Mitochondrial Function in Seasonal Acclimatization versus Latitudinal Adaptation to Cold in the Lugworm *Arenicola marina* (L.)

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ABSTRACT

Previous studies in marine ectotherms from a latitudinal cline have led to the hypothesis that eurythermal adaptation to low mean annual temperatures is energetically costly. To obtain more information on the trade-offs and with that the constraints of thermal adaptation, mitochondrial functions were studied in subpolar lugworms (*Arenicola marina* L.) adapted to summer cold at the White Sea and were compared with those in boreal specimens from the North Sea, either acclimatized to summer temperatures or to winter cold. During summer, a comparison of mitochondria from subpolar and boreal worms revealed higher succinate oxidation rates and reduced Arrhenius activation energies (E_a) in state 3 respiration at low temperatures, as well as higher proton leakage rates in subpolar lugworms. These differences reflect a higher aerobic capacity in subpolar worms, which is required to maintain motor activity at low but variable environmental temperatures—however, at the expense of an elevated metabolic rate. The lower activity of citrate synthase (CS) found in subpolar worms may indicate a shift in metabolic control within mitochondria. In contrast, acclimatization of boreal lugworms to winter conditions elicited elevated mitochondrial CS activities in parallel with enhanced mitochondrial respiration rates. With falling acclimation temperatures, the significant Arrhenius break temperature in state 3 respiration (11°C) became insignificant (5°C) or even disappeared (0°C) at lower levels of Arrhenius activation energies in the cold, similar to a phenomenon known from hibernating vertebrates. The efficiency of aerobic energy production in winter mitochondria rose as proton leakage in relation to state 3 decreased with cold acclimation, indicated by higher respiratory control ratio values and increased adenosine diphosphate/oxygen (ADP/O) ratios. These transitions indicate reduced metabolic flexibility, possibly paralleled by a loss in aerobic scope and metabolic depression during winter cold. Accordingly, these patterns contrast those found in summer-active, cold-adapted eurytherms at high latitudes.

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Introduction

Changes in habitat temperature have marked effects on biochemical and physiological processes in marine ectotherms, making adaptation critical for the maintenance of physiological functions. Previous years have shown that oxygen limitation characterizes the limits of thermal tolerance (see Pörtner 2001, 2002*a* for review). Critical temperature thresholds (T_c) have been defined for various marine invertebrate and fish species as being characterized by the transition to an anaerobic mode of metabolism, once temperatures reach low or high extremes. At these T_c's, aerobic scope is lost, and energy demand cannot be met by adequate oxygen supply. Within the envelope of T_c's, a narrower temperature window is set by limits to maximum aerobic scope (pejus temperatures, T_p; Frederich and Pörtner 2000; Mark et al. 2002). Both T_p and T_c values are determined by the capacity of ventilation and circulation for adequate oxygen supply.

In general, a rise in aerobic capacity linked to an increase in mitochondrial densities is a major feature of cold adaptation and acclimation in ectotherms. These changes include those tissues responsible for ventilation and circulation (see Pörtner et al. 1998; Pörtner 2002*a*, 2002*b* for review). Associated processes are a rise in cristae surface density of mitochondria (St-Pierre et al. 1998), alterations in membrane composition leading to an increased proportion of phosphatidylethanolamine and elevated unsaturation indices (Miranda and Hazel 1996; Logue et al. 2000), rising enzyme capacities (Crockett and Sidell 1990; Guderley 1990), or rising substrate affinities (Guderley and St-Pierre 1999). The adjustment of both mitochondrial density and function is involved in defining and adjusting the

thermal tolerance window to the environmental temperature regime, as shown for boreal and subpolar lugworms *Arenicola marina* (Sommer and Pörtner 2002).

According to a recent model, the cost of improved cold tolerance, especially in eurytherms, is partly linked to rising mitochondrial maintenance costs reflected by rising proton leakage rates as a trade-off (e.g., Pörtner et al. 1998). The original and long-debated concept of metabolic cold adaptation implies that metabolic rates of organisms living under different temperature regimes are maintained regardless of acclimatization temperature, leading to elevated temperature-specific metabolic rates in cold-adapted species (Hochachka and Somero 2002). Although more recent evidence suggests that this pattern is not evident in stenothermal Antarctic species (Clarke and Johnston 1999; Peck and Conway 2000), such a phenomenon can be seen in various species, especially sub-Arctic and Arctic marine eurytherms (van Dijk et al. 1999; Sommer and Pörtner 2002; T. Fischer, R. Knust, and H. O. Pörtner, unpublished data). The hypothesis arose that the level of metabolic cold adaptation depends on the extent of diurnal and seasonal temperature fluctuations, leading to higher costs of mitochondrial maintenance in eurythermal than in stenothermal animals in the cold (Pörtner et al. 2000; Pörtner 2002*a*).

With this hypothesis in mind, the question arose whether both seasonal and latitudinal cold adjustments are based on the same mechanisms or whether the trade-offs between achieving cold tolerance and the associated costs of living in the cold imply different trends in cold acclimatization (seasonal) versus cold adaptation (latitudinal). Full specialization on permanently cold temperatures may support energy savings. In contrast, considerable temperature fluctuations may still occur in subpolar areas, for example, in the sub-Arctic in summer. These animals maintain high activity in the cold but also at warmer summer temperatures, which is associated with enhanced cost of living (Pörtner et al. 2000). In a continuous cline between high subpolar and lower boreal latitudes, seasonally cold-acclimatized specimens may only adjust their metabolic machinery to withstand cold temperatures during winter periods of decreasing lengths. During winter, some processes like growth, reproduction, or even motor activity are suspended. Moreover, during winter cold in aquatic environments, the amplitude of thermal changes is smaller than during summer. During summer in subpolar areas, maintaining high levels of motor activity at lower mean annual temperatures may therefore contrast a strategy to passively tolerate winter temperatures in boreal to subpolar areas.

This study was designed as a first attempt to compare the functional capacity of mitochondria obtained from animals acclimatized to summer conditions at subpolar and boreal latitudes with those from animals acclimatized to winter cold. The study was carried out in the eurythermal lugworm *A. marina* (Polychaeta), which inhabits sandy sediments of inter- or subtidal zones between subtropical and polar regions. The lugworm is therefore a suitable model to study mechanisms of thermal tolerance (Sommer et al. 1997; Sommer and Pörtner 1999). Mitochondria were prepared from the muscle tissue of lugworms acclimatized to winter or summer conditions in the boreal North Sea and from lugworms acclimatized to the subpolar summer at the White Sea. We examined mitochondrial rates of succinate oxidation and proton leakage, their coupling ratios, and adenosine diphosphate/oxygen (ADP/O) values as well as the activity of the mitochondrial marker enzyme citrate synthase. Summer lugworms from the North and White Seas were acclimated to the same temperatures (11°C), and winter worms from the North Sea were acclimated to 5° and 0°C. Our data show that eurythermal White Sea lugworms displayed increased aerobic capacity of mitochondria at higher mitochondrial densities. In contrast, it seems that the efficiency of aerobic energy production in winter lugworms was enhanced, possibly at the expense of low metabolic flexibility during metabolic depression in winter cold.

