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Review

Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals☆

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Abstract

The physiological mechanisms limiting and adjusting cold and heat tolerance have regained interest in the light of global warming and associated shifts in the geographical distribution of ectothermic animals. Recent comparative studies, largely carried out on marine ectotherms, indicate that the processes and limits of thermal tolerance are linked with the adjustment of aerobic scope and capacity of the whole animal as a crucial step in thermal adaptation on top of parallel adjustments at the molecular or membrane level. In accordance with Shelford's law of tolerance decreasing whole animal aerobic scope characterises the onset of thermal limitation at low and high pejus thresholds (pejus = getting worse). The aerobic scope of an animal indicated by falling oxygen levels in the body fluids and or the progressively limited capacity of circulatory and ventilatory mechanisms. At high temperatures, excessive oxygen demand causes insufficient oxygen levels in the body fluids, whereas at low temperatures the aerobic capacity of mitochondria may become limiting for ventilation and circulation. Further cooling or warming beyond these limits leads to low or high critical threshold temperatures (T_c) where aerobic scope disappears and transition to an anaerobic mode of mitochondrial metabolism and progressive insufficiency of cellular energy levels occurs. The adjustments of mitochondrial densities and their functional properties appear as a critical process in defining and shifting thermal tolerance windows. The finding of an oxygen limited thermal tolerance owing to loss of aerobic scope is in line with Taylor's and Weibel's concept of symmorphosis, which implies that excess capacity of any component of the oxygen delivery system is avoided. The present study suggests that the capacity of oxygen delivery is set to a level just sufficient to meet maximum oxygen demand between the average highs and lows of environmental temperatures. At more extreme temperatures only time limited passive survival is supported by anaerobic metabolism or the protection of molecular functions by heat shock proteins and antioxidative defence. As a corollary, the first line of thermal sensitivity is due to capacity limitations at a high level of organisational complexity, i.e. the integrated function of the oxygen delivery system, before individual, molecular or membrane functions become disturbed. These interpretations are in line with the more general consideration that, as a result of the high level of complexity of metazoan organisms compared with simple eukaryotes and then prokaryotes, thermal tolerance is reduced in metazoans. A similar sequence of sensitivities prevails within the metazoan organism, with the highest sensitivity at the organismic level and wider tolerance windows at lower levels of complexity. However, the situation is different in that loss in aerobic scope and progressive hypoxia at the organismic level define the onset of thermal limitation which then transfers to lower hierarchical levels and causes cellular and molecular disturbances. Oxygen limitation contributes to oxidative stress and, finally, denaturation or malfunction of molecular repair, e.g. during suspension of protein synthesis. The sequence of thermal tolerance limits turns into a hierarchy, ranging from systemic to cellular to molecular levels. © 2002 Published by Elsevier Science Inc.

Keywords: Air breather; Cold adaptation; Critical temperatures; Geographical distribution; Law of tolerance; Mitochondria; Pejus temperatures; Proton leakage; Standard metabolic rate; Water breather; Bird; Bivalve; Crab; Fish; Mammal; Reptile; Squid; Worm

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1. Introduction

Oscillations in the earth's climate lead to associated fluctuations in the temperature regimes of many marine and terrestrial ecosystems. Consequences in many marine ecosystems include changes in the timing of reproduction as well as changes in reproductive success, recruitment, growth performance and mortality of species and finally, changes in their geographical distribution. Accordingly, impacts of decadal-scale variations by the coupled ocean–atmosphere system on marine communities and populations have been well documented (Cushing, 1982; Beamish, 1995; Bakun, 1996). Examples can be found among invertebrates (Southward et al., 1995) but also among fishes where temperature related trends in stocks of fish, e.g. North Sea cod (Dippner, 1997; O'Brien et al., 2000) or Pacific salmon (Klyashtorin, 1997) reflect the general fact that ectothermic animal species are adapted to and depend upon maintenance of the characteristic temperature window of their natural environment. Climate changes will have opposite effects on populations at the northern and southern edges of their distribution. In contrast to marine mammals and birds, no ectothermic species is known to occur over the widest temperature ranges possible across latitudes between polar and tropical areas. Accordingly, thermal tolerance windows differ between species depending on the range of environmental temperature. An overview of thermal tolerance ranges of tropical, temperate and polar bivalves and other ectotherms, compiled by Peck and Conway (2000), suggests that tolerance windows are wider in tropical and temperate species than in polar stenotherms. This leads to the question whether true stenothermality really exists except in the cold. Fig. 1 indicates that adaptation to temperatures below a threshold value of approximately 8 °C leads to a narrowing of the tolerance window. This strongly suggests that life in the cold is a severe challenge to the organism and forces it to truly specialise in living at low temperatures. Eurytherms tolerate wider temperature fluctuation and, in temperate zones, are able to dynamically shift tolerance windows between summer and winter temperature regimes. Nonetheless, they still specialise on a characteristic thermal environment. As life in warm waters is likely to reflect the original evolutionary situation (Arntz et al., 1994), the capability of surviving seasonal or permanent cold must be considered an evolutionary accomplishment rather than a general characteristic of all life forms. The contribution of temperature changes to climate dependent mass extinction and the drop in biodiversity in a temperature cline towards higher (Northern) latitudes would support this conclusion (cf. Pörtner, 2001, for review).

Physiological acclimatisation to wide temperature windows correlates with increasing genetic differentiation visible already between populations of the same species throughout a latitudinal or altitudinal cline (Hummel et al., 1997; Dahlhoff and Rank, 2000). The reasons for the thermal specialisation of animals, i.e. the factors determining and limiting temperature related geographical distribution need to be identified. The present paper summarises recent developments of a concept that may support an understanding of the physiological basis of temperature dependent biogeography, esp. in ectotherms. Previous data collected in various groups of predominantly marine water breathing but also in air breathing ectotherms (annelids, sipunculids, molluscs like bivalves and cephalopods, crustaceans, fishes, terrestrial arthropods and reptiles) indicate that limited oxygen availability and aerobic scope are crucial in limiting thermal tolerance. The relative stability of sea water compared with terrestrial and fresh water temperatures makes animals from the marine envi-

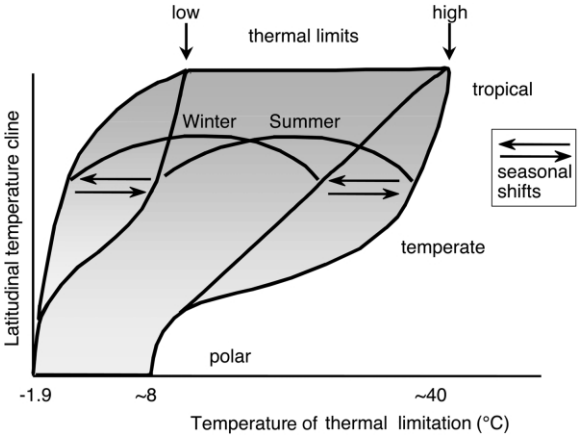

Fig. 1. Schematic depiction of the ranges of thermal tolerance in marine ectotherms (invertebrates and fish) in a latitudinal cline (modified after Pörtner, 2001). The preliminary diagram ignores the difference between climate regimes in Northern and Southern hemispheres. Seasonal shifts of tolerance windows occur in temperate zone species. Note the narrowing of the tolerance range in polar, esp. Antarctic species (see text).

ronment an excellent group to study the relevance of temperature on a large global scale and to elaborate the principles of thermal tolerance in a much clearer way than would be possible in comparisons of eurythermal species on smaller latitudinal scales. Accordingly, the present study aims at the development of a unifying hypothesis of thermal limitation and adaptation, which may explain the thermal limits of geographical distribution. As a second step this concept also attempts to explain the reasons for reproductive failure at the borders of the temperature window as well as for changes in energy allocation to growth and reproduction as a consequence of thermal acclimatisation (Pörtner et al., 2001).

Thermal sensitivity needs investigation at both the high end and the low end of the temperature spectrum. The crucial mechanism(s) of thermal limitation and adaptation should link low and high tolerance limits, since they shift unidirectionally during thermal acclimation. Also, changes occurring during acclimation to high temperatures should reverse the processes involved in low temperature acclimation. These patterns should be similar for all groups of metazoans. Within an organism the crucial mechanisms of temperature adaptation should be visible in all tissues supporting the functional co-ordination of the organismic entity. If failure of individual molecular mechanisms is involved whole animal limits and the limits of these individual molecular mechanisms should be identical. In consequence, sensitivity levels of molecules, organelles, cells, tissues and the intact organism need to be distinguished as well as the difference between mechanisms responsible for long and short-term tolerance. This includes the key question as to which physiological processes define the temperature window of growth and reproduction as required for successful long-term maintenance of a population in its natural environment. Beyond this window, passive tolerance may ensure time-limited survival of the individual animal, e.g. during unfavourable conditions like extreme seasonal cold or warm. However, such limits likely do not reflect close co-ordination between environmental temperatures and the range of optimum physiological performance and may, thus, not be closely correlated with geographical distribution limits.

2. Thermal sensitivity and the level of organisational complexity

It is widely held that heat tolerance limits for all metazoa are found at approximately 47 °C. Even the ‘Pompeii worm’, which is believed to be the ‘most thermotolerant eukaryote on earth’ (Desbruyeres et al., 1998) by being exposed to temperatures between 2 and, possibly, 105 °C in its White Smoker environment at hydrothermal deep sea vents, will likely not live long-term beyond 31 °C (Dahlhoff and Somero, 1991; for review see Desbruyeres et al., 1998). The upper thermal limit of nematodes may reach somewhat higher and beyond 45–47 °C, which is considered as the upper temperature limit of continuous inhabitation (Nicholas, 1984). Similarly, a desert ant tolerates 53.6 °C but only during short-term periods of its midday feeding excursions (Wehner et al., 1992, see below). The average upper thermal limit for insects was evaluated as 47.4 ± 0.4 °C (Addo-Bediako et al., 2000). In general, temperatures beyond 47 °C are tolerated only temporarily by metazoans (cf. Schmidt-Nielsen, 1997). This contrasts the thermal limits of bacteria some of which thrive continuously at temperatures over 90 °C and even in boiling waters. Simple eukaryotes like fungi or algae still grow at temperatures of approximately 55–60 °C (Tansey and Brock, 1972).

The question arises at which organisational level, molecular, cellular or organismic, the limits of thermal tolerance are likely to be found. According to an early hypothesis by Tansey and Brock (1972), there should be no difference in the thermal stability of aerobic heterotrophic metabolism between pro- and eukaryotes. Eukaryotes, however, are characterised by the formation of intracellular organelles like nuclei and mitochondria, a process which might have reduced the capacity to tolerate extreme temperatures. Selective transport of macromolecules across organellar membranes occurs and, according to Tansey and Brock (1972), these large pore membranes may be leakier and less thermostable than plasma membranes. However, the further reduction in heat tolerance from simple eukaryotes to metazoans (see above) remains unexplained by this interpretation. This problem disappears when it is considered, that the formation of intracellular organelles reflects a gain in structural and functional complexity between pro- and eukaryotes. The limits of tolerance would be set at the level of the organelles and their

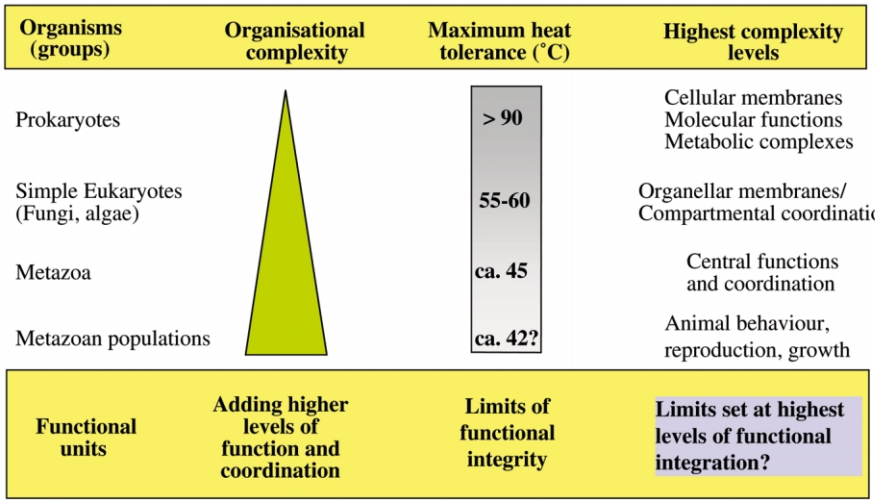

Fig. 2. Heat sensitivity depending on cellular and organismic complexity. The rise in organisational complexity between prokaryotes, unicellular eukaryotes and metazoa is interpreted to cause a drop in thermal tolerance. For further explanations see text.

functional co-ordination with the rest of the cell, i.e. at a higher level of complexity (cellular compartmentation) than in prokaryotes (Fig. 2).

