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Metabolic plasticity and critical temperatures for aerobic scope in a eurythermal marine invertebrate (*Littorina saxatilis*, Gastropoda: Littorinidae) from different latitudes

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Summary

Effects of latitudinal cold adaptation and cold acclimation on metabolic rates and aerobic scope were studied in the eurythermal marine gastropod *Littorina saxatilis* from temperate North Sea and sub-arctic White Sea areas. Animals were acclimated for 6–8 weeks at control temperature (13°C) or at 4°C, and their respiration rates were measured during acute temperature change (1–1.5°C h^{-1}) in a range between 0°C and 32°C. In parallel, the accumulation of anaerobic end products and changes in energy status were monitored. Starting from 0°C, aerobic metabolic rates of *L. saxatilis* rose quickly with increasing temperatures up to a point at or slightly above the respective acclimation temperature. Beyond this value, thermal sensitivity of oxygen consumption rate ($\dot{V}_{O_2}$) greatly decreased in a wide, 15°C range of experimental temperatures. This change in metabolic regulation was also reflected in the activation energy of aerobic metabolism (E_a), which was approximately seven times lower at temperatures above Arrhenius breakpoint temperatures (ABTs) than at temperatures below ABTs. Warming progressively led to a discrepancy between energy demand and energy production, as demonstrated by a decrease in the levels of high-energy phosphates [phosho-L-arginine (PLA) and ATP], and resulted in the onset of anaerobiosis at critically high temperatures, indicating a limitation of aerobic scope. The comparison of aerobic and anaerobic metabolic rates in *L. saxatilis* in air and water suggests that the heat-induced onset of anaerobiosis is due to the insufficient oxygen supply to tissues at high temperatures. Cold acclimation led to an increase in aerobic metabolic rates and a considerable downward shift of the upper critical temperature in North Sea *L. saxatilis* but not in White Sea *L. saxatilis*. Limited metabolic plasticity in response to cold acclimation in sub-arctic White Sea snails as compared with their temperate North Sea counterparts suggests that metabolic depression occurs during overwintering under the more extreme winter conditions at the White Sea.

Key words: temperature adaptation, aerobic scope, critical temperatures, respiration, metabolic cold compensation, *Littorina*.

Introduction

Temperature is an important environmental factor influencing all life functions of an organism through changes in the rates of biochemical and physiological processes and in the stability of biomolecules (reviewed by Hochachka and Somero, 1973; Hoffmann, 1983; Prosser, 1991). Adaptation to environmental temperature is recognized as one of the evolutionary mainstreams and is thought to be dependent, to a large extent, on the organism's ability for metabolic adjustments on both short-term and evolutionary time scales (Hochachka and Somero, 1973; Hoffmann, 1983; Prosser, 1991; Clarke, 1998). Metabolic adjustments in response to temperature change are especially crucial for aquatic ectotherms, whose body temperature fluctuates over the full range of their habitat temperatures.

The study of the effects of environmental temperature on metabolic rates of aquatic ectotherms has a very long history, and an enormous body of data on this topic has been accumulated so far (reviewed by Clarke, 1980, 1998). However, recent studies have revealed that environmental temperature not only influences the total metabolic rate in aquatic ectotherms but can also significantly affect metabolic regulation, eliciting transition to anaerobiosis even in fully oxygenated waters (reviewed by Pörtner, 2001; Pörtner et al., 2001). Beyond the thermal optimum, whole-animal aerobic scope falls and eventually vanishes at low or high critical temperatures (T_c I and T_c II, respectively), when transition to anaerobic mitochondrial metabolism occurs (Sommer et al., 1997; van Dijk et al., 1999; Frederich and Pörtner, 2000).

Presumably, the temperature-induced transition to partial anaerobiosis is caused by the progressively insufficient capacity of circulation and ventilation and can provide only time-limited survival of the animals at and beyond critical temperatures (T_cs; Pörtner, 2001). Comparisons between cold-adapted Antarctic species and their relatives (within a family or a class) from temperate waters have shown that the T_cs in Antarctic ecthotherms are much lower than in their temperate counterparts (Pörtner et al., 1998; Pörtner and Zielinski, 1998; van Dijk et al., 1999). This downward shift of the T_cs is brought about by increased mitochondrial densities and increased thermal sensitivity of mitochondrial oxygen demand, which may reduce the organism's performance at high temperatures due to the high costs of mitochondrial maintenance (Pörtner, 2001). However, in Antarctic marine stenotherms, metabolic oxygen demand is downregulated despite hugely elevated mitochondrial densities (Pörtner et al., 1998), so that little or no metabolic cold adaptation of the metabolic rates is observed (reviewed by Clarke, 1980, 1998; Clarke and Johnston, 1999).

Despite the importance of temperature-induced limitations of aerobic scope for the metabolic physiology of animals, it is much less studied compared with other aspects of the metabolic response to temperature. In particular, the amount of data about the effects of latitudinal cold adaptation on the temperature window of aerobic scope is very limited and practically absent for eurythermal ectotherms. The only eurythermal species studied in this respect is the intertidal polychaete *Arenicola marina*, which demonstrated similar downward shifts of the T_cs as a result of latitudinal cold adaptation and seasonal cold acclimatisation (Sommer et al., 1997). However, in the latter study, animals from different latitudes were acclimated at their respective environmental temperatures (12°C and 6°C), thereby confounding the effects of latitudinal adaptation and temperature acclimation on T_cs. In order to yield a better understanding of the broad applicability of the concept of T_cs, further studies on organisms with different strategies of temperature adaptation are needed. It is also very important to estimate the degree of phenotypic plasticity of T_cs and the thermal window of aerobic scope in response to temperature acclimation or acclimatisation, which are likely to be crucial for eurythermal organisms that have to cope with considerable seasonal and circadian temperature variation.

The intertidal Atlantic gastropod *Littorina saxatilis* is a uniquely convenient object for the study of latitudinal cold adaptation. Its distribution area covers more than 50° by latitude, ranging from North Africa up to Svalbard (Reid, 1996). This allows us to avoid 'apples with oranges' comparisons of different species and to study warm- and cold-adapted populations within an ecologically, morphologically and genetically cohesive unit such as a single species. Moreover, *L. saxatilis*, as with many intertidal species, is at the eurythermal extreme of the eurytherm–stenotherm continuum and is able to cope with wide and frequent temperature fluctuations typical for intertidal habitats (Sokolova et al., 2000a). *L. saxatilis* can withstand temperatures of up to 35°C for at least several hours during summer low tides and up to 47°C during short-term acute heating events (Sokolova et al., 2000a). This species is also extremely cold- and freeze-tolerant. It can survive sub-zero temperatures for several months and can tolerate temperatures as low as –20°C for at least several hours (Matveeva, 1974; Bourget, 1983; Sokolova and Berger, 2000).

The aim of the present study was to analyse the effects of latitudinal cold adaptation on metabolic rate and critical temperatures in *L. saxatilis* from the temperate North Sea and sub-arctic White Sea. We measured aerobic metabolic rates in animals from different latitudes over a wide range of experimental temperatures and analysed the accumulation of end products of anaerobic metabolism at different temperatures. Concentrations of high-energy phosphates (ATP and phospho-L-arginine) were measured simultaneously in order to establish whether the transition to partial anaerobiosis was correlated with impaired intracellular energy status. In order to estimate the phenotypic plasticity of the metabolic response to the temperature change, rates of aerobic metabolism and critical temperatures were compared in snails from different latitudes acclimated to 4°C and 13°C. Analysis of the effects of latitudinal cold adaptation and temperature acclimation on metabolic rates and the temperature range of aerobic scope in *L. saxatilis* provides important insights into the metabolic responses to short-term temperature change *vs* long-term effects of acclimation or latitudinal adaptation in a eurythermal intertidal invertebrate. It also widens our perspective of the mechanisms of metabolic adaptation and regulation in response to temperature change in ectotherms. This study also complements our broader research concerning physiological, biochemical and populational mechanisms of adaptation of *L. saxatilis* to environmental stresses (temperature, salinity, oxygen deficiency) and addresses the basis of the extremely eurybiont life modus of this species.

