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How does oxidative stress relate to thermal tolerance in the Antarctic bivalve *Yoldia eightsi*?

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Abstract: Short and long-term exposure to elevated temperatures were studied in the Antarctic stenothermal protobranch bivalve *Yoldia eightsi* (Courthouy) from Potter Cove, King George Island (South Shetland Islands, Antarctica). Above a breakpoint temperature of 2°C – the upper habitat temperature for the Potter Cove *Y. eightsi* stock – both routine (RMR) and standard metabolic rate (SMR) increased steeply. The fraction of metabolism allocated to SMR, as well as the number of intervals of elevated activity per hour increased significantly with temperature. During acute exposure, ATP concentrations in the foot muscle peaked at 2°C and fell at 5°C, whereas superoxide dismutase (SOD) activity decreased upon warming. Slow stepwise warming to a final temperature of 11°C resulted in a significant decrease of SOD activity. Malondialdehyde concentration increased compared with controls at 0°C. In contrast to the effect of short-term exposure, tissue adenylate concentrations displayed a mild increase at higher temperatures during slow warming, indicating an acclimation response. A switch to anaerobic energy production could not be observed up to 11°C, demonstrating a higher level of thermal tolerance than in other Antarctic ectotherms, or a failure of the relevant pathways in *Y. eightsi*. The imbalance between pro- and antioxidant processes upon warming indicate oxidative stress to be one feature accompanying early heat stress in *Y. eightsi*.

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Introduction

Temperature is one of the major factors modifying metabolic rates of marine ectotherms (Hochachka & Somero 1984, Kawall & Somero 1996). More specifically, an exponential rise of overall metabolic rate represents a typical response to progressive warming. As an exception to this rule, maintenance metabolism (standard metabolic rate, SMR) can be less dependent on temperature especially in intertidal benthic invertebrates which experience extreme variations of ambient temperature *in situ* (Newell & Pye 1970, Widdows 1973, Newell & Branch 1980). Outside the thermal range of a given species, both routine and maintenance metabolism are bound to increase due to compensatory biochemical reactions of the animals, as well as to elevated costs of metabolic maintenance at high temperatures (Tremblay *et al.* 1998).

In the present study, exposure to unnaturally high temperatures has been undertaken with the Antarctic stenothermal bivalve *Yoldia eightsi* to determine if thermal stress induces oxidative stress. Oxidative stress could be a factor that, in combination with energetic constraints resulting from the oxygen limitation of thermal tolerance (Pörtner *et al.* 1998, 2000, Frederich & Pörtner 2000), might contribute to thermal limitations in ectothermal organisms. Oxidative stress has frequently been related to thermal stress (Lesser *et al.* 1990, Lesser 1997, Nii & Muscatine 1997, Pellerine-Massicotte & Araujo 1997, Abele *et al.* 1998a, 1998b). The mechanisms linking both forms of stress probably relate to elevated oxygen consumption in ectothermal animals upon warming and also to the possible development of hypoxia in body fluids (e.g. Frederich & Pörtner 2000). Elevated metabolic rates reflect an increase in mitochondrial oxygen turnover (Shick & Dykens 1985). Mitochondria have generally been accepted as major cellular sources of reactive oxygen species (primarily superoxide anion radicals and hydrogen peroxide) and have been estimated to convert between 1 and 3% of their entire oxygen consumption to these highly reactive toxic oxygen derivatives (Richter 1995, Sohal & Weindruch 1996). At the same time, hypoxia has been found to increase oxidative stress owing to enhanced autooxidation of highly reduced mitochondrial electron transporters (Boveris & Chance 1973). Since warming elicits increased mitochondrial oxygen turnover and progressive hypoxia in body fluids it is therefore bound to enhance oxidative stress.

If oxidative stress occurs, this may be due either to metabolic formation of active oxygen species, or to thermal inactivation of the cellular antioxidant defence system (Storey 1996, Abele *et al.* 1998a). Oxidative stress is critical to membrane integrity and may cause a disturbance of subcellular structures and of cellular homeostasis in general (Halliwell & Gutteridge 1985). As a consequence, oxidative damage promotes cell

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death and eventually limits the survival of an animal under stressful conditions. A general review of oxidative stress in cold environments is given by Viarengo *et al.* (1998).