Even higher levels of organisational complexity are added in metazoans, for example by the specialisation of tissues, the establishment of central control and the requirement to circulate and move body fluids and air or water for gas exchange and homeostatic maintenance. Associated with this gain in complexity is an increase in metabolic rate reflecting a performance increment between unicellular organisms and animals (Hemmingsen, 1960, as depicted by Schmidt-Nielsen, 1997). It is intriguing to assume that the rise in performance seen between pro- and unicellular eukaryotes as well as between simple eukaryotes and metazoa occurs at the expense of greater thermal sensitivity. The limits of tolerance would be set at the highest level of organisational complexity, i.e. the functional co-ordination of organismic components towards a larger unit. Within the metazoan organism, lower thermal sensitivities would again be expected at lower complexity levels, i.e. at cellular and molecular levels. A sequence of thermal sensitivities results which relates to the level of organisational complexity of the system (Fig. 2).

A concept that considers the combination of functional elements towards higher levels of integration is the concept of symmorphosis as developed for the mammalian respiratory system (Taylor and Weibel, 1981). This concept states that animals maintain just enough structure to support oxygen flux rates at their maximum oxygen uptake rates. The design of all components of a system match functional demand in a way that excess capacity of any single component is avoided (Hoppeler and Weibel, 1998). This concept becomes important in the context of temperature adaptation and limitation, since oxygen limitation at extreme temperatures (both low and high) is most likely a unifying principle in animals, which defines the first lines of both heat and cold intolerance (Pörtner et al., 1998, 2000; Pörtner, 2001). More recent evidence discussed for an oxygen limitation of thermal tolerance supports earlier, more descriptive work, where improved survival of fish at high temperatures was found in normoxia compared with hypoxia in the case of trout and roach (Alabaster and Welcomme, 1962) or in hyperbaric hyperoxia compared with normoxia in the case of goldfish (Weatherley, 1970).

Evidence is discussed that the first line of thermal sensitivity becomes apparent at the highest functional level possible, in this case the integrated function of ventilation and circulation. It is hypothesised that a shift of thermal tolerance windows is predominantly caused by the observed changes in tissue mitochondrial densities and/or functional properties and the respective consequences for cell and tissue function as well as central functions like nervous control, circulation and respiration. Molecular and membrane adjustments support those at the organellar, cellular and tissue level leading to the maintenance and sensible adjustment

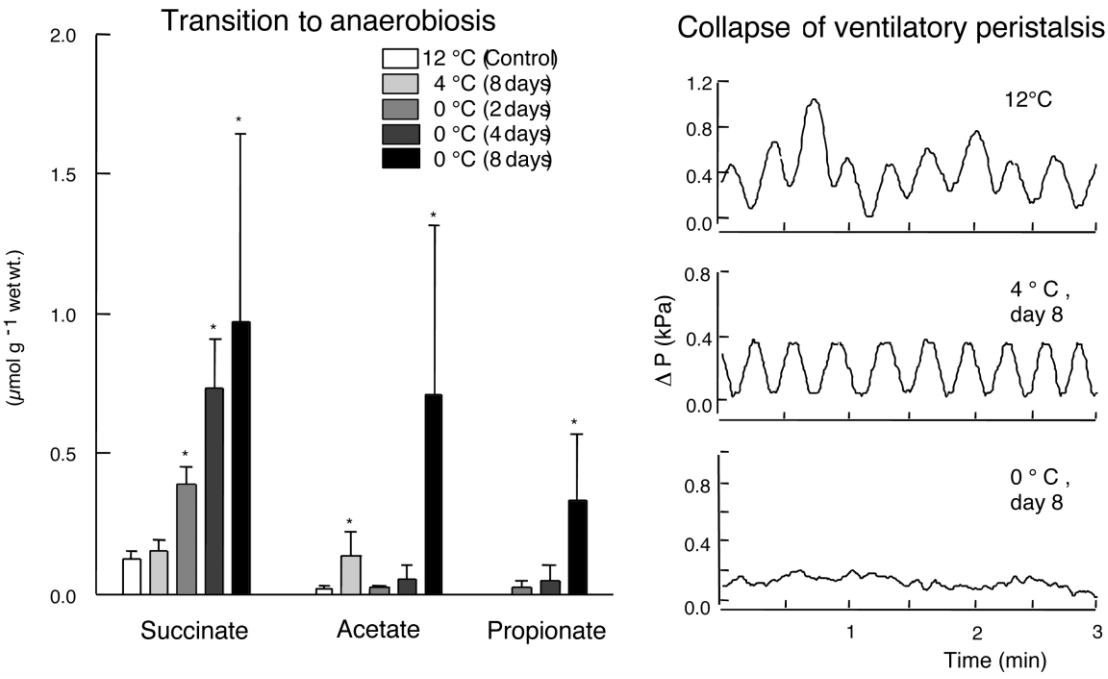

Fig. 3. Cold induced onset of anaerobic mitochondrial metabolism in the temperate zone intertidal worm *Sipunculus nudus* is indicated by the accumulation of succinate, propionate and acetate in the body wall musculature (left). Transition to cold induced anaerobiosis appears as the final result of a cold induced collapse of ventilatory peristalsis below 4 °C (right, after Zielinski and Pörtner, 1996).

of central functions. The changes in mitochondrial functions would not only explain a shift in thermal tolerance limits defined at high levels of organisational complexity, but also contribute to trade-offs within energy budgets with the respective consequences for temperature dependent changes in growth, fecundity and recruitment, ecological key processes which determine population structure and dynamics.

3. Oxygen limitation of thermal tolerance: a unifying principle?

3.1. Water breathers

Recent evidence for oxygen limited thermal tolerance arose from the observation of low and high thermal tolerance thresholds in various marine invertebrate species and, most recently, high tolerance limits in fish, which were associated with the transition to an anaerobic mode of mitochondrial metabolism. These low and high temperature thresholds had been defined as critical temperatures (T_c, for review see Pörtner et al., 1998, 2000; Pörtner, 2001). In all studies reported the onset of mitochondrial anaerobiosis indicated transition to passive, time limited cold or warm tolerance.

Cold or warm induced onset of mitochondrial anaerobic metabolism in fully aerated water indicated a mismatch of oxygen supply and demand and critically low tissue oxygen levels. Investigations of oxygen consumption during warming suggested that high critical temperatures were associated with the upper limit of a heat induced rise in oxygen demand (cf. Pörtner, 2001). Cold exposure led to a progressive failure of ventilatory peristalsis, as shown in a marine worm, *Sipunculus nudus*. Below a threshold temperature of 4 °C this process led to progressive hypoxia in the body fluids and finally transition to anaerobic mitochondrial metabolism (Fig. 3, Zielinski and Pörtner, 1996). The picture became even more complete for the spider crab, *Maja squinado*, where continuous analysis of haemolymph P_{O_2} during progressive warming or cooling (Fig. 4) revealed maintenance of high oxygen levels and maximum oxygen supply only within an optimum temperature window. Major changes in haemolymph P_{O_2} occurred outside of this window, until finally, oxygen partial pressure had fallen to extremely

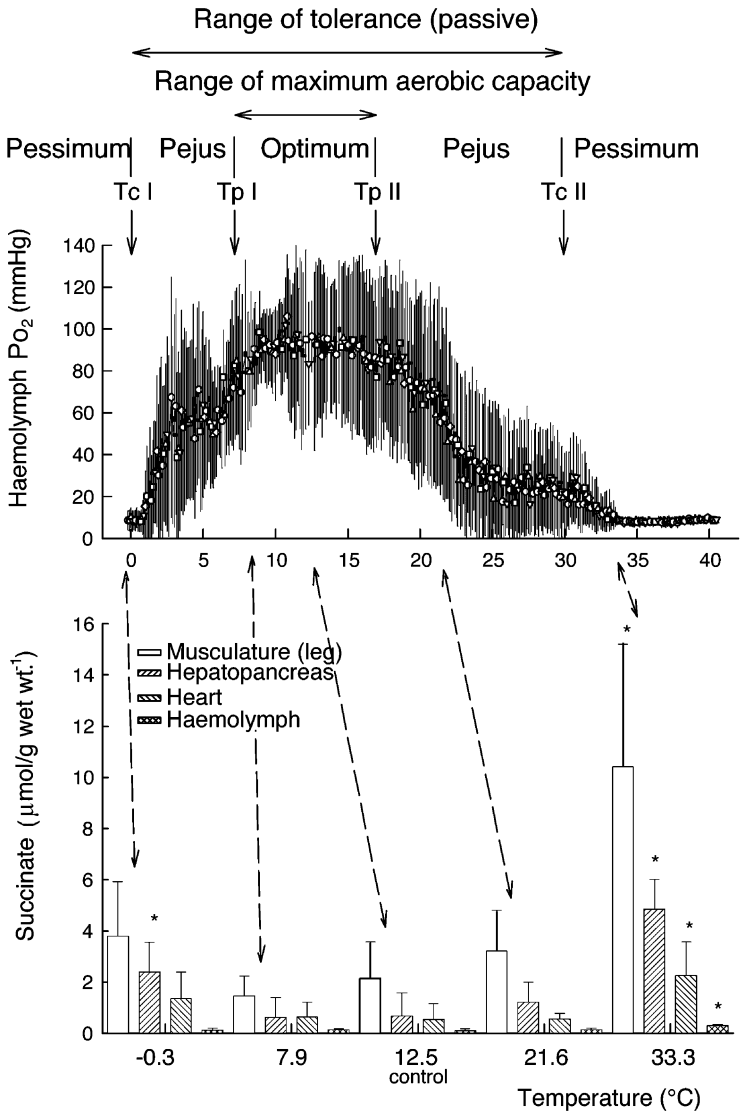

Fig. 4. Correlation of haemolymph oxygen tensions and transition to anaerobic metabolism in the spider crab, *Maja squinado* (data by Frederich and Pörtner, 2000). The P_{O_2} and metabolite profiles recorded during progressive cooling from 12 to 0 °C (at 1 °C/h) or warming from 12 to 40 °C (at 2 °C/h, tissues were sampled after the final temperatures were reached) characterises aerobic scope available to the animals and, finally, tissue hypoxia at extreme temperatures. The profiles match optimum, pejus and pessimum ranges with respect to temperature, as adopted from the law of tolerance (Shelford, 1931; Schwerdtfeger, 1977; Frederich and Pörtner, 2000).

low levels and concentrations of lactate and succinate rose in tissues and haemolymph (Frederich and Pörtner, 2000). The transition from a temperature range with maximum arterial P_{O_2} to low or high ranges of temperature characterised by progressive hypoxia in the body fluids identified a second set of low and high threshold temperatures well within the range encompassed by critical temperatures. Adopting Shelford's law of tolerance (Shelford, 1931) these threshold temperatures were called pejus temperatures, T_p I and T_p II (pejus = getting worse), since they indicate the limits of optimum haemolymph oxygenation and, thereby, onset of a worsening of oxygen supply to the organism (Frederich and Pörtner, 2000; Fig. 4).