Materials and methods

Collection sites and animals

Littorina saxatilis (Olivi) were collected in the intertidal zones of the Kandalaksha Bay of the sub-arctic White Sea (66°20′ N, 33°39′ E) in September 1999 and of Helgoland Island in the North Sea (54°11′ N, 7°53′ E) in October 1999. The surface water temperature was 10–13°C at both the White Sea and North Sea sites at the time of collection. The tidal range is 1.8–2.2 m at the White Sea sampling site and 1.6–2.8 m at the North Sea sampling site. In general, sub-arctic White Sea populations of *L. saxatilis* experience extreme seasonal variations of temperature, from freezing (from below –1.5°C in brackish seawater to –10–15°C in air) in late autumn and winter to 15–20°C in summer (Babkov, 1998; Sokolova et al., 2000a). During summer low tide, environmental temperatures in the intertidal zones may reach extreme values (35–45°C) due to solar irradiation in the continental White Sea (Sokolova et al., 2000a). By contrast, both temperature fluctuations and temperature extremes are much less

pronounced in the temperate North Sea, which is characterized by a temperate marine climate. Surface water temperatures range from 3–6°C in winter to 15–18°C in summer (R. Scharek, M. Gillbricht, E. Hagmeier, W. Hickel, H. Kayser, K. Reise, unpublished data available at www.pangaea.de; Hickel et al., 1997). The monthly average temperature of surface water rarely ever drops below zero, and an ice cover is rarely formed at the North Sea site. By contrast, the ice cover at the White Sea may persist for approximately 6 months each year, and the monthly average temperature of surface water is negative for 5–6 months (Babkov, 1998). The annual average temperatures of surface water are 4.3°C and 10.4°C at the White and the North Sea sites, respectively.

In each study area, adult *L. saxatilis* (6–11 mm shell diameter) were collected from small stones and gravel patches in the low intertidal zone, within the brown macrophyte belt (*Ascophyllum nodosum* and *Fucus vesiculosus* in the White Sea and *F. vesiculosus* and *Fucus serratus* in the North Sea). Snails were transported alive to the Alfred-Wegener-Institute (AWI) in Bremerhaven, Germany and acclimated in aquaria with recirculated seawater set to the salinity of the respective sampling sites (33.2–33.4‰ for the North Sea and 24.6–24.7‰ for the White Sea) for at least 6–8 weeks prior to experimentation. In order to reduce the effects of the circadian rhythm on the oxygen consumption of *L. saxatilis*, snails were acclimated under conditions of constant dim light and all experiments were started at the same time of day (Sandison, 1966; Shirley and Findley, 1978). Prolonged laboratory acclimation was used to eliminate the potential differences in metabolic physiology among the animals from populations, which were due to their recent acclimatisation history in the field (Hawkins, 1995). This allowed us to analyse irreversible (presumably, genetic) physiological differences among the compared groups of snails. Two acclimation temperatures were used: 13°C, which was close to the respective field temperature at the time of collection, and 4°C. Two aquaria were randomly assigned to each combination of acclimation temperature and population of origin, and samples for experimental incubations and measurements were withdrawn from them at random. Water in the aquaria was continuously aerated, passed through a filter and changed once every two weeks. Seawater from the Helgoland area was used, and the required salinity was adjusted by adding artificial sea salt (Tropic Marin, Wartenberg, Germany) or diluting with distilled water. Brown macroalgae (*F. vesiculosus*) from Helgoland Island were added as a food source *ad libitum*. No mortality was detected during transportation, and only minimum mortality (<5%) was detected during laboratory acclimation.

Respiration rates

The routine rates of oxygen consumption in water were measured in closed respiration chambers with Clarke-type electrodes connected to an oxygen partial pressure monitor (P_{O_2} monitor, Eschweiler, Kiel, Germany). Two-point calibrations of temperature-equilibrated electrodes (in air-saturated seawater for 100% readings and in Na_2SO_3-saturated water for 0% readings) were performed prior to and after each measurement.

Five to six animals of similar size [ranging from 40 mg to 50 mg (White Sea populations) and from 80 mg to 120 mg wet tissue mass (North Sea populations)] were used for each measurement. Prior to the experiments, the snails were fasted for 72 h. Shells of the experimental snails were carefully scraped and cleaned with 95% ethanol to remove potential microfouling. Snails were then allowed to quickly recover in seawater, were blotted dry with tissue paper and were put in the chamber filled with seawater at the respective acclimation temperature (4°C or 13°C). Water in the chambers was continuously mixed with magnetic stirring rods, which were placed under the nylon net 'floor' (mesh size, 1 mm) of the animal-containing main chamber. Respiration chambers were placed in a temperature-controlled aquarium (total capacity, 75 l) maintained at the respective control temperature and allowed to equilibrate for 30 min in order to minimize any effects of handling. Preliminary experiments showed that 30 min habituation of experimental chambers was sufficient to eliminate the overshoot effects of handling. Temperature was raised by 1.5°C h^{-1} or lowered by 1°C h^{-1}. The rate of the temperature change was set by the capacity of the water bath (HC F30, Julabo Labortechnik, Seelbach/Schwarzwald, Germany). During this preliminary acclimation, free water exchange between the experimental chambers and the surrounding water was allowed. After the temperature reached the desired value, the respiration chambers were closed and oxygen concentrations were monitored online for 15–25 min at the constant temperature (±0.1°C) with continuous mixing. Decline in the oxygen concentration was linear over this period, and repeated measurements of the respiration rates of the same batch of animals at the respective control temperatures indicated no change over at least 2 h. Oxygen tension was not allowed to fall by more than 15% during each measurement. After each measurement, blanks were run in the same chambers without animals in order to account for the drift of electrodes and respiration by any microorganisms in the experimental chamber and seawater. In order to minimize handling of the experimental animals, they were placed in small cages made of plastic mesh (mesh size, 1 mm) tightly fitted into the respiration chambers. During the measurement of oxygen consumption, the cages were placed into the respiration chambers, and during the blank reading they were carefully removed from the chamber and placed on the bottom of the experimental aquarium. It should be noted that the thermal limits and the rate of the temperature change used in our experiments resembled the environmental values reported for temperate and sub-arctic intertidal habitats (Sokolova et al., 2000a). Water oxygen contents were calculated as μmol l^{-1} O_2 from oxygen P_{O_2} readings (measured in Torr) using temperature-dependent solubility coefficients for oxygen. The determination of the chamber volume considered the volumes of snails, stirring rods and nylon nets determined by fluid displacement. After the experiments, animals were dissected in order to determine tissue wet mass and the level of trematode

infection. Respiration rates were expressed as $\mu mol\ O_2\ h^{-1}\ g^{-1}$ tissue wet mass. In the determinations of the respiration rates, infested and uninfested animals were pooled, as it has previously been demonstrated that infection by microphallids (Trematoda) does not influence the respiration rate of *Littorina* spp. (Lyzen et al., 1992; Sokolova, 1997). There was also no effect of the proportion of infested animals in a batch (which varied from 0% to 60%) on the rates of oxygen consumption in the present experiment (data not shown). Average trematode infestation rate across all samples was 24% (N=168) and 20% (N=129) in White Sea and North Sea snails, respectively.

In this study, the temperature dependence of aerobic metabolism was analysed by measuring the routine respiration rate in unfed *L. saxatilis*. Measurements of the basal metabolic rate (BMR) in marine gastropods is very difficult, because the animals tend to move continuously unless anesthetized. We restricted the range of movements of the snails in our experiments by providing them with minimum space so that the animals could remain open and attached with their feet to the substratum but could not move more than a few mm during the oxygen consumption rate ($\dot{V}_{O_2}$) measurements. Earlier studies had demonstrated that moderate crowding does not affect the metabolic rates of *Littorina* (Vilenkin and Vilenkina, 1973). Thus, the routine metabolic rates we have measured in our experiments are a good approximation of the resting metabolic rates of *L. saxatilis*.