During two Antarctic summer expeditions in 1996/97 and 1998/99, we investigated the effect of temperature increments on the protobranch bivalve *Yoldia eightsi*. We focused on respiration and energy metabolism, as well as on oxidative stress parameters as acute and acclimated responses to heat stress. *Yoldia eightsi* is a bioturbate deposit feeder, which ingests sediment particles into the mantle cavity with the help of the palp probosces (Davenport 1988a). According to that author's investigations at Signy Island (South Orkney Islands), *Y. eightsi* can alternatively turn to the suspension feeding mode if phytoplankton is available. During deposit feeding, the animals carry out extended vertical feeding migrations in the sediment, involving intensive locomotory activity. This obviously energy consuming feeding mode distinguishes *Y. eightsi* from other bivalves (cf. Pörtner *et al.* 1999). Routine metabolism of active *Y. eightsi* specimens is characterized by alternating intervals of high and low respiration rates. This enabled us to determine routine metabolic activity (RMR) over the experimental temperature range and to compare this to the standard metabolic activity (SMR) of the animals during inactive resting periods, where metabolic rates were minimal. Standard metabolic rate at a given temperature was thus estimated to reflect the energetic costs of the resting animal at that particular temperature.

The study investigates the thermal sensitivity of *Y. eightsi* from the changes in routine and standard metabolism, as well as the changes in the mode of energy production (aerobic vs anaerobic). Environmental anaerobiosis induces accumulation of succinate and of volatile fatty acids in invertebrate and especially in mollusc tissues (De Zwaan & Wijsman 1976). Analyses of selected parameters characterizing pro-oxidative processes (malondialdehyde) as well as for tissue antioxidant status (superoxide dismutase and catalase) were performed in order to see, whether oxidative stress might exacerbate or even be a first indicator of physiological disorder during the onset of heat stress in cold adapted invertebrates. Malondialdehyde (MDA) is one of the first products of lipid peroxidation and a widely used indicator of oxidative stress in general (Viarengo *et al.* 1991). Although MDA is by no means a terminal end product of the lipid peroxidation process, it has been found to accumulate in tissues of marine invertebrates under oxidative stress, before it is incorporated into lipofuscin particles, more generally known as ageing pigments.

Material and methods

Experimental animals: collection, maintenance and transport of samples

The protobranch bivalve *Y. eightsi* is one of the dominant macrofaunal components in the muddy sediments of Potter Cove, King George Island, South Shetlands (62°14′S, 58°40′W), where it occurs at maximal densities between 5 m (796 ind m^{-2}) and 10 m (753 ind m^{-2}) water depth (Kowalke & Abele 1998). Annual water temperatures in Potter Cove range from -1.3 to +1.4°C (Schloss *et al.* 1998).

Experimental animals were collected during two summer expeditions between January and March 1997 and 1999 with a grab sampler, deployed from an inflatable boat at between 5 and 8 m water depth. Animals were immediately sorted from the muddy sediment and transferred to seawater aquaria at the Argentinean–German research station Jubany. The bottom of each aquarium was covered with a 2 cm thick layer of sediment from the sampling site, and the aquaria were supplied with water from the cove at 34‰ salinity and temperatures between -1° and +1°C. All experiments, as well as respiratory measurements and antioxidant enzyme assays, were carried out at Jubany during the two summer field seasons. Short-term warming implied 48 h of maintenance of experimental animals at the indicated temperature. Animals warmed to 5°C were previously kept for 48 h at 2°C and then heated directly to 5°C.

During the second summer (January–April 1999) the effects of long-term temperature incubations on high energy phosphates were studied in more detail. Animals were acclimated to elevated temperatures between 2° and 9°C in thermostatted aquaria with sediment from the sampling site and unfiltered water from the cove, to prevent food limitation. Temperatures were increased stepwise at 2°C increments at intervals of 10 to 14 days, over a total period of 55 days. Additionally, a control experiment was run at 0°C covering the same time frame (60 days). Tissues were collected from recently dissected animals and frozen by use of liquid nitrogen or dry ice.

In March 1999, foot tissue samples from both experimental and control animals were shipped to Bremerhaven at -80 °C, with the support of British Antarctic Survey, for the analysis of the high energy phosphates and of volatile fatty acids. The ratio of ATP over ADP and AMP levels showed that adenylates were well preserved in the frozen materials during transport to Bremerhaven (cf. Figs 2 & 4).

Respirometry

Measurements of aerobic metabolic rates in response to short-term temperature increments were carried out in an Eschweiler flow-through respirometer in 1997. The respirometer was run in a thermostatted water bath with filtered (0.2 µm) seawater at 34‰; temperature could be increased in 1°C steps.

