and is based on the idea that adjustment of oxygen provision

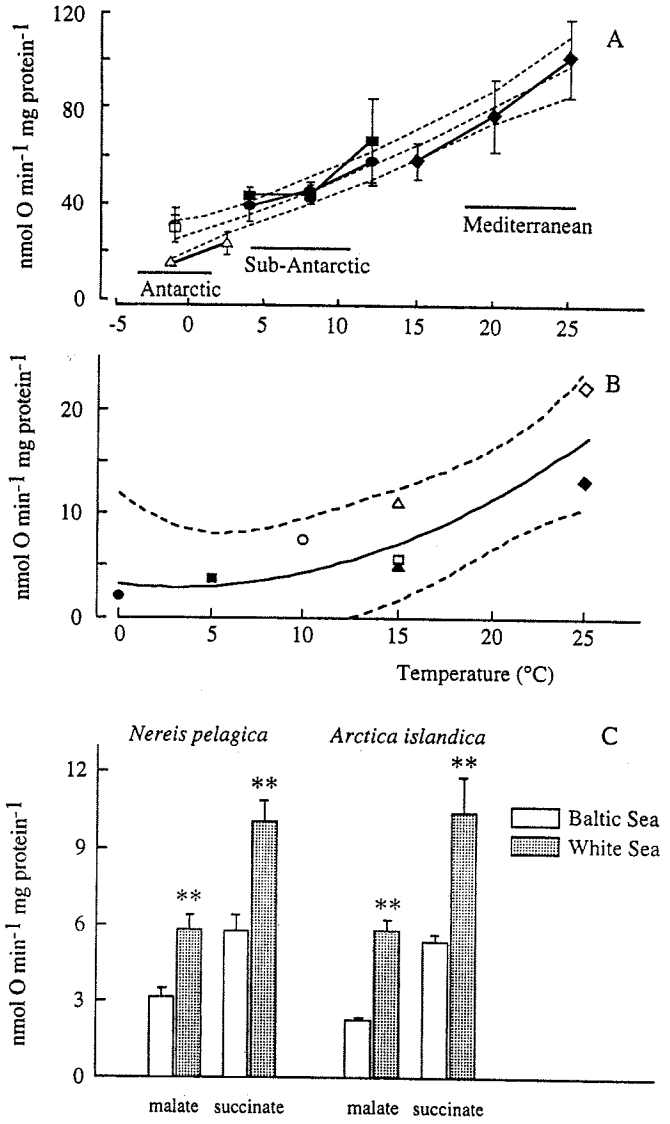

Fig 3. **(A) State III respiration rates at various temperatures of isolated mitochondria from red musculature of Antarctic (open symbols), sub-Antarctic (South American, filled circles and squares) and Meditteranean fish species (filled diamonds, redrawn from Johnston *et al.* 1998). (B) State III respiration rates at various temperatures of isolated mitochondria from bivalve tissues with glutamate as substrate (unless stated otherwise): filled circle, Antarctic bivalve *Laternula elliptica* gill (Pörtner et al., 1999b); open circle, oyster gill (Ballantyne and Moyes, 1987); filled diamond, oyster *Crassostrea viginica* gill (Burcham *et al.*, 1983), open diamond, ribbed mussel *Modiolus demissus* gill (Burcham *et al.*, 1984); filled square, mussel *Mytilus edulis* hepatopancreas (Ballantyne and Moon, 1985); open square, *Mya arenaria* mantle (Moyes et al., 1985); filled triangle, succinate oxidation in mantle mitochondria of Baltic *Sea Artica islandica*; open triangle, idem dito for specimen from the White Sea at the same temperature (Tschischka *et al.*, 1998). For comparison squid heart mitochondria reached mean values of 33 nmol O mg^{-1} mitochondrial protein min^{-1} with glutamate and pyruvate, and of 81 with succinate as a substrate (Mommsen and Hochachka, 1981) which indicates that the metabolic that the metabolic capacity of bivalve mitochondria is rather low. (C) Comparison of mitchondrial capacities in state III respiration in White Sea and Baltic Sea populations of *Nereis pelagica* and *Artica islandica* using malate or succinate as substrates (T=15ûC; after K. Tschishka, D. Abele, H. O. Pörtner, unpublished).**

and metabolism will provide an important clue to the understanding of energetics in eurythermal and stenothermal ectotherms (Pörtner *et al.* 1998). It also assumes that processes responsible for adaptation to low or high temperature extremes are linked, i.e. mechanisms causing a shift of the low critical temperature will simultaneously elicit a shift of the upper tolerance limit and vice versa.

Adjustment to temperature extremes

Both the low and the high Tc can be shifted by acclimation or acclimatisation of individual ectotherms with the possible exception of extremely stenothermal polar, especially Antarctic species (see below).

Acclimatisation to seasonal cold is well known to cause a rise in mitochondrial density which is reversed during seasonal warming. High mitochondrial densities have also been observed in polar ectotherms adapted to permanent cold. A wealth of information has been collected in fish (for review see Guderley 1998) and only recently has cold induced mitochondrial proliferation been confirmed for a marine invertebrate (Sommer and Pörtner 1999). At first sight, cooling causes aerobic mitochondrial capacity to drop in a similar way as whole animal oxygen demand, thereby maintaining the balance between energy supply and demand (see Guderley 1998). However, since the fractional cost of ion regulation rises owing to lower Q_{10} values for this process, a lower fraction of mitochondrial capacity remains for other functions (see Pörtner *et al.* 1998). In consequence, a downward shift of the low Tc would require a rise in aerobic capacity. Energy limitations may therefore be one reason why mitochondrial proliferation occurs in the cold. Reduced aerobic capacity of mitochondria in the cold may contribute to the loss of function, e.g. in circulation and ventilation. Since mitochondrial densities rise in the cold and are reduced in the warm, the adjustment of mitochondrial densities and properties may be the mechanism that causes the simultaneous change in both low and high Tcs and thereby links the critical temperatures.

The question arises what a high mitochondrial density suitable in the cold means for the animal if it is exposed to high temperatures. Acclimation to temperatures above the high Tc is associated with the reversal of an initial transition to anaerobiosis (Sommer and Pörtner 1999) and a drop in citric synthase activity, a marker enzyme of mitochondrial density. The similarity of the time course of acclimation with the half life of mitochondria also suggests that a drop in mitochondrial density is related to an upward shift of the high Tc. The reduction of mitochondrial density implies a reduction of oxygen demand that can then be covered by ventilation and circulation allowing the upper Tc to shift to higher values. It will be discussed in detail below how oxygen demand is correlated with the number of mitochondria present.

As a corollary, the shift in both low and high critical temperatures between temperate and subpolar populations may be associated with a shift in mitochondrial density, which should drop as the temperature rises and increase as ambient temperature falls. According to this rationale the drop in the low critical temperature to below polar temperatures is also caused by rising mitochondrial densities and a concomitant drop in the high critical temperature (Pörtner *et al.* 1998). It remains to be investigated whether not only mitochondrial density but also the performance of individual mitochondria is a characteristic element in defining thermal tolerance.

FURTHER MECHANISMS LIMITING THERMAL TOLERANCE?

These considerations strongly suggest that the limits of thermal tolerance are related to oxygen limitations owing to an imbalance of oxygen supply and demand. The question arises whether oxygen metabolism is really responsible for the limits of thermal tolerance of the whole organism. For the sake of completeness, other mechanisms which might contribute to set the lethal temperatures are outlined briefly and it will be discussed here and later in the text where oxygen metabolism and these mechanisms may be linked. As far as the effects on the whole organism is concerned the picture is more complete for thermal limitations at high than at low temperatures

Magnesium anaesthesia in the cold

Currently, a picture emerges which is suitable to explain the absence of marine reptant decapod crustaceans from the Antarctic and also from the low (i.e. below 0°C) temperature regions of the Arctic (Frederich *et al.* 2000). High levels of magnesium in the haemolymph of reptant decapods exert an anaesthetizing effect, which is enhanced in the cold and restricts ventilatory, circulatory and thereby aerobic capacity in this animal group. In contrast, natant decapods like shrimp have conquered these areas, being able to do so owing to their lower haemolymph magnesium levels. The general importance of these relationships for other marine ectotherms needs to be elaborated.

Loss of protein function and Arrhenius break temperatures

Individual proteins may lose their functional integrity close to the critical temperature of the whole organism. This process may be most important at the upper critical temperature. The thermal inactivation of intestinal Na^+-D-glucose cotransport occurs at 6°C in *Trematomus bernacchii* (cf. Storelli *et al.* 1998), close to the critical temperature of notothenioid fish (Table 1). Loss of enzyme activity was observed at the Tc in the Antarctic octopod *Pareledone charcoti* (Zielinski and Pörtner unpublished).

