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Temperature induced anaerobiosis in two populations of the polychaete worm *Arenicola marina* (L.)

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Abstract Temperature dependent changes in the mode of energy metabolism and in acid-base status were studied in the range from −1.7 to 26 °C in two populations of *Arenicola marina* collected in summer as well as in winter from intertidal flats of the North Sea (boreal) and the White Sea (subpolar). Extreme temperatures led to an accumulation of anaerobic end products, indicating the existence of both a low and a high critical temperature, beyond which anaerobic metabolism becomes involved in energy production. In summer animals from the North Sea the high critical temperature was found at temperatures above 20 °C, and the low critical temperature below 5 °C. Latitudinal or seasonal cold adaptation lead to a more or less parallel shift of both high and low critical temperature values to lower values. Between critical temperatures intracellular pH declined with rising temperature. Slopes varied between −0.012 and −0.022 pH-units/°C. In summer animals from the North Sea, the slope was slightly less than in White Sea animals, but differences appeared independent of the season. However, slopes were no longer linear beyond critical temperatures. A drop in intracellular pH at low temperatures coincided with the accumulation of volatile fatty acids in the body wall tissue of North Sea animals. A failure of active pH_i adjustment is held responsible for the reduced $\Delta pH_i/\Delta T$ at temperatures above the high critical temperature. Extracellular pH was kept constant over the whole temperature range investigated. The ability of North Sea animals to adapt to temperatures beyond the critical temperature is poor compared to White Sea specimens. The larger range of temperature fluctuations at the White Sea is seen as a reason for the higher adaptational capacity of the subpolar animals. A hypothesis is proposed that among other mechanisms critical temperature values are set by an adjustment of mitochondrial density and thus, aerobic capacity.

Key words Acid-base status · Alpha-stat regulation · Anaerobic metabolism · Cold adaptation · Critical temperatures · Intracellular pH · Temperature adaptation · Volatile fatty acids

Abbreviations *PCA* perchloric acid · *T*c critical temperature · Tc_I lower critical temperature · Tc_{II} high critical temperature · pH_i · intracellular · pH_e extracellular pH

Introduction

Animals are directly influenced by temperature since the rates of biochemical and physiological processes change and extreme temperatures or temperature variations can cause lethal injuries [reviews: Hochachka and Somero (1980); Prosser (1991)]. Recently, the effects of temperature on marine coastal ecosystems have come into focus owing to the process of global warming (Beukema 1979, 1992; Southward et al. 1995). An increase in mean global temperature is paralleled by a rise in the frequency and magnitude of thermal fluctuations. Therefore, animals must not only adapt to changing temperature ranges but also have to cope with extreme events. If the limits of adaptability are passed, the borders of distribution of a species will change.

Temperature effects on biochemical and physiological processes likely relate to changes in the protonation of macro- and micromolecules and therewith also to the acid-base status. The details of these interrelations are not very well understood. Based on his work with bullfrogs, Reeves (1972) postulated that pH in various body compartments changes with temperature at −0.017 pH units per °C (ΔpH/°C), such that the protonation of histidine imidazole residues in enzymes is kept constant (α-stat hypothesis). The constant degree of protonation is interpreted to maintain the functional properties of a

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protein. However, values of ΔpH/ºC differ between plasma and tissue and between different tissues. Therefore, Butler and Day (1993) concluded that intra- and extracellular pH are regulated independently. Since pH and metabolic regulation are interdependent processes (Pörtner 1989), the effects of temperature induced changes in intra- and extracellular acid-base status on metabolic regulation need to be addressed.

Most studies of the influence of temperature on metabolism have focussed primarily on O_2 consumption. For many invertebrates it has been shown that an increase in temperature leads to a rising O_2 consumption until a temperature threshold is surpassed, above which O_2 consumption decreases (Kristensen 1983). For some invertebrates of the intertidal zone, the relationship between temperature and O_2 consumption rate is peculiar in that the metabolic rate is independent of ambient temperature over a wide temperature range (Vernberg and Vernberg 1964; Newell and Pye 1970a,b).

The lugworm *Arenicola marina* can be found in the coastal area of nearly the whole northern hemisphere, from Siberia via Western Greenland to the pacific coast of North America, from Iceland and Spitsbergen via the North Sea up to the Mediterranean (Hartmann-Schröder 1971; Wesenberg Lund 1953), where it builds L-shaped burrows in the sediment of the inter- or subtidal zone (Dörjes 1972). In this habitat *A. marina* is not only exposed to different climates – from polar to subtropic – but also to seasonal and diurnal temperature changes. The metabolic rate of the lugworm remains nearly constant between 10 and 20 ºC and therefore a higher rate prevails at lower temperatures than expected from the reaction velocity-temperature relationship (Krüger 1964). On the other hand, the rate of ventilation, which is a main energy consuming process (Toulmond and Dejours 1994), is reduced at low temperatures (Seymour 1972). The question therefore arises whether the balance between the changes in O_2 delivering mechanisms and in O_2 consuming processes is disturbed beyond specific temperature values, which may lead to hypoxia in the tissue and the onset of anaerobic metabolism.