Individual animals (0.9 ± 0.4g fresh mass) were placed in a respirometer chamber of 18 ml volume at a constant flow rate of 0.9 ml min^{-1}. Oxygen concentrations were recorded at the entrance and the exit of the chamber with two polarographic electrodes connected to an M200 oxymeter (Fa. Eschweiler, Germany) and to a Linseis two-channel chart recorder. The electrodes were calibrated in a saturated Na_2SO_3 solution (0% oxygen) and in fully aerated seawater at a constant temperature

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of 1°C. Before and after each measurement, the system was checked for electrode drift and for microbial oxygen consumption in the empty chamber. Pre-experiments were run with a larger chamber (25 ml volume) to check for the effect of sediment (quartz sand) on animal respiration. Burial in the sediment had no distinct effect on respiration rates of individual specimens so that in all subsequent measurements animals were placed into the chamber without sediment.

Oxygen consumption was recorded in response to short-term temperature increments of 1°C. Each individual was measured at all temperatures, beginning at -1° and ending at +5°C. Each measuring phase lasted approximately 10 h and the first 2 h of recordings after reaching a new temperature in the system were discarded as acclimation time.

Some animals displayed very stable respiration curves with no major changes over time, while other specimens showed rhythmic sinus-shaped oscillations of the oxygen consumption, reflecting periodic activity peaks. These periodic increases in respiratory activity are related to horizontal movements, carried out by this species in the surface sediment, as observed by Davenport (1988a, 1988b) under field conditions. For these locomotory cycles, a standard metabolism in the resting state could be distinguished from active metabolism. As the horizontal movements are part of the routine activity of unstressed *Y. eightsi*, the overall mean rate of oxygen consumption, comprising both resting and activity metabolism was termed "routine metabolic rate" (RMR). In contrast, SMR refers only to the minimal metabolic activity between locomotory bouts at a given temperature.

Antioxidant enzyme activity and MDA tissue concentrations

Superoxide dismutase (SOD) and catalase activity as well as malondialdehyde (MDA) concentrations in tissue were measured in two metabolically active tissues: the palp probosces tissue which the animal can extend from the shell to search for food particles in the sediment surface, and the foot muscle.

SOD was extracted into 50 mmol l^{-1} Tris-succinate buffer (pH 8.2, 1:3/ w:v) and the activity determined according to Marklund & Marklund (1974). Catalase was extracted into 50 mmol l^{-1} KPi-buffer (pH 7.0, 1:10/ w:v) and measured according to Aebi (1985). All measurements were carried out at a common assay temperature of 20°C as a temperature controlled photometer was not available at Jubany. The data thus represent absolute changes in enzyme activities in animals from different temperature treatments, independent of Q_{10} effects.

Malondialdehyde concentration in tissue was measured spectrophotometrically, using a Calbiochem® Lipid Peroxidation Assay Kit. Freshly obtained tissue was preweighed and minced on ice. Homogenisation was carried out in a small glass homogenizer in ice-cold 20 mmol l^{-1} Tris-HCl buffer (pH 7.4) at a 1: 10 (w:v) tissue to buffer ratio. Subsequently, the homogenates were centrifuged in an Eppendorf 5401 centrifuge at 3000 g and 4°C for 10 min. Duplicate 200 µl samples of the resulting supernatant were used for the MDA assay as described in the kit. Measurements were carried out at 586 nm after a 60 min reaction time at 45°C and subsequent high speed centrifugation (15000 g, 5 min, 4°C). MDA concentrations present in the homogenate were calculated from a 5-point calibration curve.

PCA extracts and measurements of adenylates and anaerobic metabolites

For the determination of adenylates and fatty acid concentrations in *Y. eightsi* foot muscle, PCA (perchloric acid) extracts were prepared following a procedure by Beis & Newsholme (1975). As liquid nitrogen was not available at Jubany in the first field season, tissues were minced with small scissors and homogenized in a glass homogenizer of 2 ml volume on ice. In the second season, aliquots of around 300 mg tissue were ground to a fine powder using a mortar, previously cooled with liquid nitrogen. After addition of 3 volumes precooled 0.6 mol l^{-1} PCA, the tissue was homogenized with a microhomogenizer (Proxxon Minimot 100/P). Subsequent centrifugation in an Eppendorf 5401 centrifuge at 14000 g and 2°C for 4 min removed all precipitated protein. After neutralisation of the supernatant with 5 mol l^{-1} KOH, a second centrifugation removed precipitated potassium perchlorate. Extracts were stored at -80°C until analysis. Both extraction procedures, with and without liquid N_2, were compared and no significant difference was found for test samples between the two methods.

