Trade-Offs in Thermal Adaptation: The Need for a Molecular to Ecological Integration[*]

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ABSTRACT

Through functional analyses, integrative physiology is able to link molecular biology with ecology as well as evolutionary biology and is thereby expected to provide access to the evolution of molecular, cellular, and organismic functions; the genetic basis of adaptability; and the shaping of ecological patterns. This paper compiles several exemplary studies of thermal physiology and ecology, carried out at various levels of biological organization from single genes (proteins) to ecosystems.

In each of those examples, trade-offs and constraints in thermal adaptation are addressed; these trade-offs and constraints may limit species' distribution and define their level of fitness. For a more comprehensive understanding, the paper sets out to elaborate the functional and conceptual connections among these independent studies and the various organizational levels addressed. This effort illustrates the need for an overarching concept of thermal adaptation that encompasses molecular, organellar, cellular, and whole-organism information as well as the mechanistic links between fitness, ecological success, and organismal physiology. For this data, the hypothesis of oxygen- and capacity-limited thermal tolerance in animals provides such a conceptual framework and allows interpreting the mechanisms of thermal limitation of animals as relevant at the ecological level. While, ideally, evolutionary studies over multiple generations, illustrated by an example study in bacteria, are necessary to test the validity of such complex concepts and underlying hypotheses, animal physiology frequently is constrained to functional studies within one generation. Comparisons of populations in a latitudinal cline, closely related species from different climates, and ontogenetic stages from riverine clines illustrate how evolutionary information can still be gained. An understanding of temperature-dependent shifts in energy turnover, associated with adjustments in aerobic scope and performance, will result. This understanding builds on a mechanistic analysis of the width and location of thermal windows on the temperature scale and also on study of the functional properties of relevant proteins and associated gene expression mechanisms.

Introduction

Habitat temperature is a critically important environmental factor for ectothermal organisms because of the direct effects of temperature on all biological processes. Direct as well as evolutionary responses of organisms to changes in temperature have been shown at a wide range of levels of biological organization, from molecular to physiological to behavioral. Changes in habitat temperature can occur on a wide temporal range, from fluctuations within hours as a result of day-night or tidal cycles to those over many years as a result of global climate change. The magnitude of effects of global warming on species distribution will finally be a result of the proximity of organismal performance maxima and thermal limits to mean

and extreme habitat temperatures. Such effects will also depend on the capacity of those organisms to adjust their performance windows and thermal limits through acclimatization.

Understanding the response of organisms to thermal challenges requires an understanding of their acute responses and then their later, potentially compensatory, acclimatization responses to temperature. The latter may be thought of as phenotypic adaptations, or rearrangements of form and function, presumably to improve function in the new thermal environment (but see also Leroi et al. 1994). Early work in thermal physiology has been largely descriptive and has provided patterns that set the framework for many studies carried out today. Classical work by August Krogh in the early twentieth century outlined a fundamental response of ectothermal organisms in response to variation in temperature. Ege and Krogh (1914) studied the resting metabolism of a single goldfish *Carassius auratus*, at a range of temperatures from 30°C to 0°C. Because he was interested in determining resting rather than routine or active metabolism, Krogh compared these data with the metabolism of the same fish under light narcosis. The data were identical and, when combined with data for frogs and dog (using a normalization procedure we would today regard as unsatisfactory), produced what became known as Krogh's normal curve (Fig. 1). Krogh's seminal experiment led to three paradigms in thermal physiology, namely: (1) resting metabolism increases with temperature in ectotherms; (2) the evolutionary and acute responses to temperature are different; and (3) organisms that have evolved at low temperature show elevated resting metabolic rates when compared with eurythermal species measured acutely at the same low temperatures (metabolic cold adaptation).

The first two paradigms remain central to thermal physiology. The concept of metabolic cold adaptation was important in introducing the concept of evolutionary thermal compensation, a theme that has permeated subsequent work on ectothermic thermal physiology. The general validity of this concept has been rejected for marine ectotherms (Holeton 1974; Clarke and Johnston 1999), although exceptions exist among cold-adapted eurytherms (Pörtner et al. 2000). The degree of cold eurythermy appears crucial in understanding the level of metabolic cold adaptation (Pörtner 2004) and may well explain why metabolic cold adaptation phenomena are frequently found in air breathers (Pörtner 2002a), including insects (Addo-Bediako et al. 2002), as these live in a much more unstable thermal environment than water breathers.

The simplest definition of thermal compensation is probably the maintenance of physiological rate and scope in the face of temperature change. Starting with this definition, we can then ask which physiological processes show thermal compensation and how this is achieved. While we can pose this question most meaningfully for cellular processes such as adenosine 5′-triphosphate (ATP) generation or the maintenance of ion gradients across membranes, we can also in-
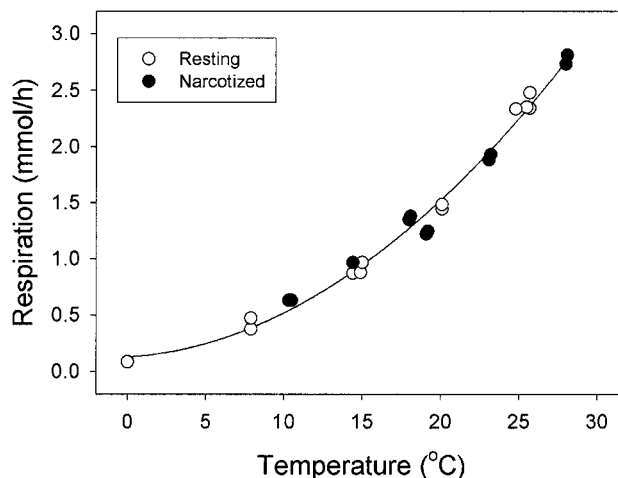

Figure 1. A classic study in thermal physiology: August Krogh's study of resting metabolism in goldfish *Carassius auratus*. The data are for a single goldfish of wet mass 9.3 g, whose temperature varied over the range 0°–30°C. To check that a true measure of resting metabolism had been made, Krogh compared data from the same fish in resting and lightly narcotized conditions; the results were indistinguishable. Plotted from original data from Ege and Krogh (1914). Krogh later added data for a range of other vertebrates (notably frog and dog) and normalized these for mass (by scaling the respiration data to match that from goldfish) to produce what became known as Krogh's normal curve.

vestigate compensation at higher levels of organismal integration, such as nervous conduction, or even whole-organism processes such as metabolic rate, growth, or reproductive output. For growth rate, however, we have to be careful to distinguish a slow rate at low temperatures caused by lack of evolutionary temperature compensation from rate limitation by some other processes such as resource limitation. Thus in the case of growth rate, the simple observation of a slow growth rate in polar organisms cannot of itself distinguish between temperature limitation and seasonal or absolute resource limitation (Clarke 1991).

The methodological and intellectual challenges associated with the study of thermal responses and compensation at the molecular, cellular, and whole-organism levels have frequently caused molecular biologists, biochemists, and animal physiologists to study these levels largely in isolation, rarely allowing the addressing of how these functional levels are intertwined. At the same time, although there is a broad understanding that physiological characters play a crucial role in setting ecological patterns (Kingsolver and Huey 1998; Allen et al. 2002; Angilletta et al. 2002; Clarke 2003; Chown and Nicolson 2004), the respective cause and effect understanding is equally insufficient, such that many mechanistic and, as a consequence, quantitative links between physiological and ecological characters remain to be firmly established. However, for meaningful physiological analyses, the mechanistic links between processes at the eco-

system level and at physiological levels need clear identification. Recent insight emphasizes that it is not possible to fully comprehend the scope and limits of adaptation exclusively based on molecular and biochemical analyses, on the one hand (Hochachka and Somero 2002), nor exclusively on ecological approaches, on the other. Driving forces for adaptation operate on organisms at population and ecosystem levels, whereas the scope and limits of adaptation originate from an integration of gene and molecular functions into cellular, tissue, and whole-organism functioning. An integrative approach is more and more important to environmental physiology (Prosser 1986; Spicer and Gaston 1999; Feder and Mitchell-Olds 2003; Chown and Nicolson 2004), especially one that succeeds in bridging the various fields. Physiological techniques that support such integrative studies include implanted micro-optodes for monitoring oxygen status online (Fernandez et al. 2000; Frederich and Pörtner 2000) or nuclear magnetic resonance imaging and spectroscopy for an integrated monitoring of tissue functioning in the intact organism (Pörtner et al. 2004).

How do we proceed in deepening our understanding of thermal adaptation, that is, the processes setting the rates and scopes of organismal functioning within limited thermal windows? This paper discusses various examples reported at the Ithala conference and attempts to develop such an integrative approach. This integrative approach can yield a conceptual framework for generation of testable hypotheses to determine the mechanistic links between organismal physiology, fitness, and ecological success as well as associated hypotheses of the mechanisms of thermal limitation, acclimation, and adaptation of animals. Such a framework should integrate molecular, cellular, and organismal as well as ecological information. An approach such as this also supports a mechanistic understanding of optimality models used to elaborate the benefits of thermal specialists versus generalists (Gilchrist 1995) and also of the general trade-offs involved in thermal adaptation (Huey and Kingsolver 1993). One such conceptual framework presented at the Ithala conference is that of oxygen- and capacity-limited thermal tolerance (Pörtner 2001, 2002a; explained in more detail below). This concept has recently been developed for water breathers and still needs testing in many species and phyla, especially among air breathers. Rather than addressing mechanisms supporting passive endurance of thermal extremes, it focuses on the setting of the thermal optimum range and its limits. Only within this range will the organism be able to feed, grow, reproduce, and develop fitness optima. At the same time, differentiation between latitudinal and ontogenetic patterns and their energetic correlates appears possible.