Temperature incubations and tissue collection

For the determination of the concentrations of high-energy phosphates and anaerobic end products in the tissues of *L. saxatilis* at different temperatures, samples of 400–500 animals were placed in large (10 l) mesh cages of the same temperature-controlled experimental aquarium that had been used for the measurements of the respiration rate. Incubations started at the respective control temperatures (13°C or 4°C). Experimental animals were allowed to equilibrate in the experimental aquarium overnight in order to minimize the effect of handling, and then the temperature was raised by $1.5°C\ h^{-1}$ or lowered by $1°C\ h^{-1}$. The rate of the temperature change was set by the capacity of the thermostat (HC F30, Julabo Labortechnik, Seelbach/Schwarzwald, Germany). The duration of the longest incubation was 22 h. During the preliminary acclimation and incubation periods, the seawater in the aquarium was continuously aerated, mixed and filtered by pumps. After each incubation, the water in the aquarium was changed in order to prevent the accumulation of metabolic waste products.

After the temperature reached the desired value, a random sample of animals was taken out of the aquaria, animals were blotted dry, dissected and quickly inspected for trematode infestation. Infested specimens were discarded. In uninfested specimens, the foot muscle was quickly cut, blotted dry with tissue paper and frozen immediately in liquid nitrogen for subsequent metabolite analysis. For one sample, foot muscles of 8–12 specimens were pooled. At each experimental temperature, 5–9 samples were collected. Collection time was approximately 5 min per sample. During the collection of the tissues, temperature in the aquarium was maintained constant.

In order to test for the possible effects of experimental incubation *per se* on metabolite concentrations, control incubations were performed. Animals were kept for 26 h in the experimental aquaria at their respective control temperatures (4°C or 13°C), and tissues were sampled at the beginning and the end of this incubation period, as described above.

Metabolites

For the determination of metabolite concentrations using enzymatic tests, samples of foot muscles were powdered with a pestle and mortar under liquid nitrogen. Approximately 300 mg of tissue powder was homogenized in an excess (5×) volume of precooled $0.6\ mol\ l^{-1}$ perchloric acid (PCA) with $10\ mmol\ l^{-1}$ EDTA to bind tissue calcium, which proved necessary for maximization of the measured ATP levels (Sokolova et al., 2000b). Precipitated protein was removed by centrifugation (2 min, 10 000 ***g***). The extract was neutralized with $5\ mmol\ l^{-1}$ potassium hydroxide to pH 7.0–7.5. Precipitated potassium perchloride was removed by a second centrifugation. For ion chromatography, the extraction procedure was identical except that EDTA was omitted. Preliminary experiments showed that EDTA interfered with the determination of the concentrations of short-chain organic acid by co-eluting with propionate. Extracts were stored at –80°C.

For capillary electrophoresis, samples of foot muscles were powdered under liquid nitrogen. Approximately 100 mg of tissue powder was homogenized in 400 µl of ice-cold 15% trichloracetic acid with $0.1\ g\ l^{-1}$ of tartrate used as internal standard. Samples were extracted for 5 min on ice, and precipitated protein was removed by centrifugation (2 min, 10 000 ***g***). The supernatant was neutralized with 4 volumes of the mixture tri-n-octylamine:1,1,2-trichlorotrifluoroethane (1:4). Organic phases were separated by centrifugation (3 min, 14 000 ***g***), and the upper aqueous phase was collected, diluted 1:3 with ultrapurified water and directly applied to the capillary electrophoresis system (P/ACE™ System MDQ, Beckman, Unterschleissheim, Germany) in order to determine concentrations of the short-chain organic acids (D- and L-lactate, malate, succinate and propionate). Separation was carried out in a 120 cm long eCAP capillary with a diameter of 75 µm (Beckman) at a constant temperature of 15°C in a buffer consisting of organic acid buffer (Agilent Technologies, Böblingen, Germany), acetonitrile and Brij 35 (20:5:1 v/v/v). The separation voltage of 27 kV was applied for 20 min to allow for the detection of the slowest moving component, propionate. Organic acids were detected by a photodiode array detector at 214 nm. The system was calibrated using standard mixtures of the respective organic acids subjected to the same extraction procedure as the tissue samples.

A reliable determination of acetate by capillary electrophoresis was not possible owing to the presence of background acetate in samples extracted by trichloracetic acid. Therefore, the concentration of acetate was measured in PCA

extracts according to a method modified from Hardewig et al. (1991) using an ion-exclusion column (IonPac ICE-AS 1, Dionex, Idstein, Germany) at a flow rate of 1 ml min^{-1} and 40°C with 0.125 mmol l^{-1} heptafluorobutyric acid as an eluent. Peaks were monitored with a conductivity detector. A micro membrane suppressor (AMMS-ICE, Dionex) was used to decrease background conductivity. No acetate was detected in our samples.

Concentrations of ATP, L- and D-alanine were measured spectrophotometrically using enzymatic tests (Bergmeyer, 1985). Concentrations of phospho-L-arginine and L-arginine were assayed spectrophotometrically using the enzymatic test described by Grieshaber et al. (1978). Octopine dehydrogenase for these determinations was purified from the adductor muscles of *Pecten maximus* following the procedure described by Gäde and Carlsson (1984).

Chemicals

All chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA) or Merck (Darmstadt, Germany). Enzymes were purchased from Roche Diagnostics (Mannheim, Germany).

Derived indices and statistics

The relative amount of phosphagen (R_{PLA}) in the total phosphagen/aphosphagen pool was calculated according to the formula:

$$R_{PLA} = \frac{[PLA]}{[PLA] + [Arg]}, \quad (1)$$

where [PLA] and [Arg] are tissue concentrations of phospho-L-arginine (phosphagen) and L-arginine (aphosphagen), respectively (measured in μmol g^{-1} wet mass).

Respiration rates (calculated as μg O_2 consumed per hour per g wet tissue mass) were standardized to the same wet tissue mass (50 mg) according to equation 2:

$$R_{st} = R_i \times \left(\frac{\overline{W}}{W_i} \right)^b, \quad (2)$$

where R_{st} is a standardized respiration rate (μg O_2 h^{-1} g^{-1}), R_i is the respiration rate of an average animal in i-th batch (μg O_2 h^{-1} g^{-1}), W_i is the wet tissue mass of an average animal in the i-th batch (g), $\overline{W}$ is the 'standard' wet tissue mass (0.05 g), and b is a power coefficient in equation: $R_i = aW_i^b$. This coefficient was obtained by a calculation of the linear regression relating log W_i to log R_i by the least-square method (Sokal and Rohlf, 1995). Within a batch, the size of the animals varied by less than 10%. The rate of ATP turnover during anaerobiosis (amount of ATP consumed per g wet mass per day) was calculated from end product accumulation and ATP and PLA depletion, as described by Pörtner et al. (1984). An ATP equivalent of 2.75 μmol per μmol of succinate was adopted (de Zwaan, 1983). The aerobic ATP turnover rate was calculated from routine oxygen consumption rates assuming an ATP production level of 6 mol ATP mol^{-1} O_2.

Statistical analysis was performed using mixed Model I analysis of variance (ANOVA) after testing the assumptions of normal distribution and homogeneity of variance of the data (Sokal and Rohlf, 1995). Factors 'acclimation temperature' and 'sea' were treated as fixed, while factor 'exposure temperature' was treated as random. There were no differences between animals from the replicate aquaria maintained at the same acclimation temperature in any of the studied parameters, so the data were pooled for subsequent analysis. At the first step of an analysis, three-way ANOVAs were performed estimating effects of factors 'sea', 'acclimation temperature' and 'exposure temperature'. If estimations of single factor effects were impossible due to significant factor interactions, data sets were split to perform two-way or one-way ANOVAs. Tukey's honest significant deviance (HSD) test for unequal N was used as a method of *post-hoc* comparisons, and the least-square method was employed for planned comparisons (Sokal and Rohlf, 1995).

The energy of activation (E_a) was determined from an Arrhenius plot, i.e. log $\dot{V}_{O_2}$ *vs* $1/T$ (K^{-1}). The apparent E_a value was calculated from the slope of the plot (i.e. slope = $-E_a/2.303R$) obtained by the least-square linear regression (Sokal and Rohlf, 1995). The Arrhenius breakpoint temperature (ABT) at which a significant change in the slope (i.e. in E_a) occurs was determined using an algorithm for fitting of two-segmented linear regressions described by Yeager and Ultsch (1988). Slopes of regression lines were compared according to Zar (1996). The highest experimental temperature (32°C) characterized by the onset of heat coma (see below) was excluded from the calculations of E_a and ABT.