The high energy phosphates were measured spectrophotometrically in an enzymatic test, ATP according to Trautschold *et al.* (1989), ADP and AMP following Jaworek & Welsch (1989). Concentrations of succinate and of the fatty acids propionate and acetate were measured in PCA-extracts using a Dionex LC ion chromatograph according to a method modified after Hardewig *et al.* (1991). Fatty acids were separated on an ion exchange column (Dionex Ion Pac ICE-AS6) using 0.2 mM heptafluorobutyric acid as an eluent at a flow rate of 1 ml min^{-1} at 25 °C. Peaks were monitored with a conductivity detector. A micro membrane suppressor (Dionex AMMS-ICE) regenerated with 5 mmol l^{-1} tetrabutylammonium hydroxide was used to decrease background conductivity.

Data analysis

Respirometry data of RMR and SMR, respectively, were analysed to yield the minimum pooled sum of squares for two regression lines, to identify a breakpoint temperature for both types of metabolism. For the biochemical data, the statistical significance of differences between experimental groups was tested at $P < 0.05$ (significant) and $P < 0.01$ (highly significant) using analysis of variance (two-way ANOVA) followed by a Bonferroni-Dunn post-hoc test for inhomogeneity between groups using a StatView program pack.

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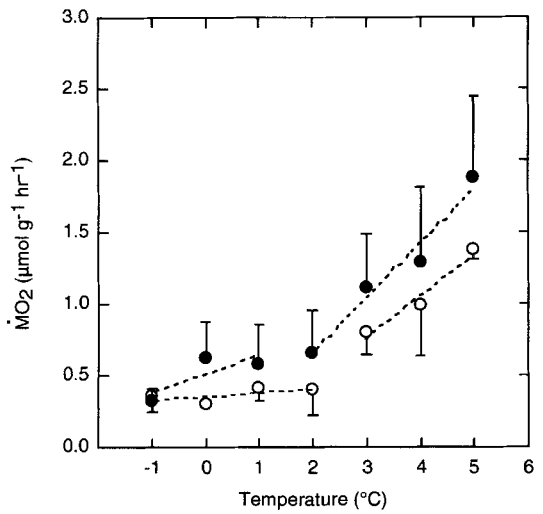


Fig. 1. Metabolic rate (MO_2) of *Y. eightsi.* (whole animals) at various temperatures, normalized to tissue wet weight (n = 5), determined by flow-through respirometry. Black circles depict RMR including phases with elevated locomotory activity, yielding elevated oxygen consumption. Open circles depict baseline metabolism representing SMR of the animals. Mean ± s d from February–March 1997. Regressions are given for the groups of temperature related data which yield the least pooled sum of squares: RMR (-1 to +1): y = 0.127x + 0.51 (r^2: 0.79); RMR (+2 to +5): y = 0.383x – 0.105 (r^2:0.983); SMR (-1 to +2): y = 0.026x + 0.355 (r^2: 0.677); SMR (+2 to +5): y = 0.291x – 0.1078 (r^2: 0.98).

Results

Respiratory and energetic responses to short-term warming

Figure 1 compares standard metabolic rate (SMR) of resting animals and routine metabolic rate (RMR) during resting and locomotory activity of *Y. eightsi* between -1° and 5°C. Low RMR and SMR Q_{10} values were found between 0 and 2°C ($Q_{10} \approx 1$). Below 0°C animals displayed the lowest level of activity and RMR and SMR were virtually identical (Table I). Beyond 2°C, i. e. outside the natural habitat temperature, both rates increased steeply yielding a Q_{10} of 32 for RMR between 2 and 5°C. In the same temperature interval standard metabolic rate (SMR) displayed a Q_{10} of 59. Q_{10} values suggest a somewhat higher thermal sensitivity for SMR than RMR. Statistical analysis of the data yielded the minimum pooled least sum of squares for two regressions, ranging from -1 to +1°C and +2 to +5°C for routine metabolism (RMR), whereas for SMR the minimum was found for the ranges -1 to 2°C and +3 to +5°C, respectively (Fig. 1). The difference between RMR and SMR was used as a measure of metabolism allocated to activity. The number of active cycles per hour (n_c in Table I), identified by periods of elevated respiration, rose significantly (y = 1.669x + 0.271 $P < 0.001$, n: 181), although very weakly ($r^2 = 0.1$) with increasing temperature. Highest inhomogeneity was found between -1° C and all other temperatures. Even between 0 and 5°C (neglecting the very low activity at -1°C) the number of activity bouts correlated positively with temperature ($P = 0.0073$). RMR–SMR divided by the number of activity cycles yields the cost per activity cycle. In accordance with the slight increase of the number of activity cycles, metabolism allocated to activity as well as the cost per activity cycle remained more or less independent of temperature; however, the fraction of SMR in total energy turnover (%SMR) correlated significantly with rising temperature between 0 and 5°C (y = 4.987x + 48.1; $r^2 = 0.176$, $P = 0.0209$, $n = 30$).