The present study was designed to compare the influence of temperature on the aerobic or anaerobic mode of energy metabolism in *A. marina* from boreal and subpolar regions, and to investigate how potential changes in metabolism are related to changes in acid-base status. Critical temperatures could be identified which were characterised by a transition to anaerobic metabolism. Therefore, the question was addressed as to whether an adaptation to changing temperatures and to latitudinal, climatic differences is associated with differences or a shift in those critical temperatures.

Materials and methods

Animals

Specimens of *Arenicola marina* were collected in 1994 from intertidal flats of the North Sea near Dorum, Germany (“North Sea animals”) and of the White Sea near Kartesh, Russia (“White Sea animals”). They were kept in the laboratory in aquaria filled with well-aerated natural brackish sea water (22%) and a 10 cm bottom layer of sediment. Temperatures varied around 2 ºC (winter) and between 10 and 12 ºC (summer) (“North Sea animals”) or was maintained at 3.5 ± 0.5 ºC and 6.4 ± 0.5 ºC (“White Sea animals”). These temperatures are referred to as control temperatures.

Experimental procedure

The animals were moved to separate -containers filled with sediment and sea water at control temperatures where they were allowed to construct burrows. After 24 h of preincubation, the containers were placed into aquaria containing sea water with different pre-set temperatures. North Sea animals collected in January were exposed to −1.7, 0, 2 (control temperature), 5, 10 or 17 ºC, specimens collected in August or September to temperatures between −1.7 and 25 ºC (North Sea) or 26 ºC (White Sea) for 10 h.

In order to compare animals exposed to temperature changes while dwelling in sediment or unburied in open sea water, winter or summer animals from the North Sea were placed into a darkened 20-container filled with 2 or 10 ºC sea water (in accordance with the control temperature maintained in holding tanks) without sediment for 24 h. Worms collected in winter were moved into aquaria containing aerated sea water at −1.5, 0, 2 (control temperature), 8 or 15 ºC, where they remained for another 24 h. Summer animals kept at 10 ºC were incubated at temperatures between 0 and 25 ºC.

Long-term incubations of summer animals from the North and the White Seas took place at −1.7 ºC, at control temperature or at temperatures 4 ºC above the respective high *T*c which had been determined in the previous experiments.

Coelomic fluid was obtained by opening the body wall at the 6th segment. The liquid was collected in ice-cold Eppendorf caps, which were immediately transferred into liquid N_2 after closing. Head and tail were dissected, the body wall opened completely and gonads as well as the digestive tract removed by use of tissue paper. The body wall musculature was blotted dry and freeze clamped in liquid N_2 by means of a precooled Wollenberger clamp (Wollenberger et al. 1960). Samples were stored in liquid N_2 until analysed.

Analyses

Analysis of intracellular pH was performed according to Pörtner et al. (1990). To reduce the period during which the tissue or tissue powder may become contaminated by CO_2 condensation, the body wall musculature was only coarsely crushed in liquid N_2 using mortar and pestle. The coarse powder was transferred into Eppendorf caps filled with 200 µl of ice-cold medium consisting of 2 $mmol \cdot l^{-1}$ nitrilotriacetic acid and 160 $mmol \cdot l^{-1}$ potassium fluoride. After filling with medium, caps were sealed air bubble free, and final extraction took place in an ultrasonic water bath at −7 ºC for 1 min. Coelomic fluid collected as described above was analysed for pH_e using a temperature-controlled capillary pH electrode (Radiometer, Copenhagen). Preliminary experiments had shown that freezing in liquid N_2 prior to analysis had no influence on coelomic fluid pH.

For the determination of metabolite concentrations body wall tissue was powdered using a mortar cooled with liquid N_2. Approximately 300 mg of powder were added to an excess (3×) volume of precooled 0.6 $mol \cdot l^{-1}$ PCA. An Ultra-Turrax T8 (Janke & Kunkel, FRG) was used to homogenize the tissue. Precipitated protein was removed by centrifugation. The extract was neutralized with 5 $mmol \cdot l^{-1}$ potassium hydroxide and a 1:6-mixture of solid $K_2CO_3/KHCO_3$. A second centrifugation withdrew precipitated potassium perchlorate.

After PCA extraction succinate was measured spectrophotometrically in an enzymatic test according to Beutler (1989), ATP according to Trautschold et al. (1989) and ADP and AMP following Jaworek and Welsch (1989). Determination of inorganic

phosphate occurred as described by Pörtner (1990). A method modified from Hardewig et al. (1991) was used to measure the concentrations of the fatty acids propionate and acetate in PCA extracts, diluted with deionized water, using a Dionex BIO LC ion chromatograph (Idstein, FRG). Fatty acids were separated on an ion exclusion column (Dionex ICE-AS 1), using 0.125 $mmol \cdot l^{-1}$ octanesulphonic acid as an eluent with a flow rate of 1 $ml \cdot min^{-1}$ at 40 °C. Peaks were monitored with a conductivity detector. A micro membrane suppressor (Dionex AMMS-ICE) regenerated with 5 $mmol \cdot l^{-1}$ tetrabutylammonium hydroxide was used to decrease background conductivity. Calibration curves were linear between acetate and propionate concentrations of 10 and 500 $\mu mol \cdot l^{-1}$.