Material and Methods

Animals and Populations

Specimens of *Arenicola marina* were collected from 1999 to 2001 from intertidal flats, near the low-water line, of the North Sea near Dorum, Germany (53°42′N, 8°35′E; hereafter called boreal or North Sea lugworms), and of the White Sea near Kartesh, Russia (66°20.8′N, 33°35.8′E; hereafter called subpolar or White Sea lugworms). The White Sea population of *A. marina* is clearly genetically distinct from the North Sea population, linked to geographical distance and physiological differences (Hummel et al. 1997). The mean annual surface water temperature in the North Sea is 10°C. Here, the boreal North Sea lugworms are rarely exposed to an ice cover in January or February, whereas in summer the burrows can reach 25°C. Depending on temperature and season, the maximum depths of the burrows range between 15 cm in summer and 70 cm in winter. At the White Sea, a thick layer of clay and rocks prevents the subpolar worms to live in burrows deeper than 10–15 cm. The average water temperature at the White Sea near Kartesh is 4°C, and the White Sea is covered with ice for about 6 mo each year (Howland et al. 1999). The temperature in the sediment can drop to −5°C (Kolyakina 1980), while during the short summer period the sediment temperature can reach 18°C (Howland et al. 1999).

Both summer populations were sampled in late summer (postspawning) when surface water temperatures were similar in both areas, despite the lower mean annual temperatures in the subpolar environment. This should allow identification of permanent functional differences between populations. North Sea winter worms were collected when temperatures reached their minimum. In January 2000, sediment temperatures were below freezing, and in February 2001 the sediment cooled down to 4°C only. The lugworms were kept in the laboratory in

aquaria filled with well-aerated natural brackish seawater (22‰ salinity, equivalent to natural conditions) and a 5–10-cm bottom layer of natural sediment from the site of collection for about 4 wk before the experiments. Temperatures were controlled at 0°, 5°, or 11°C, close to habitat temperatures prevailing during the time of collection, such that acclimation and acclimatization temperatures were virtually identical. Both terms are used throughout the text, and the data are relevant to understand seasonal cold acclimatization versus latitudinal cold adaptation.

Mitochondrial Respiration

Coupled mitochondria from the body-wall tissue of *A. marina* were isolated according to Sommer and Pörtner (2002). Mitochondrial oxygen consumption was assayed using a Clark-type oxygen electrode in a temperature-controlled respiration chamber at temperatures between −1 and +32°C (Eschweiler, Kiel). 200 μL of the mitochondrial suspension and 20 μL of a 50% (w/v) BSA solution (bovine serum albumin, Fraction V, essentially fatty acid free) were added to 780 μL of assay medium (550 mmol L^{-1} glycine, 250 mmol L^{-1} sucrose, 20 mmol L^{-1} Tris-HCl [$pH_{20°C}$ 7.5], 4 mmol L^{-1} EDTA, 5 mmol L^{-1} K_2HPO_4, 3 mmol L^{-1} $MgCl_2 \cdot 6H_2O$, 1 μg mL^{-1} aprotinin) containing 5 μmol L^{-1} Ap5A, an inhibitor of myokinase. For maximum activity, up to 10 mmol L^{-1} succinate was added as a common mitochondrial substrate in lugworms in vivo, especially after environmental hypoxia during low tide. After monitoring state 2 respiration rate, ADP (125 μmol L^{-1}) was added with a Hamilton syringe to initiate state 3 respiration (maximum oxidative capacity during ADP phosphorylation). When all ADP had been phosphorylated, the rate of state 4 respiration was determined for about 5 min before oligomycin, an inhibitor of mitochondrial F_0F_1-ATPase, was added at 2 μg mL^{-1} (state 4ol respiration reflecting proton leakage capacity). Respiratory control ratios (RCR; Estabrook 1967) were determined by dividing state 3 by state 4 respiration rates before or after the addition of oligomycin (RCRol; Pörtner et al. 1999). Effective ADP/O ratios were calculated as nanomoles ADP added divided by nanoatoms oxygen utilized during state 3 respiration (Estabrook 1967). Preliminary examinations revealed that the addition of 5 μM rotenone had minor influences on respiration rates or the ADP/O ratio. We therefore refrained from further adding this inhibitor of complex 1of the respiratory chain. The use of succinate was supported by preliminary analyses in White Sea animals, which revealed poor coupling or no respiration at all with pyruvate, glutamate, malate, acetyl-CoA, or selected combinations of either two of these substances.

Oxygen solubility in the assay medium at different temperatures was adopted from Johnston et al. (1994), taking changes in atmospheric pressure into consideration. Protein concentrations were determined by the Biuret method (Kresze 1988) using BSA as a standard.

Enzyme Activities

A method modified from Sidell et al. (1987) was used to analyze the activity of citrate synthase (CS; EC 4.1.3.7.). Approximately 200 mg of body-wall tissue was minced and homogenized in 1.5 mL ice-cold extraction buffer (75 mmol L^{-1} Tris-HCl [$pH_{20°C}$ 7.6], 1 mmol L^{-1} EDTA) using a glass pestle and tube before the tissue was finally disrupted by sonication for 5 min. The homogenate was centrifuged for 5 min at 12,000 *g*. The assay was performed with the supernatant by following changes in absorption at $\lambda = 410$ nm (Pharmacia Ultraspec 3000, Pharmacia Biotech, Freiburg). The reaction mixture consisted of 75 mmol L^{-1} Tris-HCl ($pH_{20°C}$ 8.0) with 0.25 mmol L^{-1} DTNB (5,5′-Dithio-bis-[2-nitrobenzoic acid]) and 0.6 mmol L^{-1} acetyl-CoA. The addition of 1 mmol L^{-1} oxaloacetate initiated the reaction.

Statistics

All data were checked for outliers beyond the r(99) limits of an *r*-distribution, $r_A > r(99)$, using Nalimov's test (Noack 1980). Mitochondrial respiration rates and rates of enzyme activities were plotted as a function of assay temperature. ANOVA or ANCOVA and the post hoc Student-Newman-Keuls test were used to assess the effects of assay and acclimation temperatures as well as the population studied. Arrhenius break temperatures (T_{AB}) were determined by comparing sequential linear regressions and selecting two intersecting lines when the sum of squares was minimal and regressions were significantly different. The T_{AB} was defined by midpoint approximation (Yeager and Ultsch 1989). For linear regressions with a break point, statistical significance of differences between populations and seasons was analyzed with an *F*-test according to Nickerson et al. (1989). Activation energy (E_a) for each mitochondrial or enzyme preparation was calculated according to equation (1), where k_1 and k_2 are the specific reaction rates at the lower or higher temperature (T_1, T_2) and R is the general gas constant (Segal 1976):

$$E_a = RT_1T_2 \ln (k_2/k_1)(T_2 - T_1)^{-1}. \quad (1)$$

Paired Student's *t*-test was used to compare E_a above and below T_{AB} of the same mitochondrial preparation, and an unpaired *t*-test was applied to compare E_a between populations and acclimation temperatures. Statistical significance of differences was tested at the $P \leq 0.05$ level. Data are given as means ± standard error of the mean.