Table I. Scope for activity (RMR–SMR), mean number of active cycles (n_c h^{-1}), scope for activity divided by the number of active cycles, and %SMR of total RMR (%SMR) in *Y. eightsi* during short term (24 h) exposure in the respirometer (see Fig. 1) to temperatures between -1 and +5°C. n = 5; data in mean ± s d from February–March 1997.

T/°C	RMR–SMR	n_c h^{-1}	RMR–SMR / n_c h^{-1}	%SMR
-1	-0.026	0.96 ± 1.2	0	141.7 ± 82.5
0	0.316 ± 0.29	1.72 ± 1.2	0.184	42.3 ± 31.5
1	0.198 ± 0.065	2.57 ± 1.2	0.077	42.5 ± 35.3
2	0.44 ± 0.18	1.96 ± 1.9	0.22	43.4 ± 19.0
3	0.341 ± 0.16	2.43 ± 1.74	0.14	60.4 ± 4.5
4	0.459 ± 0.258	2.82 ± 1.5	0.162	54.7 ± 11.9
5	0.460 ± 0.42	2.57 ± 1.36	0.18	57.9 ± 9.0

Acute responses to short-term warming

Significantly higher ATP concentrations in foot muscle tissues were found in animals kept at 2°C (n = 12) as compared to both higher (5°C; $P = 0.014$, n = 13) and lower (0°C; $P = 0.009$, n = 22) temperatures (Fig. 2). There was no difference between animals exposed to elevated temperatures with and without sediment. Whereas the lower ATP levels at 0°C relate to the low metabolic rates, ATP concentrations at 5°C were reduced although oxygen uptake rates remained high.

SOD activity in the probosces tissue (Fig. 3) was 0.31 ± 0.1 U mg^{-1} fresh weight in recently collected specimens (n = 8) and 0.29 ± 0.06 U mg^{-1} fresh weight in animals kept at 0°C under controlled laboratory conditions for 48 h (n = 9). Warming led to a significant decrease of SOD activity to 0.215 ± 0.06 U mg^{-1} fresh weight at 2°C ($P = 0.029$, $n = 6$) and to 0.206 ± 0.057 U mg^{-1} fresh weight at 5°C ($P = 0.014$, $n = 6$).

Catalase activity was present in probosces tissue of recently captured animals (1.14 ± 0.11 U mg^{-1} fresh weight). Only a slight, insignificant decrease was observed after 48 h of maintenance at 2°C (0.87 ± 0.068 U mg^{-1} fresh weight), as well as after a subsequent 48 h exposure at 5°C (0.92 ± 0.12 U mg^{-1} fresh weight). Control animals, which were kept at 0°C for the entire period, displayed variable catalase activities of 0.97 ± 0.24 U mg^{-1} fresh weight in probosces tissue.

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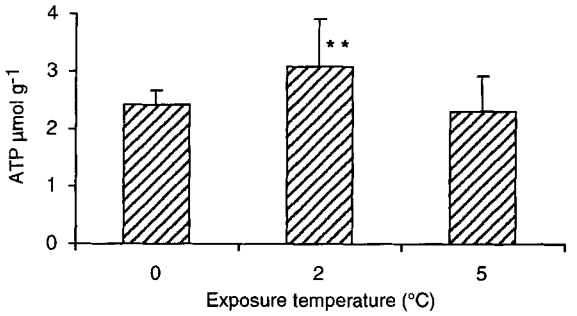


Fig. 2. ATP concentration in foot muscle tissue of *Y. eightsi* during short-term exposure to elevated temperatures for 48 h at each temperature. Samples were directly measured at Jubany. Mean ± s d from February to March 1997. ** = significantly different ($P < 0.05$) from 0° and 5°C groups, $n = 12$ (0°C), $n = 13$ (2°C), $n = 22$ (5°C).

Responses to long-term warming

In the second season, ATP concentrations in the foot muscle tissue of recently captured animals were lower than in the first season. These differences are likely to be due to interannual variations. Significantly higher ATP concentrations in control animals, as compared to recently captured individuals, were found after 20 and 30 days at 0°C ($P < 0.01$, $n = 9$–10). This increase was transient as the control group kept at 0°C for as long as 50 days displayed ATP levels (1.492 ± 0. 549 µmol g^{-1} fresh weight, Fig. 4a) almost as low as recently collected specimens.