Evolutionary experiments provide an ideal framework for testing various hypotheses of thermal adaptation and limitation. As an example, the shift in fitness optima depending on temperature is estimated from relative fitness and productivity estimates in bacterial populations over hundreds of generations (A. F. Bennett and R. E. Lenski, Ithala, 2004). However, due to long generation times, such evolutionary experiments are virtually impossible in most animals. Therefore, estimates of evolutionary success in animals must rely on an interpretation of functional information from the various levels of biological organization.

Whole-animal investigations include studies of shifting upper and lower limits in response to seasonal acclimation or evolutionary adaptation to temperature along a latitudinal cline (J. H. Stillman, Ithala, 2004). These investigations also include studies of the relationships between shifting environmental windows, depending on the season, and shifting performance windows during ontogeny (F. Schiemer, Ithala, 2004). Crucial organismal processes such as growth, reproduction, and development may help shape ecological success in that they contribute to improved fitness during thermal adaptation. The physiological principles underlying these patterns are operative in both water and air breathers and may bear important life cycle consequences (M. A. Lardies and F. Bozinovic, Ithala, 2004). Accordingly, such ecological studies set the framework for analyses of thermal effects at physiological levels. Mechanistic investigations should, for example, answer the question of why animals specialize on limited thermal windows and which environmental constraints or which constraints in adaptation would force them to do so (H. O. Pörtner and M. Lucassen, Ithala, 2004).

In this context, studies of oxygen-limited thermal tolerance indicate that the capacities of oxygen supply systems such as ventilation and circulation are crucial in thermal limitation and adaptation. In fish, circulatory systems are more important than ventilatory functions in setting thermal windows (Pörtner et al. 2004). Studies of the ontogeny of circulatory systems (B. Pelster, Ithala, 2004) are, therefore, essential for understanding how thermal adaptation windows match environmental windows in fish larvae (F. Schiemer, Ithala, 2004). These examples emphasize how many facets of thermal adaptation still need to be addressed and reinterpreted in an effort to integrate ecology and physiology (A. Clarke, Ithala, 2004). Finally, at the ecosystem level, thermal windows may differ between the various species shaping an ecosystem. Windows may differ because species display different performance levels or live in different latitudinal ranges, even if these overlap. These different windows of individual species, reflected in differential thermal sensitivities and timing of ontogeny and species productivity, will influence species interactions at the ecosystem level. Changes in these interactions are currently being investigated as crucial components of ecosystem responses to climate change (Winder and Schindler 2004). The specific role of temperature in physiological changes associated with these patterns needs to be established.

As a corollary, identifying the principles of thermal adaptation of key organisms shaping an ecosystem should lead to an understanding of the reasons that limit performance and productivity of species and communities within habitats. Such

studies at the molecular, cellular, and organismal levels will clarify the mechanistic bases of extant biogeographical patterns on large scales, for example, along latitudinal or altitudinal clines, if they are associated with shifts in the temperature regime as well as changing interactions between temperature and other environmental factors. Among the latter, water balance in changing climate and associated temperature conditions is an important issue in terrestrial animals, but it probably has little or no bearing for the specific examples addressed in this paper. Overall, we feel that an approach as developed here will give access to the mechanistic bases of organismal responses to environmental alterations, including climate change, and will provide the requested cause-and-effect understanding of observed ecological impacts (Pörtner et al. 2001; Jensen 2003).

Trade-Offs during Evolutionary Temperature Adaptation: An Experimental Test in Bacteria

Specialization on thermal windows can be understood once the trade-offs in thermal adaptation are identified. In adapting to new environments and acquiring new characters or greater performance, organisms sometimes experience a correlated decline in other structures or functions. This balance of gain and loss is termed a "trade-off." More precisely, a trade-off can be defined as a decline in nonselected characters that accompanies adaptation to new selective conditions. This correlated loss of function has been long recognized by evolutionary biologists, including Darwin, who termed it "compensation" and noted (quoting Goethe) that "in order to spend on one side, nature is forced to economize on the other side" (Darwin 1859, p. 184). This notion of a reallocation of resources persists in modern evolutionary thinking, for example, "improvements cannot occur indefinitely, because eventually organisms come up against limitations. … At that point, improvements in one trait may be achievable only at the expense of others—there is a trade-off between the traits" (Sibly 2002, p. 625). The recognition that concentration of resources and abilities is necessary extends even into commonsensical thought, with the proverbial jack-of-all-trades being a master of none of them.

A reallocation of a limited resource is certainly one potential cause of trade-offs: for example, energy allocated to reproduction is unavailable for somatic growth (Angilletta et al. 2003). However, trade-offs can also have more direct functional bases. The evolution of wings by vertebrates, for instance, has precluded the efficient use of these limbs for their historical use for terrestrial locomotion. Even within the structure/function of a single molecule, there may be trade-offs associated with environmental adaptation. Structure and function of a single enzyme, for instance, may be adapted to a particular temperature (Somero 1997; Hochachka and Somero 2002) and may lose catalytic efficiency or even functionality at other temperatures. An apparent trade-off may also occur due to mutation accumulation in nonselected structures such as vestigial organs.

While this decline is not functionally linked, either directly or through resource allocation, to adaptive characters, it would nevertheless be perceived as a trade-off (Cooper et al. 2001 for a direct test and rejection of the mutation accumulation hypothesis as an explanation for the presence of a thermal trade-off).

While it is certainly possible to point to many instances of biological trade-offs, it is not possible to say how common or necessary they are. Numerous traits and functions do not change during adaptation to a new environment, and all of these could be regarded as counterexamples and evidence that trade-offs are not universal or even usual. Even in cases of presumptive trade-offs during adaptation in nature, each evolutionary event is unique and unreplicated. Therefore, it cannot be determined how usual or necessary the observed change would have been if the event had happened repeatedly. One of the advantages of experimental evolution (Rose et al. 1990; Bennett and Lenski 1999) is precisely its ability to permit replication of an adaptive event and to determine its significance statistically.

As an illustrative example, experimental evolution was used to test for a trade-off, that is, the loss of function at high temperature that accompanied evolutionary adaptation to low temperature. In this example, 24 experimental populations of the bacterium *E. coli* were adapted to 20°C, and the accompanying trade-off (decline) in fitness at high temperature (40°C) was measured. Fitness (W) was measured as differential reproduction in competition with their common ancestor (see Bennett et al. 1992 and Mongold et al. 1996 for details of line derivation, culture, preservation, and fitness assays). In paired comparisons with their progenitor lines, adaptation to 20°C (increase in W at 20°C) and the correlated trade-off at 40°C (decrease in W at 40°C) were determined. Statistical comparisons (paired t-tests) were made for all individual pairs of lines and for the entire group of 24 lines, and the correlation between improvement in W at 20°C and its decrement at 40°C for the entire group of lines was determined. Not surprisingly, W increased substantially at 20°C as a result of adaptation to that temperature: 22 of 24 lines had a significant increment in W (2 NS), and the group of all 24 lines had a very significant improvement in W ($P < 0.0001$). Nearly two-thirds of the lines (15 of 24) had a significant decline in W at 40°C (8 NS, 1 significant increase). For all lines considered together, W declined significantly ($P = 0.0002$). However, there was no significant quantitative relationship between the magnitude of W increment at 20°C and decline at 40°C ($P = 0.49$).

Within this experimental series, a trade-off in performance at high temperature generally occurred during adaptation to low temperature. While this correlation was usual, it was not universal: some lines did not change high-temperature performance and one even increased. It is possible that the occurrence of a trade-off or lack of one was dependent on the exact mechanism by which low-temperature adaptation was achieved. The

exact genetic changes that underlie these differences are unknown at present. Apparently, most pathways to low-temperature adaptation in these populations involve a simultaneous decline in ability to handle high temperature. However, other less common pathways may exist that do not involve a loss of high-temperature competence, and these apparently were used in some of the populations. The ad hoc character of the trade-off association is further supported by the lack of a quantitative relationship between the magnitude of the adaptive increment and the trade-off decrement. High-temperature trade-offs may thus be regarded as usual but not universal or necessary in these populations. Even in this relatively simple and genetically homogeneous experimental system, trade-offs are not necessary adjuncts of adaptation, but they are common ones.

Toward a Unifying Concept: Functional Hypotheses for Thermal Trade-Offs and Adaptation?

Clearly, the interpretation of such evolutionary analyses of fitness in response to thermal adaptation would benefit from knowledge of the physiological, that is, functional, background of the trade-offs observed. Vice versa, such evolutionary experiments can test for the validity and generality of any concept in thermal adaptation and its underlying functional hypotheses. Specifically, relevant functional questions arise for the bacterial experiments about whether the mechanisms of thermal adaptation involve a cost, which is expressed in the trade-offs observed. Is there a disadvantage in not shifting both upper and lower thermal limits simultaneously? Do culture conditions fully or partially alleviate the pressure for such trade-offs that might be more severe in nature, possibly due to resource limitations or other nonideal environmental conditions? How do temperature fluctuations influence the picture? All of these questions would be enriched by an identification of the underlying functional mechanisms that underlie the trade-offs observed.

Accordingly, development of functional hypotheses is required to understand the gains and losses decisive in thermal adaptation and associated fitness (Angilletta et al. 2003). This demands investigations at a high level of functional complexity because there is no single mechanism responsible for the complete suite of events called thermal adaptation (Pörtner 2002a). This point is generally relevant in all organisms specializing on limited thermal windows. Metazoa, due to their inherently high levels of organizational complexity, specialize on environmental temperature much more than unicellular bacteria and algae. In contrast to animals, some representatives of these groups thrive at temperatures well beyond 50°–60°C. Higher levels of functional complexity in animals than in bacteria probably set heat limits in animals to lower values and thus involve mechanisms in addition to those operative in, for example, bacteria or unicellular eukaryotes (Pörtner 2002a). The critical thermal maximum and temperature-dependent performance relate to the

environmental temperature regime; however, a consistent picture of how thermal-tolerance windows develop in relation to environmental patterns does not yet exist. There is some evidence that these patterns are different toward colder regions/higher latitudes between the Northern and Southern Hemispheres; different trends in thermal adaptation may be shaped by the largely different patterns of temperature variability between hemispheres (Gaston and Chown 1999; Pörtner 2004).