In the spider crab the high P_{O_2} between T_p I and T_p II was maintained by a temperature-dependent increase in heart and ventilation rates as required to compensate for the rise in oxygen demand (Fig. 5). However, beyond the high pejus

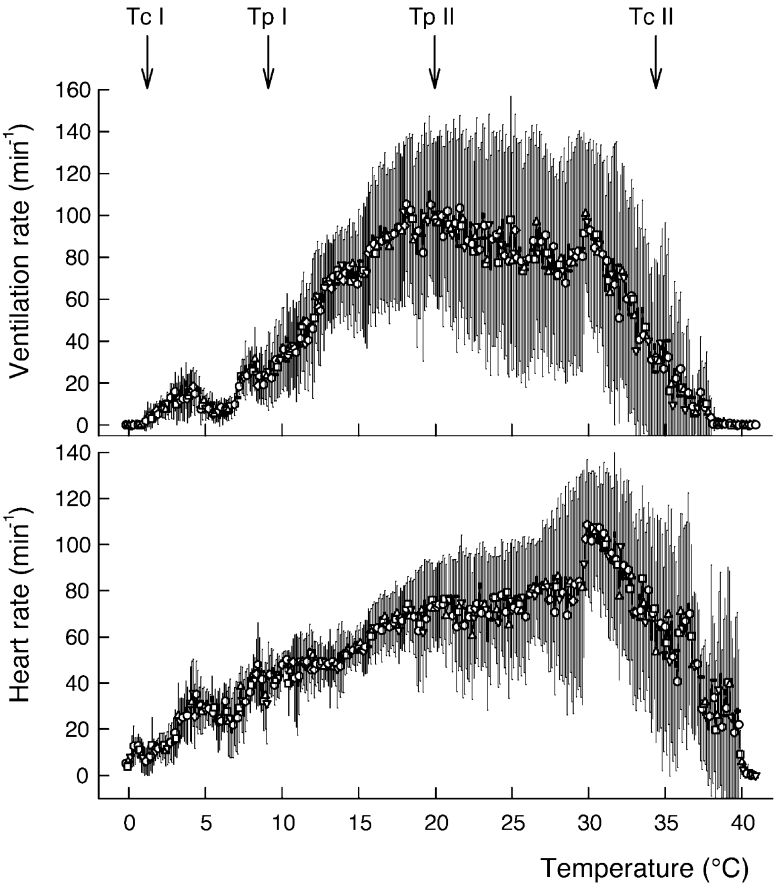

Fig. 5. Correlated changes in ventilation and heart rates in the spider crab *Maja squinado* between 0 and 40 °C. Threshold temperatures (Tc I, Tp I, Tp II and Tc II) are identified by breakpoints in ventilation rate and corresponding changes in P_{O_2} and heart rate (cf. Fig. 2, after Frederich and Pörtner, 2000).

temperature (T_p II) ventilation and heart rates became more or less constant and independent of temperature, indicating capacity limitation. The onset of a decrease in P_{O_2} then reflects a rise in oxygen demand which was no longer compensated for by an increase in ventilation and circulation. Beyond the upper critical temperature (T_c II) ventilatory and circulatory activity collapsed. As in *Maja squinado*, collapse of circulatory activity was seen during long term recordings in heat stressed Antarctic clams, *Laternula elliptica* accompanied by a decrease in oxygen uptake (Peck et al., 2001). In cold exposed spider crabs, a further decrease in ventilation and heart rates beyond the low pejus temperature (T_p I) was again followed by a decrease in P_{O_2}, also indicating insufficient oxygen uptake and transport (Fig. 5; Frederich and Pörtner, 2000). Collapse of ventilatory activity finally occurred, similar to the results obtained earlier in sipunculids (Fig. 3; Zielinski and Pörtner, 1996).

A capacity limitation of ventilation at the upper critical temperature threshold was also indicated by a progressive deviation of ventilation frequency from that dictated by the normal Q_{10} relationship in the notothenioid fish, *Lepidonotothen nudifrons* (Hardewig et al., 1999). Studies summarised 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. Accordingly, heat stress in cod was shown to elicit a temperature-dependent decrease in venous, but not arterial oxygen tensions (Pörtner et al., 2001). Non-invasive magnetic resonance imaging data obtained in polar and temperate eelpout support these conclusions by identifying a high pejus temperature where maximum blood flow is reached well below the critical temperature.

Unchanged white muscle blood oxygen levels during an exponential rise in oxygen demand reflect a loss in aerobic scope and emphasise that the changing relationships between metabolic oxygen demand and maintained or changing tissue P_{O_2} are important to indicate onset of changes in aerobic scope (F. Mark, C. Bock and H.O. Pörtner, unpublished). Finally, the fish heart may be unable to circulate blood at a sufficiently high rate to maintain the arteriovenous difference in blood P_{O_2} and a venous reserve. Progressive hypoxia and, finally, anaerobic metabolism results at the tissue level, at first in tissues with a high oxygen demand such as liver (van Dijk et al., 1999). The fish heart itself may be very resistant since it pumps venous blood and is specialised to operate at low venous P_{O_2}. Myocardial oxygen consumption removes only a small fraction of venous oxygen content (Farrell, 1996; Block, 1990). This statement is valid until venous P_{O_2} reaches a low value or oxygen demand rises to a level in the warm at which the oxygen partial pressure head required for diffusion is no longer sufficient (cf. Steffensen and Farrell, 1998). In terms of tissue design, stenothermal Antarctic notothenioid fishes possess type I heart ventricles, which are characterised by full trabeculation and the absence of a coronary circulation (Axelsson et al., 1998) and reflect a restricted dynamic range of the circulatory system as well as the narrow thermal tolerance windows characteristic for this group (Somero and DeVries, 1967).

Ventilatory organs actively and continuously involved in oxygen uptake may also be among the first tissues to experience the drop in oxygen supply. In the body wall musculature of worms and the mantle musculature of cephalopods, which are responsible for ventilation, anaerobic glycolysis was observed in addition to mitochondrial anaerobic metabolism (cf. Pörtner, 2001). Finally, a drop in energy levels (Gibbs free energy of ATP hydrolysis) to critically low levels characterised functional failure in cold exposed ventilating body wall musculature of *S. nudus* (Zielinski and Pörtner, 1996). The development of oxygen deficiency and onset of functional insufficiency and finally failure of any tissue involved in homeostatic maintenance obviously go hand in hand (Zielinski and Pörtner, 1996; Sommer et al., 1997; Sommer and Pörtner, 1999; cf. Figs. 3–5).

Prior to anaerobiosis the progressive drop in arterial P_{O_2} in the cold and in the warm reflects a reduction of oxygen availability to mitochondria. At reduced P_{O_2} mitochondria will no longer achieve maximum rates of oxygen consumption. This apparent reduction of mitochondrial aerobic capacity indicates a reduced scope for aerobic activity of the whole animal as defined by measurements of oxygen consumption in fish (Bennett, 1978). The scope for aerobic activity extends from the whole animal to tissue to mitochondrial levels (Lindstedt et al., 1998), since it reflects the range between minimum and maximum oxygen consumption by mitochondria and depends upon sufficient oxygen delivery by ventilation and circulation. Evidently, the slowing of ventilation and circulation in the cold and the insufficient increase in the warm cause a mismatch between oxygen delivery and demand which finally leads to the collapse of physiological function (Pörtner et al., 1998, 2000; Pörtner, 2001). Limited functional capacity leading to oxygen limitation and finally, functional failure are most likely interrelated processes, since transition to anaerobiosis always indicates time limited tolerance. In accordance with the argument developed above, oxygen uptake and distribution by ventilation and circulation are most likely those processes of a high organisational complexity which were predicted to set the limits of thermal tolerance.

As a corollary, the data indicate that the temperature range characterised by maximum body fluid P_{O_2} represents the temperature window of maximum scope for aerobic activity. It therefore indicates the temperature range of optimum performance of the animal. Within this thermal range the animal is fully operative and availability of aerobic energy is maximal for all physiological functions including growth and reproduction to support long-term survival in the natural environment. Thermal limitation already sets in at the transition from optimum to pejus range, characterised by progressively insufficient oxygen supply owing to temperature limited ventilatory and cardiac capacity (Figs. 3 and 5).

3.2. Air breathers

According to Section 3.1 evidence is strong for water breathers that thermal tolerance limits are set by limited oxygen supply before disturbance of molecular functions sets in. The question arises as to whether the transition to air breathing may

have changed the sequence of thermal tolerance limits (from loss of aerobic scope to onset of anaerobic metabolism and then molecular denaturation). Transition to air breathing implies a reduction by one order of magnitude in the energy cost of ventilation at approximately 30 times higher levels of ambient oxygen. This may have allowed air breathers to be more eurythermal at a reduced cost of ventilation. This advantage of breathing air rather than water is counterbalanced by the fact that thermal buffering is insignificant in the terrestrial compared to the aquatic environment.

Nonetheless, specialisation of physiological function to a thermal window is still observed in air breathers. Even endotherms, although they have optimised functions at constant high body temperatures, are adapted to a given climate, either by insulation from the environment or by mechanisms of improved heat transfer. However, the mechanisms limiting heat tolerance have not really been established in air breathers. Nonetheless, some reports indicate that thermal tolerance may be oxygen limited and allow vague estimates of the critical temperature, mostly in ectothermic species adapted to temperate or warmer climates. In the cold below 15 °C, malfunction of the circulatory system and acetate accumulation developing in warm adapted air breathing arthropods, scorpions and tarantulas (Bridges et al., 1997, R.J. Paul, unpublished) indicates oxygen limited cold tolerance (cf. Pörtner, 2001). In the alligator, *Alligator mississipiensis*, the critical P_{O_2} as identified by the onset of anaerobic metabolism (Pörtner and Grieshaber, 1993), increases as temperature rises and reaches normoxic values at approximately 45 °C indicating elimination of aerobic scope in accordance with the above definition of the T_c (Branco et al., 1993, cf. Pörtner, 2001). Temperature dependent changes in the ratio of ventilatory volume and oxygen consumption in the fresh water turtle, *Pseudemys scripta elegans* (Jackson, 1971) suggest that alveolar oxygen extraction in *Pseudemys scripta elegans* becomes insufficient at the T_c between 35 and 40 °C. Further studies in fresh water turtles demonstrated that alveolar oxygen levels in the lung decrease and alveolar, critical P_{O_2} increases exponentially at high temperatures (White and Somero, 1982; Fuster et al., 1997). Both curves would intersect between 40 and 45 °C, again indicating full elimination of the aerobic scope at the high critical temperature (Fig. 6).

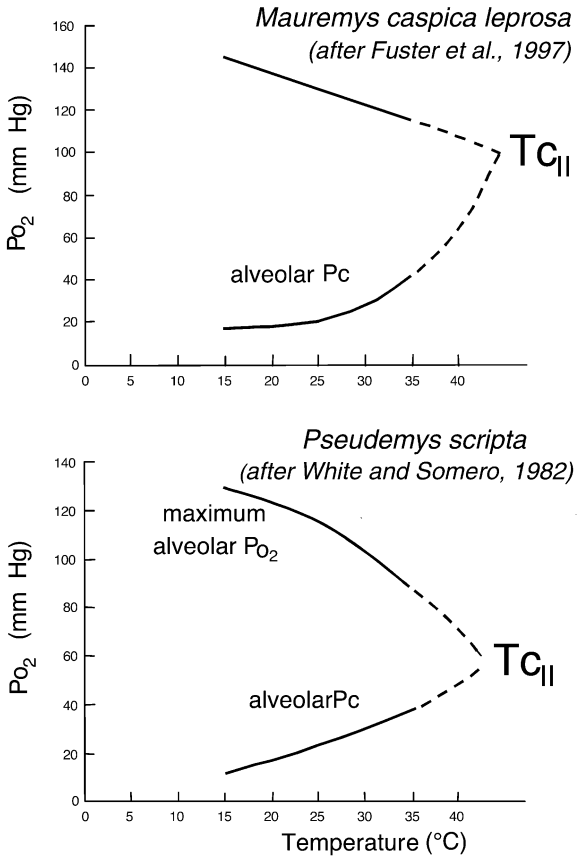

Fig. 6. Development of alveolar critical P_{O_2} (P_c) and maximum alveolar P_{O_2} in two species of fresh water turtles, data adopted from the indicated literature studies. Intersection of the extrapolated curves (dotted lines) quantifies the critical temperature (T_c), where no aerobic scope for spontaneous activity is left (see text). Similar conclusions were drawn for alligators (*Alligator mississipiensis*) by Pörtner (2001).