Results

General Mitochondrial Characteristics

In general, body-wall mitochondria of *Arenicola marina* were highly coupled at low temperatures, but RCR and RCRol values

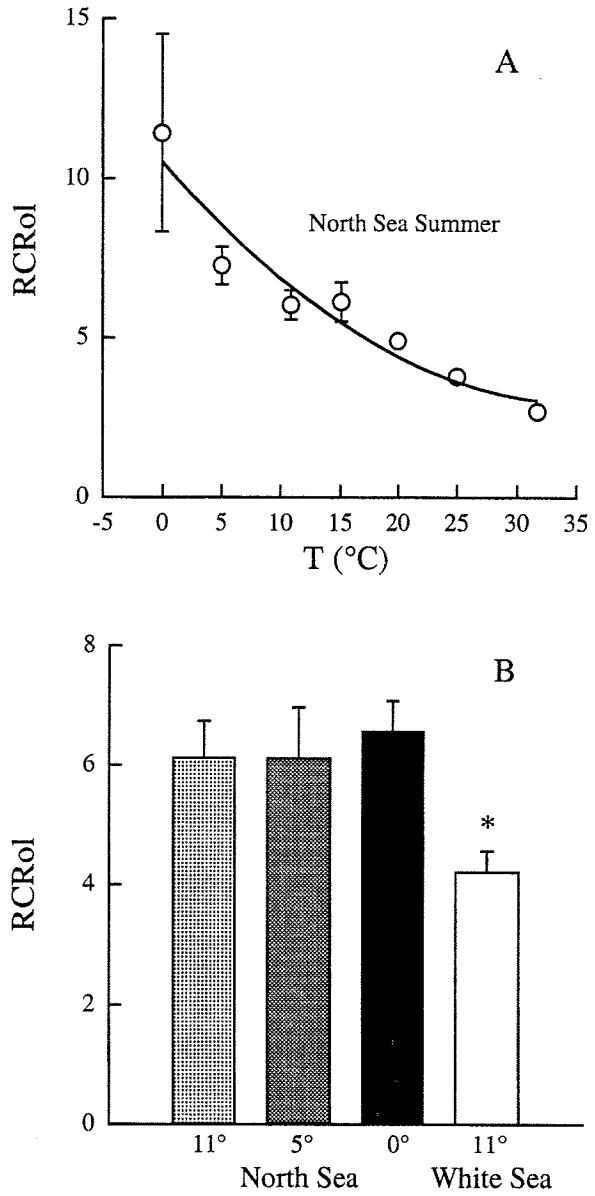


Figure 1. *A*, Mitochondrial respiratory control ratios determined from state 3 and state 4 respiration rates after the addition of oligomycin (RCRol), exemplified in North Sea summer lugworms as a function of temperature. The decrease with rising assay temperature is significant. Similar patterns were seen for White Sea summer and North Sea winter lugworms. *B*, RCRol values differed significantly between populations but not between acclimation temperatures in North Sea worms. Values are exemplified for the assay temperature of 15°C (mean ± SE; n = 7–8; *asterisk* = significantly different from North Sea lugworms).

decreased with rising assay temperatures (see Fig. 1*A* for RCRol of North Sea lugworms). In mitochondria prepared from subpolar lugworms, RCRol values were significantly lower than the values seen in mitochondria from North Sea individuals acclimated to the same temperature (Fig. 1*B*). RCR values were significantly lower than RCRol values and were significantly lower in 11°C-acclimated lugworms compared with 0°C-acclimated worms.

Determination of classical ADP/O ratios yielded numbers between 1.4 and 2.3 (Fig. 2*A*). In summer lugworms from the North Sea, values were significantly higher at −1°C compared with values obtained at higher temperatures. This temperature dependence was not found in lugworms from the White Sea, where ADP/O values remained below 1.9 at all assay temperatures. However, differences between both populations in summer were not significant. Winter North Sea worms at 0°C displayed significantly higher ADP/O ratios than animals acclimated to 5°C (winter) or 11°C (summer; Fig. 2*B*).

Thermal Sensitivity of Mitochondrial Respiration

Maximum oxidative capacity during ADP phosphorylation (nanomoles oxygen per minute per milligram mitochondrial protein) in the presence of succinate did not differ between boreal lugworms acclimated to 11° or 5°C. Winter acclimatization to 0°C, however, resulted in a significant rise in mitochondrial oxygen consumption rates (Fig. 3*A*), even beyond the oxidative capacity in White Sea worms, which was found to be significantly higher than in boreal individuals acclimated to the same temperature of 11°C (Fig. 3*A*). Arrhenius plots of respiration rates showed significant discontinuities between 5° and 11°C in summer *A. marina* from the North and the White Seas (Fig. 3*B*). In winter worms acclimated to 5°C, a discontinuity between 5° and 11°C was still apparent but no longer significant (Fig. 3*C*). The trend to develop an Arrhenius break temperature was even less in mitochondria from winter animals acclimated to 0°C. The rise in mitochondrial capacities at 0°C (strong winter) was associated with Arrhenius activation energies elevated significantly above those found in summer mitochondria from the North Sea (when compared at acclimation temperatures; Fig. 4*A*), with an intermediate value found after acclimation to 5°C (mild winter). This may indicate that mitochondria long-term adjusted to 5°C are functionally at an intermediate stage between 11° and 0°C. E_a at acclimation temperatures and above tended to be only slightly higher in summer worms from the White Sea than from the North Sea, whereas analysis at low temperatures revealed a significantly lower E_a of mitochondrial state 3 respiration in mitochondria of subpolar lugworms compared with mitochondria from boreal lugworms (Fig. 4*A*).

Proton Leakiness

Assay as well as seasonal acclimation temperatures significantly influenced residual mitochondrial oxygen consumption under oligomycin (state 4ol respiration indicating proton leakage capacity; Fig. 5*A*). State 4 or state 4ol respiration rates did not differ between cold-adapted White Sea worms and cold-