A first sign indicating loss of protein function may be a change in the thermal properties of the protein itself or the metabolic unit to which it belongs. This change is visible as a discontinuity in Arrhenius plots. Arrhenius break temperatures (ABT) of mitochondrial respiration have been compared to the thermal sensitivity of whole animals (Somero *et al.* 1998; Weinstein and Somero 1998). In this case, ABT may reflect a change in the pattern of mitochondrial regulation or even identify the temperature at which thermal inactivation of one or several mitochondrial components occurs. Accordingly, O'Brien *et al.* (1991) demonstrated that heat inactivation of membrane bound succinate dehydrogenase and the matrix enzyme malate dehydrogenase set in at about the same temperature (when cytochrome oxidase showed transient activation). The authors concluded that sharp changes in membrane fluidity are not involved and suggested that a disruption of hydrophobic interactions between proteins and associated lipids alters the temperature dependence of functions of membrane bound proteins.

Arrhenius break temperatures of uncoupled respiration in mitochondria from various fish and invertebrate species were shown to vary depending on maximal habitat or adaptation temperature, however, at values far above the habitat temperature and also above the upper critical temperature discussed in this paper. The difference between ABT and maximum habitat temperature appeared to rise with falling ambient temperatures, from an average difference of 15°C at 25°C to a difference of 25 at 5°C (Weinstein and Somero 1998). The mitochondria of the Antarctic fish *T. bernacchii*, however, did not fit this general pattern but the difference between its habitat temperature and the ABT was less (about 18°C) than expected from the correlation (Somero *et al.* 1998; Weinstein and Somero 1998) but still higher than

the Tc. In a comparative study on the notothenioid fish, *L. nudifrons*, the ABT of mitochondrial respiration was also much higher than the Tc (Hardewig *et al.* 1999a). These relationships may be different in invertebrates. In mitochondria of the Antarctic bivalve *Laternula elliptica* a small difference of about 9°C was found. In *L. elliptica* the critical temperature coincides with the ABT of mitochondrial state III respiration and isocitrate dehydrogenase (IDH) (Pörtner *et al.* 1999b).

This comparison confirms that ABTs of mitochondrial respiration and critical temperatures do not necessarily go hand in hand (they may in Antarctic invertebrates but not in fish) leading to the conclusion that ABTs reflect some functional adjustment or failure in response to temperature but do not necessarily indicate the critical, lethal temperature limits for the whole animal.

Neuromuscular failure in the heat

An important mechanism related to the loss of protein function includes the failure of synaptic transmission at high, possibly lethal temperatures. Owing to a drastic drop in substrate-binding ability at high temperature an imbalance may arise between the quantities of acetylcholine released and the ability of acetylcholine esterase to degrade this transmitter (Baldwin 1971; MacDonald *et al.* 1988; as discussed by Somero 1997). The loss of nervous control, which is discussed to cause a loss of balance in fish close to the limits of heat tolerance, may actually contribute to ventilatory failure.

Conclusions

The question arises whether the mechanisms outlined in this section become effective before oxygen limitation sets in at the critical temperature or whether they contribute to setting anaerobic Tcs. Among those reported most likely the mechanisms that affect nervous control and thereby the thermal sensitivity of ventilation or circulation will influence the critical temperature. However, ventilation and circulation depend on muscle contraction and, therefore, not only on nervous control but also on mitochondrial capacity and adjustments of the contractile machinery. In general, limitations in aerobic capacity and oxygen supply are among those closest to the tolerance limits of the animals. On the one hand, the transition to an anaerobic mode of mitochondrial metabolism appears to be a general phenomenon characterizing thermal tolerance limits. On the other hand, the adjustment of mitochondrial densities and properties seems to be crucial for temperature acclimation and adaptation. Thus, the following analysis focusses in more detail on the contribution of mitochondria to metabolic temperature adaptation.

MITOCHONDRIAL CAPACITY

Metabolic adjustment to cold in polar species has so far mostly been studied in Antarctic fish, largely from the notothenioid family. Although cold adaptation in Antarctic fish appears to be characterized by a compensatory increase in aerobic capacity, reflected in higher mitochondrial densities (Dunn 1988; Johnston *et al.* 1998) and the 1.5 - 5 fold rise in the capacity of some oxidative enzymes (Crocket and Sidell 1990, for a comparison of confamilial species see below), resting rates of oxygen consumption remain low (Clarke 1998). According to

Number of miochondria (mitochondrial components)
- △ Aerobic capacity
- △ Rate of standard metabolism
- △ Aerobic scope

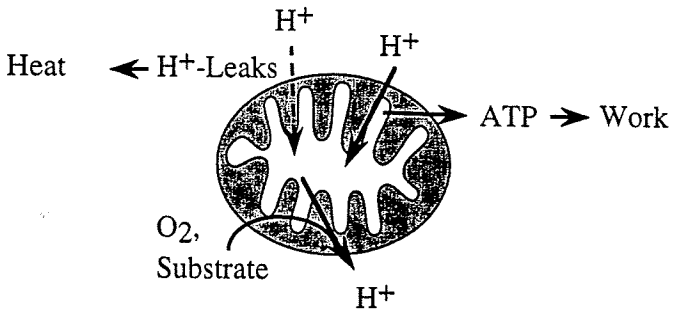

Fig 4. Generation of a proton gradient, its use in ATP synthesis and its dissipation and association heat generation in the mitochondrial proton leak. Proton leak and ATP turnover define standard metabolism. Maximum ATP production defines aerobic scope and capacity. For a suggested mechansim of the proton leak see Figure 5.

the scenario outlined above, the rise in mitochondrial density in the cold not only causes a decline in the low critical temperature to below polar temperatures, but it also leads to a drop in the upper critical temperature (Pörtner *et al.* 1998).

Whilst cold adaptation leads to higher mitochondrial densities, comparison of a variety of perciform fish species from temperate (southern South American), Mediterranean and Antarctic waters led to the conclusion that the specific oxidative capacity of the individual mitochondrion (state III respiration = the maximum metabolic rate of mitochondria at saturating substrate and oxygen levels) remains largely unaffected (Johnston *et al.* 1994; 1998; Guderley 1998; Fig. 3 A). These authors found that maximal respiration rates of mitochondria from Antarctic species at -1°C were close to those expected from a comparison with the temperate and warm water species after extrapolation with a Q_{10} of about 2. Similarly, the specific aerobic capacity of mitochondria isolated from the gills of *Laternula elliptica* was of the same order of magnitude as the capacity of mitochondria from other bivalves when extrapolated to 0°C with a Q_{10} of 2.2 to 2.5 (Fig. 3 B). However, a detailed comparison of respiratory capacities in invertebrate mitochondria at different temperatures is not feasible to date since the animals used in the cited studies originated from diverse environments or even food suppliers, various seasons and acclimation regimes. Nonetheless, the preliminary comparison depicted in Fig. 3B does not lead us to expect significant cold compensation in Antarctic bivalve mitochondria. Since specific mitochondrial capacity and the degree of metabolic cold compensation may go hand in hand, this conclusion is in accordance with Houlihan and Allan (1982) who did not find significant cold compensation in the metabolic rate of Antarctic gastropods. This may be true for Antarctic invertebrates in general (see Clarke 1991, for review).

In contrast to the data obtained in fish from different latitudes (Fig. 3A) seasonal cold acclimation of eurythermal fish does cause an increase in the specific aerobic capacity of mitochondria (see Guderley 1998 for review). Moreover, a study on bivalve and polychaete mitochondria (Fig. 3C) revealed that the specific respiratory rates in all functional states were by a factor of 2 higher in White Sea compared

to Baltic Sea animals. The comparison of White Sea and North Sea *Arenicola marina* demonstrated that whole animal resting metabolic rates are higher in White Sea than in North Sea specimens, indicating significant cold compensation, to some extent even over-compensation. Invertebrate life appears to be more costly at the higher latitudes of the White Sea, evident not only from elevated oxygen consumption rates, but also from mitochondrial densities (Sommer and Pörtner 1999; Fig. 3C). The question arises how the apparent discrepancy between intraspecific and interspecific studies on animals from different latitudes can be explained (Fig. 3C, comparing invertebrate populations of the same species, and Johnston *et al.* 1998, comparing various species of perciform fish, Fig. 3A).

The difference between these findings may be related to the differences in the temperature regime characterizing the environment of the animals. Antarctic ectotherms experience minor temperature fluctuations of up to 3°C per year. The sub-Antarctic and Mediterranean fish species studied by Johnston *et al.* (1998) are subjected to annual temperature fluctuations of around 5°C with a relatively short winter period. In contrast, some North Sea and White Sea species experience seasonal temperature fluctuations of more than 15°C. Moreover, White Sea (intertidal) species are exposed to rapid temperature changes during short periods in spring and in fall with a long winter period at temperatures close to freezing (cf. Sommer *et al.* 1997). This comparison strongly suggests that metabolic cold compensation and a potential increase in the specific capacity of individual mitochondria may be negligible in more stenothermal animals living at permanently low temperatures throughout the year. Living at extremely fluctuating temperatures like in the intertial zone of the White Sea may not only require mitochondrial proliferation but may also require the specific capacity of the individual mitochondrion to increase. In this context, the ability to adjust to temperature extremes is enhanced in White Sea compared to North Sea specimens (Sommer and Pörtner 1999), an observation possibly linked to the enhanced aerobic capacity. In contrast, the low aerobic capacity in stenothermal polar species may be associated with the loss of ability of these animals to acclimate to changing temperatures (cf. Weinstein and Somero 1998).