Although it has been recognized for a long time that different functions have different thermal windows, with active behaviors such as reproduction occurring in narrower windows than passive tolerance, much mechanistic work has emphasized passive survival at temperatures where active behaviors stall. For example, heat shock proteins appear as prime candidates for understanding thermal limitation in bacteria (Riehle et al. 2003) or in animals (Tomanek and Somero 1999; for a review, see Feder and Hofmann 1999). Further stress and stress protection mechanism are being elaborated. This focus on protection mechanisms during "passive" endurance will be relevant in providing access to understanding critical thermal limits and associated mortality.

A mechanistic background is also required for an understanding of thermal windows of ecologically relevant "active" lifestyle or performance measures such as growth, development, reproduction, and foraging or other behaviors. Adaptive mechanisms that cause shifts in the width and location of such performance windows need elaboration. Studies of mechanisms supporting the adjustments of functional scope or rate to temperature would, in fact, address the more direct link between organismal and ecosystem functioning. Little effort has been invested so far into an integrative understanding of the various functional levels involved.

As a direct link between thermal physiology and ecology of animals, the recent concept of oxygen- or capacity-limited thermal tolerance (Pörtner 2001, 2002a; elements displayed in Fig. 2) focuses on the maintenance of functions and scope and integrates molecular to cellular information into a whole-organism functional picture. According to this concept, onset of a mismatch between oxygen demand and the capacity of supply mechanisms sets the thermal-tolerance windows of metazoans, first by limiting aerobic scope at both upper and lower pejus temperatures (T_p; pejus = getting worse). Within the pejus envelope, the level of aerobic scope links to ecological functions or fitness through rates of growth, reproduction, development, and behaviors and thereby integrates the organism into ecosystem functioning. Toward low- or high-temperature extremes beyond pejus temperatures, the organism becomes more passive until critical temperatures (T_c) indicate onset of anaerobic metabolism.

Evidence available across various aquatic phyla, namely, annelids, sipunculids, molluscs (bivalves, cephalopods), crustaceans, and fish studied across latitudes, supports the concept
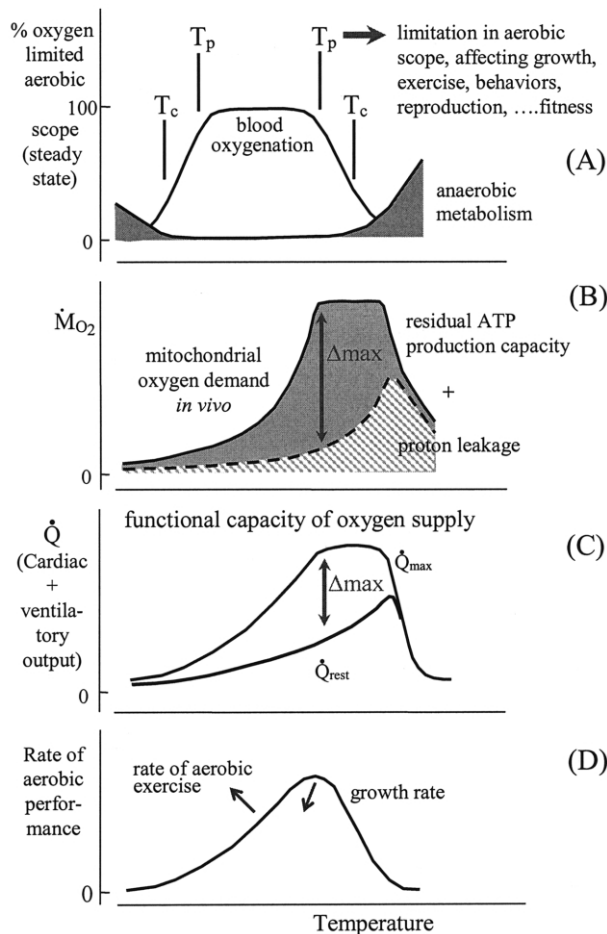
Figure 2. Elements of the concept of oxygen- and capacity-limited thermal tolerance in metazoans (modified after Pörtner 2001, 2002a; Pörtner et al. 2004, 2005). Onset of a mismatch between oxygen demand and supply mechanisms set the thermal-tolerance windows of metazoans by limiting aerobic scope (A), delineated by both upper and lower pejus temperatures (T_p). Critical temperatures (T_c) indicate onset of anaerobic metabolism. In both cold and warm temperatures (B), the functional capacities of mitochondria and, consecutively, cells and tissues (including ventilation and circulation) become insufficient. In the cold, this occurs partially through insufficiently low mitochondrial ATP formation capacity. In warm temperatures, the rising baseline oxygen demand of organismic and cellular maintenance (Fig. 1), caused in part by a rising fraction of mitochondrial proton leakage, elicits a mismatch between oxygen supply and demand. According to capacity limits of ventilatory and circulatory organs, the residual capacity of mitochondria to produce ATP is reduced beyond pejus temperatures in the warm, when at the same time, energy demand rises exponentially. Accordingly, the temperature-dependent capacity of ventilation and circulation, especially the difference between maximum and resting output, characterizes limits to oxygen supply and aerobic scope (C). At the upper pejus temperature, maximum functional reserves in mitochondrial ATP generation (B) and, thus, in oxygen supply (C) support an asymmetric performance curve (D), with optimal performance (e.g., growth, exercise) close to upper pejus levels. Here functions are supported by both high temperatures and optimum oxygen supply. As a trade-off in eurythermal cold adaptation (indicated

of oxygen- and capacity-limited thermal tolerance. Among air breathers, there is indirect evidence among vertebrates (for review, see Pörtner 2001, 2002a; Pörtner et al. 2004). Recently, the concept has been supported for a terrestrial isopod but questioned for insects, based on results obtained by thermal-limit respirometry (Klok et al. 2004). In insects, diffusive oxygen supply to tissues through tracheal systems may alleviate a limiting role for ventilation and circulation with respect to oxygen supply. However, oxygen concentration in air has been suggested to impose a limit on body size and flight metabolism in insects, suggesting some oxygen limitation of functioning in this group (Dudley 1998; Harrison and Lighton 1998; Frazier et al. 2001). In fact, hyperoxia generally increased mass and growth rate at higher rearing temperatures in *Drosophila melanogaster* (Frazier et al. 2001). This relates to improved aerobic scope for growth, and, in turn, aerobic scope is temperature dependent, even in insects. Aerobic scope has not been investigated by Klok et al. (2004), nor have pejus or critical temperatures according to Figure 2 been identified, so their conclusions may be premature. Moreover, the finding of significant metabolic cold adaptation in insects (Addo-Bediako et al. 2002), especially in Northern Hemisphere eurytherms (Pörtner 2004), strongly suggests that capacity compensation occurs and that energetic consequences of thermal adjustments of aerobic metabolism probably apply as outlined below.

At the cellular level in both heat and cold, the functional capacities of mitochondria and, consecutively, of cells and tissues (including ventilation and circulation) in vivo become insufficient. In cold temperatures, this occurs partially through insufficiently low mitochondrial ATP formation capacity. In warm temperatures, the rising baseline oxygen demand of organismic and cellular maintenance, caused in part by a rising fraction of mitochondrial proton leakage, elicits a mismatch between oxygen supply and demand (Fig. 2). Capacity limits of ventilatory and circulatory organs are reached and limit oxygen supply to mitochondria. This limits the residual capacity of mitochondria to produce ATP beyond pejus temperatures in warm temperatures, when at the same time energy demand rises exponentially. Accordingly, the temperature-dependent capacity of ventilation and circulation, especially the difference between maximum and resting output, characterizes limits to oxygen supply and aerobic scope. This capacity, in turn, depends on the capacity of tissue mitochondria, and this again

by arrows), standard metabolism and aerobic exercise capacity increase in the cold (D; Pörtner 2002b), while temperature-specific growth performance is reduced. As a corollary, the figure illustrates how mitochondrial to ecological functions are intertwined. Mechanisms setting aerobic scope (Fig. 7) are crucial in thermal adaptation that leads to a shift of the window of aerobic scope (not shown).

depends on the functional capacity of their protein "equipment."

At the upper pejus temperature, maximum functional reserves are available to the organism in mitochondrial ATP generation and, thus, in oxygen supply and aerobic scope. Accordingly, in an asymmetric performance curve (Fig. 2), aerobic performance (e.g., in growth, exercise, reproduction, behaviors) results optimal close to upper pejus levels. Evidently, functions at the upper T_p are supported by both high temperatures and optimum oxygen supply.

Within the limits of environmental and phylogenetic constraints, thermal adaptation also sets the width of thermal windows, with important implications for aerobic energy turnover and standard metabolic rates (SMRs). Trade-offs in eurythermal cold adaptation affect standard metabolism and aerobic exercise capacity (Pörtner 2002b) as well as temperature-specific growth performance.

All of these considerations illustrate how molecular, organismal, and ecological levels of functioning are intertwined. As a corollary, thermal adaptation essentially occurs at a high level of organizational complexity and implies a shifting window and level of thermally limited aerobic scope. Mechanisms setting aerobic scope, that is, molecular to cellular mechanisms setting aerobic tissue functioning, among these the capacities of ventilatory and circulatory organs, are thus crucial to thermal adaptation. Accordingly, analyses of adaptation, functional genomics, and gene expression in aerobic metabolism are key to an understanding of these processes (Pörtner et al. 2005). As mentioned above, these mechanisms have overarching relevance in the context of ecosystem change, and the quantitative relationships need to be elaborated.