If long-term heat tolerance limits in metazoa are generally found at approximately 45 °C, air breathers, for the reasons discussed above, seem to approach that limit more closely than water breathers. A recent review of upper thermal limits in insects (on average 47.4 °C) revealed that upper lethal limits are not correlated with ambient extremes or latitude (Addo-Bediako et al., 2000). It needs to be emphasised here that these limits have been determined during experimental short-term temperature increments leading to limits beyond upper pejus and, most likely, critical temperatures. Evidently, short-term tolerance limits are not significantly correlated with geographical distribution, at least not in insects.

In endotherms studies addressing the question whether a mismatch of oxygen supply and demand

is involved in thermal tolerance limits are rare. For example, data provided by Jourdan et al. (1989) suggest that progressive circulatory failure below 20 °C body temperature in the rat leads to progressive energy deficiency and elevated lactate levels. Accordingly, functional failure (capacity limitation) of circulation and tissue perfusion is likely to limit cold tolerance. The role of oxygen demand in heat tolerance is not clear. However, many events discussed for both cold and heat damage are similar to those elicited by hypoxia or ischemia including the formation of oxygen radicals (Rifkind et al., 1993, Ivanov 2000). In general, studies of thermal tolerance usually focus on lower complexity levels, the pathology of heat shock and cold injury, including the cellular mechanisms of heat or cold hardening by heat shock proteins and related chaperones (e.g. Burdon, 1987; Fujita, 1999) as well as temperature induced injuries by oxygen radical formation (Ando et al., 1994; Bhaumik et al., 1995), endotoxemia (Caputa et al., 2000) or elevated calcium levels (Ivanov, 2000). As a corollary, further investigations at the level of aerobic scope and ventilatory and circulatory capacity depending on body temperature are highly needed in mammals and birds.

3.3. Synopsis

In both air and water breathers exposure to extreme heat or cold appears to involve elimination of aerobic scope and finally oxygen deficiency. For a final judgement of whether oxygen limitation really limits thermal tolerance the situation becomes much clearer for air breathers if it is considered that the window delimited by pejus temperatures will be smaller than the one defined by critical temperatures and closer to environmental temperature oscillations. Since pejus temperatures characterise the onset of a reduction in aerobic scope the conclusion arises that onset of a loss in aerobic scope sets the long-term limits of thermal tolerance in air breathing ectotherms as much as it does in water breathers.

Finally, if oxygen limitation characterises thermal intolerance, it is no longer surprising that lactate as an end product of anaerobic metabolism is able to elicit behavioural hypothermia in aquatic and terrestrial ectotherms. In vertebrates and some invertebrates lactate indicates extreme oxygen limitation and, being formed at the high T_c, would stimulate the organism to move to cooler temperatures (Pörtner et al., 1994; De Wachter et al., 1997; Branco and Steiner, 1999). Optimised functions depending on temperature in the sense that maximum aerobic scope is maintained, are supported by behavioural thermoregulation in both ecto- and endotherms. If different thermal niches are accessible to an individual, they are chosen either to maximise activity and reactivity, e.g. by warming up in the sun, or they are chosen to escape from daily or seasonal temperature extremes by hiding in the shade or underground.

Considering that the rise in the performance of ventilatory and circulatory structures matches the rise in whole organism performance throughout the animal kingdom (e.g. between amphibians and mammals) a limiting role of ventilation and circulation becomes understandable when the capacity of oxygen uptake and transport systems is set to a minimally required level in accordance with metabolic requirements. These considerations are in line with the concept of symmorphosis (see above). In the context of thermal adaptation and limitation this means that the functional capacities of oxygen delivery systems are set to be optimal between the average highs and lows of environmental temperatures. This immediately leads to the conclusion that the specific design constraints of a phylum together with the lifestyle dependent metabolic rate of a species in relation to the oxygen content of ambient media will determine the tolerated temperature extremes. Accordingly, Fig. 7 emphasises that limiting temperature maxima and constraining factors shift between animal groups but all relate to oxygen provision. Owing to the variability in lifestyles and designs relatively safe comparisons appear possible especially at the high end of metazoan body temperatures and between groups from similar environments. Comparing a desert pupfish and a warm water squid, which are both water breathers, the higher metabolic rate and poorer oxygen transport of the squid (cf. Pörtner, in press) may explain, why maximum heat tolerance in this group is lower than in fish. Comparing a desert lizard and a desert ant the design of the respiratory system of the insect would support its slightly higher heat tolerance, however, only at a body size several orders of magnitude smaller than the reptile. The assumption of an oxygen limited design in insects is supported by the observation, that only the elevation of atmospheric oxygen levels (35% atmospheric oxygen in the Phanerozoic compared with 20.9% of today) allowed for

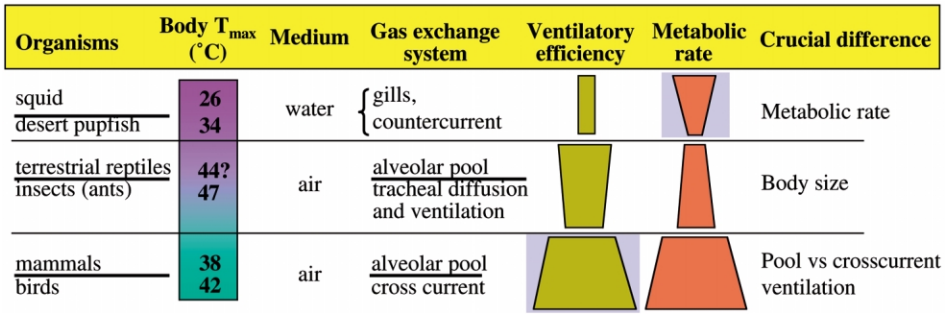

Fig. 7. Comparisons of maximum sustained body temperatures in warm adapted highly organised animal groups identifying metabolic rate, body size or the efficiency of ventilatory systems as the crucial differences (see lightly shaded areas) between paired species. Gas exchange systems are countercurrent gills in the water breathers, lungs in reptiles and trachea in insects, as well as lungs operating either as a pool exchange system in mammals or as a cross current system in birds. Low oxygen availability in water likely explains the lower heat tolerance of squid and fish compared with air breathers.

the evolution of large insects, namely giant dragon flies (Dudley, 1998; Berner et al., 2000). Finally, when comparing mammals and birds, the improved ventilatory efficiency of the bird lung with its cross-current gas exchange system would suggest a lesser degree of oxygen limitation in birds allowing for higher defended body temperatures than in mammals and a more active lifestyle in hot terrestrial environments (Fig. 7).

In general and as a corollary, the three-dimensional interaction between oxygen limitation, environmental temperature and also performance levels of the whole animal needs to be considered as an important constraint in animal evolution. Recent studies emphasised that high oxygen concentrations in ambient media supported the evolution of larger species. This trend becomes visible (e.g. among amphipods; Chapelle and Peck, 1999) in cold, polar and Lake Baikal waters characterised by elevated oxygen solubilities compared with warmer waters and also in earth history with the presence of giant dragon flies at high atmospheric oxygen contents in the warm (see above). In a trade-off between maximum aerobic scope for activity, ambient oxygen levels and temperature, maximum body size within a group is limited by each of these factors, a suggestion which seems most unequivocal in aquatic invertebrate athletes with a high oxygen demand, squid (Pörtner and Zielinski, 1998; Pörtner, in press). Since the maximum levels of muscular activity appear generally lower in the permanent cold (Clarke, 1998), high oxygen levels in cold waters would then allow for giant life forms to develop. Accordingly, giant and more or less sluggish cephalopods are confined to cold ocean environments, the deep sea in the case of giant squid (supported by neutral buoyancy of ammoniacal tissues) or the cold Northern Pacific in the case of the giant octopus. In warm waters the necessity to maximise aerobic scope at reduced ambient oxygen levels would then limit maximum body size in cephalopods. This comparison already shows the principle difference between terrestrial and aquatic environments in that oxygen levels in terrestrial environments are independent of temperature. The mitochondrial story told below suggests that cost efficiency, i.e. maximum output of aerobic energy at minimum mitochondrial density increases with rising temperature, however, only as long as sufficient oxygen can be provided. Therefore, metabolic efficiencies rise at high body temperatures (see below). These relationships become effective, especially in air and support the development of larger forms of terrestrial ectotherms in the warm.

4. Hierarchy and time windows of thermal limits

Oxygen limitation of thermal tolerance, visible as limitation in aerobic scope, appears as a unifying principle at temperatures above freezing (cf. Pörtner, 2001). It is set as the first line of thermal sensitivity at the whole organism level, i.e. at a high level of organisational complexity, due to capacity limitations of ventilation and circulation. The initial limiting effect of temperature is a reduction in aerobic scope and oxygen supply beyond pejus temperatures (T_p in Fig. 8). A sequence of thermal limits results, where oxygen

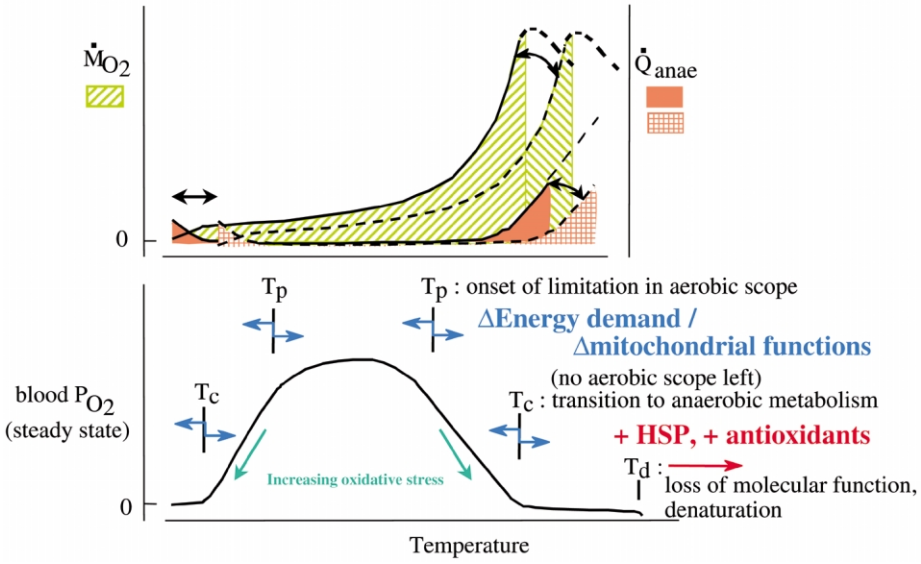

Fig. 8. Modelled hierarchy of thermal tolerance thresholds in animals (modified after Pörtner, 2001) considering changes in metabolic heat production according to patterns of oxygen consumption ($\dot{M}_{O_2}$), or anaerobic heat production (Q_{anae}). The model is predominantly based on data for water breathers (T_c critical, T_p, pejus, T_d denaturation temperatures) but likely extends to air breathers (see text). Mechanisms shifting the respective tolerance thresholds include overall mitochondrial functional capacity, which causes a shift in both upper and lower T_c and T_p, at the expense of significant metabolic cold adaptation in eurytherms (see text). Downward pointing linear arrows qualify increased oxidative stress during progressive hypoxia. A drop in oxygen demand as well as anaerobic heat production is expected from a decrease in mitochondrial densities and capacities during warming. The upper denaturation temperature likely shifts with the presence of heat shock proteins (HSPs, cf. Tomanek and Somero, 2000) or antioxidants (Abele et al., 1998b). A lower denaturation temperature is not considered, but the same mechanisms may be effective. Antifreeze protection or supercooling as well as reduced Mg^{2+} levels in body fluids extend the tolerance range below the freezing point of body fluids (not shown).

limitation in the sense of a reduction in aerobic scope indicates the earliest limits reached, namely the limits of long term tolerance. According to the early work by J.R. Brett, activity and even more so reproduction (which includes associated spontaneous activity) of sockeye salmon (*Oncorhynchus nerca*) occurs within much narrower thermal windows than metabolic maintenance (for review see Cossins and Bowler, 1987). Once aerobic scope starts to be diminished beyond pejus thresholds growth and reproduction would be the first processes to be suspended (Fig. 9). This causes a decline in population but has limited effects on individual survival except for a temperature-dependent change in individual life span (rate of living).