If (short term) cold exposure leads to a larger increase of mitochondrial capacities in eurytherms than in stenotherms (on an evolutionary time scale) this should also be visible at the level of mitochondrial enzymes. A comparison of closely related species will provide the least equivocal insight. In fact, cold acclimation (to 0°C) of eurythermal North Sea eelpout (*Zoarces viviparus*) caused a 1.7-fold rise in cytochrome c oxidase (COX) activity in the white musculature. Compensation was less pronounced in the liver which, however, increases in size during cold adaptation. Compared to warm acclimated *Zoarces viviparus*, cytochrome c oxidase activity was not enhanced in cold adapted confamilial Antarctic eelpout *Pachycara brachycephalum* (Hardewig *et al.* 1999b), despite the higher mitochondrial density expected for the Antarctic species. Lower COX levels in Antarctic specimens are correlated with lower levels of COX specific mRNA, indicating that the lower expression of COX in Antarctic eelpout is engineered at the transcriptional level (Hardewig *et al.* 1999b).

In general, it appears that the degree of eurythermality exhibited by an animal has not been considered to the extent required for a complete story of metabolic temperature adaptation. Further comparative study is required for a more complete understanding of eurythermal versus stenothermal adjustment to cold.

Mitochondrial proton leakage and SMR

How can mitochondria contribute to a mismatch of energy demand and aerobic supply beyond both critical temperatures? At the low Tc the insufficient capacity of mitochondria may contribute to functional failure, among others of ventilation and circulation, thereby leading to a breakdown of oxygen transport and aerobic metabolism (e.g. Zielinski and Pörtner 1996). Mitochondrial proliferation in the cold provides a rise in aerobic capacity as required to meet energy demands. This will ultimately cause a rise in oxygen demand which becomes detrimental at the upper Tc. How can this increase in oxygen demand be explained?

It has only recently become clear that oxygen demand is not only related to cellular energy requirements but also to the number of mitochondria and their properties (see below). In this context it needs to be taken into account that oxygen demand of mitochondria does not only depend upon the maximum rate of coupled respiration in the presence of substrate and ADP (state III respiration) but also on the rate of proton leakage. This process is defined as the passive influx of protons through the inner mitochondrial membrane driven by the proton gradient. Maintenance of the proton gradient leads to oxygen consumption without concomitant ATP production (Fig. 4). Proton leakage comprises a large fraction of mitochondrial state IV respiration, i.e. respiration in the presence of substrate at saturated phosphorylation potentials when ATP levels are constantly high and ADP levels constantly low. State IV respiration therefore largely reflects a situation close to that in the resting cell. Thus, proton leakage reflects baseline idling of mitochondria and their metabolic activity, and may be interpreted to account for part of the cost of mitochondrial maintenance (Pörtner *et al.* 1998, Fig. 4).

Recent work on the processes contributing to mitochondrial respiration has demonstrated that proton leakage is responsible for a significant fraction of whole animal standard metabolic rate. It covers 25 to 50 % of baseline metabolic rate in rat hepatocytes and skeletal muscle (Brand *et al.* 1994; Rolfe and Brand 1996). In general, proton leakage correlates with standard metabolic rate (SMR, Brookes *et al.* 1998). This means that its % contribution to SMR is similar in ectotherms and endotherms. SMR is the oxygen consumption of the conscious unfed animal at rest, excluding the effect of spontaneous activity (Pörtner and Grieshaber 1993). SMR is related, by a more or less constant factor, to maximum aerobic capacity of the organism (Wieser 1985; Brand 1990), a ratio which likely depends upon the capacity of ventilation and circulation. The relationship between mitochondrial proton leakiness and aerobic capacity appears to be constant, even with different levels of mitochondrial capacity. In support of this conclusion, it was found that the respiratory coupling ratio (RCR = the ratio of state III over state IV respiration) of isolated mitochondria remained constant in a gradient of geographical distribution towards high latitudes despite an increase of mitochondrial capacity (K. Tschischka, pers. comm.).

Proton leakiness rises exponentially with increasing proton motive force (Δp). This relationship becomes significant when the

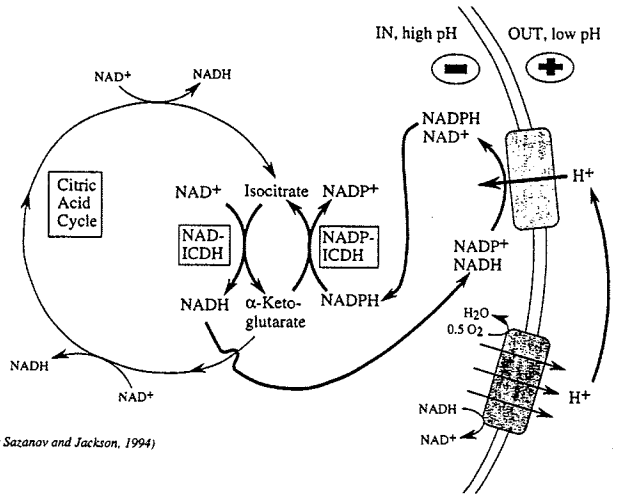

Fig 5. Scheme of the discussed substrate cycle (after Sazanov and Jackson, 1994). The transhydrogenase dissipates the proton gradient to convert NADH to NADPH, which is used by NADP-dependent IDH. The resulting futile cycle allows mitochondrial substrate oxidation and ATP production to react rapidly to changes in energy demand.

phosphorylation potential (defined as the ATP/ADP ratio) is high (Davis and Davis-van Thienen 1991). Proton leakage will reduce the P/O ratio in the living cell to values even below 1 under resting conditions (Rolfe and Brand 1996). Functional P/O ratios under resting conditions can obviously be far below the expected value depending on the magnitude of the proton leak. In consequence, we have to accept that strict coupling of whole animal oxygen consumption with ATP turnover does not exist. These considerations deviate from the traditional framework of our understanding of whole animal metabolism. Since oxygen consumption and ATP turnover of the whole animal can vary independently, changes in oxygen consumption always reflect changes in the consumption of substrate, but not necessarily in true metabolic energy (ATP) turnover. Examples abound in the literature where changes in oxygen consumption remain unexplained by theoretical evaluations of metabolic cost (e.g. during specific dynamic action or post-exercise recovery). These examples need to be re-investigated.

Recently the temperature dependence of H^+ leakage was investigated in mitochondria from tissues of two Antarctic species, liver of notothenioid fish (*Lepidonotothen nudifrons*) (Hardewig *et al.* 1999a) and bivalve gills (*Laternula elliptica*) (Pörtner *et al.* 1999b). The change in proton leakage with temperature was more or less continuous, with constant Arrhenius activation energies. The thermal sensitivity of proton leakage is not driven by a change in Δp, which hardly varies with temperature (Dufour *et al.* 1996) especially since an alphastat change in extramitochondrial pH can be expected to maintain the pH gradient across the inner mitochondrial membrane (cf. Moyes *et al.* 1988). The finding of a fairly high Q_{10}-value (4.2) for proton leakage suggests that this process involves an enzyme catalyzed process rather than simple diffusion of protons across the mitochondrial membrane which would be indicated by a Q_{10} significantly below 2 (Ellory and Hall 1987). Brand and co-workers have recently shown phospholipid composition (long chain polyunsaturates) to correlate with proton leak. Investigations on membrane vesicles, however, revealed that this effect is not a direct one but is presumably due to interaction between lipids and membrane bound proteins. The nature of the respective protein is still obscure (Brookes *et al.* 1998). The thermal sensitivity of H^+ leakage is probably not or not only correlated with changes in membrane properties but may depend upon the thermal sensitivity of the proteins involved.

Recently, Sazanov and Jackson (1994) proposed that a futile substrate cycle in the mitochondria may contribute to proton leakage. This process goes along with the transport of protons from the cytosol into the mitochondrial matrix (Fig. 5). The futile cycling between isocitrate and 2-oxoglutarate relies on both NAD^+ dependent isocitrate dehydrogenase or IDH (forward reaction) and the reduction of 2-oxoglutarate by $NADP^+$ dependent IDH (reverse reaction). The $NADP^+$-dependent enzyme is an equilibrium enzyme predominantly regulated by the NADPH/$NADP^+$ ratio, whereas NAD-dependent IDH is not (Alp *et al.* 1976). A proton-translocating transhydrogenase may operate in this futile cycle which is thought to enhance the regulatory flexibility of the citric acid cycle. Generally, NAD^+ dependent IDH activity is about 10 to 100 times lower than that of the $NADP^+$ dependent enzyme in the forward reaction and of the order of magnitude of the reversed reaction (Crabtree and Newsholme 1970, Alp *et al.* 1976; Howlett and Willis 1998). The forward reaction of $NADP^+$-dependent IDH, however, will be brought to a standstill or even reversed by high NADPH/$NADP^+$ ratios provided by the transhydrogenase such that the regulatory influence of $NADP^+$ dependent IDH on the citric acid cycle flux is mediated by the capacity of transhydrogenase and NAD dependent IDH. Thus the higher capacity of $NADP^+$ dependent isocitrate dehydrogenase (forward reaction) reflects its nature as an equilibrium enzyme and not the magnitude of its contribution to metabolite flux (Fig. 5).