The following sections will provide examples at various levels of biological organization that further illustrate the requirement of studying thermal adaptation within such an integrating concept. Such effort not only will provide a framework of testable hypotheses but also will provide a context for the interpretation of isolated phenomena published in the literature. Details can be evaluated according to whether they match or do not match with the hypothetical framework.

Analyses of the Plasticity of Thermal Windows across Thermal Clines: Animal Examples

The examples compiled here illustrate how comparisons of populations from latitudinal/altitudinal/intertidal/riverine clines can reveal functional mechanisms and the evolutionary processes involved in thermal adaptation. Such studies also suggest which fitness measures or lifestyle characteristics may be influenced by thermal adaptation.

Thermal Limits in Intertidal and Latitudinal Clines: Porcelain Crabs in Marine Shallow Waters

Traditional analyses of thermal-tolerance ranges are carried out as determinations of upper and lower thermal-tolerance limits, CT_{max} and CT_{min}, respectively, defined as the onset of spasms (at CT_{max}) in the whole organism (Lutterschmidt and Hutchison 1997). These extreme limits to thermal stress are found beyond the window of aerobic scope for activity (Zakhartsev et al. 2003a). When interpreted from the point of view of Figure 2, these extreme thermal limits mean complete cessation of functional capacity, reaching close to or beyond critical temperatures where aerobic scope is largely reduced and survival is passively sustained.

Comparative studies by Stillman and Somero (Stillman and Somero 1996; Stillman 2003) have used a definition of critical thermal limits that differs from the classical approach of the onset of spasms. They examined thermal limits of cardiac performance (monitored using impedance electrodes during thermal ramp experiments in which temperature was increased for CT_{max} or decreased for CT_{min} at 0.1°C min^{-1}) in multiple species of porcelain crabs, genus *Petrolisthes*, with respect to thermal habitat and thermal acclimation. Arrhenius break temperatures of cardiac performance (for details, see Stillman 2003) were used as indicators for thermal limitation and were found to shift during thermal acclimation. In general, the degree to which organisms are able to adjust CT_{max} and CT_{min} has been referred to as acclimation flexibility (Kingsolver and Huey 1998) or capacity for acclimation (Stillman 2003). Within the concept of oxygen-limited thermal tolerance, applicable to water breathers, cardiac performance at thermal limits is a most suitable parameter to study since the onset of thermal limitation in crustaceans (seen in the spider crab *Maja squinado*) is expressed through limited capacity to supply oxygen, namely, the combined capacity of ventilatory and circulatory performance (Frederich and Pörtner 2000; Fig. 2). As a note of caution, CT_{max} and CT_{min} as determined for the porcelain crabs likely reach beyond the respective points of capacity limitation in both cold and warm temperatures.

Porcelain crabs are an excellent group of organisms for comparative studies of temperature adaptation because, as a result of differences in latitudinal and vertical (intertidal zone) distributions, various species exist across a large range in thermal microhabitat conditions. Phylogenetic information for these crabs facilitates selection of closely related species that live in different thermal microhabitats (Stillman and Reeb 2001; Stillman 2002). Six species of *Petrolisthes* were selected for study: *Petrolisthes cinctipes* and *Petrolisthes cabrilloi* (temperate, middle to high intertidal zone), *Petrolisthes eriomerus* and *Petrolisthes manimaculis* (temperate, low intertidal, and subtidal zones), *Petrolisthes gracilis* and *Petrolisthes hirtipes* (northern Gulf of California, high and middle intertidal zones, respectively). Crabs from the northern Gulf of California live in an extreme
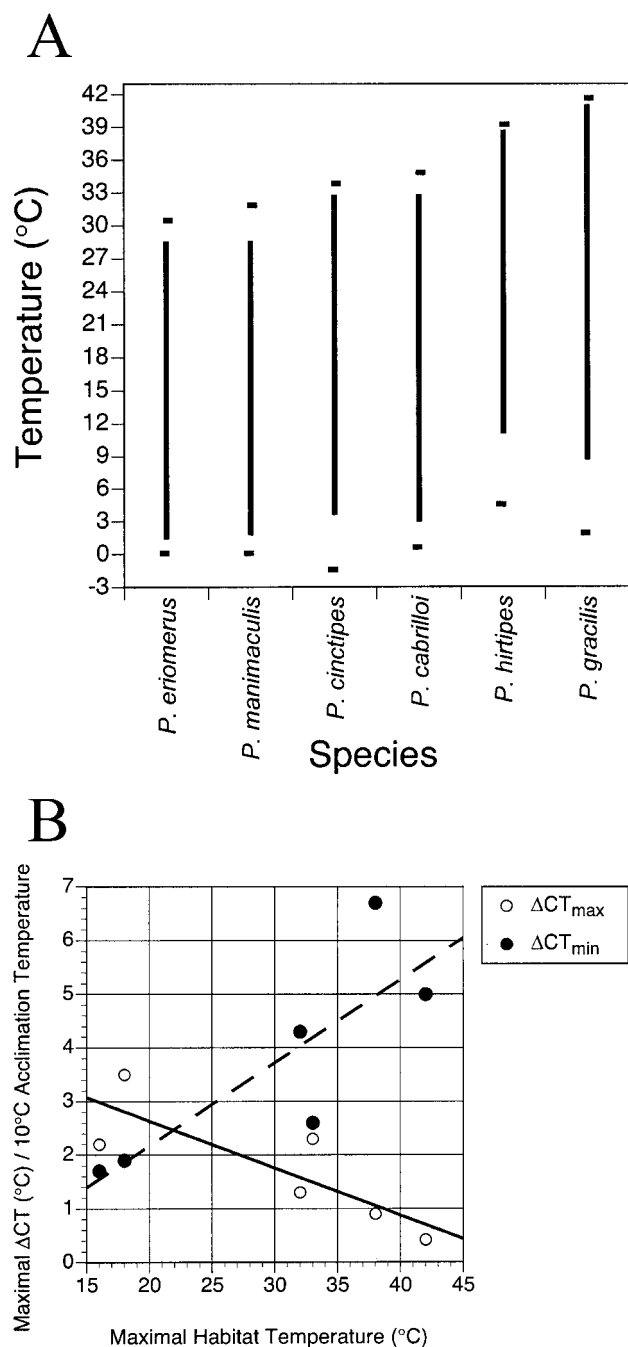
A

B

thermal habitat where water temperatures can be as cold as 12°C in winter and as warm as 30°C in summer, and crab microhabitat temperatures warmer than 40°C have been measured during summertime low tide periods. In the cold north temperate zone, water temperatures fluctuate between 7° and 13°C, and intertidal species of *Petrolisthes* experience fluctuating microhabitat temperatures that can be above 30°C in summer months. Thus, the species selected for study were from a great range of thermal microhabitats, from *P. eriomerus*, which sees the coolest and least variable habitat temperatures, to *P. gracilis*, which sees the warmest and most variable thermal habitat (Stillman 2004).

Acclimation for a minimum of 4 wk to various temperatures from within each species' natural thermal range affected upper and lower thermal limits of cardiac function in all species; however, the magnitude of change of CT_{max} and CT_{min} differed among species (Fig. 3; Stillman 2004). The temperate subtidal zone species, *P. eriomerus* and *P. manimaculis*, showed the greatest change in CT_{max} and the smallest change in CT_{min} of all of the species tested (Fig. 3A). Conversely, the warm-adapted crabs *P. gracilis* and *P. hirtipes* showed the smallest changes in CT_{max} and the largest changes in CT_{min} during thermal acclimation (Fig. 3A). Thus, in porcelain crabs from different thermal habitats, flexibility of CT_{max} is inversely correlated with maximal habitat temperature, and the opposite is true for CT_{min} (Fig. 3B).

Functional analyses of the concomitant effects of thermal acclimation on both heat and cold tolerance have seldom been reported in multiple species adapted to different thermal habitats. Overall, the data reflect limited capability to shift thermal limits when close to ambient thermal maxima and minima, but they also reflect the capacity to maintain the width of the thermal window with a slight trend to wider windows at higher acclimation temperatures. The results suggest an evolutionary and/or functional trade-off between achieving extreme-tolerance limits and maintaining plasticity of tolerance limits. Evolutionary trade-offs between extreme tolerance and plasticity have been demonstrated in *Drosophila* (e.g., desiccation tolerance; Hoffmann et al. 2003), and thus

Figure 3. *A*, Windows of critical thermal limits of cardiac function in six species of porcelain crabs (genus *Petrolisthes*) from different thermal habitats. The bar and dashes for each species represent its thermal window and the effects of thermal acclimation. For each bar, the top of the bar is the heat-tolerance limit of cold-acclimated specimens, and the dash above the bar is the maximal heat-tolerance measured (generally, the warm-acclimated specimens). Likewise, the bottom of the bar is the cold-tolerance limit of the warm-acclimated specimens, and the dash below is the minimum cold tolerance measured in the cold-acclimated specimens. Variances are not included, for the sake of clarity (based on data from Stillman 2003, 2004). *B*, Maximal plasticity of critical thermal limits of cardiac function during thermal acclimation in six species of porcelain crabs (genus *Petrolisthes*) from different thermal habitats. For each species, the maximal change in CT was calculated over a 10°C acclimation temperature range. From lowest to highest maximal habitat temperature, pairs of points for ΔCT are for *Petrolisthes eriomerus*, *Petrolisthes manimaculis*, *Petrolisthes cinctipes*, *Petrolisthes cabrilloi*, *Petrolisthes hirtipes*, and *Petrolisthes gracilis*. Linear regression indicated a significant relationship between ΔCT and maximal habitat temperature for both CT_{max} ($P = 0.040$, $r^2 = 0.692$) and CT_{min} ($P = 0.0396$, $r^2 = 0.694$).

evolutionary trade-offs are a reasonable hypothesis in thermal adaptation of porcelain crabs.