Alanopine and strombine were measured by ion exclusion chromatography using a High Performance Liquid Chromatograph (Beckman Instruments GmbH, Munich). Separation was carried out at 45 °C on a Polyspher ARAC column for organic acids (Merck, Darmstadt), using $5 \cdot 10^{-5}$ $mol \cdot l^{-1}$ HCl as an eluent with a flow rate of 0.6 $ml \cdot min^{-1}$. A conductivity detector was utilized to monitor the peaks. Standard opine solution for calibration was obtained by means of in vitro synthesis following a method modified from Siegmund and Grieshaber (1983).

Statistics

Data were checked for outliers beyond the r(95) limits of an *r*-distribution ($r_A > r(95)$) using Nalimov's test (Noack 1980). Statistical significance of differences was tested at the $p \leq 0.05$ level using analysis of variance (ANOVA) or Covariance (ANCOVA) and the post-hoc Student-Newman-Keuls test for independent samples. Data are given as means ± standard deviation.

Results

In summer animals from the North Sea, anaerobic metabolites accumulated at both very low and very high temperatures (Fig. 1a). The *T*c for the onset of anaerobic metabolism were between 2 and 5 °C (low *T*c) and above 20 °C (high *T*c). Acetate concentrations in the body wall musculature increased significantly within 10 h after temperature was elevated to 26 °C or lowered to −1.7 °C, while propionate concentration rose only with falling temperatures. An increase in the levels of both acetate and propionate, although not yet significant, became apparent when the incubation water was cooled from 12 °C (control temperature) to 2 °C. In North Sea animals collected in January the low *T*c was found between 0 and 2 °C (Fig. 1b) somewhat lower than in summer animals where the levels of acetate and propionate were already slightly elevated at 2 °C. In these lugworms acetate accumulated to a level of 1.30 ± 0.37 $\mu mol \cdot g^{-1}$ fresh wt ($n = 3$) after a small temperature drop from 2 to −1.7 °C for 24 h. Thus, the rate of acetate accumulation at −1.7 °C did not differ significantly between animals collected in summer (0.07 ± 0.03 $\mu mol \cdot g^{-1}$ fresh $wt \cdot h^{-1}$) and in winter (0.05 ± 0.01 $\mu mol \cdot g^{-1}$ fresh $wt \cdot h^{-1}$). In contrast to summer animals, no propionate accumulated in winter. Changes in succinate levels were insignificant in winter as well as in summer animals (Table 1).

Lugworms from the subpolar region of the White Sea showed a similar temperature dependence of the transition to anaerobic metabolism as North Sea lugworms. However, a low *T*c could not be clearly identified, since concentration changes were too small. Propionate and succinate concentrations reached significantly higher values when White Sea animals were exposed to 26 °C for 10 h than in North Sea worms exposed to 25 °C (Fig. 1a, c; Table 1). Overall, the high temperature threshold for the onset of anaerobic metabolism was found slightly above 17 °C in animals from the White Sea. Changes in metabolite levels were already significant at 21 °C in these animals, whereas North Sea specimens were still completely aerobic at 20 °C. This observation suggests that the *T*c is lower in White Sea animals than in lugworms from the North Sea.

Anaerobic metabolism occurred at both very high and very low temperatures also when North Sea animals were permanently exposed to normoxia in open sea

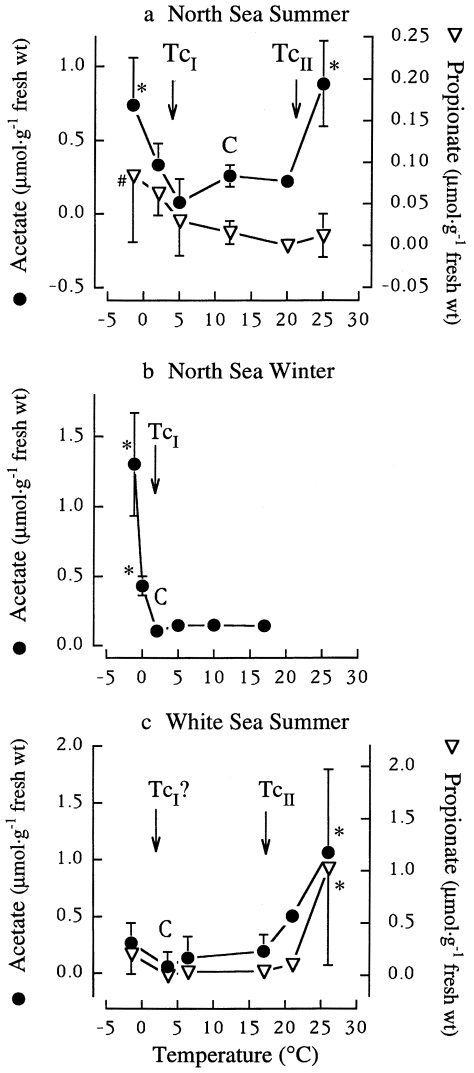

Fig. 1a–c Concentrations of volatile fatty acids in the body wall tissue of *Arenicola marina* dwelling in sediment at different temperatures: **a** North Sea summer animals, after 10 h; **b** North Sea winter animals, after 24 h; **c** White Sea animals, after 10 h. No propionate could be detected in the body wall tissue of winter animals from the North Sea ($n = 5$; mean ± SD; • Acetate; ∇ Propionate; * = significantly different from the control value; # = significantly different from the value at 20 °C)