The characteristics of this substrate cycle lead to predictions, which are in agreement with the features of the proton leak. Designed to support the flexibility of flux through the citric acid cycle, the futile cycle is correlated with aerobic capacity and, thus, with inner membrane surface area. Like proton leakiness, it would correlate with standard metabolic rate. Thus the contribution of this futile cycle to metabolic rate will be large under resting conditions (standard metabolic rate). Since it requires $NADP^+$-dependent IDH to operate in reverse the rate of cycling can be predicted to depend upon the NADPH/$NADP^+$ ratio. The non-linear relationship between this ratio and proton motive force is similar to that between proton motive force and proton leak (Brookes *et al.* 1998). A high proton-motive force leads to a high NADPH/$NADP^+$ ratio which stimulates the turnover of the substrate cycle and, thus, proton translocation, i.e. proton leakage. Both $NADP^+$- and NAD^+-dependent isocitrate dehydrogenases have similar functional characteristics in vertebrate and invertebrate (bivalve) tissues (Head and Gabbot 1980, 1981; Kargbo and Swift 1983) such that this principle may be operative in a wide range of animal mitochondria.

Thermal characteristics of enzymes and mitochondria

The activation energy of the proton leak being high, it is not surprising that the activation energy of isocitrate dehydrogenase ranges high as well. In consequence, the thermal dependence of flow through isocitrate dehydrogenase and transhydrogenase may determine, or at least contribute, to the high thermal sensitivity of mitochondrial proton leakage found in Antarctic mitochondria (see above). Further evidence

indicates that NADP$^+$ dependent IDH is subject to selection according to the thermal conditions of the habitat. Recently, Hummel *et al.* (1997) demonstrated a clear correlation between mean habitat temperature and a change in the isoenzyme pattern of mitochondrial NADP$^+$ dependent IDH in the lugworm *Arenicola marina*. Further study demonstrated that the thermal sensitivity or Arrhenius activation energy (Ea) of NADP$^+$-dependent isocitrate dehydrogenase was higher in a population from the subpolar White Sea than in a North Sea population (100 vs. 64 kJ mol^{-1}, Sommer and Pörtner unpublished). This is quite unexpected in the light of other literature data and the long held assumption that Ea values of enzymatic reactions in general are lower in ectotherms than in endotherms and lower in cold than in warm adapted species. Such a generalization may not be possible since only a limited number of enzymes like lactic dehydrogenase has so far been studied (Hochachka and Somero 1984). In fact, cytochrome c oxidase in cold adapted (White Sea) lugworms exhibited the expected downward shift in activation energies from 31 to 14.9 kJ mol^{-1} (Sommer and Pörtner unpublished). A small drop in the Ea of cytosolic MDH with falling habitat temperature was found by Dahlhoff and Somero (1993) in different species of abalone.

Although the picture is not yet clear some literature data indicate that the rise in Ea for NADP$^+$- dependent IDH observed in cold adapted *Arenicola marina* may not only be relevant for the mitochondrial but also for the cytosolic isozyme. Calculations of Ea-values from data by Alp *et al.* (1976) reveal that Ea-values of NADP$^+$ dependent IDH (mostly cytosolic) are higher in ectotherms (a gastropod, *Buccinum undatum*; Ea = 88; a swimming crab, Ea = 71; a dogfish, Ea = 73; a locust, Ea = 72 kJ mol^{-1}) than in endotherms (a rat, Ea = 38; a pigeon, Ea = 45 kJ mol^{-1}). Such a trend is visible also for citrate synthase, but not for NAD$^+$-dependent IDH. Ea values of NADP$^+$ dependent IDH from mitochondrial or cytosolic compartments of various ectotherms are high (*Fundulus heteroclitus*: 55 kJ mol^{-1}, Gonzalez-Villasenor and Powers 1986; rainbow trout: 76 kJ mol^{-1}, Moon and Hochacka 1971; *Mytilus edulis*: 70 to 90 kJ mol^{-1}, calculated from data by Head and Gabbott 1980), but below the level for the mitochondrial enzyme in the cold adapted Antarctic notothenioid, *Lepidonotothen nudifrons* (91 kJ mol^{-1}, Hardewig *et al.* 1999a) or in the Antarctic bivalve *L. elliptica* (138 kJ mol^{-1}, Pörtner *et al.* 1993). It remains to be seen whether this is a universal principle. In rainbow trout four weeks of cold acclimation caused a shift in the isoenzyme pattern but no significant increase in the activation energy of (mostly cytosolic) NADP$^+$ dependent IDH (Moon and Hochachka 1971). Further studies should address whether permanent cold adaptation (to lower mean temperatures at high latitudes) is required for the increase in Ea of this IDH to take place.

Other examples from the literature also support the conclusion that a cold induced drop in Ea is not obligatory and that there may be exceptions with unchanged or even higher Eas (Alexandrov 1977). In mammals, birds and marsupials mitochondrial membrane enzymes show a higher Ea in those species that are characterized by the capacity to reduce body temperature during hibernation (Geiser and McMurchie 1985). Somero (1997) assumed that these examples may reflect an adaptation to metabolic depression during hibernation. In cold acclimated carp the Ea of phosphoglyceraldehyde dehydrogenase was found to increase (Marcinkowski *et al.* 1990). Recently, PFK activities

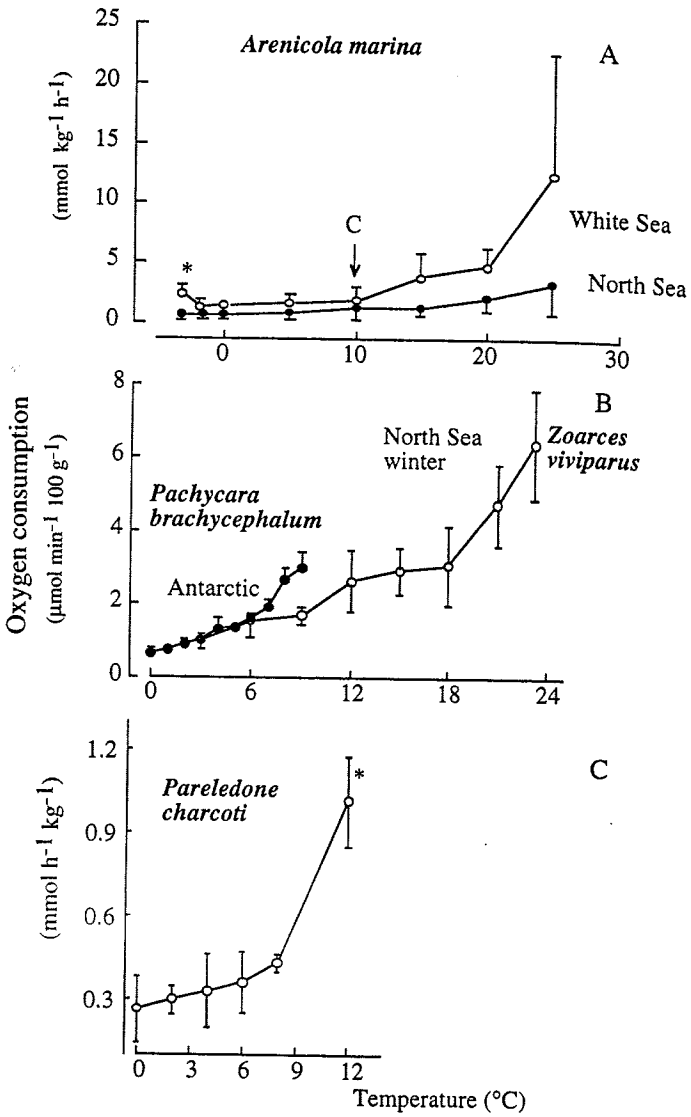

Fig 6. **Temeprature-dependent oxygen consumption (A) in North Sea and White Sea lugworms, *Arenicola marina* (Sommer and Pörtner, 1999b); (B) in the common eelpout, Zoarces viviparus, from the North Sea caught in winter and acclimated to 3°C; in *Pachycara brachycephalum* from the Antarctic (van Dijk et al., 1999) and (C) in the Antarctic octopod, *Pareledone charcoti* (Zielinski et al., 1999). Oxygen uptake rises more steeply with temperature in cold adapted populations of a species (A) or in cold stenothermal representatives of a family (B) or a phylum (C). These differences are reflected by a higher value of the apparent Arrhenius activation energy Ea for cold adapted animals (Table 2).**

were identified in Antarctic octopods and squid with high Ea-values (160 - 200 kJ mol^{-1}) in the arms and low values in the mantle musculature (40 - 100 kJ mol^{-1}, Zielinski and Pörtner, unpublished). Since GAPDH and PFK are key regulatory enzymes of glycolysis it should be investigated whether a cold induced increase in Ea is typical in key regulatory enzymes of a pathway or, as in the case of IDH, in other enzymes present in low quantities as a means to minimize turnover at high enzyme levels.