To assess potential ecological consequences of the relationship between acclimation capacity of CT_{max} and maximal habitat temperature, it is also necessary to consider the proximity of thermal performance limits to maximal habitat temperatures. In the most heat-tolerant species, *P. gracilis*, CT_{max} of summer-acclimatized crabs (25°–30°C) is within 1°C of its maximal habitat temperature, whereas in the least heat-tolerant species, *P. eriomerus*, CT_{max} of summer-acclimatized crabs (13°C) is more than 15°C away from its maximal habitat temperature. The acclimation capacity of CT_{max} of *P. gracilis* is only 1°C across a 20°C range of acclimation temperatures. If maximal habitat temperatures increase by more than 2°C as a result of global climate change, it is likely that there will be a decline in abundance of this species in its present habitat. Thus, it is the most heat-tolerant species that will be most susceptible to global warming. This result may seem counterintuitive, but it reflects the fact that the species has already stretched toward the most heat-exposed habitat available and may have to retreat to higher latitudes during warming. However, these findings also suggest that the Arrhenius break temperatures of cardiac performance do not always closely mirror habitat limitations. It will be challenging in the future to elaborate whether further limitations, for example, at the ecosystem level, are constraining a species to narrower windows and whether these are close to as yet unidentified physiological limits. It would be most interesting to investigate how the cardiac limits as defined in the porcelain crab study match the window of aerobic scope as defined in the crustacean study by Frederich and Pörtner (2000).

Matching Ontogeny and Environmental Temperature Trends: Fish Larvae in Large Rivers

Along the course of rivers in central Europe, characteristic fish associations are adapted to prevailing temperature regimes. The longitudinal thermal profile of rivers resembles to some extent the latitudinal geographical pattern. Local success of species is dependent on the match or mismatch between requirements and field conditions during early life history, which may be the critical phase that determines the success or failure of a species. Early life-history larval periods from the onset of feeding until full yolk resorption (mixed feeding period) and entirely exogenous feeding are the most critical (Blaxter 1992; Kamler 1992; Wieser 1995).

During early ontogenetic development, rheophilic fish species characteristic for large river systems are finely tuned to the seasonal temperature regime of their main nursery habitats in the inshore zones of rivers (Fig. 4). Faster runoff in such rivers is observed currently because of river regulation and the construction of hydropower dams. Temperatures may fall below the optimal range for the development of fish (Schiemer et al. 2003). Under such conditions, growth during the "critical pe-
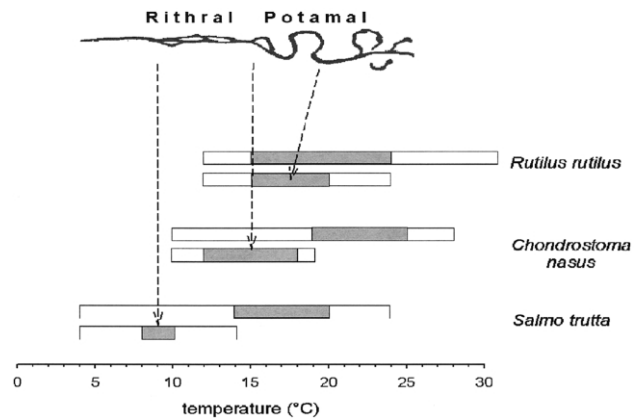

Figure 4. Viable (*white*) and the optimal (*gray*) temperature ranges of embryogenesis (*lower bar*) and larval development (*upper bar*) of three species characteristic for different fish regions along the longitudinal range of rivers. The upper part of the figure is a sketch of the hydro-morphological and biocoenotic zonation, rithral, and potamal of rivers as physiographically determined by current velocity or substrate. Data on thermal ranges according to *Salmo trutta fario*, embryogenesis (Ojanguren and Brana 2003) and early juvenile period (Ojanguren et al. 2001); *Chondrostoma nasus*, embryogenesis (Kamler et al. 1998) and larval period (Keckeis et al. 2001); and *Rutilus rutilus*, embryogenesis (Wieser et al. 1988) and larval period (Mooij and van Tongeren 1990).

riod" of life history is retarded, resulting in high mortality rates at insufficient body size. This is one main reason for the decline of stocks and for critically low recruitment of many riverine species. Additional trends caused by long-term climatic changes have to be similarly addressed from the point of view of match or mismatch between requirements and the predicted changes in environmental conditions.

For example, long-term trends in temperature development have been analyzed for the Austrian Danube. The potential effect of such trends was tested against the temperature dependence of embryogenesis and a growth model developed for one target species, the cyprinid *Chondrostoma nasus*. Field data on individual growth rates of fish, based on otolith analysis, provide information on daily growth increments in the larval and early juvenile phases. The study analyzed the thermal ranges and ontogenetic niche shifts during early life history in relation to potential constraints in temperature conditions. The study also identified long-term trends in the temperature regime of the Danube and analyzed the potential effects of such trends on characteristic fish species.

In the cyprinid *C. nasus*, spawning characteristically occurs in April at river temperatures between 8° and 12°C (Keckeis 2001). Embryogenesis under constant conditions takes place between 10° and 19°C. Thermal windows shift and expand during larval and early juvenile periods (Fig. 5). After hatching, the required larval microhabitats are richly structured inshore zones with higher temperatures, low current velocity, high production of zooplankton as larval food, and refuge possibilities
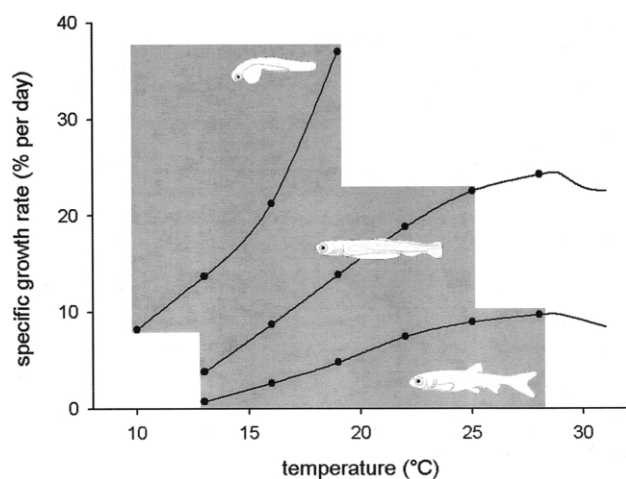
Figure 5. Specific growth rates during embryogenesis and larval and early juvenile periods of *Chondrostoma nasus* grown at constant temperatures between 10° and 28°C and with ad lib. food supply. Growth rates are particularly high during the egg development phase (i.e., the conversion from yolk into tissue; data from Kamler et al. 1998 and Keckeis et al. 2001) and decrease with size and stage. The figure illustrates both the shift and the expansion of the thermal niche from larval to early juvenile periods.

in the case of floods. With increasing larval size, the preferred microhabitats shift toward more lotic conditions.

At higher temperatures, the hatching of the embryos, the up-swimming of larvae, and the onset of exogenous feeding occur at distinctly smaller body size (tissue weight) and at a less advanced stage of morphological differentiation, including a larger yolk reserve. Evidently, within the viable temperature range, warming accelerates the various functions to different degrees (Lindsay and Arnason 1981). This strategic shift indicates trade-offs between size, duration of development, and the conservation of energy reserves. At present, it is unclear whether this shift is adaptive in the sense that it matches the shift in stochastic environmental conditions during warming (higher temperature, earlier food availability, lower oxygen supply, reduced water velocity), which will have cumulative effects.

Temperature and the Functional Development of the Circulatory System in Fish Larvae

Understanding the critical influences of temperature on larval ontogeny in relation to shifting ambient regimes requires an analysis of crucial functions defining thermal windows. Studies in adult fish demonstrated that circulatory performance rather than ventilation is crucial in the thermal limitation of fish (for review, see Pörtner et al. 2004). In light of shifting thermal windows during larval ontogeny of riverine fish, this leads to the question of how these crucial processes develop in the context of thermal adaptation.

Before now, however, studies in larval fish have investigated only the influence of temperature on growth (Fig. 5) and on aerobic metabolism. A comparison of Q_{10} data for larval oxygen consumption from about 20 species clearly demonstrated that the Q_{10} of oxygen consumption in fish larvae is quite variable, ranging from 1.49 in *Coregonus lavaretus* to 6.4 in *Pleuronectes platessa* (Rombough 1988). The overall average Q_{10} of about 3 is significantly higher than the much less variable mean Q_{10} of about 2, typically obtained for adult fish (Fry 1971). In addition, temperature sensitivity of larvae varies during development. The earliest stages of zebra fish, for example, are quite sensitive to temperature changes, and metabolism may even have a Q_{10} of 4–5. The Q_{10} then decreases to values between 2 and 3 and increases again in later stages (Barrionuevo and Burggren 1999). Similar results were obtained in the minnow *Phoxinus phoxinus*, although Q_{10} values were not quite as variable as the ones recorded for zebra fish larvae (Schönweger et al. 2000). By way of explanation, it has been proposed that early life stages are more stenothermal than adults (Rombough 1988). It has also been discussed that temperature-related changes in activity may contribute to these Q_{10} values (Fuiman and Batty 1997; Wieser and Kaufmann 1998). Enhanced growth or growth efficiency may play a role, and at least in some cases, metabolic activity appears to be somehow correlated to growth rates (Johns and Howell 1980; Rombough 1988).