Table 1 Succinate concentrations in the body wall of *Arenicola marina* dwelling in sediment after 10 h of exposure to different temperatures ($\mu mol \cdot g^{-1}$ fresh wt; mean ± SD)

North Sea animals						White Sea animals		
Summer			Winter			Summer		
−1.7 °C	0.38 ± 0.28	(n = 4)	−1.7 °C	0.04 ±0.07	(n = 4)	−1.7 °C	0.71 ±0.62	(n = 4)
2 °C	0.53 ± 0.61	(n = 4)	0 °C	0.00 ± 0.00	(n = 3)	**3.6 °C**	0.47 ± 0.57	(n = 5)
5 °C	0.01 ± 0.01	(n = 4)	**2 °C**	0.03 ± 0.05	(n = 4)	6.4 °C	< 0.01	(n = 3)
12 °C	0.03 ± 0.12	(n = 4)	5 °C	0.10 ± 0.10	(n = 5)	17 °C	0.68 ± 0.54	(n = 5)
20 °C	0.03 ± 0.06	(n = 4)	10 °C	< 0.01	(n = 4)	21 °C	2.45 ± 0.37[a]	(n = 4)
25 °C	0.28 ± 0.37	(n = 5)	17 °C	≤ 0.01	(n = 5)	26 °C	3.67 ± 2.30[a]	(n = 5)

[a]significantly different from the control value

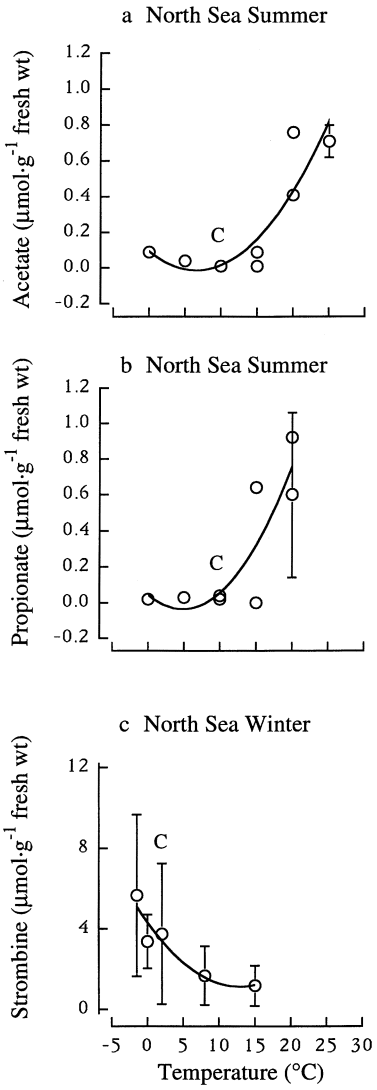

Fig. 2a–c Anaerobic metabolism in the body wall tissue of summer (**a, b**) and winter (**c**) animals from the North Sea after 24 h of incubation in sea water without sediment at different temperatures. Curves of regression (2nd order) were significant (r = 0.85 for acetate, r = 0.71 for propionate, r = 0.5 for strombine; (**a, b**) data of individual animals, except for those collected at 0 °C; mean ± SD, n = 3; and 25 °C: mean ± SD, n = 4; (**c**) mean ± SD; n = 6 for 2 and −1.5 °C, n = 4 for 0, 8 and 15 °C)

water (Fig. 2). After a temperature rise in the sea water from 10 °C (control temperature) to 25 °C, acetate and propionate concentrations in the body wall musculature of summer animals increased significantly. When the sea water was cooled to below 10 °C changes in volatile fatty acid levels were not indicative of a low Tc, however, the opine strombine rose in winter animals incubated for 24 h at −1.5 °C. When dwelling in sediment at low temperatures, there was only a non-significant trend for strombine to accumulate in winter animals (Table 2). The higher level of muscular activity in animals without sediment is probably the reason for the higher level of strombine, which is known to accumulate during functional anaerobiosis. Alanopine levels neither changed with temperature in animals incubated in open sea water nor in those dwelling in sediment (Table 2).

The concentrations of ATP, ADP and AMP remained unaffected by temperature in the body wall tissue of animals incubated in open sea water, while the level of inorganic phosphate (P_i) rose slightly at higher temperatures (Fig. 3). Obviously, ATP-producing and ATP-consuming processes were more or less in equilibrium under those conditions.

Lugworms incubated in open sea water showed a linear pH_i/temperature relationship over the whole temperature range investigated (Fig. 4). pH_i fell with rising temperature in both summer ($\Delta pH_i/\Delta t$ = −0.019 pH-units/°C) and winter animals ($\Delta pH_i/\Delta t$ = −0.012 pH-units/°C) during 24 h. In contrast, pH_e showed no dependence on temperature. Therefore, the values of pH_i and pH_e became significantly different at higher temperature.