However, enzymes with a low activity, some of which are flux limiting, have hardly been considered and this may be the reason why such a rise in Ea in some enzymes (like glyceraldehydephosphate dehydrogenase, phosphofructokinase, isocitrate dehydrogenase) has been overlooked. A rise in Ea may be restricted to and typical for low activity, potentially flux limiting enzymes of a pathway.

process	endotherm	temperate, e.g. North Sea	subpolar, e.g. White Sea	polar, e.g.Antarctic
whole animal respiration	-	53 (zoarcid)[h]	-	105 (zoarcid)[h]
		66 (polychaete)**[g]	89 (polychaete)**[g]	81.5 (bivalve L.)[i]
				181 (bivalve Y.)[j]
mitochondrial state IV respiration	8.1 (rat liver)[a]	28.6 (trout liver)[a]		86.7 (bivalve L.)[f*]
		23.0 (frog muscle)[b]		92.5 (notothenioid)[e*]
		27.4 (blowfly muscle)[c]		
		65.9 (turtle heart)[d]		
isocitrate dehydrogenase (NADP)		64 (polychaete muscle)[g]	100 (polychaete muscle)[g]	138 (bivalve L.)[f]
				91 (notothenioid)[e]

* indicates a specific analysis of proton leak
** close to the high Tc
References: [a]Lyons and Raison, 1970, [b]Pye, 1973, [c]El-Wadawi and Bowler, 1995; [d]Almeida-Val *et al.*, [e]Hardewig, *et al.*, [f]Pörtner al. 1999; L. = *Laternula elliptica*; [g]Sommer and Pörtner, 1999a; hvan Dijk *et al.*, 1999, [h]L.S. Peck, I. Hardewig, H.O. Pörtner, unpublished; L. = *Laternula elliptica*, [i]Abele *et al.*, 1998, Y = *Yoldia eightsi*. Note: The Ea of whole animal respiration did not vary with temperature in North Sea and Antarctic zoarcids, whereas a maximum Ea of 142 kJ mol^{-1} was observed at temperatures close to critical in Antarctic octopods (calculated based on Zielinski *et al.*, 1999) and in the polychaete *Arenicola marina*.

Table 2: Arrhenius activation energies (kJ mol^{-1}) of mitochondrial enzymes and processes associated with standard metabolic rate (state IV respiration) or proton leakage (at or close to habitat or body temperature).

The elevation of the Ea of IDH in the cold provokes the question whether the Ea of the proton leak is higher in cold adapted than in warm adapted mitochondria. Unfortunately, unequivocal determinations of the thermal dependence of proton leak in ectothermal animals are not available except in our studies on *L. nudifrons* and *L. elliptica*. However, state IV respiration is usually close to proton leakage rates and available data suggest that the thermal sensitivity of state IV respiration is less in temperate or warm acclimated than in polar species (Table 2). Only turtle heart mitochondria show a relatively high Ea, possibly linked to metabolic depression in the cold as discussed above. Overall, the comparison is in line with the observed rise in the Ea of proton leak and isocitrate dehydrogenase activity in cold adapted species. Under conditions of high proton motive force as prevailing in the resting cell, this means that the thermal sensitivity of the proton leak may significantly influence the temperature dependence of resting or standard metabolic rate (see below).

Values of Arrhenius activation energy of state III respiration rates available from the literature and our own studies do not reveal a clear dependence on habitat or body temperature. This is in line with the assumption that proton leakage is minimized under conditions of maximum ATP turnover and that metabolic control is likely to shift to processes associated with ATP formation. It is important to emphasize here that the thermal sensitivity of state IV respiration or proton leak relates to the resting rate of oxygen consumption, whereas the thermal sensitivity of state III respiration relates to maximum respiration at maximum ATP turnover, e.g. during exercise.

Tradeoffs in eurytherms and stenotherms

Why should it be useful to possess key enzymes with high activation energies in the cold? Considering again that evolution started in warm waters two explanations appear possible:

1. It has been emphasized that the level of enzymatic binding sites in relation to the concentration of metabolites is relevant for metabolic maintenance (Sols and Marco 1970). Cold temperatures may disturb this balance by restricting the diffusion of substrates and oxygen (Sidell and Hazel 1987). Metabolic cold compensation therefore implies an increase in the quantities of metabolic enzymes and mitochondria to begin with. The secondary reduction of metabolic flux in polar species, which can be interpreted as a compensation for metabolic cold adjustment, will then alleviate the requirement for an enhanced number of enzyme molecules. However, the diffusion distance for substrates between enzyme molecules might become too large. A logical strategy would therefore imply the production of more enzyme molecules with high Ea-values and thus less activity in the cold. In this way enzyme "proliferation" would increase the number of enzyme molecules without causing an increased flux through the reaction. This mechanism appears effective mostly for enzymes present at low activities, which according to the classical understanding of metabolic control is typical for some regulatory enzymes ("bottlenecks") catalyzing reactions in disequilibrium and limiting the flux through metabolic pathways (Newsholme and Crabtree 1981). It may hold also for other low flux, but equilibrium enzymes (like IDH). Those enzymes of a pathway which show high maximum activities and molecule numbers like LDH will show the expected decrease in Ea (Hochachka and Somero 1984), thereby allowing for the respective reactions to remain in equilibrium.

A consequence of producing enzymes with higher Ea-values would be an increased temperature dependence of a pathway (see below). In the case of $NADP^+$-dependent isocitrate dehydrogenase a very high activation energy, as observed in polar mitochondria, may support the reduction of flux through the substrate cycle (Fig. 5) in the cold and thus a reduction of proton leakage compared to mitochondria of animals subjected to seasonal cold (Pörtner *et al.* 1998). The rise in Ea in some enzymes would actually support metabolic depression in polar species below the rate otherwise expected from the effect of mitochondrial proliferation. Similarly, expression of enzymes with high Ea values in

hibernators undergoing metabolic depression or in the arms of Antarctic octopods may reflect the necessity to maintain a high enough enzyme concentration at minimized metabolic flux.

2. GAPDH and PFK are flux limiting enzymes of glycolysis. Enhancing their Ea might not only reflect the overall downregulation of metabolic activity, but also support a selective reduction in the use of anaerobic glycolysis as visible in some polar species (Dunn and Johnston 1986). Alternatively, it might indicate differences in the use of a pathway between tissues, like in the case of cephalopod arms and mantle (see above).

The two explanations suggested above appear complementary. Having a sufficiently high concentration of an enzyme at minimized substrate flux allows a reduction of metabolic expenditure and supports the controlled use of metabolic pathways. Stenothermal animals can exploit this mechanism to a larger extent than eurythermal species since a high Ea means a high thermal sensitivity, reflected in a large increase in metabolic flux with a small rise in temperature. This is problematic for eurytherms but not for stenotherms since they experience only small temperature variations in their habitat. This may also transfer to the whole animal level since a large response to rising temperature in polar compared to temperate zone species may not only be visible in IDH activity or in proton leakage but also in the pattern of resting oxygen consumption (SMR) of the whole animal. This was actually observed in North Sea and Antarctic eelpout, in Antarctic octopods and a mobile Antarctic bivalve, *Yoldia eightsi* (Fig. 6, Table 2). In principle, the rise in oxygen consumption in these animals follows the Q_{10} relationship as reported in many textbooks, however, owing to higher Q_{10} values and Arrhenius activation energies oxygen consumption increases more steeply with rising temperature reaching maximum values at lower temperatures in polar than in temperate species. In *Arenicola marina* like in other intertidal animals MO_2 is constant over a wide range of temperatures. An increase in oxygen consumption occurs at temperatures above the thermoneutral range, close to the upper Tc. Owing to higher Arrhenius activation energies this increase was also larger in cold adapted sub-polar (White Sea) compared to temperate (North Sea) *Arenicola marina* (Figure 6, Table 2). All of these observations lead us to ask for the processes which might determine the large thermal sensitivity of metabolic rate.

As outlined above, the mitochondrial proton leak accounts for a large fraction of SMR. If cellular phosphorylation potential remains high and constant in the aerobic temperature range the thermal sensitivity of uncoupled mitochondrial respiration should have a direct influence on the thermal sensitivity of cellular, tissue and whole animal oxygen consumption. Thus the high thermal sensitivity of the proton leak and an associated dissipative heat loss is likely to account for much of the high thermal sensitivity of oxygen consumption (standard metabolism) in cold adapted, especially polar stenothermal species. The resulting oxygen demand can be met until a discrepancy develops between oxygen demand and supply at the upper critical temperature. The large thermal sensitivity of oxygen demand causes this mismatch to develop at low temperatures in polar species owing to an early limitation by ventilation and circulation. In other words, the apparent activation energy of aerobic metabolic rate may be a correlate of both cold adaptation and, in addition, the degree of stenothermality of a species (Table 2).