Differences were considered significant if the probability level of Type I error was <0.05. Results are expressed as percentages or mean values ± S.E. unless indicated otherwise.

Results

Temperature dependence of aerobic metabolism

Effects of acclimation to 4°C and 13°C on the rates of oxygen consumption were different between White Sea and North Sea snails. In North Sea *L. saxatilis*, cold acclimation led to a significant increase of routine respiration rates ($F_{1,7}$=12.40, P=0.01), whereas in White Sea *L. saxatilis* no differences were observed between cold- and warm-acclimated snails in the rates of aerobic metabolism ($F_{1,7}$=2.52, P=0.11) (Fig. 1). In both populations, respiration rates increased with temperature in the range between 0°C and 28°C. At the highest experimental temperature of 32°C, a significant decrease of respiration rates was observed in all experimental groups except for the cold-acclimated North Sea snails (Fig. 1). In all experimental groups, exposure to 32°C led to the onset of heat coma characterized by the loss of contractility of the foot muscle and the characteristic inward curling of the lateral foot area (Clarke et al., 2000a,b). Heat coma was fully reversible upon return of the snails to their respective control conditions (I. M. Sokolova and H.-O. Pörtner, personal observations).

Between-sea comparisons of the rates of aerobic metabolism

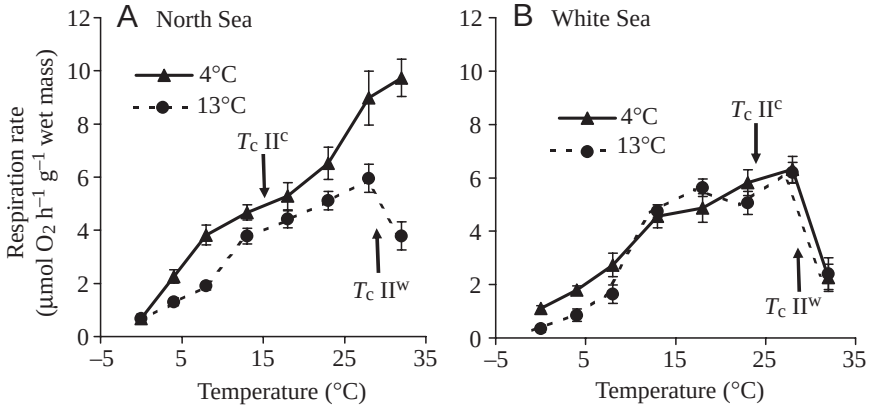

Fig. 1. Temperature dependence of the respiration rates in (A) North Sea and (B) White Sea *Littorina saxatilis* acclimated to 13°C and 4°C. High critical temperatures (T_c II^c and T_c II^w in cold- and warm-acclimated animals, respectively) are indicated by arrows. These are the temperatures where an onset of anaerobiosis occurred as determined by significant succinate accumulation compared with the respective control level (see Fig. 3). N=6–16 for each data point.

revealed a complex picture in *L. saxatilis*. The two-way mixed model ANOVA demonstrated a significant effect of interaction of the factors 'sea' and 'exposure temperature' on the respiration rate, thus preventing further analysis of the single-factor effects. Instead, *post-hoc* tests were used to compare metabolic rates in White Sea and North Sea animals at each of the test temperatures. In general, differences in aerobic metabolic rates between White and North Sea snails acclimated to 13°C were small and statistically insignificant (P>0.05). After the acclimation to 4°C, North Sea animals tended to have higher rates of aerobic metabolism at all test temperatures compared with White Sea snails. However, due to the high variation of respiration rates, these differences were only significant at 8°C, 28°C and 32°C (P<0.03). It is worth noting that at the highest temperatures (28–32°C and 32°C in cold- and warm-acclimated animals, respectively), White Sea animals had significantly lower rates of oxygen consumption than did their North Sea counterparts (P=0.03–0.0001).

Arrhenius breakpoint temperatures (ABTs), indicating discontinuity in the temperature dependence of aerobic metabolic rates, varied between 8°C and 18°C in different experimental groups. In North Sea snails, the ABTs were 8°C and 13°C in cold- and warm-acclimated animals, respectively. In White Sea snails, ABTs were 18°C and 10.5°C for cold- and warm-acclimated animals, respectively. The considerable difference in the ABTs between warm- and cold-acclimated groups from the White Sea was unexpected because the respiration rates did not differ significantly between these two groups at any experimental temperature (see above). This probably reflects a lower activation energy of aerobic respiration (E_a) in the range of 0–8°C in the cold-acclimated animals from the White Sea and a less sharp transition to the 'temperature-independent' zone of metabolism (approximately 13–28°C; Fig. 1). The ABT calculated for the pooled data, including the warm- and cold-acclimated animals from the White Sea, was 10.5°C. In general, the increase of respiration rates with temperature was considerably slower beyond the ABT in all experimental groups from both locations. This was reflected in a significantly lower E_a at higher temperatures (P<0.05, d.f.=37–55): the E_a comprised 70–140 kJ mol^{-1} in the temperature range between 0°C and the respective ABT and 10–35 kJ mol^{-1} beyond the ABT (Fig. 2).

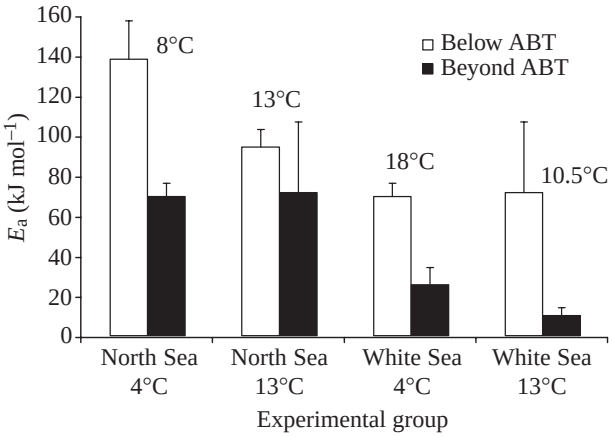

Fig. 2. Activation energy of aerobic respiration in North Sea and White Sea *Littorina saxatilis* acclimated to 13° and 4°C. Arrhenius breakpoint temperatures (ABTs) are given above the respective columns. Activation energy (E_a) values below and above the respective ABTs were determined in the temperature range 0–28°C. E_a values beyond ABT were significantly lower than E_a values above the ABT in all experimental groups (P<0.05). On the x-axis, the population of origin (North Sea *vs* White Sea) and acclimation temperatures (4°C *vs* 13°C) are indicated.

There was a trend for lower E_a values in White Sea animals as compared with their North Sea counterparts (Fig. 2).

Critical temperatures: onset of anaerobiosis and changes of cellular energy status

Exposure to high temperatures led to an onset of anaerobic metabolism in *L. saxatilis*, as indicated by considerable succinate accumulation in the foot muscle tissue (Fig. 3). Succinate was the only anaerobic end product accumulated in *L. saxatilis* under these conditions. No lactate, acetate, propionate or alanine accumulation was found. Incubation for 10 h or 26 h at the respective control temperatures did not lead to changes in the concentrations of succinate in White Sea or North Sea *L. saxatilis* (t-tests, P>0.14 in all comparisons, N=4–6, data not shown), suggesting that the temperature increase and not the incubation *per se* was responsible for the switch to anaerobiosis. Thus, the temperatures at which