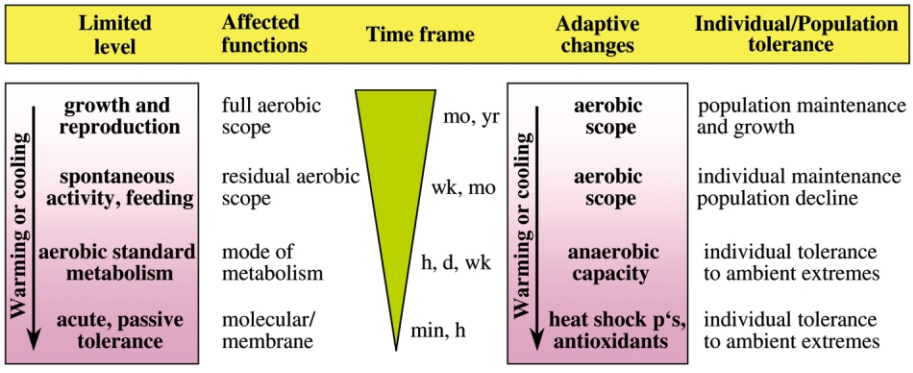

Fig. 9. Mechanisms defining the time limits of thermal tolerance (see text).

As temperature rises further beyond pejus levels, individual tolerance but becomes progressively time limited (Fig. 9). Spontaneous activity is reduced as aerobic scope falls such that food uptake is finally prevented towards the critical temperature (Fig. 8). Survival becomes a question of hours, days or weeks, depending on individual resistance to starvation. Once critical temperatures are surpassed, the transition to extreme hypoxia and, finally, an anaerobic mode of mitochondrial metabolism characterises passive tolerance limits (T_c). Since maintenance of aerobic standard metabolism is no longer possible, the capacity of anaerobic metabolism and, thus, hypoxia tolerance or metabolic depression strategies will determine individual survival on a time scale reduced further compared with the pejus range of temperatures between T_c and T_p (Fig. 9).

Oxidative stress may already increase when oxygen limitation sets in at pejus and even more so during extreme hypoxia beyond critical temperatures. Since mitochondria are considered as major cellular sources of reactive oxygen species at between 1% and 3% of their rate of oxygen consumption (Sohal and Weindruch, 1996) warming will not only cause an increase in metabolic rate but also an associated increase in oxygen radical production possibly linked to enhanced oxidative stress. At the same time, hypoxia develops during warming and cooling (see above). Hypoxia has been found to cause excess production of oxygen radicals owing to facilitated autooxidation of haem groups in mitochondria or haemoglobin (Boveris and Chance, 1973; Rifkind et al., 1993). These processes are also found in marine invertebrates (Abele et al., 1998a). A moderate accumulation of malondialdehyde as a product of lipid peroxidation, was found in the Antarctic bivalve, *Yoldia eightsi* at 5 °C and above (Abele et al., 2001) and a progressive destabilisation of membranes occurred in the Antarctic limpet *Nacella concinna* (Abele et al., 1998b). SOD forms the first line of defence against oxidative stress, as it catalyses the removal of oxygen radicals at the expense of generating H_2O_2, which is then removed by catalase. Increased availability of SOD either by chemical modification or owing to overexpression alleviates oxidative stress and minimises hypoxia induced post-ischemic injury (Wang et al., 1998, Nakajima et al., 2000). During warming above ambient temperature maxima, superoxide dismutase (SOD) levels decreased in *Yoldia eightsi* and in *N. concinna* (Abele et al., 1998a,b, 2001). The reasons for the inactivation of SOD at elevated temperatures remain unclear, however, progressive hypoxia elicited by the drastic increase in oxygen demand at high temperatures may hamper protein synthesis (see below) and thereby, SOD expression. In similar ways as in the Antarctic molluscs, oxidative stress mechanisms appear to contribute to thermal stress in temperate zone invertebrates (Tremblay et al., 1998) and also to thermal stress and bleaching in tropical corals (Lesser et al., 1990; Lesser, 1997). In the sea anemone *Aiptasia pulchella* (Nii and Muscatine, 1997) oxidative stress occurs during heating regardless of the presence of endosymbiotic algae. As a corollary, the progressive development of hypoxia and oxidative stress very likely go hand in hand, at least during heat stress. Functional consequences of oxidative stress have not been investigated, however, damage by oxygen radicals may support progressive loss of function already beyond pejus and, even more so, critical temperatures. Short-term tolerance to oxidative stress will support tolerance to extreme thermal events (Fig. 8) and is likely to be most expressed in the intertidal, a conclusion supported by the relatively higher stability of antioxidative enzymes in intertidal compared with subtidal invertebrates (Regoli et al., 1997).

In the sequence of thermal limits, finally, loss of protein function occurs at denaturation temperatures (T_d in Fig. 8), possibly indicated by an Arrhenius break temperatures of molecular, organellar or cellular functions or by neuromuscular failure in the heat. At lower levels of complexity, each individual molecular or membrane function has been optimised with respect to ambient temperature. Therefore, functional failure of these mechanisms (T_d in Fig. 8) likely correlates with whole animal tolerance thresholds, however, at temperatures beyond pejus or even critical values. For example, the Arrhenius break temperature of state 3 respiration in isolated mitochondria correlates with habitat temperature but is usually situated above experimental tolerance limits of the whole organism (Weinstein and Somero, 1998; Somero et al., 1998), even above critical temperatures (Pörtner et al., 2000).

A cellular defence mechanism discussed to contribute to thermal tolerance is the so-called heat shock response, which involves the increased synthesis of heat shock proteins (HSP) under condi-

tions of cold or warm stress (for review see Feder and Hofmann, 1999). A role for heat shock proteins (HSPs) in thermal protection is suggested by a comparison of congeneric snail species from different tidal heights (Tomanek and Somero, 1999, 2000). Rapid expression of HSPs to moderate levels is correlated with increased hardiness against extreme thermal events (shifting T_d in Fig. 8). Expression is also correlated with the improved capability to recover from heat stress. Similarly, a desert ant (*Cataglyphis*) that regularly undergoes temperatures beyond 50 °C during feeding excursions, synthesises heat shock proteins as a protective measure against these temperature extremes which nonetheless are lethal during exposure longer than several minutes (Gehring and Wehner, 1995). However, excess synthesis of heat shock proteins seems harmful since it occurs at the expense of other protein synthesis (Tomanek and Somero, 2000).

HSPs likely protect cellular molecules especially in situations which require time-limited tolerance (hardening), e.g. in the intertidal zone, and beyond the limits of long-term thermal tolerance as defined above. This thinking is in line with the conclusion that temperature ranges between pejus and critical temperatures and even more so beyond critical temperatures are accessible for the organism only during limited time periods. It is also in line with the observation that HSP expression is triggered by hypoxia events (see Burdon, 1987; Feder and Hofmann, 1999, for review). Extreme hypoxia at the T_c associated with increased oxygen radical production may thus contribute to elicit HSP gene expression. At more extreme temperatures HSP synthesis stops (cf. Tomanek and Somero, 1999). On the one hand, the frequency of extreme temperature events in the natural environment will be important in whether adequate use of this response is crucial for wider distribution patterns in such environments (cf. Tomanek and Somero, 1999). Such a correlation still needs to be established in thermal tolerance analyses. On the other hand, life in thermally stable natural environments may eliminate the necessity to use the heat shock response. This thinking is supported by the finding that stenothermal Antarctic fish but also cod do not show a temperature induced heat shock response in vivo (Hofmann et al., 2000; Pörtner et al., 2001). Despite existence of the gene family some species may not use the response and have lost tolerance to extreme temperatures beyond the range of long-term fluctuations. In those species, which express the heat shock response, the correlation of the threshold temperatures for HSP induction with the thermal niche of a species or with seasonal acclimatisation (cf. Feder and Hofmann, 1999) will likely be influenced by a shift in thermal tolerance windows defined by oxygen limitation.

According to the links outlined between oxygen deficiency, protein synthesis, oxidative stress and heat shock protein expression, the picture arises that not only a sequence but a hierarchy of thermal limits exists in metazoa (Fig. 8). In the whole organism, disintegration of mechanisms at a lower level of complexity may at least be favoured if not elicited by oxygen deficiency beyond pejus or critical thresholds (T_p or T_c in Fig. 8) and may, thus, not only result from direct thermal denaturation. Damage to membranes or proteins may occur as a consequence of rising oxidative stress. Protein synthesis may be reduced or completely collapsed during temperature induced hypoxia, thereby preventing molecular repair such that limits become effective depending on the duration of exposure to thermal stress. Whereas the concept of symmorphosis is an approach to understand how individual components match the design of the system the concept of a hierarchy of thermal tolerance emphasises that insufficient capacity of the overall system will affect the functional and structural integrity of individual components, through insufficient oxygen supply.

It is important to note in this context that thermal tolerance is frequently investigated during short-term progressive heat increments and defined as lethal limits. However, these short-term protocols will only provide information on the tolerance to short-term extreme temperature events that very likely go far beyond the thresholds defined by the onset of oxygen limitation (Fig. 8). The presence of heat shock proteins and antioxidants will cause more or less efficient molecular protection (Fig. 8) at extreme temperatures and, thereby, influence the period of time-limited survival. These considerations explains why acute survival thresholds identified by the typical procedure of progressive short-term heating or cooling within minutes or hours (cf. Elliott and Elliott, 1995) are higher in the warm (lower in the cold) than the limits of longer term thermal tolerance for the resting indi-

vidual. This extends to Antarctic species (Lahdes 1995; Urban, 1998). For example, the upper lethal temperature (LT_{50}) of the Antarctic bivalve *Laternula elliptica* has been determined at 14.9 °C after 24 h of acute temperature exposure (Urban, 1998). However, oxygen limitation sets in already at a pejus temperature close to 4 °C (H.O. Pörtner, L.S. Peck, T. Hirse, unpublished). Interestingly, this is very close to the temperature (3.6 °C) at which 50% of the animals lost the ability to rebury in the sediment (Urban, 1998).

Fig. 9 illustrates how the hierarchy of thermal tolerance limits is reflected in an increasingly reduced tolerance over time to extreme thermal events. Limits are found at progressively lower organisational levels, and malfunction of the higher levels would set the time limits (Fig. 8). Nonetheless, even the short-term lethal limits may shift with long term acclimation to higher or lower temperatures (e.g. Tomanek and Somero, 1999; Stillman and Somero, 2000). Upward shifts are observed in temperate but no longer in tropical species where further heat acclimation is no longer possible. As a precondition for the shifts of short-term tolerance limits during long term acclimation, pejus and critical thresholds should shift as well, such that the full array of organismic to molecular tolerance limits moves more or less in parallel. Key mechanisms causing such long-term shifts in pejus and critical temperatures need to be discussed.

From an ecological perspective, pejus rather than critical temperatures are likely to reflect the upper and lower temperature limits determining geographical distribution. In the future, this conclusion needs to be tested and quantified for both air and water breathing species in a latitudinal cline, in a collaborative effort of physiologists and ecologists. In this context, the hierarchy of pejus, critical and denaturation temperatures and the distances between these thresholds await further quantification in water breathers and even more so in air breathers, where a limitation of thermal tolerance by loss in aerobic scope still needs unequivocal verification for many groups including mammals and birds. These investigations should also address the trade-offs involved in thermal adaptation and the consequences for the success of species in thermally stable vs. unstable environments.

5. Temperature adaptation: overcoming aerobic scope limitations in eurytherms and stenotherms

It became evident early on that critical temperatures differ between species and populations depending on latitude or seasonal temperature acclimatisation and are therefore related to geographical distribution. For example, a within species comparison of *Arenicola marina* populations in a latitudinal cline revealed that both low and high T_c values were lower in cold adapted, sub-Arctic animals from the Russian White Sea than in boreal, North Sea specimens (Sommer et al., 1997). High critical temperatures close to 23 °C were found in eelpout from the North Sea and close to 9 °C in eelpout from the Antarctic (van Dijk et al., 1999). Even lower 'heat tolerance' limits of 2–3 °C were found in an Antarctic bivalve, *Limopsis marionensis* (Pörtner et al., 1999a).