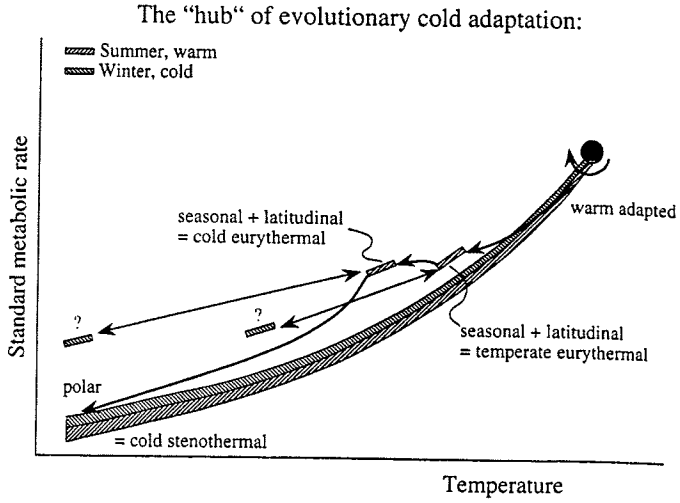

Fig 7. A modelled depiction of the effects of seasonal and permanent adjustments to cold on standard metabolic rate based on research in marine stenotherms and eurytherms. Starting from a warm adapted situation the polar stenotherms reach low standard metabolic rates close to those expected according to a regular Q10 effect. SMR increases and reflects metabolic cold compensation in those species subjected to seasonal temperature fluctuations. Cold compensation is illustrated by a progressive turn around a symbolic hub which reflects the original warm situation. The effect is maximum at high latitudes characterized by low temperature means and high and rapid seasonal temperature fluctuations as observed at the White Sea.

Within these general constraints, the lifestyle and the associated overall aerobic scope of an organism may also influence the Tc owing to its dependence on the scopes for ventilation and circulation. The flexibility of ventilatory or circulatory systems needs to be large enough to allow for the temperature dependence of mitochondrial oxygen requirements to become visible over the whole temperature range. This appears to be the case in higher organisms like fish or cephalopods (Fig. 6). In others like *Limopsis marionensis* (Pörtner *et al.* 1999a) ventilation or circulation may be limiting at much lower temperatures (such that the aerobic scope of the whole animal may be less than that of isolated mitochondria). If progressive hypoxia develops early at high temperatures, a down-regulation of metabolic rate typical for hypoxia tolerant animals may reduce energy demands and may thus influence the whole animal picture such that the typical rise in metabolic rate with temperature is not always visible or its thermal sensitivity is not fully expressed (cf. Wieser, 1998 for a treatment of tradeoffs in complex dynamic systems).

A COMPREHENSIVE MODEL

These considerations may lead to a more comprehensive picture of eurythermal versus stenothermal adaptation to cold (Fig. 7). A compensatory increase in standard metabolic rate owing to mitochondrial proliferation seems to be the typical short term answer to cooling (Fig. 7). Permanently stenothermal polar ectotherms compensate for this process by reversing this increase in standard metabolic rate, whereas extremely eurythermal animals may have to maximize aerobic capacity for being able to tolerate a wide range of temperatures. On the one hand, capacity must be sufficiently high to maintain oxidative metabolism at the lowest experienced temperature. On the other hand, thermal sensitivity of enzymes and mitochondria must be minimized to allow minimal oxygen demand at the highest

experienced temperature. As a benefit the rise in metabolic capacity depending on the degree of eurythermality may allow eurythermal species to profit from a more active and flexible lifestyle, whereas the reversal of metabolic cold compensation in marine polar ectotherms (Fig. 7) supports a less active lifestyle. In other words being eurythermal and leading a high activity mode of life may be two sides of the same coin.

This may also extend to terrestrial animals an aspect beyond the scope of this review. We are not aware of examples where the relationships presently evolving from marine studies have been investigated in terrestrial ectotherms, possibly because extreme stenothermality is very rare, at least on the cold side. An anecdotal example was presented by Marshall and Coetzee (1998). Terrestrial mites are exposed to larger diurnal temperature fluctuations (-2 to 30°C in summer) under the clear skies of the continental Antarctic than under the clouds of the more maritime (Northern) Antarctic (0 to 20°C). Possibly as a consequence of a higher degree of eurythermality and with no other differences in mode of life being apparent, the continental mites turned out to be more active than their maritime counterparts.

PREDICTIONS AND SUMMARY

In conclusion, some baseline constraints in cold adaptation could be identified that animals had to cope with on the way from stenothermal warm via eurythermal cold to stenothermal cold considering that polar life forms originated in warmer waters (Arntz *et al.* 1994). On the basis of available information the following statements and predictions appear to be justified. They all emphasize that oxygen limitation characterizes thermal tolerance limits. Tolerance thresholds and standard metabolic rates are related to an adjustment of mitochondrial densities, mitochondrial oxygen demand and the thermal properties of mitochondria:

1. Critical temperatures (Tc) are characterized by the transition to anaerobic metabolism owing to insufficient ventilation and/or circulation and aerobic energy provision. Insufficient aerobic capacity of mitochondria may contribute to set the low Tc, whereas the upper Tc is set by a mismatch of excessive oxygen demand by mitochondria and insufficient oxygen uptake and distribution by ventilation and circulation. Eurythermal species are able to shift critical temperatures by adjusting mitochondrial density and capacity according to the environmental temperature regime. Metabolic cold compensation by a rise in standard metabolic rate appears as the short term and intermediate term response to cooling (Fig. 7). The elevation of mitochondrial density causes a rise in energy expenditure (cost of mitochondrial maintenance). Cost is highest at low ambient mean temperatures linked to large temperature amplitudes both of which determine the degree of mitochondrial proliferation and the potential upregulation of mitochondrial aerobic capacity.
2. The elevation of mitochondrial density is required to cause a downward shift of Tc values. Critical temperatures in general are shifted by an adjustment of mitochondrial density since the energetic deficiency developing beyond tolerance limits causes a requirement for more mitochondria at cold and less mitochondria at high temperatures. These shifts occur on both a long term evolutionary and a seasonal time scale.
3. The cold induced rise in energy turnover elicited by the proliferation of mitochondria and their enzymes is compensated for on a long term evolutionary time scale in stenothermal polar and even more so, in deep sea species. Maintenance of high mitochondrial densities and capacities with comparatively low activation energies cause the maintenance cost to be higher in cold adaptated eurytherms than in stenotherms. In contrast to polar stenotherms, eurythermal animals may not be able to minimize the increment in metabolic rate elicited by cold adaptation (Fig. 7).
4. A rise in the Arrhenius activation energy of processes determining metabolic flux and mitochondrial costs may contribute to the low standard metabolic rates in stenothermal polar species. Compared to animals from warmer climates standard metabolic rate follows Q_{10} (polar) or is further reduced (negatively compensated) in the deep sea. This drop in SMR will require a reduction in ATP-dependent cellular functions like ionic or acid-base regulation (e.g. Sartoris and Pörtner 1997; Pörtner *et al.* 1998a) with a concomitant reduction in locomotor activity. This tradeoff may also contribute to the drop of the high Tc since the functional capacity of oxygen providing mechanisms drops. These considerations may be a key to an understanding of why "there are no polar tuna" (Clarke 1998).
5. Expression of flux limiting enzymes with high activation energies and therefore low specific activities may ensure that the number of enzymes can be kept sufficiently high in cold stenotherms to avoid a limitation of substrate diffusion. High activation energies, however, lead to strong flux stimulation with increasing temperature. The large thermal sensitivity of the mitochondrial proton leak contributes to a high oxygen demand at high temperatures and to setting the high critical temperature to low values in polar ectotherms. As a corollary, energy savings at low temperature to the extent suggested for polar species (Hochachka 1988) appear to reflect full scale adaptation and a specific advantage developed by polar marine stenotherms. In this light large thermal sensitivity and cold stenothermality appear as the consequence of sustaining an efficient low expenditure lifestyle at permanently low temperature.

Future investigations on temperature adaptation must emphasize the link between oxygen providing processes and the development of metabolic and life style strategies in closely related animal species from various environments and characteristic thermal conditions in a latitudinal gradient. These studies must include the temperature dependent modulation of energy partitioning between cellular functions like ion- and acid-base regulation, protein synthesis (growth) and the maintenance of mitochondria. This must extend to an analysis of the degree of heterocygosity as well as the energy partitioning between systemic functions like behaviour, reproduction and growth, as well as the consequences for stress resistance and tolerance limits.

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REFERENCES

Abele D., Wencke P. and Pörtner H.O. (1998). Oxidative stress and temperature acclimation in Antarctic shallow water molluscs. Ber. zur Polarforschung **299**, 237-242.

Alexandrov V.Ya. (1977). Cells, molecules and temperature. Springer Verlag, Berlin, Heidelberg, New York.