The comparison with patterns of stenothermy in polar fish may help to elucidate some of these relationships. Here specialization on narrow thermal windows, that is, stenothermy, is mirrored in low SMRs and high Q_{10} values of baseline mitochondrial oxygen demand set by proton leakage. These patterns were interpreted to reflect high activation enthalpies and, thus, high kinetic barriers to metabolic flux at reduced maintenance costs (Pörtner et al. 2000, 2005). Reduced SMRs result, with the benefit of enhanced growth rates as a trade-off. Similar mechanistic relationships may also support high growth at enhanced degrees of stenothermy during early development (Fig. 5).

Only recently has the development of cardiac activity in fish larvae been studied with respect to the influence of temperature. In the minnow, larval heart rate increased with increasing temperature. The Q_{10} values revealed a strong temperature dependency in the low-temperature range, while at higher temperatures the Q_{10} was about 2 (Schönweger et al. 2000). For rainbow trout, Mirkovic and Rombough (1998) reported a Q_{10} value of 2.4 between 5° and 15°C, and values around 2 have also been reported for zebra fish larvae. These Q_{10} values were determined in larvae raised at different temperatures, that is, after chronic exposure. In adult fish, chronic temperature acclimation typically results in Q_{10} values around or even below 1.5, lower than values close to 2 found in response to acute temperature change (Graham and Farrell 1989; Farrell and Jones 1992). Again, larval Q_{10} values appear high and mirror the high Q_{10} of SMR, although their Q_{10} values for heart rate certainly are much lower than the values for SMR. These high

Q_{10} values may also indicate that, at this stage, the extrinsic mechanisms responsible for the temperature compensation in adult fish hearts are not yet functional.

Both heart rate and stroke volume determine cardiac output. For adult fish, it is well established that stroke volume can be modified in response to environmental challenges, much more so than, for example, in mammals, and several studies reported temperature-related changes in stroke volume. In winter flounder, for example, stroke volume increased with heart rate during temperature acclimation (Cech et al. 1976). This variability, however, is not yet seen in larval stages. In the tropical zebra fish as well as in the cold-water trout, stroke volume was almost constant irrespective of the experimental temperature, and the Q_{10} was close to 1.0 (Mirkovic and Rombough 1998; Jacob et al. 2002). In *P. phoxinus*, at the onset of cardiac contraction, stroke volume was almost the same between 12.5° and 25°C, and the Q_{10} was 0.8. At the time of swim bladder filling, stroke volume even decreased with increasing temperature, resulting in a Q_{10} of 0.6 between 15° and 25°C (Schönweger et al. 2000).

As expected from increasing heart rate and constant stroke volume, cardiac output increases with increasing temperatures in larval fish, and Figure 6 shows a typical data set of Q_{10} values adopted from a study with zebra fish (Jacob et al. 2002). For trout larvae, an overall Q_{10} value of 3.06 has been calculated for cardiac output (Mirkovic and Rombough 1998). In zebra fish, the respective Q_{10} was around 2, lower than in trout. In the minnow, the influence of temperature on cardiac output varied with development. At the onset of cardiac activity, cardiac output was very similar at all temperatures, and the Q_{10} was close to 1.2. At the time of swim bladder filling, the Q_{10} was close to a value of 2.2, which is in line with the data for zebra fish.

In summary, temperature sensitivity of oxygen uptake is higher and more variable in larvae than in adult fish. High thermal dependence as a potentially stenothermic character may be important in reducing energy expenditure in larval fish. Heart rate shows a clear temperature dependence, with a Q_{10} of about 2–2.5, while stroke volume is almost temperature independent. Due to the temperature effect on heart rate, cardiac output is also temperature dependent. The Q_{10} for cardiac output appears to be lower than the Q_{10} for oxygen consumption, which indicates that cardiac activity and metabolic activity are not yet closely coupled in early larval stages of fish (Pelster 2002). In light of oxygen- and capacity-limited thermal tolerance, these patterns also result in stenothermy because of an early oxygen deficit during warming (Mark et al. 2002). Overall, narrow windows of thermal tolerance result in the larvae, thereby explaining the patterns depicted in Figure 5.

Cellular, Molecular, and Gene Expression Aspects in Thermal Sensitivity and Adaptation

Changing temperature sensitivities during ontogeny of fish (Fig. 5) and different sensitivities between larvae and adults lead to
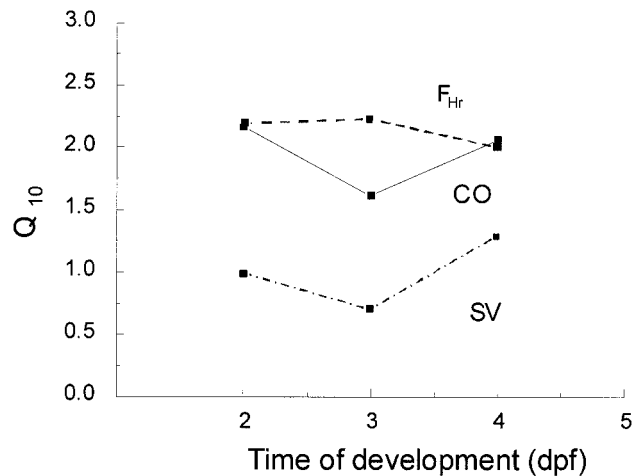

Figure 6. Q_{10} values for cardiac activity calculated for the zebra fish *Danio rerio* raised at 25° and 31°C (mean data from Jacob et al. 2002). While the Q_{10} values for heart rate (F_{Hr}) and cardiac output (*CO*) indicate significant temperature dependence, stroke volume (*SV*) appears to be hardly influenced by temperature. Note that Q_{10} values vary with development. *dpf* = days postfertilization.

the question about the mechanisms at the cellular level that contribute to thermal sensitivity or temperature compensation. Once again, very few data are available. Recent experiments with the break-dance mutant of zebra fish (identified in the Tübingen screen; Chen et al. 1996) suggest that ion channels may contribute to temperature sensitivity at the cellular level. The name "break-dance" refers to the observation that the ventricle of these mutants beats only once for every two atrial contractions. There is a similar arrhythmia in mammals, and this mutation is due to an alteration in the delayed rectifier potassium channel, coded by the so-called *erg* gene (ether-a-go-go-related gene). Langheinrich et al. (2003) identified a mutation in break-dance mutants of zebra fish at amino acid position 59 in this gene and suggested that this mutation is the cause of the break-dance mutation. This conclusion was supported by experiments using the morpholino approach. By injection of morpholinos, the expression of this gene was impaired in wild-type animals, and this resulted in the appearance of a 2 : 1 rhythm.

Heart rate analyses in these break-dance mutants at different temperatures revealed a profound change in the thermal sensitivity of the heart as compared with wild-type animals. While in the wild type, heart rate showed a Q_{10} of about 2, the ventricular rate of the mutants was almost independent of temperature and remained close to about 80 beats per minute between 25° and 31°C (S. Grillitsch, T. Schwerte, and B. Pelster, unpublished data). In conclusion, mutation of an ion channel in the heart may completely abolish temperature sensitivity over this range. Therefore, besides metabolic homeostasis, ion channels and thus ion homeostasis may significantly contribute to

temperature effects on cardiac activity and in consequence to whole-animal, that is, larval, thermal tolerance.

The consideration of ion channels in the function of fish hearts illustrates how investigations of mutants may help to elucidate the functional relevance of individual molecules in shaping the thermal response of a tissue and the whole animal. Adjustment in one individual molecular process does not, however, explain the full scope of thermal adjustments from cellular to tissue to whole-organism levels. Rather, the functional integration of numerous molecules, pathways, and compartments into the functional entity will be required. The concept of symmorphosis, as developed for the mammalian respiratory system (Taylor and Weibel 1981), states that animals maintain just enough structure to support oxygen flux rates at their maximum oxygen uptake rates. According to this concept, the design of all components of a system match functional demand in such a way that excess capacity of any single component is minimized (Hoppeler and Weibel 1998). This concept, albeit still hypothetical, would also provide a framework for understanding the integration of individual molecular, organellar, and cellular functional capacities into whole-organ and finally whole-animal capacity. In fact, this concept has already helped to qualify a systemic to molecular hierarchy of thermal-tolerance limits in animals, where the first line of thermal sensitivity becomes apparent at the highest functional level possible, which in animals was found to depend on the integrated function of ventilation and circulation (Pörtner 2002a).

Nonetheless, the complexity of cellular processes and the large number of proteins involved pose the question of whether we should expect the cell to exhibit thermal compensation in all processes, only in key processes, in strongly rate-limiting steps in those processes, or perhaps in some form of homeostatic response spanning many different individual processes (Podrabsky et al. 2000; Podrabsky and Somero 2004). Evidence for homeostasis is strong in membranes, where various aspects of lipid composition are adjusted in response to temperature change (Hazel 1995). There is also some evidence for homeostatic adjustment of the cellular milieu through pH, though here the picture is less complete (Pörtner and Sartoris 1999). The vast range of proteins produced by a typical cell means that relatively few have been examined in terms of evolutionary temperature compensation, and even fewer have been quantified with respect to the relevance of their contribution to the tissue or whole-organism response. Again, the concept of capacity-limited thermal tolerance helps to evaluate the relative importance of observed changes in setting the window of whole-organism functioning, for example, through changes in mitochondrial densities and capacities (Fig. 2; Pörtner 2004; Pörtner et al. 2005).

Among the few enzyme examples investigated, evidence suggests that key enzymes involved in intermediary metabolism

exhibit a strong degree of temperature compensation, with a classical study being that of Graves and Somero (1982) on muscle lactate dehydrogenase (LDH) in barracuda (Sphyraena). This study demonstrated maintenance of substrate affinity over a wide range of temperatures. Later efforts found that minimal or no changes in amino acid sequences were associated with these functional changes. Since differences in kinetic properties could be overcome through partial denaturation and renaturation, it was concluded that different conformations do in fact exist for the same enzyme protein (Ozernyuk et al. 1994; Fields and Somero 1997). These findings indicate that temperature-dependent protein folding also exerts control over functional properties.