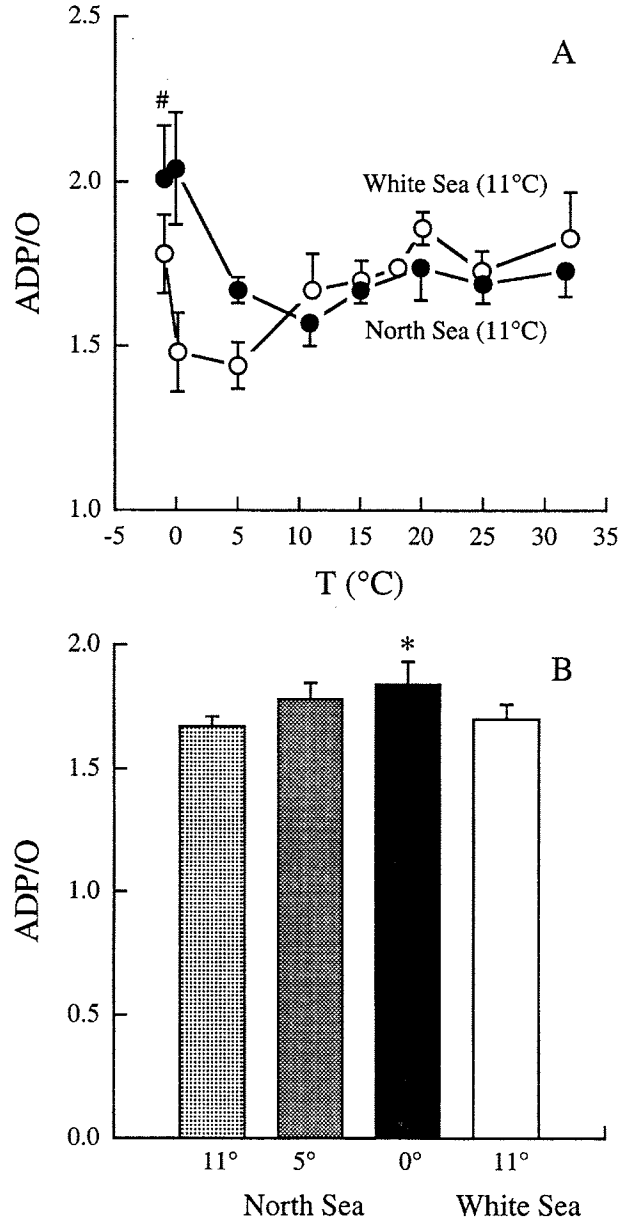


Figure 2. *A*, ADP/O ratios of succinate-oxidizing mitochondria isolated from body-wall tissue of North and White Sea lugworms acclimated to 11°C and assayed at various temperatures. *B*, Mitochondrial ADP/O ratio in North and White Sea lugworms acclimated to 0°, 5°, or 11°C. Values are depicted for the assay temperature of 15°C (mean ± SE; n = 7–8; *pound sign* = significantly higher than the values at assay temperatures above 5°C; *asterisk* = significantly higher than in worms acclimated to 11°C).

acclimatized (0°C) winter lugworms from the North Sea. However, leakage rates found in boreal worms acclimated to 5° (winter) or 11°C (summer) were significantly lower. Arrhenius plots of proton leakage rates could not reveal any significant discontinuity for North or White Sea mitochondria (Fig. 5*B*, 5*C*), although slopes appeared very high in North Sea mitochondria acclimated to 11°C when analyzed between −2° and 1°C. A change in the characteristics of these mitochondria in the cold is also suggested by the finding of high ADP/O values at low temperatures in North Sea mitochondria acclimated to 11°C. Although E_a of mitochondrial proton leakage in White Sea mitochondria at 11°C appeared slightly lower than in North Sea mitochondria at the same temperature (Fig. 4*B*), values did not differ significantly between populations or with seasonal acclimation temperature, with the exception of a significant drop observed in boreal lugworms acclimated to 5°C (Fig. 4*B*).

The different thermal sensitivities of state 3 respiration and proton leakage capacity (state 4ol) led to a progressive uncoupling of mitochondria at high temperatures, seen not only in decreasing RCRol values (Fig. 1) but also in a rising percentage of oxygen needed to compensate for proton leakage during state 3 respiration. (Owing to its dependence on proton motive force, the actual percentage fraction of proton leakage in phosphorylating state 3 mitochondria is likely to be lower since proton motive force in state 3 is somewhat below the one under state 4 conditions.) The higher proton leakage rate in *A. marina* adapted or acclimated to cold went hand in hand with elevated state 3 respiration rates, such that the picture of a higher rate of phosphorylating respiration (elevated state 3–4ol values) remained unchanged (Fig. 6).

Citrate Synthase

As seen in Figure 7, the activity of citrate synthase (CS) increased with assay temperatures between −1° and 29°C, reflecting similar Q_{10} values, regardless of population origin or acclimation temperature (Table 1). Above 29°C, the rise in activity was either reduced (in the case of lugworms from the North Sea) or activity was lost to a value below the one at 29°C, as seen in White Sea worms. The highest activities were seen in winter lugworms (0°C) from the North Sea, whereas latitudinal cold adaptation (summer) was accompanied by a reduction in CS activity, especially when compared at habitat temperatures. Differences between seasons and populations were significant. No discontinuities were seen in Arrhenius plots between −1° and 29°C (not shown). E_a values were 32.02 ± 1.91 (White Sea), 35.84 ± 2.77 (North Sea, 11°C), or 37.53 ± 1.63 (North Sea, 0°C) kJ mol^{-1} and were not significantly different between populations and seasonal temperatures (for Q_{10} values, see Table 1).

Discussion

We have previously argued that the adjustment of mitochondrial density and function in boreal and subpolar lugworms is involved in defining and adjusting the thermal tolerance window to the environmental temperature regime (Sommer and Pörtner 2002). This study shows that not only latitudinal cold

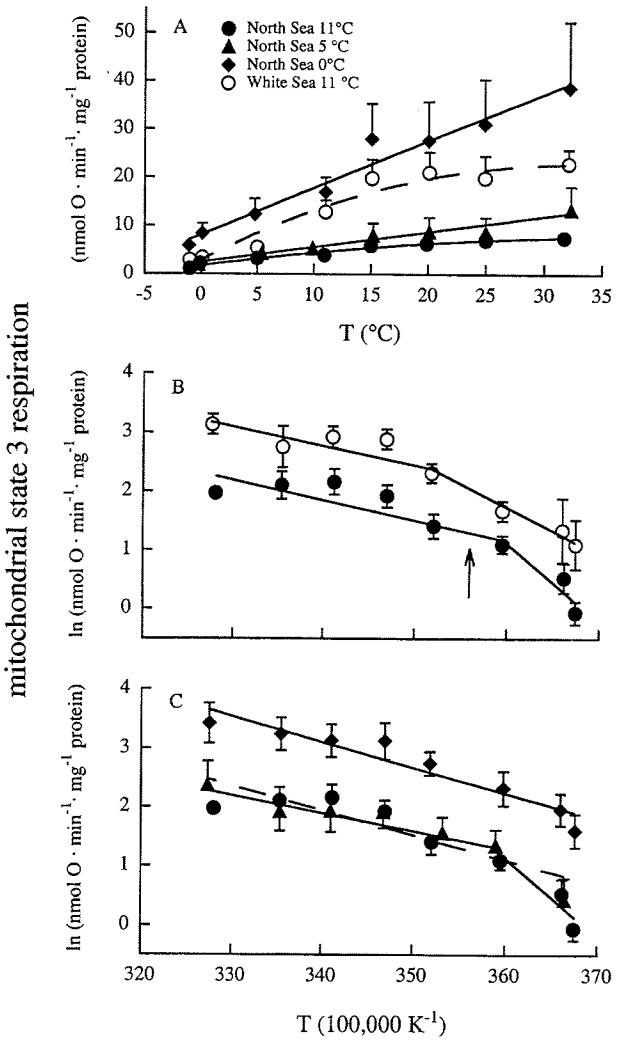


Figure 3. *A*, Temperature dependence of state 3 respiration rates, determined in mitochondria from North Sea lugworms (*filled symbols*) acclimated to 0°, 5°, or 11°C and in White Sea lugworms (*open symbols*) acclimated to 11°C. Mitochondrial respiration rates of boreal lugworms acclimated to 0°C and of subpolar lugworms were significantly higher than in boreal specimens acclimated to 5° or 11°C. *B*, Arrhenius plots (ln respiration rate vs. inverse temperature) of mitochondrial state 3 respiration from White and North Sea summer lugworms and from (*C*) North sea lugworms acclimated to different temperatures. Slopes reveal Arrhenius activation energies (Fig. 4*A*; *arrow* = significant Arrhenius break temperature in boreal and subpolar summer animals; means ± SE; n = 4–8).

adaptation but also seasonal cold acclimatization causes mitochondrial capacities to become elevated, with maximum activities at habitat temperatures above those found in boreal summer worms acclimated to 11°C (Fig. 3*A*). However, the data obtained suggest that latitudinal cold adaptation and seasonal cold acclimatization do not necessarily follow identical ways.