Almeida-Val V.M., Buck L.T. and Hochachka P.W. (1994). Substrate and acute temperature effects on turtle heart and liver mitochondria. Am. J. Physiol. **35**, R858-R862.

Alp P.R., Newsholme E.A. and Zammit V.A. (1976). Activities of citrate synthase and NAD^+-linked and $NADP^+$-linked isocitrate dehydrogenase in muscle from vertebrates and invertebrates. Biochem. J. **154**, 689-700.

Arntz W.E., Brey T. and Gallardo V.A. (1994). Antarctic Zoobenthos. Oceanography and Marine biology: An annual Review **32**, 241-304.

Baldwin J. (1971). Adaptation of enzymes to temperature: acetylcholinesterase in the central nervous system of fishes. Comp. Biochem. Physiol. **40B**, 181-187.

Ballantyne J.S. and Moon T.W. (1985). Hepatopancreas mitochondria from *Mytilus edulis*: substrate preferences and effects of pH and osmolarity. Mar. Biol. **87**, 239-244.

Ballantyne J.S. and Moyes C.D. (1987). The effects of salinity acclimation on the osmotic properties of mitochondria from the gill of *Crassostrea gigas*. J. exp. Biol. **133**, 449-456.

Brand M.D. (1990). The contribution of the leak of protons across the mitochondrial inner membrane to standard metabolic rate. J. Theor. Biol. **145**, 267-286.

Brand M.D., Chien L.-F., Ainscow E.K., Rolfe D.F.S. and Rolfe R.K. (1994). The causes and functions of mitochondrial proton leak. Biochim. Biophys. Acta **1187**, 132-139.

Brookes P.S., Buckingham J.A., Tenreiro A.M., Hulbert A.J. and Brand M.D. (1998). The proton permeability of the inner membrane of liver mitochondria from ectothermic and endothermic vertebrates and from obese rats: correlations with standard metabolic rate and phospholipid fatty acid composition. Comp. Biochem. Physiol. **119B**, 325-334.

Brown, G.C. (1991). Total cell protein concentration as an evolutionary constraint on the metabolic control distribution in cells. J. theor. Biol. **153**, 195-203.

Burcham J.M., Paynter K.T. and Bishop, S.H. (1983). Coupled mitochondria from oyster gill tissue. Mar. Biol. Lett. **4**, 349-356.

Burcham J.M., Ritchie A.L. and Bishop S.H. (1984). Preparation and some respiratory properties of coupled mitochondria from ribbed mussel (*Modiolus demissus*) gill tissue. J. exp. Zool. **229**, 55-67.

Clarke A. (1991). What is cold adaptation and how should we measure it? Am. Zool. **31**, 81-92.

Clarke A. (1998). Temperature and energetics: an intoduction to cold ocean physiology. In: *Cold ocean physiology* (eds. Pörtner H.O. and Playle R.) Cambridge, University Press, pp 3-30.

Crockett E.L. and Sidell B.D. (1990). Some pathways of energy metabolism are cold adapted in Antarctic fishes. Physiol. Zool. **63**, 472-488.

Dahlhoff E. and Somero G.N. (1993). Kinetic and structural adaptations of cytoplasmatic malate dehydrogenases of eastern pacific abalone (genus *Haliotis*) from different thermal habitats: biochemical correlates of biogeographical patterning. J. exp. Biol. **185**, 137-150.

Davis J.E. and Davis-van Thienen W.I.A. (1991). An assessment of the role of proton leaks in the mechanistic stoichiometry of oxidative phosphorylation. Arch. Biochem. Biophys. **289**, 184-186.

Dufour, S., Rousse N., Canioni P. and Diolez P. (1996). Top-down control analysis of temperature effect on oxidative phosphorylation. Biochem. J. **314**, 743-751.

Dunn J.F. (1988). Low-temperature adaptation of oxidative energy production in cold-water fishes. Can. J. Zool. **66**, 1098-1104.

Dunn J.F. and Johnston I.A. (1986). Metabolic constraints on burst-swimming in the Antarctic teleost *Notothenia neglecta*. Mar. Biol. **91**, 433-440.

Ellory J.C. and Hall A.C. (1987). Temperature effects on red cell membrane transport processes. In: *Temperature and Animal Cells* (eds. Bowler K. and Fuller B.J.) Symposia of the Society of Experimental Biology, **21**, pp. 53-66.

El-Wadawi R. and Bowler K. (1995). The development of thermotolerance protects blowfly flight muscle mitochondria function from heat damage. J. Exp. Biol. **198**, 2413-2421.

Farrell A.P. (1997). Effects of temperature on cardiovascular performance. In: *Global warming: implications for freshwater and marine fish* (eds. Wood C.M. and McDonald D.G.) Cambridge University Press, pp. 135-158.

Frederich M. and Pörtner H.O. (2000). Thermal Tolerance defined by cardiac and ventilatory performance in the spider crab, *Maja squinado* (in review).

Frederich M., Sartoris, F.J., Arntz, W. and Pörtner, H.O. (2000). Haemolymph magnesium regulation in decapod crustaceans: physiological correlates and ecological consequences in polar areas. J. exp. Biol, in press.

Geiser F. and McMurchie E.J. (1985). Arrhenius parameters of mitochondrial membrane respiratory enzymes in relation to thermoregulation in endotherms. J. comp. Physiol. **155B**, 711-715.

Gonzalez-Villasenor L.I. and Powers D.A. (1986). A multilocus system for studying tissue and subcellular specialization. The pH and temperature dependence of the two major NADP-dependent isocitrate dehydrogenase isozymes of the fish *Fundulus heteroclitus*. J. Biol. Chem. **261**, 11471-11477.

Grieshaber M.K., Hardewig I., Kreutzer U. and Pörtner H.O. (1994). Physiological and metabolic responses to hypoxia in invertebrates. Rev. Physiol. Biochem. Pharmacol. **125**, 43-147.

Guderley H. (1998). Temperature and growth rates as modulators of the metabolic capacities of fish muscle. In: *Cold ocean physiology* (eds. Pörtner H.O. and Playle R.) Cambridge, University Press, pp 58-87.

Hardewig I., van Dijk P.L.M. and Pörtner H.O. (1998). High-energy turnover at low temperatures: recovery from exhaustive exercise in Antarctic and temperate eelpouts. Am. J. Physiol. **274**, R1789-R1796.

Hardewig I., Peck L.S. and Pörtner H.O. (1999a). Thermal sensitivity of mitochondrial function in the Antarctic Notothenioid *Lepidonotothen nudifrons*. J. Comp. Physiol. B 169, 597-604.

Hardewig I., van Dijk P.L.M., Moyes C.D. and Pörtner H.O. (1999b). Temperature-dependent expression of cytochrome c oxidase in Antarctic and temperate fish. Am. J. Physiol. 277, R508-R516.

Head E.J.H. and Gabbot P.A. (1980). Properties of NADP-dependent isocitrate dehydrogenase from the mussel *Mytilus edulis* L. Comp. Biochem. Physiol. **66B**, 285-289.

Head E.J.H. and Gabbot P.A. (1981). Kinetic control of NADP-dependent isocitrate dehydrogenase activity in the mussel *Mytilus edulis* L. Comp. Biochem. Physiol. **68B**, 383-388.

Hoberman H.D. and Prosky L. (1967). Evidence of reduction of fumarate to succinate in perfused rat liver under conditions of reduced O_2 tension. Biochem. Biophys. Acta **148**, 392-399.

Hochachka P.W. (1988). Channels and pumps - determinants of metabolic cold adaptation strategies. Comp. Biochem. Physiol. **93**, 515-529.

Hochachka P. W. and Somero G. N. (1984). Biochemical adaptation. Princeton, University Press, 537 pp.

Houlihan D. F. and Allan D. (1982). Oxygen consumption of some Antarctic and British gastropods: an evaluation of cold adaptation. Comp Biochem Physiol **73A**: 383-387

Howlett R.A. and Willis W.T. (1998). Fiber-type-related differences in the enzymes of a proposed substrate cycle. Biochim. Biophys. Acta **1363**, 224-230.

Hummel H., Sommer A., Bogaards R. and Pörtner H.O. (1997). Variation in genetic traits of the lugworm *Arenicola marina*: temperature related expression of mitochondrial allozymes? Mar. Ecol. Prog. Ser. **159**, 189-195.

Johnston I.A. (1975). Anaerobic metabolism in the carp *(Carassius carassius* L.). Comp Biochem. Physiol. **51B**, 235-241.

Johnston I.A., Guderley H., Franklin C.E., Crockford T. and Kamunde C. (1994). Are mitochondria subject to evolutionary temperature adaptation? J. exp. Biol. **195**, 293-306.

Johnston I.A., Calvo J., Guderley H., Fernandez D. and Palmer L. (1998). Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. J. Exp. Biol. **201**, 1-12.

Kargbo A.O. and Swift M.L. (1983). NAD$^+$-dependent isocitrate dehydrogenase from the oyster, *Crassostrea virginica* GMELIN. Comp. Biochem. Physiol. **76B**, 123-126.