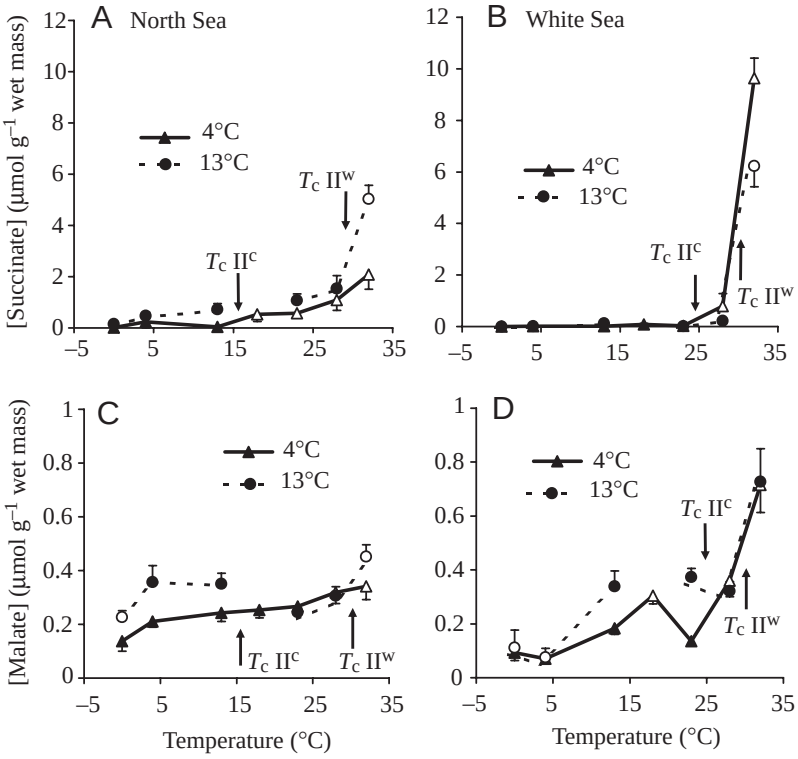

Fig. 3. Changes in (A,B) succinate and (C,D) malate levels in foot muscle tissue of *Littorina saxatilis* during temperature incubations. High critical temperatures (T_c II^c and T_c II^w in cold- and warm-acclimated animals, respectively) are indicated by arrows. These are the temperatures where an onset of anaerobiosis occurred as determined by significant succinate accumulation compared with the respective control level. Open symbols denote values that are significantly different from the respective controls (P<0.05). N=5–10 for each data point.

succinate accumulation significantly exceeded the respective control levels were designated as the high critical temperatures (T_c II). No low critical temperatures (T_c I) were detected within the studied temperature range in *L. saxatilis*.

Acclimation to low temperatures resulted in a considerable downward shift of the critical temperatures (T_cs) in North Sea *L. saxatilis* from 28°C in the warm-acclimated animals to 15°C in the cold-acclimated animals (Fig. 3A). In the White Sea animals, the T_cs changed only slightly with cold acclimation from 32°C to 28°C (Fig. 3B). It is worth noting that at and above the respective T_cs, the level of succinate accumulation was higher in White Sea animals compared with their North Sea counterparts. In addition to succinate accumulation, an increase in malate levels was observed at higher temperatures in *L. saxatilis* (Fig. 3C,D). By contrast, low temperatures (0–4°C) caused the malate concentrations to fall below the respective control levels in warm-acclimated animals from the two studied sites. In the cold-acclimated animals, the malate content of the foot muscle was similar in the control and at 0°C.

Incubation at high temperatures resulted in an impairment of cellular energy status, as visualized by the depletion of high-energy phosphates [the phosphagen phospho-L-arginine (PLA) and ATP] in *Littorina*. In general, North Sea animals tended to have higher control levels of PLA compared with their White Sea counterparts (3.3–3.1 μmol g^{-1} wet mass *vs* 2.7–2.8 μmol g^{-1} wet mass in North Sea and White Sea snails, respectively). However, these differences were only statistically significant in the cold-acclimated group (P=0.007). The concentrations of PLA were higher in the warm-acclimated animals from the White Sea compared with their cold-acclimated counterparts ($F_{1,5}$=16.98, P=0.009). In North Sea animals, acclimation temperature did not significantly affect PLA concentrations ($F_{1,5}$=2.93, P=0.15), although a similar trend is visible. The PLA concentration decreased significantly below the respective control level at or just below the critical temperatures (Fig. 4A,B). In parallel with the decrease of the PLA levels, the concentration of the respective aphosphagen (L-arginine) increased (Fig. 4C,D). The relative proportion of PLA (R_{PLA}) in the total phosphagen/aphosphagen pool declined at or just below the T_cs, reflecting PLA depletion (Fig. 5A,B). Characteristically, the R_{PLA} increased at low temperatures (0–4°C), probably due to the lower L-arginine concentrations in the animals incubated in the cold (Fig. 4C,D).

ATP concentrations were similar in control animals from the White Sea and the North Sea (P=0.10–0.50) and ranged between 1.7 μmol g^{-1} wet mass and 2.2 μmol g^{-1} wet mass. Similar to PLA, and as found in a previous study on fish (cf. van Dijk et al., 1999), ATP concentrations were significantly higher in warm-acclimated snails compared with their cold-acclimated counterparts ($F_{1,5}$=7.91, P=0.04, and $F_{1,5}$=273.7, P<0.001, for North Sea and White Sea animals, respectively). Changes in intracellular ATP concentration in *L. saxatilis* closely followed the depletion of the phosphagen pool during temperature incubations (Fig. 5C,D), but a significant drop in ATP levels generally occurred later than the decrease of PLA concentrations (cf. Figs 4, 5).

Discussion

Due to their semi-terrestrial mode of life, intertidal marine gastropods *Littorina saxatilis* experience fast and frequent temperature changes, both predictable (e.g. associated with the circadian, circatidal and seasonal variations) and unpredictable (i.e. irregular short-term changes that are especially pronounced during low tide due to the low thermal buffering capacity of the air). Besides, foraging times and food abundance are frequently limiting in the intertidal compared with subtidal levels, posing energy constraints and requiring a tight matching of energy demand and energy supply in intertidal inhabitants (Branch et al., 1988). As a result,

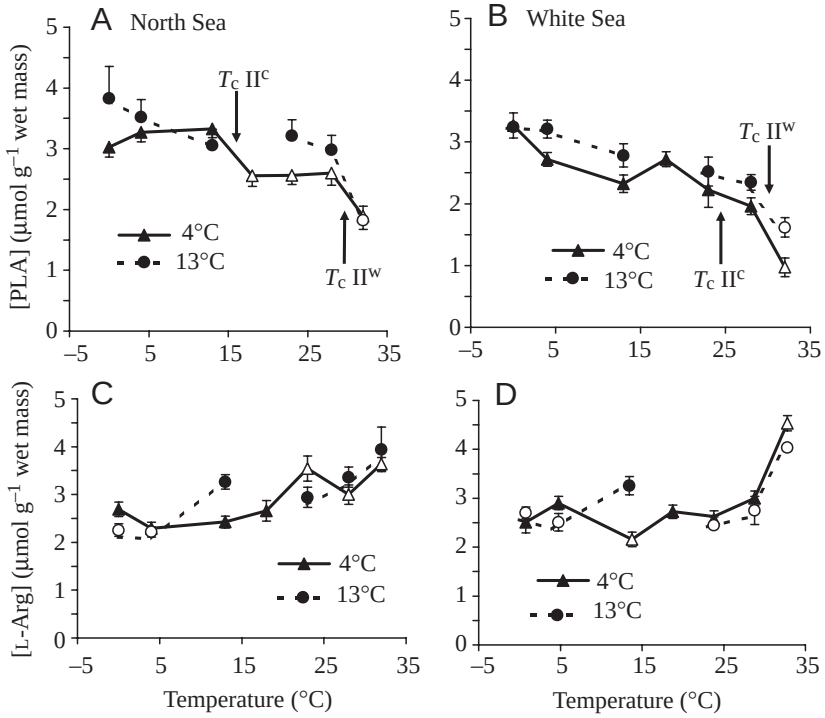

Fig. 4. Changes in (A,B) phospho-L-arginine (PLA) and (C,D) L-arginine (L-Arg) levels in foot muscle tissue of *Littorina saxatilis* during temperature incubations. High critical temperatures (T_c II^c and T_c II^w in cold- and warm-acclimated animals, respectively) are indicated by arrows. These are the temperatures where an onset of anaerobiosis occurred as determined by significant succinate accumulation compared with the respective control level. Open symbols denote values that are significantly different from the respective controls (P<0.05). N=5–10 for each data point.