Mitochondrial Function and Thermal Adjustments of the Whole Organism: Latitudinal Cold Adaptation

On the one hand, latitudinal cold adaptation in White Sea *Arenicola marina* was mirrored in a rise of the maximum rate of mitochondrial succinate oxidation and a decreased E_a of state 3 respiration at low temperatures. Additionally, mitochondrial density in the body-wall tissue as well as the activity of mitochondrial enzymes like cytochrome c-oxidase (CYTOX) and NADP-dependent isocitrate dehydrogenase is increased in subpolar compared with boreal lugworms during summer (Sommer and Pörtner 2002). On the other hand, subpolar lugworm mitochondria displayed a lower degree of mitochondrial coupling, higher proton leakage rates, and reduced activity of CS (this study). The latter is surprising in the light of an increased aerobic capacity in cold-adapted mitochondria; however, changing ratios of CYTOX and CS capacities may also be related to a changing use of lipid biosynthesis (cf. Pörtner 2002*b*). In this case, it needs to be investigated whether the drop in CS capacity indicates a shift in metabolic control away from lipid biosynthesis in White Sea eurytherms during summer. Otherwise, enhanced mitochondrial phosphorylation and proton

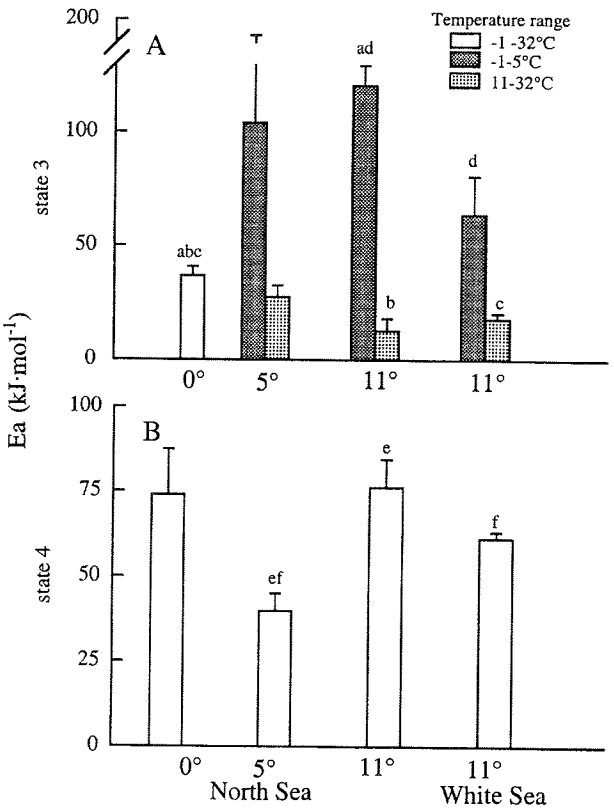


Figure 4. Arrhenius activation energies (E_a) for (*A*) mitochondrial state 3 and (*B*) state 4ol respiration rates in White and North Sea lugworms acclimated to different temperatures (mean ± SE; same letters indicate significant differences).

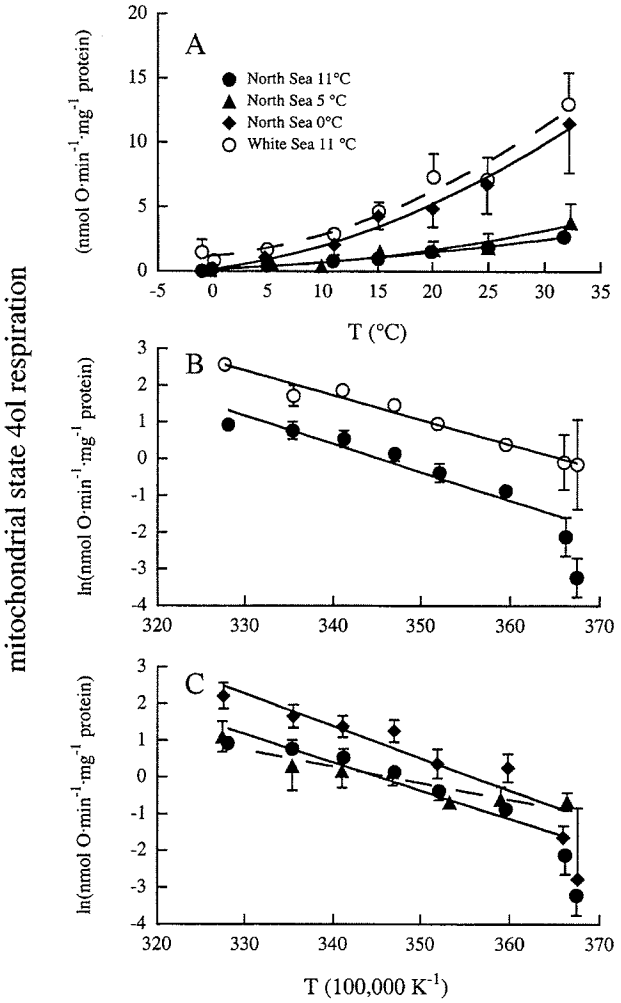


Figure 5. *A*, Temperature dependence of state 4ol respiration rates, determined in mitochondria from North Sea lugworms (*filled symbols*) acclimated to 0°, 5°, or 11°C and in White Sea lugworms (*open symbols*) acclimated to 11°C. Mitochondrial respiration rates of subpolar lugworms and of boreal lugworms acclimated to 0°C were the same, and both differed significantly from North Sea specimens acclimated to 5° or 11°C. *B*, Linear Arrhenius plots of mitochondrial state 4ol respiration in summer animals from the North and White Seas and from (*C*) North Sea animals acclimated to different temperatures. Slopes reveal E_a values (Fig. 4*B*; means ± SE; n = 4–8).

leakage capacities have not only been observed in subpolar lugworms but also in subpolar compared with boreal bivalves and nereids (Tschischka et al. 2000).

Consideration of the thermal influence on whole-animal metabolism may support an understanding of these patterns. Various studies now emphasize the correlated failure of circulatory and ventilatory mechanisms and the onset of temperature-dependent anaerobiosis in ectotherms at both low and high temperature extremes (for review see Pörtner 2001, 2002*a*). In the cold, loss of aerobic scope is compensated for by a rise in aerobic capacity, as seen in subpolar lugworms. This process enhances aerobic energy production and functional capacity of ventilation and circulation and, thereby, shifts the limits of cold tolerance to lower temperatures.

As a drawback, the combination of both elevated mitochondrial capacity and density reflects a higher cellular and organismic oxygen demand, partly due to rising mitochondrial maintenance costs (e.g., for proton and ion gradients and protein synthesis; Pörtner et al. 1998). Accordingly, elevated oxygen consumption rates have been found in cold-adapted lugworms (Sommer and Pörtner 2002), as well as in cold-adapted or acclimated eelpouts (van Dijk et al. 1999) or cod (T. Fischer, R. Knust, and H. O. Pörtner, unpublished data), compared with warm-adapted or acclimated specimens. As a trade-off, the balance between oxygen supply to tissues and oxygen consumption during warming is disturbed at lower temperatures in subpolar rather than in boreal lugworms, causing a drop in heat tolerance.