Lyons J.M. and Raison J.K. (1970). A temperature-induced transition in mitochondrial oxidation: contrasts between cold and warm-blooded animals. Comp. Biochem. Physiol. **37**, 405-411.

MacDonald J.A., Montgomery, J.C. and Wells R.M.C. (1988). The physiology of McMurdo Sound fishes: current New Zealand research. Comp. Biochem. Physiol. **90B**, 567-578.

Marcinkowski A., Danielewicz R. and Wolny M. (1990). The effect of temperature on catalytic function of glyceraldehyde-3-phosphate dehydrogenase from muscle of pig and carp *Cyprinus carpio*. Comp. Biochem. Physiol. **97B**, 49-54.

Marshall D.J. and Coetzee L. (1998). Historical biogeographic and ecological evidence for the gondwanian origin and pliocene-pleistocene speciation of continental Antartic mites. New Zealand Nat. Sci. **23** (Suppl.), 118.

Moon T.W. and Hochachka P.W. (1971). Temperature and enzyme activity in poikilotherms. *Isocitrate dehydrogenases* in rainbow-trout liver. Biochem. J. **123**, 695-705.

Mommsen T.P. and Hochachka P.W. (1981). Respiratory and enzymatic properties of squid heart mitochondria. Eur. J. Biochem. **120**, 345-350.

Moyes C. D., Moon T. W. and Ballantyne J. S. (1985). Glutamate metabolism in mitochondria from *Mya arenaria* mantle: Effects of pH on the role of glutamate dehydrogenase. J. exp. Zool. **236**, 293-301.

Moyes C.D., Buck L.T. and Hochachka P.W. (1988). Temperature effects on pH of mitochondria isolated from carp red muscle. Am. J. Physiol. **254**, R611-R615.

Newsholme E.A. and Crabtree B. (1981). Flux-generating and regulatory steps in metabolic control. Trends Biochem. Sci. **6**, 53-56.

O'Brien J., Dahlhoff E. and Somero G.N. (1991). Thermal resistance of mitochondrial respiration: hydrophobic interactions of membrane proteins may limit thermal resistance. Physiol. Zool. **64**, 1509-1526.

Peck L.S. (1989). Temperature and basal metabolism in two Antarctic marine herbivores. J Exp Mar Biol Ecol **127**: 1-12

Peck L.S. (1998). Feeding, metabolism and metabolic scope in Antarctic marine ectotherms. In: *Cold Ocean Physiology*. (eds. Pörtner H.O., and Playle R.) Cambridge University Press, pp. 365-390

Pörtner H.O., and Grieshaber M.K. (1993). Characteristics of the critical Po_2(s): gas exchange, metabolic rate and the mode of energy production. In: *The vertebrate gas transport cascade: adaptations to environment and mode of life* (ed. Bicudo, J.E.P.W.). CRC Press Inc., Boca Raton FL, U.S.A, pp. 330-357.

Pörtner H.O., MacLatchy L.M., and Toews D.P. (1991). Metabolic responses of the toad *Bufo marinus* to environmental hypoxia: An analysis of the critical Po_2. Physiol. Zool. **64**, 836-849.

Pörtner H. O., Hardewig I., Sartoris F. J. and van Dijk P. L. M. (1998a). Energetic aspects of cold adaptation: critical temperatures in metabolic, ionic and acid-base regulation? In: *Cold ocean physiology* (eds. Pörtner H.O. and Playle R.) Cambridge University Press. pp 88-120.

Pörtner H.O., Peck L., Zielinski S. and Conway L.Z. (1999a). Intracellular pH and energy metabolism in the highly stenothermal Antarctic bivalve *Limopsis marionensis* as a function of ambient temperature. Polar Biology in press

Pörtner H.O., Hardewig I. and Peck L.S. (1999b). Mitochondrial function and critical temperature in the Antarctic bivalve *Laternula elliptica*. Comp. Biochem. Physiol. 124a, 179-189.

Pörtner H.O. and Sartoris F.J. (1999). Invasive studies of intracellular acid-base parameters: environmental and functional aspects. In: *Regulation of tissue pH in plants and animals: A reappraisal of current techniques.* (eds. Taylor, E.W., Egginton, S. and Raven J.A.). Cambridge University Press, pp 69-98.

Pörtner H.O and Zielinski S. (1998). Environmental constraints and the physiology of performance in squids. CIAC Symposium proceedings, Cape Town, South African Journal of Marine Science, 20, 207-221.

Rolfe D.F.S. and Brand M.D. (1996). Contribution of mitochondrial proton leak to skeletal muscle respiration and to standard metabolic rate. Am. J. Physiol. **271**, C1380-1389.

Sazanov L.A. and Jackson J.B. (1994). Proton-translocating transhydrogenase and NAD and NADP- linked isocitrate dehydrogenase operate in a substrate cycle which contributes to fine regulation of the tricarboxylic acid cycle activity in mitochondria. FEBS Letters **344**, 109-116.

Sartoris F.J. and Pörtner H.O. (1997). Temperature dependence of ionic and acid-base regulation in boreal and arctic *Crangon crangon* and *Pandalis borealis*. J. Exp. Mar .Biol. Ecol. **211**, 69-83.

Sidell B.D. and Hazel J.R. (1987). Temperature effects the diffusion of small molecules through cytosol of fish muscle. J. exp. Biol. **129**, 191-203.

Smith M.J. and Heath A.G. (1980). Responses to acute anoxia and prolonged hypoxia by rainbow trout (*Salmo gairdneri*) and mirror carp (*Cyprinus carpio*) red and white muscle: use of conventional and modified metabolic pathways. Comp. Biochem. Physiol. **66B**, 267-272.

Sols A. and Marco R. (1970). Concentrations of metabolites and binding sites. Implications in metabolic regulation. Curr. Top. Cell. Regul. **2**, 227-270.

Somero G.N. (1997). Temperature relationships: from molecules to biogeography. Handbook of Physiology, Section 13: Comparative Physiology Ed. William H. Dantzler, Oxford University Press, New York/Oxford.

Somero G.N. and DeVries A.L. (1967). Temperature tolerance of some Antarctic fishes. Science **156**, 257-258.

Somero G.N., Dahlhoff E. and Lin J.J. (1996). Stenotherms and eurytherms: mechanisms establishing thermal optima and tolerance ranges. In: *Animals and temperature* (eds. Johnston I.A. and Bennett A.F.) Cambridge University Press, pp 53-78.

Somero G.N., Fields P.A., Hofmann G.E., Weinstein R.B. and Kawall H. (1998). Cold adaptation and stenothermy in Antarctic notothenioid fishes: What has been gained and what has been lost? In: *Fishes of Antarctica.* A biological overview (eds. Di Prisco G., Pisano E. and Clarke A.) Milano, Springer-Verlag, pp 97-109.

Sommer A., Klein B. and Pörtner H.O. (1997). Temperature induced anaerobiosis in two populations of the polychaete worm *Arenicola marina* (L.). J. Comp. Physiol. **167B**, 25-35.

Sommer A. and Pörtner H.O. (1999). Exposure of *Arenicola marina* (L.) to extreme temperatures: adaptive flexibility of a boreal and a subpolar population. Mar. Ecol. Prog. Ser. 181, 215-226.

Storelli C., Acierno R. and Maffia M. (1998). Membrane lipids and protein adaptations in Antarctic fish. In: *Cold ocean physiology* (eds. Pörtner H.O. and Playle R.) Cambridge University Press. pp. 166-189.

van Dijk P.L.M., Hardewig I., Tesch C. and Pörtner H.O. (1999). Physiological disturbances at critically high temperatures. A comparison between stenothermal Antarctic, and eurythermal temperate eelpouts (Zoarcidae).J. exp. Biol. 202, 3611-3621.

van Waarde A., van den Thillart G. and Kesbeke F. (1983). Anaerobic metabolism of the Eurpean eel, *Anguilla anguilla*. J. Comp. Physiol. **149**, 469-475.

Weinstein R.B. and Somero, G.N. (1998). Effects of temperature on mitochondrial function in the Antarctic fish *Trematomus bernacchii*. J. Comp Physiol. **168B**, 190-196.

Wieser W. (1985). Developmental and metabolic constraints of the scope of activity in young rainbow trout (*Salmo gairdneri*). J. Exp. Biol. **118**, 133-142.

Wieser W. (1998). Optimality in complex dynamic systems: constraints, trade-offs, priorities. In: *Principles of animal design* (eds. Weibel E.R., Taylor C.R. and Bolis L.) Cambridge University Press. pp 230-238.

Zielinski S. and Pörtner H.O. (1996). Energy metabolism and ATP free-energy change of the intertidal worm, *Sipunculus nudus*, below a critical temperature. J. comp. Physiol. **166B**, 492-500.

Zielinski S. Hirse T, Klein B, Finke E., Wegewitz V. and Pörtner H.O. (1999). Temperature as a critical constraint in warm water and Anatrctic cephalopods. (in review)