During long-term acclimation to stepwise temperature increments, adenylate levels increased in control as well as in experimental animals (Fig. 4). In contrast to short-term exposure, energy depletion was not obvious above 2°C in the acclimated animals (Fig. 4b). ATP, ADP and AMP levels of the control group, maintained at 0°C for up to 50 days, were in the same range as in animals exposed to temperatures between 2° and 9°C. During the same period no switch to anaerobic energy production was observed. All samples analysed contained more or less constant succinate levels between 3 and 6 µmol g^{-1} fresh weight, whereas concentrations of acetate and propionate were essentially below detection limits in experimental as well as in control animals.

In 1999 MDA formation and SOD activity were measured in the foot muscle for comparison with the changes in tissue energetics. Twenty-four hours after collection from the cove, MDA concentrations appeared highly variable with a mean concentration of 25.25 ± 13 nmol g^{-1} fresh weight, ($n = 6$). After 2 days of acclimation to controlled laboratory conditions at 0°C, MDA levels were reduced by approximately 20% and continued to decrease to 10.32 ± 6.25 nmol g^{-1} fresh weight ($n = 5$) after 50 days (Fig. 5a). Long-term acclimation to 5°, 7° and 9°C resulted in a significant increase ($P < 0.01$) of MDA tissue concentrations with respect to the control group

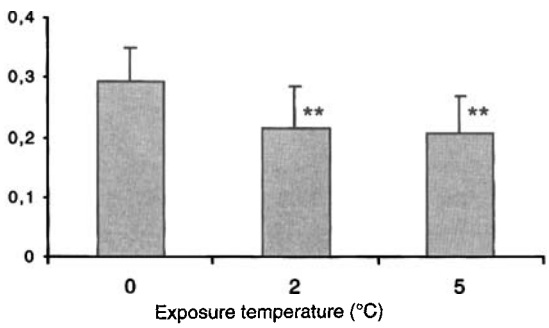


Fig. 3. SOD activity in palp probosces tissue of *Y. eightsi* during short-term exposure to elevated temperatures ($n = 6$–9), measured at a common assay temperature of 20°C. ** = significantly different ($P < 0.05$) from control animals at 0°C under laboratory conditions. Mean ± s d from February to March 1997.

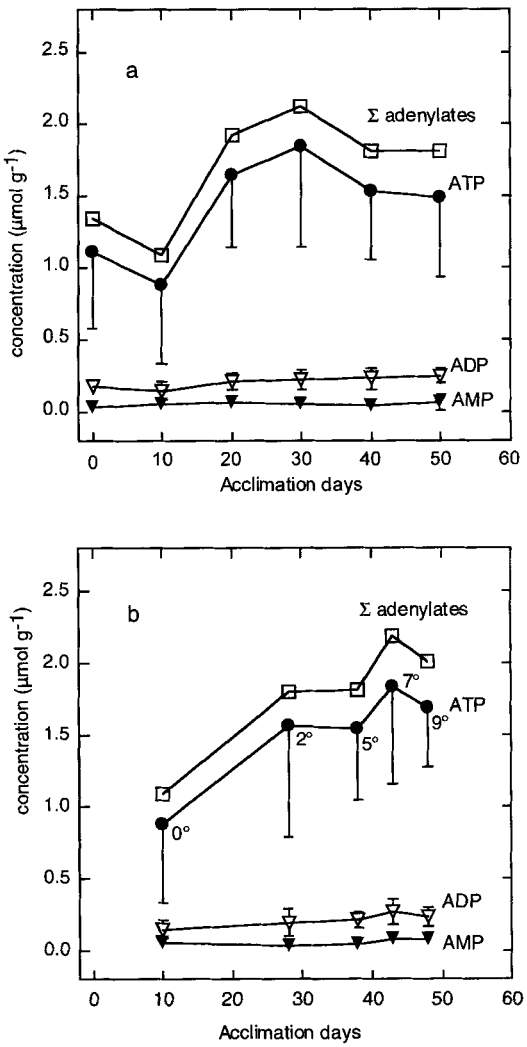


Fig. 4. Concentrations of high energy phosphates (ATP, ADP and AMP) and of total adenylates in foot muscle tissues of **a.** control animals, and **b.** of animals acclimated to higher temperatures, versus experimental time in days ($n = 8$–10). Mean ± s d from January to March 1999.

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kept at 0° C and also compared with animals kept at 2°C for a period of 12 days.