Cellular oxygen demand under resting conditions (standard metabolic rate) is significantly influenced by the rate of proton leakage in both ecto- and endotherms (Brand 1990; Brookes et al. 1998). The capacity for mitochondrial proton leakage is reflected in the level of state 4 respiration, determined by the addition of saturating amounts of oligomycin, an inhibitor of the mitochondrial F_0F_1-ATPase (Brand et al. 1994). Proton leakage capacities were increased at all temperatures in mitochondria of subpolar compared with boreal summer lugworms (Fig. 5). A fraction of this increase is likely involved in the cold-induced elevation in standard metabolic rate. High capacity of proton leakage is adaptive, as it not only reduces harmful oxygen radical production (Brand 2000; Echtay et al. 2002) but, as outlined earlier in the context of thermal adaptation (Pörtner et al. 2000; Sommer and Pörtner 2002), it reflects the baseline idling of aerobic metabolism, which goes hand in hand with enhanced mitochondrial phosphorylation capacity and with that the flexibility and scope of aerobic metabolism in response to environmental or internal stimuli like muscular activity (Brand et al. 1994). Such flexibility is required for maintaining an active lifestyle not only at low mean annual temperatures but even more so under the effect of large environmental temperature fluctuations in the cold, like in the White Sea habitat (Sommer and Pörtner 1999, 2002). These conclusions are supported by recent findings of interdependent changes in mitochondrial capacity and flexibility in hibernating frogs (St-Pierre et al. 2000).

In general, between 15% and 35% of mitochondrial respiration is needed to compensate for the energy-dissipating futile proton cycling across the inner mitochondrial membrane (Brand 2000). However, nonphosphorylating respiration (state 4ol) was significantly more temperature sensitive than phosphorylating respiration (state 3) at temperatures above 5°C (Table 1; Fig. 4), reflecting an increased percentage of mitochondrial oxygen consumption to compensate for proton leak-

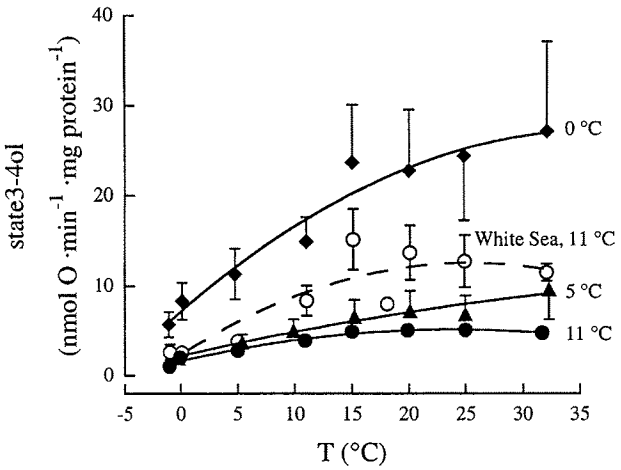


Figure 6. Temperature dependence of the difference between the rates of state 3 and state 4ol respiration in mitochondria of North (*filled symbols*) and White Sea (*open symbols*) lugworms acclimated to 0°, 5°, or 11°C. Values were significantly higher in subpolar animals and in boreal specimens acclimated to 0°C than in North Sea worms acclimated to 5° or 11°C (means ± SE; n = 4–8).

age and a progressive uncoupling of mitochondria with rising temperatures (Fig. 1*A*). The same phenomenon was seen in mitochondria from Antarctic ectotherms, the notothenioid *Lepidonotothen nudifrons* (Hardewig et al. 1999), and the bivalve *Laternula elliptica* (Pörtner et al. 1999). The proton leakage capacity was much higher in mitochondria from White Sea than in those from North Sea lugworms acclimated to the same summer temperature of 11°C, reflected by significantly lower RCRol values (Fig. 1*B*). However, the maximum capacity of state 3 respiration rate was also higher in subpolar worms, so that higher state 3–4ol rates resulted (Fig. 6). Together with the level of RCRol, these findings emphasize that high aerobic capacity (state 3 respiration) and high proton leakage capacities go hand in hand in cold-adapted worms at high latitudes, corroborating our previous results (Tschischka et al. 2000).

As enhanced mitochondrial proton leakage capacities in vitro mirror enhanced standard metabolic rate and contributing proton leakage at elevated mitochondrial densities in vivo, the question arises how excessive mitochondrial idling and thus baseline metabolic costs can be reduced during cold adaptation. Membranes and their composition have a strong influence on the activity of membrane-bound enzymes (Wodtke 1981; Paradies et al. 1994) and the maintenance of ion gradients (Brookes et al. 1998; Else and Wu 1999). In this manner, they can act as pacemakers of metabolism (Hulbert and Else 1999, 2000). Changing mitochondrial membrane properties to compensate for increased metabolic costs by reducing the rate of proton leakage might therefore compromise mitochondrial capacity.

Seasonal Cold Acclimatization

Like latitudinal cold adaptation, seasonal cold acclimatization in *A. marina* from the North Sea was associated with rises in maximum mitochondrial succinate oxidation and proton leakage capacities. Winter lugworms acclimatized to 0°C displayed even higher rates of state 3 respiration than subpolar summer worms. However, at the lowest temperature tested, proton leakage rate decreased in relation to state 3 respiration rate in cold-acclimated North Sea lugworms to values below 5% of their maximum oxygen consumption in state 3. Accordingly, the efficiency of mitochondrial energy production rose, indicated by higher ADP/O ratios (Fig. 2) and state 3–4ol respiration rates (Fig. 6) in mitochondria of winter- compared with summer-acclimatized North Sea lugworms. With a higher efficiency, mitochondrial maintenance costs likely decrease in vivo, despite high aerobic capacity at 0°C during winter.

Rising muscle aerobic capacities (as reflected by CS activities) with cold acclimatization (8° vs. 23°C) were also found in the threespine stickleback (*Gasterosteus aculeatus*; Guderley and Leroy 2001). Since the thermal sensitivity of CS activity remained unchanged at rising CS capacities with seasonal acclimatization in lugworms (Fig. 7), a rise in enzyme levels in the body-wall tissue probably caused the activity increment, similar to observations in winter-acclimatized rainbow trout (St-Pierre et al. 1998). The improved efficiency of aerobic energy production in winter lugworms at low temperatures likely supported a shift of the low critical temperature to a lower value

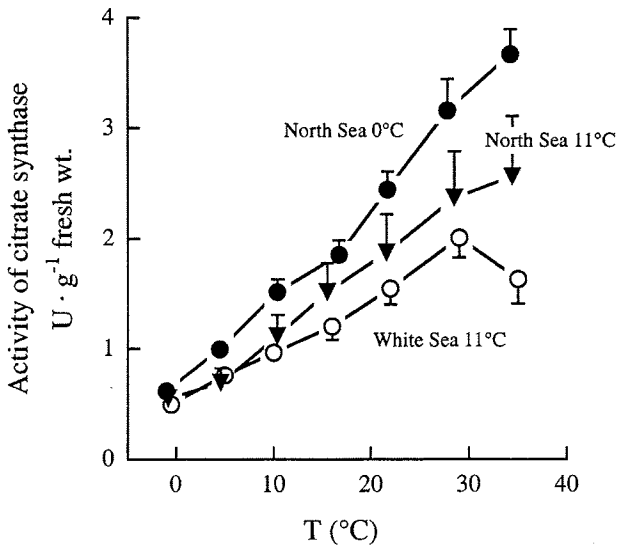


Figure 7. Activities of citrate synthase in the body-wall tissue of *Arenicola marina* from the North (*filled symbols*) or White Sea (*open symbols*) at various temperatures (−1° to 35°C). Activities differed significantly between both populations and with acclimation temperature (n = 5–7; mean ± SE). For the respective Q_{10} values, see Table 1.