The width of the window between the two pejus temperatures as well as between critical temperatures reflects the amplitude of temperature fluctuations in the habitat of a species. The tolerance window is large in tropical and temperate species and narrow especially in polar areas (Fig. 1) and most notably 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 (for review see Pörtner et al., 2000). A preliminary comparison of the lifestyles of these species suggests that sessile epifauna species are characterised by lower critical temperatures compared with those of mobile species. The capacity of ventilation and circulation is higher in active fish or octopods than in sessile bivalves, and this may be related to the higher pejus and critical temperatures in more mobile compared to sessile Antarctic species.

The question arises which are the mechanisms determining the position and width of thermal tolerance windows. This involves the question to what extent the mechanisms used during cold adaptation are similar or different in stenotherms adapted to permanent cold and in eurytherms adapted to tolerate seasonal cold. Especially Northern hemisphere species possess the ability to acclimatise and shift both low and high tolerance thresholds (T_p and T_c) between seasons and in a latitudinal cline (cf. Pörtner, 2001). On evolution-

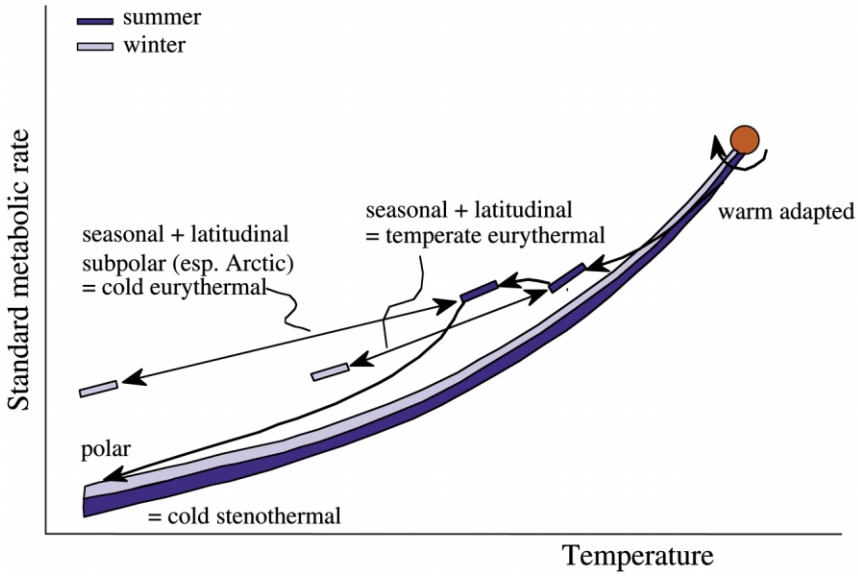

Fig. 10. Model depicting the 'hub' of seasonal and permanent cold adaptation (modified after Pörtner et al., 2000). Eurythermal cold adaptation causes standard metabolic rate to rise (cf. Fig. 8) in relation to the degree of ambient temperature fluctuations and the cold compensation of mitochondrial aerobic capacity and proton leakage. Adaptation to the permanent cold of Antarctic and high Arctic areas does not show this phenomenon owing to a compensatory reduction in energy demand and a rise in kinetic barriers (Arrhenius activation energies) which compensate for the energy demand elicited by cold induced mitochondrial proliferation (cf. Pörtner et al., 2000).

ary timescales baseline constraints and trade-offs in cold adaptation, with which animals had to cope on the way from permanently warm conditions via eurythermal to stenothermal cold need to be identified.

Evidently, adaptation to changing temperatures involves escaping the threat of temperature induced hypoxia (Pörtner et al., 1998, 2000). Acclimation to seasonal cold is well known to cause a rise in mitochondrial density or mitochondrial aerobic capacity in fish (e.g. St.-Pierre et al., 1998; Guderley, 1998). This process is reversed during seasonal warming. High mitochondrial densities have also been observed in Antarctic ectotherms adapted to permanent cold, however, at respiratory capacities of individual mitochondria which were found reduced in the cold, according to the effect of Q_{10}. Only recently has cold-induced mitochondrial proliferation been confirmed for a marine invertebrate, *Arenicola marina*, in a comparison of sub-Arctic and temperate populations (Sommer and Pörtner, submitted). In contrast to Antarctic species, cold-adapted Arctic eurytherms displayed a rise in mitochondrial aerobic capacity, which compensates for the temperature dependent decrement (Tschischka et al., 2000; Sommer and Pörtner, submitted). These findings are in accordance with high costs of eurythermal vs. stenothermal cold adaptation and significant metabolic cold adaptation in eurytherms (Pörtner et al., 2000, 2001; Fig. 10).

The interpretation that temperature-induced changes in mitochondrial densities and functional capacities are crucial in causing a shift of oxygen limited thermal tolerance windows casts new light on the functional role of these processes. In the sense that a function of high organisational complexity defines the limits of thermal tolerance the key role of mitochondria does not neglect the importance of integrated modifications in lipid saturation, kinetic properties of metabolic enzymes, contractile proteins and transmembrane transporters (cf. Johnston, 1990; Hazel, 1995; Tiku et al., 1996; Storelli et al., 1998; Pörtner et al., 1998), all of which will contribute to the optimisation of higher functions within the window of thermal tolerance. Since each of these mechanisms is also found in unicellular pro- and eukaryotes, the larger tolerance windows of these groups compared with metazoa (see above) suggests that the individual mechanism alone does not provide a key to understanding thermal limits in metazoa.

Only the combination of these mechanisms including the adjustment of mitochondria enables higher functions to take place. Mitochondrial changes are only part of the overall picture but crucial to understand the aerobic capacity adjustments in cold and warm adapted animals. In the context of an oxygen limitation of thermal tolerance mitochondrial adjustments are closest to setting the level of aerobic energy available to the organism, especially if it concerns mitochondrial contents of tissues involved in circulatory and ventilatory performance and its neural control.

5.1. Mitochondria in the cold providing insufficient aerobic energy

Mitochondrial density and/or capacity reflect the level of aerobic scope of the whole animal. On the one hand, this capacity can only be made available if P_{O_2} is kept high in the body fluids which requires sufficient ventilation and circulation. On the other hand, limited capacity of mitochondria to produce energy in the cold is likely to contribute to the loss of function and scope, e.g. in circulation and ventilation. Limitations in the availability of aerobic energy may therefore be the key to understand why an increase in mitochondrial aerobic capacity occurs during resistance adaptation to cold (Pörtner et al., 1998, 2000), in parallel with a temperature-dependent adjustment of contractile functions. These processes lead to a downward shift of the low temperature thresholds (critical and pejus). Cold adaptation of metabolic parameters as observed in water breathers also occurs in air breathers such as weevils and mountain beetles (Chown et al., 1997; Dahlhoff and Rank, 2000, for review see Chown and Gaston, 1999).

5.2. Limits to cold adaptation

The capacity to overcome the oxygen limitation of cold tolerance by mitochondrial adjustments will be limited by the freezing of body fluids or, as found in the case of marine reptant decapod crustaceans, by high magnesium levels in the body fluids. The latter constraint is discussed to explain why this group is almost completely absent in areas of the Antarctic and the Arctic with permanent subzero water temperatures (Frederich et al., 2000a). Since magnesium acts as a natural calcium antagonist at neuromuscular junctions it exerts an anaesthetising effect, especially in the cold, and thereby restricts ventilatory and circulatory capacities (Frederich et al., 2000b). In consequence, magnesium limits aerobic scope and thereby exacerbates the oxygen limitation of cold tolerance. Obviously, reptant decapods could not overcome this limitation by cold-induced mitochondrial proliferation. Only a reduction in haemolymph magnesium levels enabled other crustaceans like amphipods, isopods and shrimp to occupy those niches in polar areas which in other oceans are usually dominated by benthic crabs (Frederich et al., 2001).

At temperatures below freezing the presence of antifreeze protection or passive freeze tolerance extends the range of cold tolerance. Polar fishes face the problem of sea water temperatures below the freezing point of their body fluids (-1.9 °C vs. -0.9 °C). Elimination of the threat of cold induced hypoxia by cold adaptation of the metabolic machinery (see above) could, therefore, only become effective in combination with the development of antifreeze proteins, as an evolutionary precondition for fish life in the Antarctic and in the Arctic (for review see DeVries, 1988; Fletcher et al., 1998; Wöhrmann, 1998). Whereas antifreeze proteins in polar fish support an active mode of life at permanently low water temperatures, antifreeze protection or freeze tolerance in terrestrial environments also becomes important at temperatures well below the range of optimum physiological performance, e.g. during seasonal cold. Accordingly, supercooling points and lower lethal limits in insects were found to be correlated with geographical distribution (Addo-Bediako et al., 2000) since such mechanisms guarantee passive survival during unfavourable seasonal conditions.

5.3. Mitochondria in the warm contributing to high oxygen demand

What are the consequences of a mitochondrial density adjusted high in the cold when the animal is exposed to high temperatures? As outlined above, the increase in oxygen demand during warming may become a problem at high temperature. It has only recently become clear that oxygen demand is not only related to cellular energy requirements but some baseline oxygen demand is already caused by the mitochondria present and their functional properties. Oxygen demand of mitochondria is not only defined by the maximum

rate of coupled respiration in the presence of substrate and ADP (state 3 respiration) but also by the rate of proton leakage across the inner mitochondrial membrane. Mitochondrial proton leakage in the resting cell comprises a constantly large fraction of standard metabolic rate (Brand, 1990; Brand et al., 1994). It accounts for 25% of baseline metabolism in rat hepatocytes, for 50% in skeletal muscle and for 20–30% in the whole animal (Brand et al., 1994; Rolfe and Brand, 1996). Proton leakage reflects the baseline idling of mitochondria, and on top of the cost of biosynthesis and degradation accounts for the cost of mitochondrial maintenance (Pörtner et al., 1998). The relative contribution of proton leakage to standard metabolic rate (SMR) is similar in ectotherms and endotherms (Brookes et al., 1998). Moreover, SMR is related, by a more or less constant factor, to maximum aerobic capacity of the organism (Wieser, 1985; Brand, 1990; Lindstedt et al., 1998). Proton leakage will therefore contribute to the warm induced increase in SMR.

Recently, the temperature dependence of H^+ leakage was investigated in mitochondria from tissues of two Antarctic species, gills of the bivalve, *Laternula elliptica* (Pörtner et al., 1999b) and liver of the notothenioid fish, *Lepidonotothen nudifrons* (Hardewig et al., 1999). The change in proton leakage with temperature was more or less continuous, with constant but very high Arrhenius activation energies. The thermal sensitivity of proton leakage cannot be explained by a change in proton motive force (Δp), which scarcely varies with temperature (investigated in mammals by Dufour et al., 1996, and in Antarctic bivalves and fish by H.O. Pörtner and T. Hirse, unpublished). Whatever the mechanism of proton leakage, its high thermal sensitivity suggests that the futile cycling of protons may increase to a similar or even larger extent than resting ATP turnover at high temperatures. Thereby it likely contributes to the exponential rise in oxygen demand during warming. Owing to the progressive limitation of ventilatory and circulatory capacity (Figs. 3 and 5) this rise in oxygen demand would contribute to the drop in haemolymph P_{O_2} beyond T_p as observed in *Maja squinado* (Frederich and Pörtner, 2000) and *Laternula elliptica* (H.O. Pörtner, L.S. Peck and T. Hirse, unpublished) or in venous blood of the cod (*Gadus morhua*, I. Serendero, G. Lannig, F.J. Sartoris, H.O. Pörtner, unpublished). Finally at T_c, ventilation and circulation fail to meet baseline oxygen requirements. Accordingly, mitochondrial baseline oxygen demand will contribute to the limited aerobic performance beyond the upper pejus temperature (T_p II) and become detrimental at the critical temperature (T_c II). As a corollary, the reduction of mitochondrial density observed during warming leads to a reduction of baseline oxygen demand, thereby allowing the upper T_c to shift to higher values. These considerations have been considered in the model outlined in Fig. 8. Some increase in oxygen demand will also result from the exponential increase in cell membrane and epithelial permeability upon warming (Moseley et al., 1994), which requires energy for the compensation of dissipative ion movements.