In animals dwelling in sediment intracellular pH declined with rising temperature only between 0 and 20 °C. Slopes varied around −0.020 pH-units/°C. In summer animals from the North Sea the slope of −0.016 pH-units/°C was slightly less than in White Sea animals with −0.019 pH-units/°C, but differences in slopes were not significant and appeared independent of the season and the length of the incubation period. With one exception, however, slopes were no longer linear beyond Tc. Only in White Sea animals was the slope seen to remain constant above Tc_{II}. Below Tc_I the pH_i/temperature

Table 2 Opine concentrations in the body wall musculature of *Arenicola marina* (winter animals) dwelling in sediment or incubated in open sea water at different temperatures for 24 h. Strombine levels in animals incubated without sediment are depicted Fig. 2 ($\mu mol \cdot g^{-1}$ fresh wt; mean ± SD; none were significantly different from the control value at 2 °C)

In sediment Temperature	Strombine	Alanopine	In open sea water Temperature	Alanopine
−1.7 °C	1.35 ± 1.23 (n = 4)	0.22 ± 0.32 (n = 5)	−1.5 °C	0.54 ± 0.70 (n = 7)
0 °C	0.34 ± 0.19 (n = 4)	< 0.01 (n = 4)	0 °C	0.01 ± 0.01 (n = 4)
2 °C	0.24 ± 0.06 (n = 4)	< 0.01 (n = 5)	2 °C	1.22 ± 1.12 (n = 6)
5 °C	1.04 ± 0.71 (n = 4)	0.06 ± 0.05 (n = 4)	8 °C	0.30 ± 0.36 (n = 4)
10 °C	0.81 ± 0.28 (n = 4)	0.10 ± 0.02 (n = 4)	15 °C	0.16 ± 0.05 (n = 4)
17 °C	0.42 ± 0.25 (n = 5)	0.13 ± 0.02 (n = 4)		

relationship showed a significant deviation from linearity. In summer animals, e.g. from the North Sea, pH_i no longer rose but fell from 7.25 ± 0.01 (n = 4) at 5 °C to 7.14 ± 0.10 (n = 5) at −1.7 °C and in winter animals pH_i fell from 7.38 ± 0.03 (n = 4) at 0 °C to 7.29 ± 0.11 (n = 5) at −1.7 °C (Fig. 5). In lugworms from the North Sea the decrease in pH_i at low temperatures coincided with the accumulation of acetate in the body wall.

Latitudinal and seasonal adaptation

The presence and extent of adaptational processes during exposure to extreme temperatures was studied in summer animals from the North and the White Seas during long-term incubations. The ability to adapt is interpreted to be associated with the reversal of an initial transition to anaerobiosis during long-term exposure to cold or heat. After a temperature drop from 12 to −1.7 °C the concentrations of succinate, propionate and acetate rose in the body wall musculature of lugworms from the North Sea, but acetate only reached a significantly higher level after 7 days. There were no significant changes in the levels of the other metabolites and in intracellular pH (Table 3). However, no adaptive reversal of acetate accumulation could be detected, although all animals survived cold exposure for 7 days, a

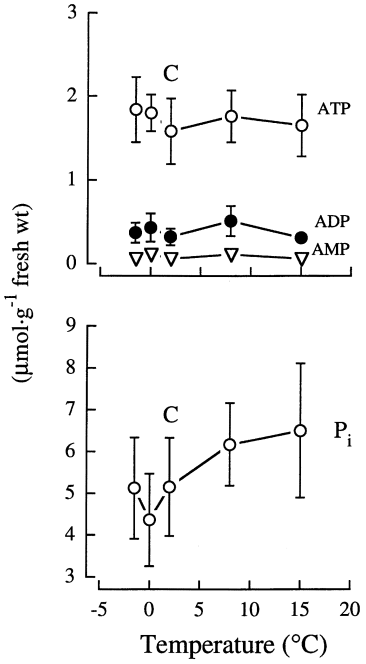

Fig. 3 Adenylate concentrations and the concentration of inorganic phosphate in body wall tissue of winter animals from the North Sea at different temperatures after a 24 h period of incubation in open sea water (mean ± SD; n = 7 for 2 and −1.5 °C, n = 4 for 0, 8 and 15 °C; * = significantly different from the control value at 2 °C)