SOD activity in foot muscle was lower than the activity found in the probosces tissue in 1997. Moreover, the activity remained fairly constant in animals kept for 24 hrs at 0°C after capture and in 0°C control animals between day 1 and day 30 of the incubations (139–144 U g^{-1} fresh weight, n = 7–8, Fig. 5b). Above 2°C, SOD activities began to decline and were significantly lower after 48 days of warming to 9°C and also after 51 days of warming to a final temperature of 11°C, compared to controls after 50 or 53 days at 0°C ($P < 0.01$). After 43 days, SOD activity rose sharply in control animals, but was decreased again to below initial levels within the next two samplings on days 48 and 51. When compared with the bulk of control animals, kept at 0°C between 30 and 55 days (neglecting the extremely high value after 40 days at 0°C), SOD activity in *Y. eightsi* foot muscle decreased significantly when the bivalves were acclimated to elevated temperatures.

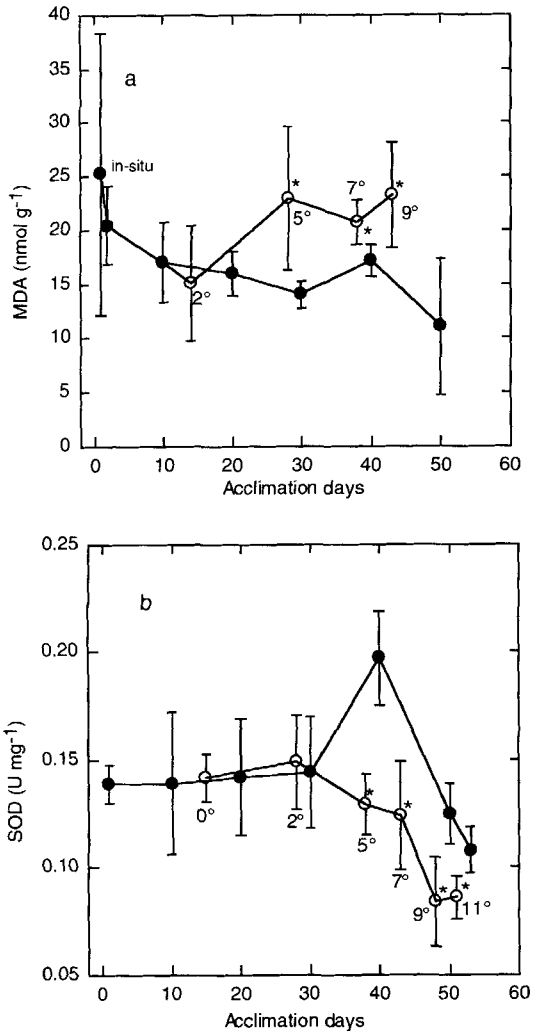


Fig. 5a. Malondialdehyde (MDA) concentrations, and **b.** superoxide dismutase (SOD) activities in foot muscle tissue of *Y. eightsi* in control animals (●) and in animals acclimated to elevated temperatures (○) vs time of incubation (n = 8–10). Numbers indicate incubation temperature in °C in each group of acclimated bivalves. * = significant difference to respective control group ($P < 0.01$). Mean ± s d from January–March 1999.

Discussion

Ventilation rates and oxygen uptake of *Y. eightsi* from Signy Island, South OrkneyIslands were studied by Davenport (1988b) at two temperatures (+0.2 and +2.5°C) within the range which the animals encounter in their natural habitat. At Signy Island, water temperatures range from -1.8° C in winter to +3°C in late summer. Davenport found both physiological parameters to be largely independent of acute temperature fluctuations within the margins of the habitat temperatures of the stock.

By contrast, habitat temperatures at King George Island display lower annual variability and a maximum temperature of +1.4°C is given by Schloss *et al.* (1998). To investigate the effects of temperature stress, we chose an experimental temperature range beginning at -1°C and ending at +5°C for the short-term temperature incubations.

After animals had become more active at 0° compared to -1°C, RMR and SMR appeared largely insensitive to temperature increments between 0° and +2°C. These data confirm the findings of Davenport for *Y. eightsi*, as well as other literature on the thermal independence of SMR in molluscs (e.g. Widdows 1973). For *Y. eightsi* 2°C seemingly marks a threshold for the respiratory response to short-term warming. At the lowest temperature of -1°C animals were inactive and RMR and SMR were virtually identical. At higher temperatures the level of activity, as deduced from the number of activity cycles per hour, increased significantly. The fraction of SMR (%SMR in Table I) of the total metabolic rate increased only above +2°C. Obviously, maintenance costs are kept constant within the habitat temperature range of the animals. According to Davenport (1988b), temperature independent low metabolic rates within the habitat temperature range also minimise loss of energy reserves during times of limited food supply.