6. Perspectives: the hub of metabolic cold adaptation

According to the foregoing chapter the adjustment of mitochondrial densities and properties appears as one key mechanism contributing to the unidirectional shift in both low and high pejus and critical temperatures. In accordance with the prediction mentioned earlier this mechanism links upper and lower thermal tolerance thresholds. Enhancing mitochondrial capacity in the cold eliminates the capacity limitations of ventilation and circulation. Reduction of mitochondrial capacity in the warm reduces the oxygen demand of mitochondrial maintenance and leaves enough aerobic energy for ventilation and circulation to maintain aerobic scope.

These considerations lead to the conclusion that a cold-induced rise in mitochondrial densities should cause elevated costs of mitochondrial maintenance and a rise in standard metabolic rate as seen in temperate ectotherms during winter cold or in eurythermal, esp. Northern hemisphere populations along a latitudinal cline. However, adaptation to the permanent cold of polar areas and the deep sea has led to life forms that do not display a significant cold induced rise in metabolic rate (e.g. Clarke and Johnston, 1999). Using the baseline considerations of mitochondrial contributions to standard metabolism outlined briefly above, Pörtner et al. (2000) elaborated a model (Fig. 10) that would explain the differences between cold stenotherms and eurytherms and that would also explain why adaptation to permanent cold caused Antarctic species to become obligatory stenoth-

erms. Compared with eurythermal cold this may be a secondary situation linked to reduced mitochondrial capacities and increased Arrhenius activation energies (E_a) of mitochondrial oxygen demand, especially proton leakage, and of flux limiting enzymes in metabolism like isocitrate dehydrogenase (Pörtner et al., 2000). A high kinetic barrier may support low metabolic flux in cold stenotherms despite mitochondrial proliferation. In contrast, temperature dependence is reduced and flux is facilitated in other, e.g. equilibrium enzymes like lactic dehydrogenase (Hochachka and Somero, 1984). While E_a of overall metabolism appears to be reduced in active winter acclimated animals (cf. Pörtner, 2001), high E_a values in Antarctic species reflect a high temperature dependence of metabolism and, in consequence, a reduced heat tolerance of the whole organism. According to Fig. 10 cost of maintenance rises in cold adapted eurytherms to levels higher than in cold stenothermal animals. As a trade-off growth and reproduction are reduced or suspended in cold eurytherms, whereas in stenothermal animals aerobic capacity and even more so baseline energy expenditure are minimised to free metabolic energy for growth and reproduction processes in the cold.

Finally, recruitment failure of a species depending on climate change appears as a consequence of the thermal limitations (loss in aerobic scope) discussed in the present review. The prediction also arises that in those cases, where juvenile stages may prove more sensitive to temperature change than adults of the same species, the principles outlined in the current paper will still be valid, i.e. thermal sensitivity likely results at the highest level of complexity and relates to the capacity of the oxygen supply system, which may be more sensitive to environmental changes during the period of embryonic and larval development than during the period of adult growth and reproductive activity. These relationships need to be addressed in future efforts to extend our knowledge on the role of aerobic scope in limiting thermal tolerance and geographic distribution in various groups of animals.

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References

Abele, D., Großpietsch, H., Pörtner, H.O., 1998. Temporal fluctuations and spatial gradients of environmental PO_2, temperature, H_2O_2 and H_2S in its intertidal habitat trigger enzymatic antioxidant protection in the capitellide worm *Heteromastus filiformis*. Mar. Ecol. Prog. Ser. 163, 179–191.

Abele, D., Burlando, B., Viarengo, A., Pörtner, H.O., 1998. Exposure to elevated temperatures and hydrogen peroxide elicits oxidative stress and antioxidant response in the Antarctic intertidal limpet *Nacella concinna*. Comp. Biochem. Physiol. B 120, 425–435.

Abele, D., Tesch, C., Wencke, P., Pörtner, H.O., 2001. How does oxidative stress relate to thermal tolerance in the Antarctic bivalve *Yoldia eightsi*? Antarctic Sci. 13, 111–118.

Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. Proc. R. Soc. Lond. B 267, 739–745.

Alabaster, J.S., Welcomme, R.L., 1962. Effect of concentration of dissolved oxygen on survival of trout and roach in lethal temperatures. Nature 194, 107.

Ando, M., Katagiri, K., Yamomoto, S., Asunama, S., Usuda, M., Kawahara, I., Wakamutsu, K., 1994. Effect of hyperthermia in glutathione peroxidase and lipid peroxidative damage in liver. J. Therm. Biol. 19, 177–1285.

Arntz, W.E., Brey, T., Gallardo, V.A., 1994. Antarctic Zoobenthos. Oceanography and Marine biology: an Annual Review, Vol. 32. pp. 241–304.

Axelsson, M., Agnisola, C., Nilsson, S., Tota, B., 1998. Fish cardio-circulatory function in the cold. In: Pörtner, H.O., Playle, R. (Eds.), Cold Ocean Physiology. Cambridge University Press, Cambridge, pp. 326–364.

Bakun, A., 1996. Patterns in the Ocean—Ocean Processes and Marine Population Dynamics. California Sea Grant College System, La Jolla, CA 323 pp.

Beamish, R.J., 1995. Climate change and northern fish populations. In: Beamish, R.J. (Ed.), Canadian Special Publications on Fish and Aquatic Science, Vol. 121. 739 pp.

Bennett, A.F., 1978. Activity metabolism of the lower vertebrates. Ann. Rev. Physiol. 400, 447–469.

Berner, R.A., Petsch, S.T., Lake, J.A., et al., 2000. Isotope fractionation and atmospheric oxygen: implications for phanerozoic O_2 evolution. Science 287, 1630–1633.

Bhaumik, G., Srivastava, K.K., Selvamurthy, W., Purkayastha, S.S., 1995. The role of free radicals in cold injuries. Int. J. Biometereol. 38, 171–175.

Block, W., 1990. Cold tolerance of insects and other arthropods. Phil. Trans. R. Soc. Lond. B 326, 613–633.

Boveris, A., Chance, B., 1973. The mitochondrial generation of hydrogen peroxide. Biochem. J. 134, 707–716.

Branco, L.G.S., Steiner, A.A., 1999. Central thermoregulatory effects of lactate in the toad *Bufo paracnemis*. Comp. Biochem. Physiol. A 122, 457–461.

Branco, L.G.S., Pörtner, H.O., Wood, S.C., 1993. Interaction between temperature and hypoxia in the alligator. Am. J. Physiol. 265, R1339–R1343.

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., Rolfe, R.K., 1994. The causes and functions of mitochondrial proton leak. Biochim. Biophys. Acta 1187, 132–139.

Bridges, C.R., le Roux, J.M., van Aardt, W.J., 1997. Ecophysiological adaptations to dry thermal environments measured in two unrestrained *Naminian scorpions*, *Parabuthus villosus* (Buthidae) and *Opisthophthalmus flavescens* (Scorpionidae). Physiol. Zool. 70, 244–256.

Brookes, P.S., Buckingham, J.A., Tenreiro, A.M., Hulbert, A.J., 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. B 119, 325–334.

Burdon, R.H., 1987. Thermotolerance and the heat shock proteins. In: Bowler, K., Fuller, B.J. (Eds.), Temperature and Animal Cells. Symposia of the Society of Experimental Biology, Vol. 21. pp. 269–283.

Caputa, M., Dokladny, K., Kurowicka, B., 2000. Endotoxemia does not limit heat tolerance in rats: the role of plasma lipoproteins. Eur. J. Appl. Physiol. 82, 142–150.

Chapelle, G., Peck, L.S., 1999. Polar gigantism dictated by oxygen availability? Nature 399, 114–115.

Chown, S.L., Gaston, K.J., 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. Biol. Rev. 74, 87–120.

Chown, S.L., van der Merwe, M., Smith, V.R., 1997. The influence of habitat and altitude on oxygen uptake in sub-Antarctic weevils. Physiol. Zool. 70, 116–124.

Clarke, A., 1998. Temperature and energetics: an introduction to cold ocean physiology. In: Pörtner, H.O., Playle, R. (Eds.), Cold Ocean Physiology. Cambridge University Press, Cambridge, pp. 3–30.

Clarke, A., Johnston, N.M., 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. J. An. Ecol. 68, 893–905.

Cossins, A.R., Bowler, K., 1987. Temperature Biology of Animals. Chapman and Hall, London 339 pp.

Cushing, D.H., 1982. Climate and Fisheries. Academic Press, London 373 pp.

Dahlhoff, E.P., Rank, N.E., 2000. Functional and physiological consequences of genetic variation at phosphoglucose isomerase: heat shock protein expression is related to enzyme genotype in a montane beetle. Proc. Natl. Acad. Sci. USA 10, 10056–10061.

Dahlhoff, E., Somero, G.N., 1991. Pressure and temperature adaptation of cytosolic malate dehydrogenases of shallow- and deep-living marine invertebrates: evidence for high body temperatures in hydrothermal vent animals. J. Exp. Biol. 159, 473–487.

Desbruyeres, D., Chevaldonne, P., Alayse, A.-M., et al., 1998. Biology and ecology of the 'Pompeii worm' (*Alvinella pompejana* Desbruyeres and Laubier) a normal dweller of an extreme deep-sea environment: a synthesis of current knowledge and recent developments. Deep-Sea Res. II 45, 383–422.

DeVries, A.L., 1988. The role of antifreeze glycopeptides and peptides in the freezing avoidance of Antarctic fishes. Comp. Biochem. Physiol. B 90, 611–621.

De Wachter, B., Sartoris, F.-J., Pörtner, H.-O., 1997. The anaerobic endproduct lactate has a behavioural and metabolic signalling function in the shore crab *Carcinus maenas*. J. Exp. Biol. 200, 1015–1024.

Dippner, J.W., 1997. Recruitment success of different fish stocks in the North Sea in relation to climate variability. Deutsche Hydrogr. Zeitschr. 49, 277–293.

Dudley, R., 1998. Atmospheric oxygen, giant paleozoic insects and the evolution of aerial locomotor performance. J. Exp. Biol. 201, 1043–1050.

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

Elliott, J.M., Elliott, J.A., 1995. The effect of the rate of temperature increase on the critical thermal maximum for parr of Atlantic salmon and brown trout. J. Fish. Biol. 47, 917–919.

Farrell, A.P., 1996. Features heightening cardiovascular performance in fishes, with special reference to tunas. Comp. Biochem. Physiol. A 113, 61–67.

Farrell, A.P., 1997. Effects of temperature on cardiovascular performance. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for Freshwater and Marine Fish. Cambridge University Press, Cambridge, pp. 135–158.

Feder, M.E., Hofmann, G.E., 1999. Heat shock proteins, molecular chaperones and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61, 243–282.

Fletcher, G.L, Goddard, S.V., Davies, P.L., Gong, Z., Ewart, K.V., Hew, C.L., 1998. New insights into fish antifreeze proteins: physiological significance and molecular regulation. In: Pörtner, H.O., Playle, R. (Eds.), Cold Ocean Physiology. Cambridge University Press, Cambridge, pp. 239–265.

Frederich, M., Pörtner, H.O., 2000. Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in the spider crab, *Maja squinado*. Am. J. Physiol. 279, R1531–R1538.

Frederich, M., Sartoris, F.J., Arntz, W., Pörtner, H.O., 2000a. Haemolymph magnesium regulation in decapod crustaceans: physiological correlates and ecological consequences in polar areas. J. Exp. Biol. 203, 1383–1393.

Frederich, M., De Wachter, B., Sartoris, F.J., Pörtner, H.O., 2000b. Cold tolerance and the regulation of cardiac performance and haemolymph distribution in *Maja squinado* (Crustacea, Decapoda). Physiol. Biochem. Zool. 73, 406–415.

Frederich, M., Sartoris, F.J., Pörtner, H.O., 2001. Distribution patterns of decapod crustaceans in polar areas: a result of magnesium regulation? Polar Biology 24, 719–723.

Fujita, J., 1999. Cold shock response in mammalian cells. J. Mol. Microbiol. Biotechnol. 1, 243–255.

Fuster, J..F, Pages, T., Palacios, L., 1997. Effect of temperature on oxygen stores during aerobic diving in the freshwater turtle *Mauremys caspica leprosa*. Physiol. Zool. 70, 7–18.

Gehring, W.J., Wehner, R., 1995. Heat shock protein synthesis and thermotolerance in *Cataglyphis*, an ant from the Sahara desert. Proc. Natl. Acad. Sci. USA 92, 2994–2998.