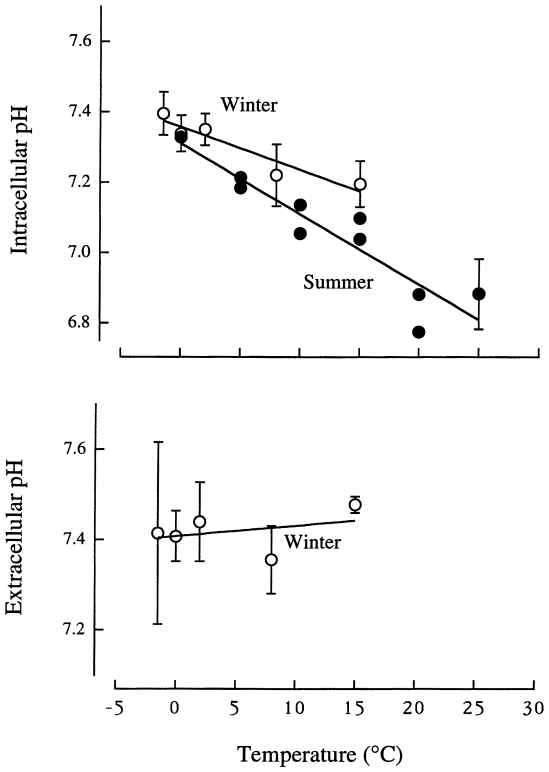

Fig. 4 Intra- and extracellular pH in North Sea animals incubated for 24 h in aerated open sea water at different temperatures (summer: data from individual animals, except for those collected at 0 °C: mean ± SD, n = 3; and 25 °C: mean ± SD, n = 4; winter: mean ± SD; n = 6 for 2 and −1.5 °C, n = 4 for 0, 8 and 15 °C)

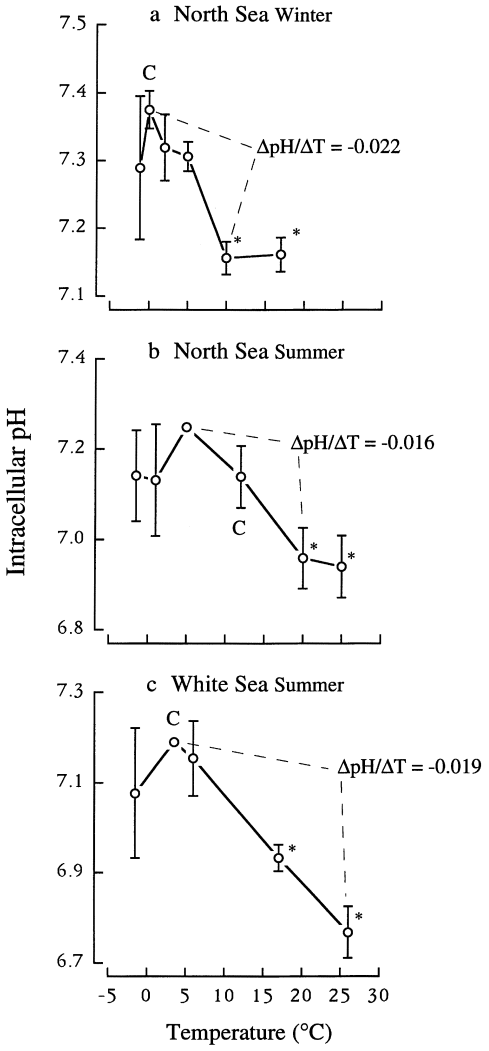

Fig. 5a–c Intracellular pH in animals from the North and the White Seas after 10 h of incubation in sediment at different temperatures (n = 5; mean ± SD; * = significantly different from the control value)

period not unusual for weather and temperature conditions in the German Bight.

When sediment was warmed to 4 °C above the high *T*c, for example from 12 to 25 °C in North Sea specimens, these animals survived for only slightly longer than 1 day. Death of all animals occurred within 3 days. The respective metabolite data are summarized in Table 4. Succinate concentrations increased significantly during the first 4 h, acetate reached a significantly higher concentration after 10 h and a significant rise in propionate concentration occurred within 24 h. Intracellular pH dropped significantly during 4 h, after which this low value was kept constant.

In contrast to North Sea animals, all White Sea lugworms not only survived long-term exposure to high temperatures but also exhibited an adaptive reversal of acetate accumulation. After a temperature increase in the sediment from 6.4 to 21.0 °C (4 °C above the high *T*c) the concentrations of acetate in the body wall musculature of White Sea animals rose significantly only during the first 4 h and decreased again thereafter (Fig. 6). Finally, control values were reached within 3 days. Propionate concentrations showed no significant changes. pH_i dropped to far lower levels in White Sea than in North Sea specimens under these conditions within 4 h but started to rise again after 1 day at 21 °C.

Discussion

Characterisation of two critical temperatures

Incubation of *A. marina* dwelling in sediment at very low temperatures led to rising acetate and propionate concentrations in the body wall, which indicated that anaerobic metabolism became involved in energy production. However, no fatty acids were accumulated in lugworms kept at low temperatures in a large volume of sea water without sediment. This can be explained by passive diffusion of acetate and propionate into the larger volume of ambient water, whereas fatty acid efflux into the sea water was reduced in animals dwelling in sediment. Moreover, the degree of hypoxia was certainly less in animals dwelling in open sea water than in those exposed to low temperatures in the sediment. Nevertheless, the accumulation of strombine in the body wall indicates that anaerobiosis is induced at low tempera-

Table 3 Anaerobic metabolites and intracellular pH in the body wall of *Arenicola marina* ("North Sea animals") in summer after a temperature drop in the sediment from 12 to −1.7 °C (mean ± SD)