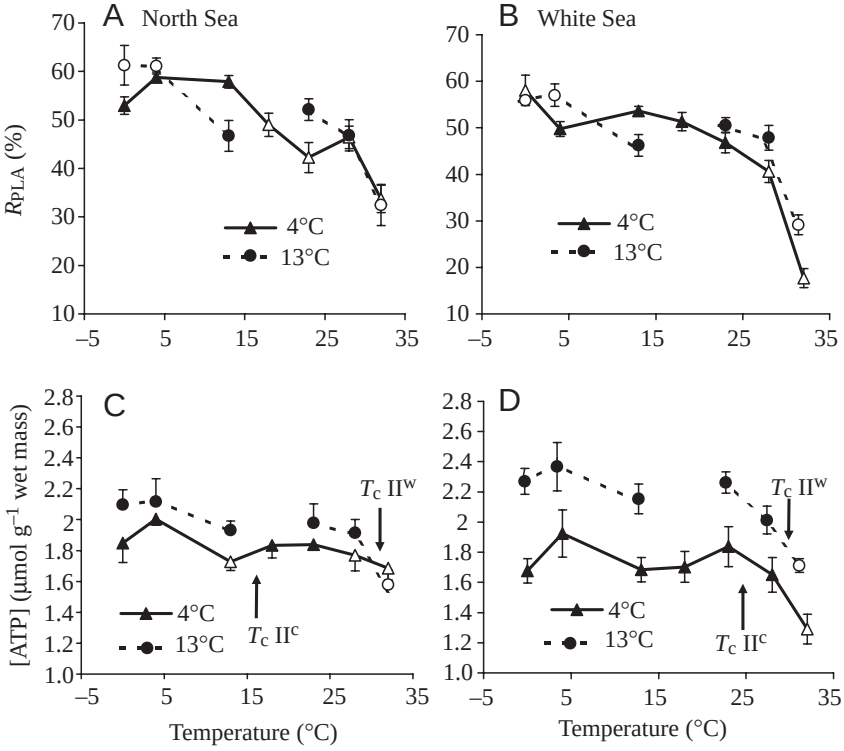

Fig. 5. Changes in the (A,B) phosphagen/aphosphagen ratio (R_{PLA}) and (C,D) ATP levels in foot muscle tissue of *Littorina saxatilis* during temperature incubations. High critical temperatures (T_c II^c and T_c II^w in cold- and warm-acclimated animals, respectively) are indicated by arrows. These are the temperatures where an onset of anaerobiosis occurred as determined by significant succinate accumulation compared with the respective control level. Open symbols denote values that are significantly different from the respective controls (P<0.05). N=5–10 for each data point.

adaptation to intertidal life in *L. saxatilis* entails high metabolic flexibility and efficient regulation of metabolism in a wide range of environmental temperatures.

Acute temperature effects and the zone of 'temperature independence' of metabolism

The respiration rate of *L. saxatilis* rose quickly with increasing temperatures until a point at or slightly above the respective acclimation temperature was reached. Beyond this value, thermal sensitivity of $\dot{V}_{O_2}$ was reduced in a wide, 15°C range of experimental temperatures. This change in metabolic regulation was also reflected in the ABTs of respiration rates (8°C for cold-acclimated North Sea animals and 11–13°C for all other groups). The E_a of aerobic metabolism was approximately seven times lower above the ABT as compared with the E_a below the ABT, reflecting diminished thermal dependence of aerobic metabolism. It should be noted that both the control temperature (13°C) used in our experiments as well as the ABTs were close to water temperatures at the time of collection (September at the White Sea and October at the North Sea) and were in the lower range of environmental temperatures experienced by the snails in early autumn. Air and substrate temperatures during low tide could be much higher (>20°C), especially on sunny days (I. M. Sokolova, personal observation). Comparison with other gastropod species suggests that a temperature insensitivity of aerobic metabolism at the high end of ambient temperatures is typical for intertidal gastropods while not observed in their subtidal counterparts. Thus, a temperate subtidal limpet *Patella oculus* demonstrates a high temperature dependence of aerobic metabolism over a wide range of environmental temperatures (10–25°C) with a Q_{10} of 2.5–3.9, whereas in the two intertidal limpets *Patella granularis* and *Patella cochlear*

the metabolic rate changes very little over the same environmental temperature range (Q_{10}=1.3–1.9) (Branch et al., 1988). In the intertidal tropical gastropods *Nodilittorina interrupta*, *Littoraria irrorata* and *Siphonaria pectinata* living at ambient water temperatures of 28–30°C, the $\dot{V}_{O_2}$ was practically temperature independent between 25°C and 40°C but changed considerably with temperature in the subtidal snail *Stramonita haemastoma* from the same area (McMahon, 1992). Interestingly, even among Antarctic species, the intertidal gastropod *Nacella concinna*, which experiences fluctuating temperatures during daytime, had a temperature-independent metabolic rate over a temperature range close to its environmental range (–1.7°C to +0.5°C), in contrast to the subtidal species *Pelilittorina setosa* and *Trophon* sp., which showed a significant increase in $\dot{V}_{O_2}$ with increasing temperature (Houlihan and Allan, 1982). Possibly, the relative thermal independence of metabolic rate at high temperatures can be adaptive for intertidal animals, allowing them to minimize energy expenditure during fast and frequent temperature fluctuations at low tide.

In molluscs, high thermal sensitivities of metabolism within the environmental range were associated with increased long-term metabolic costs and with a lower tolerance to extreme temperatures (Hawkins, 1995), suggesting that a reduced temperature dependence of metabolic rate can be selectively advantageous in thermally unstable intertidal environments. Thermal insensitivity of metabolism may also reflect progressive metabolic depression at high temperatures in *L. saxatilis*. Whichever is the case, such an 'energy conservation' strategy can be especially important for high shore species such as *L. saxatilis* that are resource limited due to the short feeding periods that are generally restricted to a short period around high tide when the substrate is wet. By contrast, subtidal species are not food limited and experience smaller temperature fluctuations than their intertidal counterparts. They adopt an 'exploitative strategy' that allows them to utilize resources at a high rate in order to maximize growth, and their metabolic rates increase with increasing temperature (Branch et al., 1988). The physiological mechanisms allowing intertidal eurytherms to maintain a temperature-independent rate of metabolism in a wide range of environmental temperatures are not known and would be an interesting subject for further studies.

Heat-induced loss of aerobic scope

Acute temperature increase resulted in a considerable metabolic disturbance in *L. saxatilis* at critically high temperatures indicated by the onset of anaerobic metabolism and the adverse changes in the cellular energy status. In warm-acclimated *L. saxatilis* from the White Sea and the North Sea, the onset of anaerobiosis between 28°C and 32°C coincided with entering heat coma, characterized by the loss of nervous integration and muscle relaxation (Clarke et al., 2000a). The onset of heat coma in gastropods can be easily determined by the loss of contractility of the foot muscle and the characteristic inward curling of the lateral foot area (Clarke et al., 2000a,b). In cold acclimated *L. saxatilis*, heat coma was also observed at 32°C, although the onset of anaerobiosis occurred at lower temperatures (18°C and 28°C in North Sea and White Sea snails, respectively). The heat coma temperature (HCT) in our experiments was within the range of the mean HCTs reported for *L. saxatilis* from some North Atlantic populations (30.8–31.7°C) (Clarke et al., 2000b).

It has been suggested that neuromuscular failure in ectotherms may become detrimental later than oxygen limitation due to more extreme denaturation temperatures of proteins in general, including those involved in neuromuscular functions (Pörtner, 2001; Sokolova and Pörtner, 2001b). On the other hand, extreme hypoxia may also contribute to limiting neural function, in line with a suggested systemic to molecular hierarchy of thermal limits (Pörtner, 2002). Our data suggest that in an extremely eurythermal species such as *L. saxatilis*, the neuromuscular failure indicated by the onset of heat coma and oxygen limitation may go hand in hand, at least in warm-acclimated animals with wide temperature windows of aerobic scope. Unlike most vertebrate ectotherms, the neuromuscular failure associated with heat coma is a fully reversible condition in gastropods (Clarke et al., 2000a,b; I. M. Sokolova, personal observations), and entry into heat coma generally occurs at temperatures well below acute lethal limits (45–47°C in *L. saxatilis*; Sokolova et al., 2000a). This may reflect the lower level of integration in invertebrates and relatively smaller role of the central nervous system in survival of acute environmental stress in these organisms.