Guderley, H., 1998. Temperature and growth rates as modulators of the metabolic capacities of fish muscle. In: Pörtner, H.O., Playle, R. (Eds.), Cold Ocean Physiology. Cambridge University Press, Cambridge, pp. 58–87.

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

Hazel, J.R., 1995. Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? Ann. Rev. Physiol. 57, 19–42.

Hochachka, P.W., Somero, G.N., 1984. Biochemical Adaptation. Princeton University Press, Princeton, NJ.

Hemmingsen, A.M., 1960. Energy metabolism as related to body size and respiratory surfaces, and its evolution. Resp. Steno Hosp. 9, 1–110.

Hofmann, G.E., Buckley, B.A., Airaksinen, S., Keen, J.E., Somero, G.N., 2000. Heat-shock protein expression is absent in the Antarctic fish *Trematomus bernacchii* (Family Nototheniidae). J. Exp. Biol. 203, 2331–2339.

Hoppeler, H., Weibel, E.R., 1998. Limits for oxygen and substrate transport in mammals. J. Exp. Biol. 201, 1051–1064.

Hummel, H., Sommer, A., Bogaards, R., 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.

Ivanov, K.P., 2000. Physiological blocking of the mechanism of cold death: theoretical and experimental considerations. J. Therm. Biol. 25, 467–479.

Jackson, D.C., 1971. The effect of temperature on ventilation in the turtle *Pseudemys scripta elegans*. Respir. Physiol. 12, 131–140.

Johnston, I.A., 1990. Cold adaptation in marine organisms. Phil. Trans. R. Soc. Lond. B 326, 655–667.

Jourdan, M.L., Hoo-Paris, R., Wang, L.C.H., 1989. Characterization of hypothermia in a non-hibernator: the rat. In: Malan, A., Canguilhem, B. (Eds.), Living in the Cold II. Colloque INSERM/John Libbey Eurotext, pp. 289–296.

Klyashtorin, L., 1997. Pacific Salmon: Climate-Linked Long-term Stock Fluctuations, Vol. 5. PICES Press, pp. 2–34.

Lahdes, E., 1995. Acute thermal tolerance of two Antarctic copepods, *Calanoides acutus* and *Calanus propinquus*. J. Therm. Biol. 20, 75–78.

Lesser, M.P., 1997. Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs 16, 187–192.

Lesser, M.P., Stochaj, W.R., Tapley, D.W., Shick, J.M., 1990. Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen species. Coral Reefs 8, 225–232.

Lindstedt, S.L., McGlothlin, T., Percy, E., Pifer, J., 1998. Task-specific design of skeletal muscle: balancing muscle structural composition. Comp. Biochem. Physiol. B 120, 35–40.

Moseley, P.L., Gapen, C., Wallen, E.S., Walter, M.E., Peterson, M.W., 1994. Thermal stress induces epithelial permeability. Am. J. Physiol. 267, C425–C434.

Nakajima, H., Ishizaka, N., Hangaishi, M., et al., 2000. Lecithinized copper, zinc-superoxide dismutase ameliorates prolonged hypoxia-induced injury of cardiomyocytes. Free Rad. Biol. Med. 29, 34–41.

Nicholas, W.L., 1984. The Biology of Free Living Nematodes. Clarendon Press, Oxford.

Nii, C.M., Muscatine, L., 1997. Oxidative stress in the symbiotic sea anemone Aiptasia pulchella (Carlgren, 1943): contribution of the animal to superoxide ion production at elevated temperatures. Biol. Bull. 192, 444–456.

O'Brien, C.M., Fox, C.J., Planque, B., Casey, J., 2000. Climate variability and North Sea cod. Nature 404, 142.

Peck, L.S., Conway, L.Z., 2000. The myth of metabolic cold adaptation: oxygen consumption in stenothermal Antarctic bivalves. In: Harper, E., Taylor, J.D., Crame, J.A. (Eds.), Evolutionary Biology of the Bivalvia, Vol. 177. Geological Society, Special Publications, London, pp. 441–450.

Peck, L.S., Hardewig, I., Pörtner, H.O. Metabolic demand, oxygen supply and critical temperatures in the Antarctic bivalve, *Laternula elliptica*, Physiol. Biochem, 2001., in press.

Pörtner, H.O., 2001. Climate change and temperature dependent biogeography: Oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88, 137–146.

Pörtner, H.O. Environmental and functional limits to muscular exercise in marine invertebrate athletes, Comp. Biochem. Physiol, in press.

Pörtner, H.O., Grieshaber, M.K., 1993. Characteristics of the critical P_{O_2}(s): gas exchange, metabolic rate and the mode of energy production. In: Bicudo, J.E.P.W. (Ed.), The Vertebrate Gas Transport Cascade: Adaptations to Environment and Mode of Life. CRC Press, Boca Raton, FL, pp. 330–357.

Pörtner, H.O., Zielinski, S., 1998. Environmental constraints and the physiology of performance in squids. In: Payne, A.I.L., Lipinski, M.R., Clarke, M.R., Roeleveld, M.A.C. (Eds.), Cephalopod Biodiversity, Ecology and Evolution, South African Journal of Marine Science, Vol. 20. pp. 207–221.

Pörtner, H.O., Branco, L.G.S., Malvin, G.M., Wood, S.C., 1994. A new function for lactate in the toad *Bufo marinus*. J. Appl. Physiol. 76, 2405–2410.

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

Pörtner, H.O., Peck, L., Zielinski, S., Conway, L.Z., 1999a. Intracellular pH and energy metabolism in the highly stenothermal Antarctic bivalve *Limopsis marionensis* as a function of ambient temperature. Polar Biol. 22, 17–30.

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

Pörtner, H.O., van Dijk, P.L.M., Hardewig, I., Sommer, A., 2000. Levels of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. In: Davison, W., Williams, C.H. (Eds.), Antarctic Ecosystems: Models for Wider Ecological Understanding. Caxton Press, Christchurch, New Zealand, pp. 109–122.

Pörtner, H.O., Berdal, B., Blust, R., et al., 2001. Climate effects on growth performance, fecundity and recruitment in marine fish: developing a hypothesis for cause and effect relationships in Atlantic cod (*Gadus morhua*) and common eelpout (*Zoarces viviparus*). Contin. Shelf Res. 21,

1975–1997.

Regoli, F., Principato, G.B., Bertoli, E., Nigro, M., Orlando, E., 1997. Biochemical characterization of the antioxidant system in the scallop *Adamussium colbecki*, a sentinel organism for monitoring the Antarctic environment. Polar Biol. 17, 251–258.

Rifkind, J.M., Abugo, O., Levy, A., Monticone, R., Heim, J., 1993. Formation of free radicals under hypoxia. In: Hochachka, P.W., Lutz, P.L., Sick, T., Rosenthal, M., van den Thillart, G. (Eds.), Surviving Hypoxia: Mechanisms of Control and Adaptation. CRC Press, Boca Raton, FL, pp. 509–525.

Rolfe, D.F.S., Brand, M.D., 1996. Contribution of mitochondrial proton leak to skeletal muscle respiration and to standard metabolic rate. Am. J. Physiol. 271, C1380–C1389.

Schmidt-Nielsen, K., 1997. Animal Physiology. Adaptation and Environment. fifth ed. Cambridge University Press, Cambridge 607 pp.

Schwerdtfeger, F., 1977. Ökologie der Tiere: Autökologie. Verlag Paul Parey, Hamburg, Berlin, pp. 1–460.

Shelford, V.E., 1931. Some concepts of bioecology. Ecology 12, 455–467.

Sohal, R.S., Weindruch, R., 1996. Oxidative stress, caloric restriction and aging. Science 273, 59–63.

Somero, G.N., DeVries, A.L., 1967. Temperature tolerance of some Antarctic fishes. Science 156, 257–258.

Somero, G.N., Fields, P.A., Hofmann, G.E., Weinstein, R.B., Kawall, H., 1998. Cold adaptation and stenothermy in Antarctic notothenioid fishes: what has been gained and what has been lost? In: Di Prisco, G., Pisano, E., Clarke, A. (Eds.), Fishes of Antarctica. A Biological Overview. Springer, Milan, pp. 97–109.

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

Sommer, A.M., Pörtner, H.O. Metabolic cold adaptation in the lugworm *Arenicola marina* (L.): comparison of a White Sea and a North Sea population, submitted.

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

Southward, A.J., Hawkins, S.J., Burrows, M.T., 1995. Seventy years' observations of changes in distribution and abundance of zooplankton and intertidal organisms in the western English Channel in relation to rising sea temperature. J. Therm. Biol. 20, 127–155.

Steffensen, J.F., Farrell, A.P., 1998. Swimming performance, venous oxygen tension and cardiac performance of coronary-ligated rainbow trout, *Oncorhynchus mykiss*, exposed to progressive hypoxia. Comp. Biochem. Physiol. A 119, 585–592.

Stillman, J.H., Somero, G.N., 2000. A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*. Influences of latitude, vertical zonation, acclimation, and phylogeny. Physiol. Biochem. Zool. 73, 200–208.

St.-Pierre, J., Charest, P.-M., Guderley, H., 1998. Relative contribution of quantitative and qualitative changes in mitochondria to metabolic compensation during seasonal acclimatisation of rainbow trout *Oncorhynchus mykiss*. J. Exp. Biol. 201, 2961–2970.

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

Tansey, M.R., Brock, T.D., 1972. The upper temperature limit for eukaryotic organisms. Proc. Natl. Acad. Sci. USA 69, 2426–2428.

Taylor, C.R., Weibel, E.R., 1981. Design of the mammalian respiratory system. I. Problem and strategy. Respir. Physiol. 44, 1–10.

Tiku, P.E., Gracey, A.Y., Macartney, A.I., Beynon, R.J., Cossins, A.R., 1996. Cold-induced expression of Δ^9-desaturase in carp by transcriptional and posttranslational mechanisms. Science 271, 815–818.

Tomanek, L., Somero, G.N., 1999. Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: implications for limits of thermotolerance and biogeography. J. Exp. Biol. 202, 2925–2936.

Tomanek, L., Somero, G.N., 2000. Time course and magnitude of synthesis of heat-shock proteins in congeneric marine snails (genus *Tegula*) from different tidal heights. Physiol. Biochem. Zool. 73, 249–256.

Tremblay, R., Myrand, B., Guderley, H., 1998. Thermal sensitivity of organismal and mitochondrial oxygen consumption in relation to susceptibility of blue mussels, *Mytilus edulis* (L.), to summer mortality. J. Shellfish Res. 17, 141–152.

Tschischka, K., Abele, D., Pörtner, H.O., 2000. Mitochondrial oxyconformity and cold adaptation in the polychaete Nereis pelagica and the bivalve Arotica islandica from the Baltic and White Seas. J. Exp. Biol. 203, 3355–3368.

Urban, H.J., 1998. Upper temperature tolerance of two Antarctic molluscs (*Laternula elliptica* and *Nacella concinna*) from Potter Cove, King George Island, Antarctic Peninsula. Ber. Polarforsch. 299, 230–236.

van Dijk, P.L.M., Hardewig, I., Tesch, C., 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.

Wang, P., Chen, H., Qin, H., Sankarapandi, S., Becher, M.W., Wong, P.C., Zweier, J.L., 1998. Overexpression of human copper, zinc-superoxide dismutase (SOD1) prevents postischemic injury. Proc. Natl. Acad. Sci. 95, 4556–4560.

Weatherley, A.H., 1970. Effects of superabundant oxygen on thermal tolerance of goldfish. Biol. Bull. 139, 229–238.

Wehner, R., Marsh, A.C., Wehner, S., 1992. Desert ants on a thermal tightrope. Nature 357, 586–587.

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

White, F.N., Somero, G., 1982. Acid-base regulation and phospholipid adaptations to temperature: time courses and physiological significance to modifying the milieu for protein function. Physiol. Rev. 62, 40–90.

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

Wöhrmann, A.P.A., 1998. Antifreeze glycopeptides and peptides in the freezing avoidance of Weddell Sea fishes: its relation to mode of life, depth distribution and evolution. In: Pörtner, H.O., Playle, R. (Eds.), Cold Ocean Physiology. Cambridge University Press, Cambridge, pp. 266–295.

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