Table 1: Q_{10} values for mitochondrial respiration and for the activity of citrate synthase (CS) in White and North Sea lugworms acclimated to 11°, 5°, or 0°C

	North Sea			White Sea
State/Temperature Range (°C)	11°C	5°C	0°C	11°C
State 3:				
−1 to 32	...	...	1.39 ± .08	...
−1 to 5	4.24 ± 1.16	5.06 ± 3.67	...	2.11 ± .56
11 to 32	1.47 ± .07	1.86 ± .08	...	1.60 ± .16
State 4:				
−1 to 32	2.41 ± .25	1.84 ± .38	2.22 ± .43	2.15 ± .15
State 4ol:				
−1 to 32	2.34 ± .19	2.03 ± .57	1.63 ± .20	2.19 ± .19
CS:				
−1 to 29	1.76 ± .08	nd	1.87 ± .12	1.71 ± .04

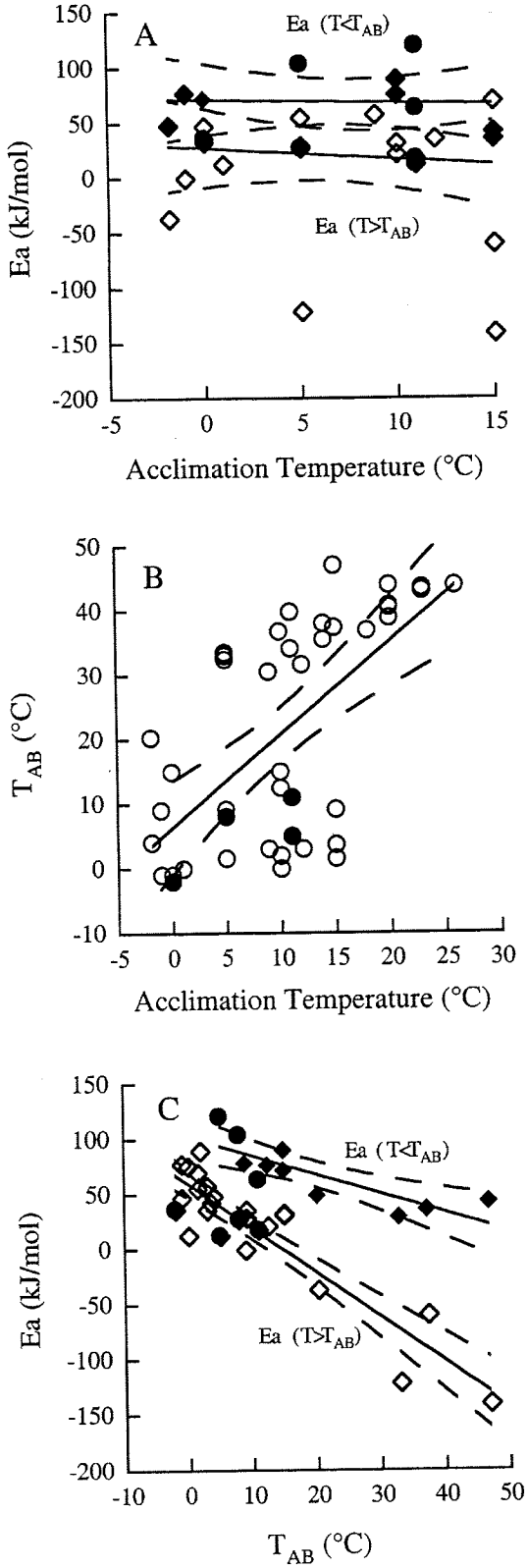
Note. Mean ± SE; nd = not determined.

(Sommer et al. 1997). The contrasting changes in CS activities in North Sea winter versus White Sea summer lugworms warrant investigation. The rise in CS capacity in response to winter cold may reflect a shift to lipid biosynthesis, as is typically seen in cold-adapted stenotherms (cf. Pörtner 2002*b*).

Under state 3 conditions, when proton leakage is minimized, the ADP/O ratio might increase as the phosphorylation rate rises (Dufour et al. 1996). In state 4, especially in the presence of oligomycin, the effective ADP/O ratio is 0, while there is no net ATP synthesis and the whole proton current is via the leakage. Although the ADP/O ratio is sensitive toward changes in the proton leakage rate (Brand et al. 1993), proton leakage rate seems not to be involved in the thermal control of oxidative phosphorylation (Dufour et al. 1996). The membrane potential in states 3 and 4 is unlikely to change with temperature (Dufour et al. 1996), a finding recently confirmed for polar and subpolar marine ectotherms, including *A. marina* (A. Sommer, H. O. Pörtner, and T. Hirse, unpublished data). However, in spite of different thermal sensitivities of state 3 and state 4ol respiration rates in lugworms, the overall impact of temperature on the effective ADP/O ratios of mitochondria from both populations was small. Only the significant rise in ADP/O ratios assayed in the cold in North Sea mitochondria acclimated to 11°C (Fig. 2*A*) would be in line with a high E_a of proton leakage and thus minimized dissipation of proton motive force.

Interestingly, changes in mitochondrial functions from those observed at 11°C were minimal when boreal *A. marina* reached only 5°C during winter and became significant only when temperature dropped further to 0°C. These findings suggest that cold acclimatization of mitochondrial functions occurs below a threshold temperature rather than progressively with falling environmental temperatures. A similar two-step process of seasonal acclimatization was seen in the thermal response of liver mitochondrial membranes from ground squirrels *Spermophilus richardsonii* (Augee et al. 1984). In five out of 11 active winter animals kept at 4°C, T_{AB} of succinate–cytochrome c reductase activity was found at 22°C, similar to animals maintained at 19°C, whereas in the other six animals, T_{AB} was lowered to below 4°C, like in hibernating specimens kept at 4°C. It was concluded that the lowering of T_{AB} is a requisite for hibernation and associated metabolic depression rather than a response to the low body temperatures.

In contrast to latitudinally cold-adapted lugworms, an increased efficiency of mitochondrial functions during winter cold (Figs. 1*B*, 2*B*, 3, 6) likely occurred at the expense of reduced metabolic flexibility, reflected in reduced proton leakage capacity and thus low futile cycling of mitochondria. Together with the disappearance of the T_{AB} and concomitant increase in the E_a of state 3 respiration seen in boreal lugworms with falling acclimatization temperatures, this pattern may indicate that metabolic depression proceeds in similar ways as in hibernating mammals (Augee et al. 1984; Geiser and McMurchie 1985). Although hibernation is more commonly described for freshwater and terrestrial species than marine invertebrates, dormancy has been found in marine polychaetes, like in *Lanice conchilega* from the North Sea (Cáceres 1997). Future studies are needed to clarify whether winter acclimatization in North Sea lugworms is really associated with metabolic depression. Such a strategy appears very likely because the animal's burrows are found considerably deeper than in summer (A. M. Sommer, unpublished observation) and a frozen sediment surface prevents cropping by birds or fish (De Vlas 1979). As glycogen and lipid stores in lugworm tissues were found to decline during winter, indicating insufficient food supply or uptake (Juretschke and Kamp 1995), energy savings during metabolic depression would extend the period during which energy stores can be used. White Sea lugworms may undergo similar strategies during winter as observed in their boreal conspecifics.