An underestimated parameter shaping the contribution of an enzyme, pathway, or functional organelle (such as the mitochondrion) to metabolic control is the level of the activation enthalpy term (Gibb's free energy of activation). Activation enthalpy of some functions is sometimes found to be decreased during cold adaptation. This decrease has traditionally been interpreted to reflect cold compensation through the facilitation of flux in the cold (reviewed by Hochachka and Somero [1984]). However, this interpretation does not consider findings of elevated activation enthalpies for other functions in the cold, even lactate dehydrogenase in some cases (Zakhartsev et al. 2003b). A trade-off between the required concentration of enzymes and the required metabolic flux was suggested to explain the patterns observed (Pörtner et al. 2000). For example, high activation enthalpies of proton leakage rates were found in mitochondria from Antarctic ectotherms. These may contribute to keeping mitochondrial maintenance costs low despite the exceptional increase in mitochondrial densities typically observed in muscle tissues of these animals (for review, see Pörtner et al. 2000, 2005). As a further trade-off, this elevation in activation enthalpy causes not only reduction in maintenance costs but also an increase in thermal sensitivity (or Q_{10}) of these costs. This may occur at the expense of narrow windows of thermal tolerance, that is, stenothermy, in Antarctic ectotherms. Such patterns may also explain the level of stenothermy in early developmental stages of fish (Fig. 5). As a trade-off, high degrees of stenothermy may serve to reduce energy costs and thereby support energy-efficient growth.

A largely unexplored field of research is the role of and limit to gene expression capacity of individual metabolic functions or pathway complexes in thermal adaptation. As mentioned above, those mechanisms that adjust aerobic scope appear most promising for study. Figure 7 illustrates how the level of gene expression differs between eelpout species in a latitudinal cline. Cold-adapted Antarctic eelpout (Pachycara brachycephalum) express similarly low levels of cytochrome c oxidase (COX) activity at similar levels of COX I mRNA as warm-acclimated temperate eelpout (Zoarces viviparus) at 18°C. This finding is in line with energy savings and narrower thermal windows in the Antarctic species. The significantly higher level of COX
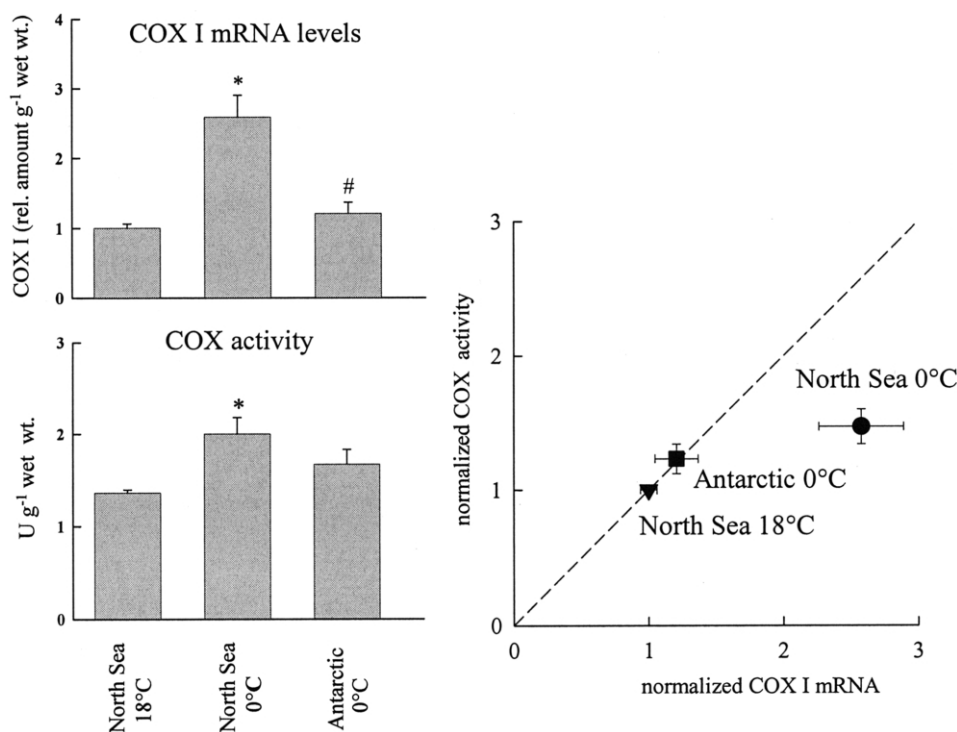
Figure 7. Differential gene expression of white muscle cytochrome c oxidase (COX) during cold acclimation of temperate zone eelpout *Zoarces viviparus* versus evolutionary cold adaptation of Antarctic eelpout *Pachycara brachycephalum*, quantified as the levels of COX activity in relation to the levels of COX I mRNA. Enzyme activities were compared at 9°C. Compared to the rise in COX activity seen in temperate eelpout at 0°C, cold acclimation leads to an overproportional rise in COX I mRNA contents over the levels found in Antarctic eelpout (redrawn from Hardewig et al. 1999).

activity reached in cold-acclimated temperate eelpout at disproportionally elevated RNA levels indicates compensation of aerobic capacity during eurythermal cold acclimation.

A later study compared gene expression capacities for aerobic enzymes (citric synthase [CS] and COX) in white muscle of two populations of cod *Gadus morhua*, from Barents Sea (North Eastern Arctic cod [NEAC]) and North Sea (NSC). Starting from levels at 10°C, which were only slightly higher in NEAC than in NSC, both CS and COX activities as well as the levels of CS and COX mRNA rose to significantly higher values in NEAC than in NSC when acclimated from 10° to 4°C. Evidently, the subarctic population of NEAC is more capable of cold acclimation than the population of NSC. In the case of CS, the increments in enzyme activity were accompanied by elevated transcript levels and indicated transcriptional control, whereas such a strong correlation was again absent for COX activities and mitochondrial-encoded COX II message (M. Lucassen, N. Koschnick, and H. O. Pörtner, unpublished data).

Overall, the mechanisms of and limits to temperature-dependent gene expression are largely unexplored. Nonetheless, these findings reflect largely different phenotypic plasticities in thermal acclimation between two populations of the same species in a latitudinal cline. These relate to permanent functional

differences between cod populations along eastern Atlantic latitudes, mirrored in permanently elevated metabolic rates and reduced growth performance of cod populations at high latitude (Pörtner et al. 2001).

Life-History Variation along a Latitudinal Cline: Trade-Offs in Energy Budgets in Air and Water

The phenotypic plasticity observed in larval fish depending on ambient temperature could lead to differential performance characteristics for the same species in river systems across a wide latitudinal cline. However, as outlined for cod populations in a latitudinal cline (Pörtner et al. 2001), this trend may even lead to functional differentiation and permanent functional differences between populations associated with genetic differentiation and different ranges of phenotypic plasticity or flexibility (Blanckenhorn 1997). Differences in physiological plasticity between populations may also result in intraspecific variation in phenotypic life-history traits (Piersma and Drent 2003; Pigliucci and Preston 2004), which in most animals, both endotherms and ectotherms, are pervasive in clinal latitudinal comparisons (Negovetic and Jokela 2001). These observed traits are called phenotypic because it is unclear whether the trait has

a genetic basis or is environmentally induced or both. Physiological variation within the life history of an individual can have profound implications on fitness (Ricklefs and Wikelski 2002).

Among ectotherms, active species have greater maintenance energy requirements than less active or sessile species, which may affect energy allocation to maintenance, growth, digestion, locomotion, and reproduction. Furthermore, energy expenditure related to maintenance and production changes in time and space in response to environmental energy availability (Sibly and Calow 1987). The energetic costs of maintenance are the major determinants of the quantity of energy available for activity and reproduction (Angilletta 2001). Thus, analyses of the role of energetics in life-history variation depending on temperature are feasible through comparisons of species populations at various temperatures in a latitudinal cline. Implicit in the covariation between reproductive output and maintenance cost is the assumption that between-population variations in metabolic traits support a higher fitness in individuals among populations. Evidence for these covariations currently is sparse (Spicer and Gaston 1999; Pörtner et al. 2001; Ricklefs and Wikelski 2002; Wikelski et al. 2003). Nevertheless, some studies have examined between-population variation in physiological and life-history (fitness) features. Ayres and Scriber (1994) found that populations of tiger swallowtail from Alaska had an estimated fitness three times greater than individuals from Michigan populations. In addition, Angilletta (2001) demonstrated that a higher SMR in a northern population of the geographically widespread lizard *Sceloporus undulates* contributed to greater reproductive effort in South Carolina than lizards from New Jersey. These observations resemble similar findings in cod (*Gadus morhua*) populations in a latitudinal cline (Pörtner et al. 2001).

Constraints on adaptive responses are established by the allocation of limited time, energy, and nutrients among competing functions (van Noordwijk and de Jong 1986). Variables such as seasonality, food, and temperature, among others, have been suggested to explain latitudinal clines in life-history traits (Fox and Czesak 2000; Ricklefs and Wikelski 2002). However, it also seems logical that latitudinal variations in life-history traits may be related to differences in metabolic rates among individuals from different populations. Metabolic costs play a major role in the network of physiological mechanisms underlying life-history traits and may mediate major life-history trade-offs (Wikelski et al. 2003). The bioenergetic consequences of thermal adaptation, as they become visible on macroscales, affect baseline and overall metabolic costs and also imply modifications in energy budgets, with consequences for growth, reproductive output, and the capacity for motor performance as well as lifestyle strategies (Pörtner et al. 2001; Pörtner 2002b). Therefore, we tested the hypothesis that reproductive output (i.e., total dry mass of eggs) is linked and negatively correlated with metabolic rate along an intraspecific latitudinal gradient

in populations of the terrestrial woodlouse *Porcellio laevis* from a geographical gradient in southern South America. *Porcellio laevis* is widely distributed in Chile, as well as throughout several other biogeographic areas around the world (Leistikow and Wägele 1999). In this study (M. A. Lardies and F. Bozinovic, unpublished data), woodlice were collected during 2002 at five localities in northern-central Chile along a latitudinal gradient. Life-history (i.e., reproductive output) and physiological (i.e., metabolic rate at 18°C) traits were recorded for all populations (see also Castañeda et al. 2004).