Tremblay *et al.* (1998) suggested that higher resistance to fluctuations in habitat temperature, i.e. better eurythermal tolerance, was reflected in a lower percentage of standard metabolic rate of the maximal aerobic capacity (estimated using the ratio of MO_2 in starved vs fed mussels). The contribution of standard metabolism to overall energy expenditures amounted to 48% in thermally resistant stocks of blue mussels, while susceptible stocks had to invest 60% of their overall metabolic capacity for maintenance of tissue homeostatic functions. In *Y. eightsi* the percent fraction of SMR in total metabolic rate was rather constant between 0 and 2°C (42%) and increased to about 60% at 3°C and higher.

Tremblay *et al.* (1998) have already pointed out that increased Mo_2 at higher environmental temperatures may give rise to excess mitochondrial reactive oxygen species (ROS)

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production in marine ectotherms. The potential to prevent oxidative damage by increasing the antioxidant defence levels during acute temperature fluctuations could thus be one feature characterising temperature tolerance. SOD forms the first line of defence against emerging oxidative stress under high temperature conditions, as it catalyses the removal of metabolically derived oxygen radicals at the expense of creating H_2O_2. Moreover, this enzyme has been shown to respond to temperature stress in several temperate and polar marine invertebrates, while temperature effects on catalase (CAT) were not as clear (Abele *et al.* 1998a, 1998b). In *Y. eightsi,* however, CAT and SOD activities did not increase during short and long-term exposure to elevated temperatures. Instead, SOD levels even decreased. Our long-term data on MDA levels demonstrate elevated oxidative stress in *Y. eightsi*, at temperatures of 5°C and above, but they do not represent a measure of the resulting oxidative damage.

During warming, increased lipid peroxidation might have occurred, because increased tissue oxygen consumption could not be counterbalanced by enzymatic antioxidant protection. Elevated rates of oxygen turnover above 2°C are indicative of higher rates of oxygen radical release from the mitochondria. Reduced SOD activities, as well as a relative increase of tissue MDA concentrations at temperatures above the habitat temperature range, indicate a negative effect of high temperature exposure on the enzymatic defence system of this polar bivalve.

Although polar species definitely display higher thermal sensitivity than temperate animals, the same mechanisms appear to contribute to thermal stress in boreal ectotherms (Tremblay *et al.* 1998, Pellerin-Massicotte & Araujo 1997). Moreover, thermal stress has been shown to cause oxidative stress and induce bleaching in symbiotic cnidarian species from tropical systems (Lesser *et al.* 1990, Lesser 1997, Nii & Muscatine 1997). In the case of *Aiptasia pulchella* (Nii & Muscatine 1997) it was explicitly shown that oxidative stress in the anemone occurred in response to elevated temperatures in the host tissue, independent of the presence or absence of photosynthesising endosymbiotic algae.

The complex nature of our data supports the view that, due to their long thermal history at low temperatures, polar species have adjusted their antioxidant system, combining maximal enzymatic activities at low temperatures with the possible trade-off of higher thermal sensitivity of their antioxidant system. This is in line with work by Regoli *et al.* (1997), who found higher levels of SOD activity in gill and digestive gland of the subtidal Antarctic clam *Adamussium colbecki* acclimated to 0°C, when compared with warm water scallops at a common assay temperature of 19°C. High thermal sensitivity of SOD activity in polar molluscs may involve protein unfolding as well as physiological disturbances at the whole animal level.

A slight, but significant ATP depletion between 2 and 5°C seen during short-term exposure could be indicative of an imbalance between ATP consumption and supply (cf. Pörtner *et al.* 1999). Compensation for this process, i.e. reconstitution of ATP levels, was visible during slow acclimation to elevated temperatures. Adenylate levels did not differ from the control groups after 50 days even when heated to 9°C. Formation of succinate and volatile fatty acids as markers for anaerobic energy production could not be detected and mitochondrial metabolism was obviously aerobic at the end of the acclimation period. In conclusion, a critical temperature marking the ultimate limitation of passive thermal tolerance owing to oxygen deficiency (Pörtner *et al.* 1998, 2000) could not be detected within the range of temperatures tested.

As a corollary, onset of thermal sensitivity of standard metabolism and inactivation of the important antioxidant SOD above 2°C relate to the narrow margins of environmental temperature fluctuations in Potter Cove. Protein synthesis might be generally impaired, preventing adjustments of the antioxidant defence. Prolonged exposure to temperatures exceeding 2°C results in a progressive failure to fully balance internal ROS formation, enhancing the process of lipid peroxidation. Evidently, *Y. eightsi* has optimized its antioxidant system to function at the permanently low habitat temperatures of Potter Cove. Thereby, the animals have acquired suitable protection from oxidative stress arising from enhanced mitochondrial ROS liberation during periodic burrowing activities, characteristic of their mode of deposit feeding.

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