Heat coma and the associated neuromuscular failure at extremely high temperature (32°C) are likely to contribute to capacity limitations and, finally, disintegration of oxygen uptake and/or delivery systems. In marine molluscs, the ventilation of the mantle cavity is maintained by ciliary activity, which may be reduced upon entry into heat coma and may decrease oxygen delivery (Sandison, 1967). It is not unreasonable to assume that the loss of neural integration would also interact with a loss in cardiac function, although direct evidence for this is absent for marine gastropods. In general, the contention that neuromuscular failure is involved in the development of oxygen limitation and associated metabolic disturbances would not be all that surprising. However, our data suggest that the heat coma *per se* is not a primary mechanism of oxygen limitation in *Littorina*. First of all, in cold acclimated *L. saxatilis*, the onset of anaerobiosis occurred at temperatures well below HCT (31–32°C), implying that other physiological limitations are involved. Secondly, the depletion of the phosphagen PLA was observed below the HCT in both warm- and cold-acclimated snails, indicating progressive discrepancy between energy demand and supply that is not related to neuromuscular failure. A decline in PLA levels was closely paralleled by the onset of anaerobiosis, suggesting that temperature-induced anaerobiosis was linked to insufficient energy production to cover high energy demands at elevated temperatures. In support of these considerations, there was a significant negative correlation between respiration rate and succinate

accumulation at 32°C in *L. saxatilis* (r=–0.971, N=4, P=0.029), supporting the notion that anaerobiosis is involved in the compensation for insufficient aerobic energy production at elevated temperatures.

It is interesting to note that at extremely high temperatures (32°C), White Sea *L. saxatilis* had significantly lower respiration rates associated with considerably higher levels of succinate accumulation as compared with their North Sea counterparts. This contrasts with findings in the polychaete *A. marina*, where both higher rates of oxygen turnover and higher levels of succinate were found in White Sea compared with North Sea specimens at extreme temperatures (Sommer and Pörtner, 2002). High levels of succinate accumulation were previously reported for three *Littorina* spp. (Sokolova et al., 2000b), the blue mussel *Mytilus edulis* (Sukhotin and Pörtner, 1999) and *A. marina* (Sommer et al., 1997) from the White Sea as compared with their temperate counterparts, indicating a higher reliance on mitochondrial anaerobic metabolism at critical temperatures in the sub-arctic White Sea animals. Nonetheless, anaerobic metabolism was not sufficient to keep up with the energy demand at high temperatures, as indicated by the progressive decline in ATP levels.

Temperature-induced mitochondrial anaerobiosis in *L. saxatilis* is probably brought about by insufficient oxygen supply to tissues before disruption of mitochondrial function occurs at critically high temperatures. At first sight, dysfunction of the electron transport systems and/or denaturation of key aerobic mitochondrial enzymes at extremely high temperatures might elicit the transition to anaerobiosis and the formation of anaerobic end products even in mitochondria that are not oxygen limited. However, in fish and bivalves, the respiration rate of isolated mitochondria was not impaired over a broad temperature range that was well beyond the critical temperatures and even lethal temperature limits, suggesting that aerobic scope at critical temperatures is not limited by the thermal intolerance of mitochondrial function (Weinstein and Somero, 1998; Pörtner et al., 1999 on *Laternula elliptica* gill mitochondria; Hardewig et al., 1999; van Dijk et al., 1999). We do not have data on the heat stability of isolated mitochondria in *L. saxatilis.* However, denaturation temperatures of key mitochondrial enzymes in this species are far beyond the critical temperatures of aerobic scope and range between 40°C and 45°C (Sokolova and Pörtner, 2001b). This agrees with the findings of high thermal tolerance of mitochondrial enzymes and isolated mitochondria in other species as compared with the whole-organism thermal limits (Weinstein and Somero, 1998; Pörtner et al., 1999; Hardewig et al., 1999; van Dijk et al., 1999).

Interestingly, indirect evidence that the heat-induced anaerobiosis in *L. saxatilis* is due to insufficient oxygen supply to tissues is also provided by the comparison of aerobic and anaerobic metabolic rates in water *vs* air in this amphibious species. This specialized high-shore gastropod possesses a vascularized area of mantle cavity that can serve as a lung in addition to a ctenidium and is equally well adapted to breathe atmospheric and water-born oxygen (Fretter and Graham, 1976; McMahon, 1988). However, ambient oxygen levels in air are approximately 30 times higher than in seawater, implying higher oxygen availability and lower costs of ventilation in air-breathing animals. During prolonged air exposure at 30°C, more than 98% of total (aerobic and anaerobic) ATP turnover in *L. saxatilis* was supplied by aerobic pathways (Sokolova and Pörtner, 2001a). Moreover, succinate accumulation, which indicates mitochondrial anaerobiosis, was very low, and the main anaerobic end product in air was alanine (Sokolova and Pörtner, 2001a), suggesting that mitochondria remained predominantly aerobic, and anaerobic metabolism was mostly restricted to the cytosolic compartment (Grieshaber et al., 1994). Succinate accumulation contributed only 5–12% to anaerobic ATP turnover during air exposure at high temperatures in *L. saxatilis*, the rest being supplied by alanine accumulation and depletion of high-energy phosphates. By contrast, exposure of *L. saxatilis* to similar temperatures in water led to enhanced levels of succinate accumulation, indicating more severe mitochondrial anaerobiosis. Anaerobic metabolism contributed approximately 2.5% to total (aerobic and anaerobic) ATP turnover in 4°C-acclimated North Sea animals and 16–43% in other experimental groups (Fig. 6). A low anaerobic contribution in cold-acclimated North Sea animals reflects exceptionally high aerobic metabolic rates at high temperatures in this experimental group. Succinate accumulation accounted for 76–97% of total anaerobic ATP turnover rate ($\dot{M}_{ATP}$) during temperature-induced anaerobiosis of *L. saxatilis* in water (Fig. 7). This comparison suggests that the temperature-induced limitation of aerobic scope in *L. saxatilis* is alleviated in air, probably due to the higher oxygen availability in this milieu. This also supports our proposition that the transition to partial mitochondrial anaerobiosis at

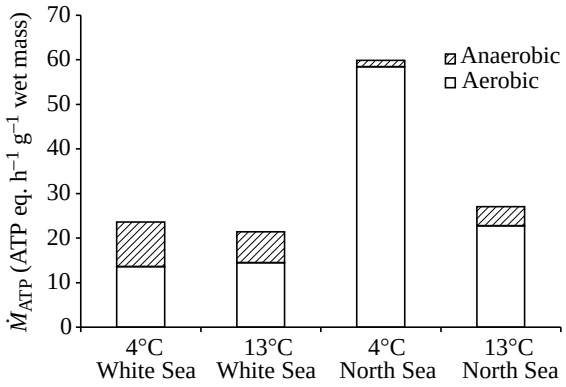

Fig. 6. Aerobic and anaerobic ATP turnover rates ($\dot{M}_{ATP}$) at 32°C in North Sea and White Sea *Littorina saxatilis* acclimated at 13°C and 4°C. The rate of ATP turnover during anaerobiosis (amount of ATP consumed per g wet mass per hour) was calculated from end product accumulation and ATP and phospho-L-arginine (PLA) depletion as described in the text, assuming an ATP equivalent of 2.75 μmol per μmol of succinate. The aerobic ATP turnover rate was calculated from routine oxygen consumption rates, assuming that 6 mol ATP produced 1 mol O_2.