Time (h)	Acetate	Propionate ($\mu mol \cdot g^{-1}$ fresh wt)	Succinate	pH_i
0	0.26 ± 0.08 (n = 5)	0.02 ± 0.01 (n = 5)	0.10 ± 0.12 (n = 4)	7.14 ± 0.07 (n = 5)
4	0.19 ± 0.10 (n = 5)	0.02 ± 0.01 (n = 5)	1.45 ± 1.28 (n = 5)	7.21 ± 0.01 (n = 5)
10	0.74 ± 0.32 (n = 5)	0.05 ± 0.04 (n = 4)	0.38 ± 0.28 (n = 4)	7.14 ± 0.10 (n = 5)
24	0.53 ± 0.73 (n = 5)	0.01 ± 0.02 (n = 5)	0.25 ± 0.34 (n = 5)	7.15 ± 0.15 (n = 5)
72	0.73 ± 0.36 (n = 5)	0.14 ± 0.29 (n = 5)	0.51 ± 0.42 (n = 4)	7.13 ± 0.10 (n = 5)
168	1.00 ± 0.64[a] (n = 5)	0.50 ± 0.69 (n = 5)	2.42 ± 3.14 (n = 5)	7.05 ± 0.29 (n = 5)

[a]Significantly different from the control value (0 h)

Table 4 Anaerobic metabolites and intracellular pH in the body wall of *Arenicola marina* ("North Sea animals") in summer after a temperature rise in the sediment from 12 to 25 °C (mean ± SD). Animals started to die after 24 h and none were alive after 3 days

Time (h)	Acetate	Propionate ($\mu mol \cdot g^{-1}$ fresh wt)	Succinate	pH_i
0	0.26 ± 0.08 (n = 5)	0.02 ± 0.01 (n = 5)	0.10 ± 0.12 (n = 4)	7.14 ± 0.07 (n = 5)
4	0.16 ± 0.15 (n = 5)	0.08 ± 0.14 (n = 5)	0.79 ± 0.29 (n = 4)	6.92 ± 0.02[a] (n = 5)
10	0.88 ± 0.29[a] (n = 5)	0.01 ± 0.03 (n = 5)	0.28 ± 0.37 (n = 5)	6.94 ± 0.07[a] (n = 5)
24	0.69 ± 0.12[a] (n = 5)	0.36 ± 0.17[a] (n = 5)	0.70 ± 0.42 (n = 4)	6.89 ± 0.05[a] (n = 5)

[a]Significantly different from the control value (0 h)

tures even in animals incubated in normoxic open sea water.

The low threshold for the onset of anaerobic metabolism was found at temperatures below 5 °C regardless of whether the animals were incubated in sediment or in open sea water. However, this threshold seemed to be lower in winter animals from the North Sea (below 2 °C) than in summer animals (between 2 and 5 °C). In summer animals from the White Sea a low threshold could not be significantly confirmed, but the trend of metabolite accumulation at −1.7 °C suggests that it may be present.

The relationship between temperature and the concentrations of free fatty acids in the body wall musculature can be described by a more or less U-shaped curve. Therefore, exposure of *A. marina* to high temperatures is also linked to a transition to anaerobic metabolism. Available evidence indicates that the reason for the onset of anaerobic metabolism at both low and high temperatures is an insufficient uptake and distribution of O_2.

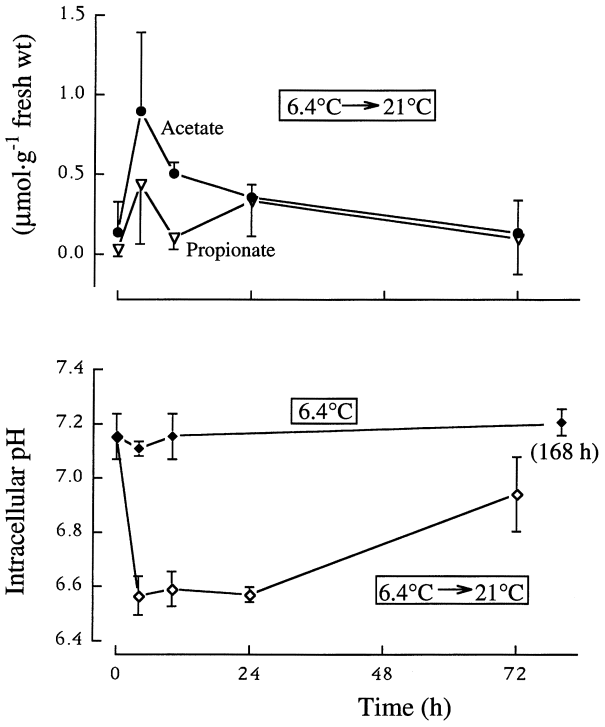

Fig. 6 Concentrations of volatile fatty acids and intracellular pH in the body wall tissue of *Arenicola marina* (White Sea animals) after an increase in temperature in the sediment from 6.4 to 21 °C (n = 5; mean ± SD; • Acetate; ∇ Propionate * = significantly different from the control value at 0 h)