Reproductive output increased gradually from low- to high-latitude populations. Metabolic rate was significantly correlated with body mass at ambient temperature (18°C). At the same time, temperature-specific oxygen consumption ($\dot{V}o_2$) of a standard-size animal increased progressively from high- to low-latitude populations. The comparison among populations revealed a significant and negative phenotypic correlation between rising reproductive output and falling $\dot{V}o_2$ toward high latitudes. The greater reproductive output of higher-latitude woodlice may be caused by a lower rate of energy expenditure. In this sense, high energy expenditure in maintenance metabolism is likely the principal cause of reduced reproductive output in *P. laevis* at low latitudes (Fig. 8).

According to Figure 8, low maintenance costs are elicited by low environmental temperature along a latitudinal gradient, and they support high reproductive output. Implicit in the link between reproductive output and maintenance cost is the assumption that among-population variation in physiological traits serves to enhance the fitness of individuals among pop-
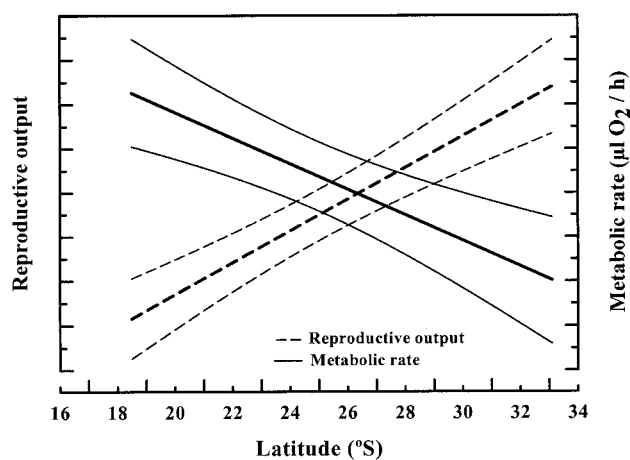

Figure 8. Regression lines and 95% confidence interval of reproductive output (*solid lines*; based on total dry weight of egg mass) and metabolic rate (*dashed lines*; for a mean-size animal of 100 mg at 18°C) in populations of woodlice (*Porcellio laevis*) along a latitudinal gradient in Chile. Populations were collected at Arica (18°30′S), Iquique (20°10′S), Antofagasta (23°38′S), La Serena (29°55′S), and Santiago (33°23′S). The correlation coefficient between traits is $r_p = -0.51$ (based on data from Lardies 2004).

ulations. Evidence for this link is currently sparse since few studies directly examine among-population variations in physiological traits and life-history (i.e., fitness) traits (Ricklefs and Wikelski 2002). This relationship suggests that individuals at high temperatures may allocate more energy toward rapid growth at high metabolic rates and invest less energy in reproduction. Inversely, the slow growth and low metabolism of species inhabiting low-temperature habitats allow for more energy investment in egg production since eggs contain larger internal reserves. In other words, individuals at low latitudes increase maintenance costs and thereby reduce investment in reproduction but still grow faster than those at high latitudes.

These observations are in line with recent conclusions from a study of scallop metabolic rates and growth performance across latitudes and temperature regimes (Fig. 9). In this case, growth performance was found to rise toward warm temperatures but displayed lower Q_{10} values compared with the temperature dependence of metabolic rate. These findings indicate reduced metabolic efficiencies in warmer regimes, similar to the metabolic background of reduced reproductive output in low-latitude woodlice. Conversely, the data in Figure 9 indicate enhanced growth efficiency in the cold; that is, a larger fraction of available energy is allocated to growth, despite falling metabolic rates. This relationship is similar to the enhanced efficiency for reproductive output seen in high-latitude woodlice. The trade-off between energy allocation to growth or to reproductive output is unclear and awaits further study. Enhanced energy allocation to reproductive output or to growth in the cold requires energy savings in maintenance metabolism, with respective consequences at all levels of biological organization, from cost reductions at levels of protein synthesis and transmembrane ion exchange to those in oxygen transport, muscular activity, and lifestyle. These interactions would have consequences for the level of thermal tolerance and the width of the thermal-tolerance window (for review, see Pörtner et al. 2005). In summary, physiological differences may have important implications for fitness, and the existence of physiological variation may serve to reduce fitness variation between populations. The role of physiology in generating geographic variation in life histories is less understood than the role of the environment, perhaps because data on the extent of physiological divergence between populations are rarely available.

Where to Go?

The examples selected for this paper illustrate the need to develop an integrative view of molecular to ecological phenomena for a comprehensive and quantitative understanding of the driving forces and the trade-offs and constraints in thermal adaptation. An overarching concept encompassing information from all levels of functioning is required to develop an integrative bigger picture. Finding and verification of such concepts involves further testing of their applicability as illustrated for
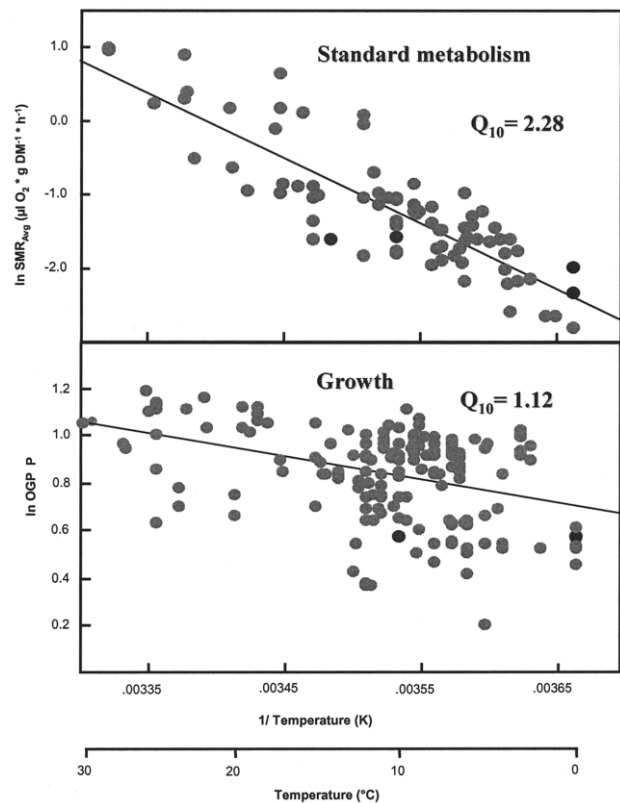

Figure 9. *Top*, metabolic rates of scallops from different latitudes fitted into an Arrhenius plot (fitted least squares regression: $\ln(\mathrm{SMR_{Avg}}) = 30.116 - 8{,}874.24 \times 1/T$ ($r^2 = 0.725$). Data are resting or standard rates for species at their normal ambient temperatures. *Bottom*, fitted Arrhenius model of the relationship between overall growth performance (OGP P) and temperature: $\ln(\mathrm{OGP\ P}) = 4.22 - 958.466 \times 1/T$ ($r^2 = 0.132$) (redrawn from Heilmayer et al. 2004).

the oxygen- and capacity-limitation hypothesis and associated energetic consequences, which contributes to explain numerous trade-offs in the thermal adaptation of animals.

For example, if we consider temperature compensation to reflect the compensation of physiological rate and scope, then the question arises whether this is true and relevant for all processes. There are many physiological processes in which evolutionary temperature compensation appears to be imperfect. An important example is the generation of ATP by mitochondria, which is strongly related to temperature in fish and other ectotherms (again with the exception of cold-adapted eurytherms). At a higher organizational level, there may also be a strong temperature constraint in the rate at which fish can digest and absorb a meal, and this also applies to terrestrial ectotherms. Answers may again arise from investigations in the context of a valid overarching concept. In the context of oxygen- and capacity-limited thermal tolerance, mitochondrial ATP formation capacity relates to the level of aerobic scope and associated temperature-dependent lifestyles. Limitations in

digestive processes may again involve limited aerobic scope due to trade-offs in the allocation of oxygen and metabolic energy to such processes. Even within the scope of the oxygen- and capacity-limitation concept, there are many important physiological and cellular processes for which we have very little data on evolutionary temperature compensation, for example, with respect to gene expression, message processing, and protein synthesis. Addressing such processes will be required for a deeper understanding of thermal adaptation and also for testing the overall relevance of the conceptual framework.

In the future, further comparisons of air and water breathers are required. These comparisons will allow testing for the general validity of patterns and underlying mechanisms in both terrestrial and aquatic realms. One important difference between the two environments is that, unlike the terrestrial taxa, water breathers live in a habitat with a large heat capacity and hence are generally exposed to relatively slow rates of temperature change. This means that it is often easier to see the evolutionary signal of adaptation against the background of short-term adjustments to environmental noise. It is also possible and needs to be clearly elaborated whether the different thermal characteristics of terrestrial and aquatic environments have selected for different physiologies (Pörtner 2004). Also, oxygen availability is about 30 times higher in air than in water. The influence of this difference as well as the role of different ventilatory systems used in air and water breathers requires investigation with respect to the suggested role of oxygen- and capacity-limitation in thermal tolerance. Finally, further overarching concepts of thermal adaptation and limitation may exist and, once identified, would require similar integrative efforts from molecular to ecosystem levels, as elaborated here.

Acknowledgments

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