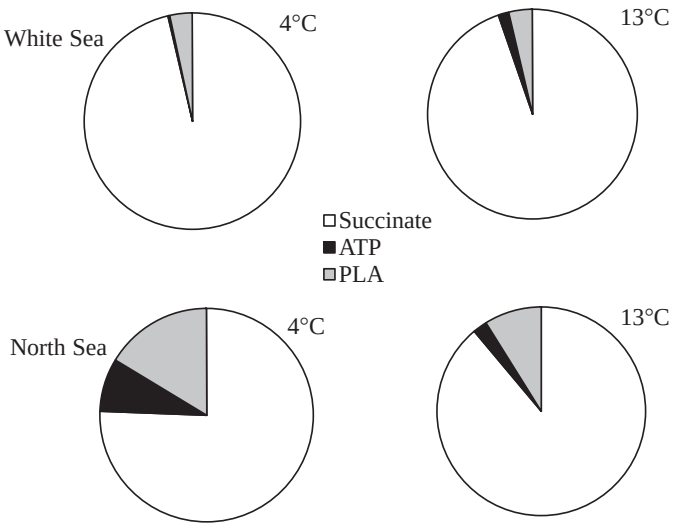

Fig. 7. Relative contribution of succinate accumulation and depletion of high-energy phosphates to anaerobic ATP turnover in *Littorina saxatilis* at 32°C in water. Upper row, White Sea snails; lower row, North Sea snails.

critically high temperatures in *L. saxatilis* is caused by insufficient oxygen uptake and/or delivery rather than by the heat-induced disruption of mitochondrial or neural function. It is worth noting that, in order to test this hypothesis and further elucidate the role of variable oxygen concentration in heat-induced anaerobiosis of this species, additional studies involving experimental manipulations of oxygen content in the medium are required.

Effects of latitudinal adaptation vs *cold acclimation on metabolism*

The rate of aerobic metabolism was similar in White Sea and North Sea animals acclimated at the control temperature (13°C), indicating no metabolic cold adaptation in respiration rates in sub-arctic *L. saxatilis*. These results contrast with our previous findings that the activity of several key metabolic enzymes is higher in White Sea *L. saxatilis* than in their North Sea counterparts (Sokolova and Pörtner, 2001b). Interestingly, similar results were reported for pectinid bivalves, where a considerably higher activity of a key aerobic enzyme, citrate synthase, in a cold-adapted Antarctic scallop (*Adamussium colbecki*) compared with its temperate counterpart (*Aequipecten opercularis*) did not result in a compensation of the aerobic metabolic rate at the whole organism level (Heilmayer et al., 2002). In the roach *Rutilus rutilus*, seasonal cold acclimatisation and cold acclimation led to a compensatory increase in the activity of several key metabolic enzymes and of Na^+/K^+-ATPase but did not result in the overall increase of the metabolic rate (Koch et al., 1992). This shows that increased enzymatic capacity is not always directly translated into increased metabolic flux and emphasizes the complex relationships between enzyme capacity and the regulation of enzyme in the whole-animal.

Metabolic responses to cold acclimation differed in *L. saxatilis* from the North and the White Seas. In the temperate North Sea animals, cold acclimation led to an increase in aerobic metabolic rates and the concordant downward shift of the critical temperature by nearly 15°C. It should be noted that an increase in the respiration rate as a result of cold acclimation is a well-documented phenomenon in aquatic ectotherms and matches an increase of mitochondrial volume density and/or capacity (Egginton and Sidell, 1989; Guderley, 1998; St-Pierre et al., 1998). At least in eurytherms, increased mitochondrial density leads to a rise in the baseline energy demand due to the high costs of mitochondrial maintenance that are no longer met by oxygen uptake at higher temperatures, and the higher the density of mitochondria (other things being equal), the lower is the critical temperature when oxygen uptake and/or delivery becomes insufficient and onset of anaerobiosis is observed (Pörtner, 2001). It is plausible to suggest that the simultaneous increase in the $\dot{V}_{O_2}$ and the downward shift in the T_c in eurythermal North Sea *L. saxatilis* may have a similar physiological background. A downward shift of T_c associated with increased mitochondrial densities has been previously shown in the cold-adapted lugworms *A. marina* (Sommer et al., 1997; Sommer and Pörtner, 2002). By contrast, cold acclimation in the White Sea *L. saxatilis* did not significantly affect $\dot{V}_{O_2}$ or T_cs of aerobic scope, suggesting that cold acclimation may have had no effect on mitochondrial oxygen demand in these animals.

As a corollary, T_cs characterized by the onset of anaerobiosis proved to be very plastic and responsive to cold acclimation in North Sea *L. saxatilis* but not in their White Sea counterparts. In White Sea snails, the temperature window of aerobic scope was very wide and remained practically unchanged by cold acclimation. There was also no effect of cold adaptation or acclimation on aerobic metabolic rates in sub-arctic *L. saxatilis* compared with their temperate counterparts. These evidently opposing strategies may be related to the differences in winter environmental conditions between the two study sites. At the North Sea site, the winter temperatures are approximately 3–6°C, and intertidal animals usually remain active, feed and grow throughout the winter (Hickel et al., 1997; Janke, 1997; I. M. Sokolova, personal observation). A compensatory increase in metabolic rate due to cold acclimatisation in these animals could help them to maintain relatively high activity levels at low temperatures. By contrast, in the White Sea, the winter is associated with extremely low temperatures (down to –1.5°C) and greatly diminished food availability due to light-limited algal growth in this sub-arctic area (Matveeva, 1974; Babkov, 1998). White Sea *Littorina* spend the winter in an inactive state in low intertidal and subtidal horizons, where they migrate during late autumn (Matveeva, 1974; Galaktionov, 1993; I. M. Sokolova, personal observations). Accordingly, they do not demonstrate a compensatory increase in respiration during cold acclimation (this study). A depression of metabolic rates, which correlates with reduced levels of feeding and general activity, was observed in many marine and freshwater molluscs in winter (Innes and Houlihan,

1985) and is consistent with an important role of hypometabolism as a survival strategy during prolonged periods of cold exposure or resource limitation. This strategy is found across a wide variety of phyla, from resting bacterial spores through estivating and dormant invertebrates to hibernating mammals (Hochachka and Guppy, 1987). In general, the intriguing differences in the metabolic response to acclimation between *L. saxatilis* from different latitudes call for further investigation in order to establish the different effects of cold acclimation on metabolic rates at the cellular and sub-cellular level, e.g. with respect to mitochondrial densities and capacities. These findings also warrant further investigations on populations from a wide range of latitudes and thermal habitats in order to allow generalizations about the effects of latitude on critical temperatures of aerobic metabolism and metabolic plasticity in eurythermal invertebrates.

In conclusion, our study demonstrates that temperature-induced oxygen limitation also occurs in an extremely eurythermal intertidal invertebrate, suggesting that mitochondrial hypoxia and anaerobiosis at high temperatures are a common feature of ectotherm physiology (cf. Pörtner, 2001, 2002). The heat-induced onset of anaerobiosis is presumably due to the limited capacity of oxygen uptake and transport mechanisms rather than a result of neural and/or mitochondrial dysfunction and can (partly) be alleviated in the presence of high ambient concentrations of oxygen, e.g. in air. The heat-induced metabolic disturbances lead to a progressive discrepancy between energy demand and energy supply and result in a time-limited situation, which can prove lethal if the adverse temperature conditions persist. It should be noted that extreme temperatures ranging between 35°C and 45°C are not uncommon in the natural habitats of *L. saxatilis* in summer even in the sub-arctic White Sea area (Sokolova et al., 2000a), suggesting that the metabolic machinery may function close to its physiological limits in this extremely eurythermal species. This also implies that *L. saxatilis* populations may become vulnerable to the global temperature change, especially in such ecologically marginal habitats as high shore levels characterized by extreme temperature fluctuations (Sokolova et al., 2000a,b). Notably, Stillman and Somero (2000) arrived at a similar conclusion in their study of upper thermal limits of 20 species of crabs of genus *Petrolisthes*, which suggested that the upper thermal tolerance limits of some intertidal species may be near current habitat temperature maxima, and global warming therefore may affect the distribution limits of intertidal species to a greater extent than their subtidal counterparts (Stillman and Somero, 2000). Differences in the metabolic response to temperature acclimation in White Sea and North Sea *Littorina* shown in this study require further investigation and emphasize the importance of taking into account other relevant factors (e.g. seasonal amplitude of temperatures, food availability, activity levels of animals) when studying metabolic adaptations to temperature.

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