Thermal Adjustments of Mitochondrial Functions

The E_a values of 64 kJ mol^{-1} found in cold-adapted White Sea and of 37 kJ mol^{-1} in cold-acclimatized North Sea mitochondrial state 3 respiration at low temperatures were in the range of values found in gill mitochondria of the Antarctic bivalve *L. elliptica* between 0° and 3°C (77.8 kJ mol^{-1}; Pörtner et al. 1999) or the Antarctic notothenioid *L. nudifrons* (47.5 kJ mol^{-1}; Hardewig et al. 1999). These values are significantly lower than seen in boreal summer mitochondria acutely exposed to cold and suggest kinetic facilitation of mitochondrial processes during cold adaptation or acclimation. However, E_a values in subpolar worms were lower than in boreal ones only in the low thermal range. At temperatures above the T_{AB}, no difference in E_a was found.

To gain further insight into this problem, we collected data from the literature and from our own published and unpublished work to see whether there is a general influence of thermal acclimation or adaptation on E_a of mitochondrial state 3 respiration in marine ectotherms across various animal groups and latitudes. Despite this nonrepresentative mix of data from ectotherms and the use of mitochondrial data obtained with different preparation techniques, the depiction would argue against an influence of acclimation temperatures on such E_a values in marine ectotherms ($r^2 \leq 0.01$; Fig. 8*A*). However, as Dahlhoff et al. (1991) pointed out, thermal history may set Arrhenius break temperatures of mitochondrial state 3 respiration, a hypothesis supported by our own study (Fig. 8*B*). T_{AB} may then in turn influence E_a below and above T_{AB} (Fig. 8*C*). In this way, the same acclimatization temperature of 11°C for summer animals in North and White Sea populations would explain similar T_{AB}'s despite different mean annual temperatures, which the worms experience in their natural habitat.

The lugworm data fit very well into the general picture seen in Figure 8. Acclimatization to winter cold caused the significant T_{AB} in state 3 respiration (11°C) to become insignificant (5°C) or to even disappear (0°C). In consequence, E_a was reduced in

Figure 8. Between-species comparisons of (*A*) apparent Arrhenius activation energies (E_a) of mitochondrial state 3 respiration at acclimation temperatures and (*B*) Arrhenius break temperatures (T_{AB}) of mitochondrial state 3 respiration depending on acclimation temperature; (*C*), E_a versus T_{AB} in various marine ectotherms. *A* and *C* used E_a in the thermal range above and below the respective T_{AB}. Values were taken from this study (*filled circles*) as well as from those of Dahlhoff et al. (1991); Guderley et al. (1997); Weinstein and Somero (1998); Guderley and St-Pierre (1999); Hardewig et al. (1999); Pörtner et al. (1999); and G. Lannig and K. Heise (personal communication). There was no clear dependence of E_a on acclimation temperature ($r^2 \leq 0.01$), but T_{AB}'s were significantly influenced ($T_{AB} = 6.24 + 1.45x$; $r^2 = 0.39$). E_a increased with decreasing T_{AB} both below and above T_{AB} ($E_a\ [T > T_{AB}] = 59.98 - 4.03x$, $r^2 = 0.824$; $E_a\ [T < T_{AB}] = 102.48 - 1.72x$, $r^2 = 0.672$). Negative E_a values indicate the effect of thermal inactivation.

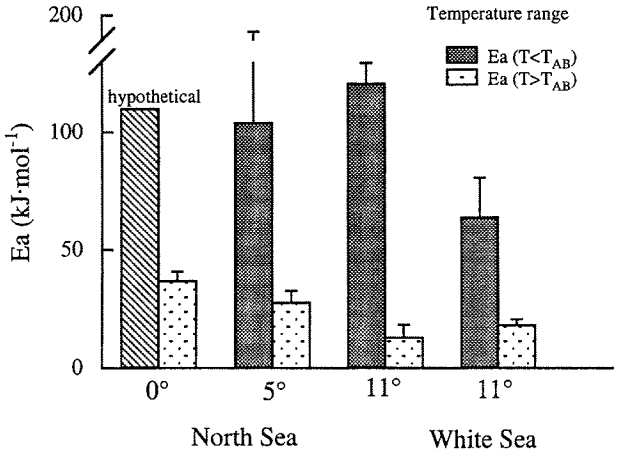


Figure 9. Arrhenius activation energies (E_a) for mitochondrial state 3 respiration rates in White and North Sea lugworms acclimated to 0°, 5°, or 11°C for temperature ranges above (*light pattern*) or below (*filled pattern*) the respective T_{AB}. The value below T_{AB} (*hatched pattern*) for North Sea animals acclimated to 0°C was estimated according to Figure 8 (mean ± SE).

the cold. At first sight, this pattern seems to contrast the results obtained in the between-species comparison (Fig. 8C). However, the disappearance of a T_{AB} can be seen as a shift of this break to below the lowest measured temperature (Geiser and McMurchie 1985). For lugworms acclimatized to 0°C, only the E_a above T_{AB} was determined (Fig. 8C). The apparent drop in the E_a (36.81 ± 3.99 kJ mol^{-1}) found at low temperatures in North Sea winter lugworms acclimatized to 0°C compared with summer worms acclimatized to 11°C (120.55 ± 8.75 kJ mol^{-1}), therefore, appears as a conversion of a high E_a (below T_{AB}) to a low E_a (above T_{AB}; Fig. 9). As a consequence, the E_a of mitochondrial state 3 respiration in North Sea lugworms acclimatized to 0°C in winter and determined at low temperature was still significantly higher than the respective E_a in summer worms of both populations determined at their ambient temperature of 11°C (Fig. 4). These findings may reflect an enhanced kinetic barrier and reduced substrate turnover in winter and may support metabolic depression during the colder months.

A close relationship between T_{AB} and E_a values like in marine ectotherms (Fig. 8C) has also been found for mitochondrial respiration in mammalian hibernators. Mammalian torpor or hibernation is also characterized by a lowering or even disappearance of the T_{AB} and a concomitant increase in the apparent E_a of mitochondrial membrane-associated respiratory enzymes, when compared with normothermic or homeothermic species (Augee et al. 1984; Geiser and McMurchie 1985). These findings support the general contention by Somero (1997) that temperature may exert similar selective pressures in hibernators and ectothermic animals. The mechanisms setting T_{AB} are obscure but may be associated with the modification of lipid unsaturation levels (Hazel 1995) or cholesterol content (Geiser et al. 1997).

Conclusions

The observed changes in mitochondrial functions during both latitudinal cold adaptation and seasonal cold acclimatization support a downward shift of cold-tolerance thresholds (Sommer et al. 1997). However, eurythermal cold adaptation of White Sea lugworms is reflected by an increased aerobic capacity at the expense of elevated mitochondrial proton leakage and, in consequence, a higher standard metabolic rate to maintain functional capacity like motor activity. In contrast, it seems that, although aerobic capacity rose in winter individuals, seasonal cold acclimatization occurs with an enhanced efficiency of aerobic energy production and, possibly, metabolic depression. This mechanism is only suitable for a time-limited situation. Mitochondrial functional parameters therefore reflect some of the trade-offs involved in adaptation to cold temperatures. Future work is required to elaborate how such fine tuning of mitochondrial functions occurs and how the trade-offs involved affect the animal's performance in its thermal environment.

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