Mangum (1978) reported that the haemoglobin in the blood of *A. marina* shows a Bohr effect and that blood pH rises with declining temperature. At low temperatures this would reduce O_2 transport via the blood since O_2 affinity rises with decreasing temperature. A 50% reduction in oxygen transport could be elicited by a temperature drop from 23 to 6 °C. Moreover, Baumfalk (1979) observed a decrease in peristaltic movements in *A. marina* with declining temperature and Seymour (1972) added that pulsation of the dorsal vein also diminishes. As a corollary, O_2 uptake and distribution in *A. marina* was not sufficient to meet O_2 demand at low temperatures even under normoxia, e.g. in well-oxygenated open sea water. This conclusion is supported by recent findings in *Sipunculus nudus* dwelling in sediment, where a decrease in ventilation resulted in insufficient O_2 supply and transition to anaerobiosis below a low Tc (Zielinski and Pörtner 1996). However, the findings in *A. marina* are different from those in *Sipunculus nudus*, in that neither opines nor volatile fatty acids accumulated in *Sipunculus nudus* incubated in cold open sea water. This may be due to the fact that *A. marina* possesses a circulatory system, whereas *Sipunculus nudus* does not, emphasising that insufficient ventilation and circulation of body fluids are setting the low Tc.

At high temperatures in *A. marina* the temperature-dependent rise in energy demand can also not be met by the ventilatory and circulatory mechanisms of O_2 supply, especially, since ventilation rate drops sharply at high temperatures (Seymour 1972). Additional, anaerobic energy production is therefore required. Consequently, a low and a high critical temperature (T_{c_I} and $T_{c_{II}}$) can be defined, which are characterised by the onset of anaerobic metabolism (Fig. 1).

The question remains open why propionate accumulation is less in North Sea than in White Sea animals under temperature induced hypoxia. In lugworms from the North Sea, Schmidt et al. (1992) found a uniform Q_{10} as high as 4 for propionate formation during extreme hypoxia in a temperature range from 2 to 20 °C.

The high Q_{10} might be the reason for the low accumulation rate of propionate below Tc_l in North Sea specimens, but this does not explain the higher rate of propionate accumulation in White Sea than in North Sea animals. It is hypothesised (see below) that lugworms from the White Sea possess a higher mitochondrial density in their body wall musculature, explaining elevated succinate and propionate levels and a higher anaerobic mitochondrial metabolic rate.

Acid-base status

Intracellular pH in the body wall musculature of lugworms incubated at different temperatures changed in accordance with the α-stat hypothesis ($\Delta pH_i/\Delta T = -0.017$) such that changes in the dissociation equilibria of functional groups in enzymes would be kept in a narrow range (Reeves 1972; Howell et al. 1973; Walsh and Moon 1982). Values of $\Delta pH_i/\Delta T$ varied between −0.012 and −0.022. Similar values are reported by Toulmond (1977) for the blood of *Arenicola* and some other marine invertebrates. However, coelomic fluid is thought to represent the true extracellular fluid of the tissues in *A. marina* and coelomic fluid pH remained constant, such that extra- and intracellular pH changed independently in *A. marina*. A significant pH difference between body wall tissue and coelomic fluid resulted at rising temperatures, but the pH difference was still in the typical range of 0.1–0.2 units for *A. marina* (Pörtner 1993). In contrast to *A. marina*, pH_i in different tissues of the brown trout *Salmo trutta* remained constant and unaffected by rising temperature, whereas blood pH declined (Butler and Day 1993). In the sipunculid *Sipunculus nudus* pH_e could be identified as a key parameter controlling metabolic rate (Reipschläger and Pörtner 1996). If this is also true for vertebrates and other invertebrates like *A. marina*, a constant pH_e at changing pH_i could be one reason why metabolic rate is relatively temperature independent in the lugworm between 10 and 25 °C, as well as between 2 and 5 °C (Krüger 1964). These questions certainly require further investigation.

In water breathing animals intra- and extracellular pH are adjusted by ion exchange mechanisms with some respiratory influence involved. Accordingly, the regulation of pH_i with changing temperature is attributed not only to a passive but also to an active component, which is high in eurythermal species (Sartoris and Pörtner 1996; van Dijk et al. 1996). However, the slope of the pH_i/temperature relationship deviates from linearity beyond the critical temperatures in animals dwelling in sediment, indicating transition to a non-steady state situation. On the one hand, gas exchange declines with reduced ventilation leading to respiratory acidification. On the other hand, anaerobic metabolic reactions influence pH by releasing or binding protons (Pörtner 1987a,b; Pörtner et al. 1984b, 1986a,b). For example, propionate and acetate accumulation cause pH to fall and, actually, in North Sea animals an accumulation of volatile fatty acids at low temperatures was accompanied by the development of an acidosis, seen as a significant reduction in the temperature induced pH rise (Fig. 5). Above the high Tc, a failure of active pH_i adjustment might be a reason for the slope of $\Delta pH_i/\Delta T$ being reduced in North Sea animals. This deviation from the linear slope beyond Tc_{II} was not seen in lugworms from the White Sea. Here the higher extent of volatile fatty acid accumulation compared with North Sea specimens might have caused the higher extent of acidification above Tc_{II}. Alternatively, cold adaptation increases the velocity and capacity of pH regulatory mechanisms (Sartoris and Pörtner 1996). The reason for the lower intracellular pH in the body wall musculature of White Sea than of North Sea lugworms (Fig. 1) remains obscure. Similar results were obtained in *Crangon crangon* from the White and the North Seas (Sartoris and Pörtner 1996). This may indicate that some animals exposed to latitudial cold have a lower intracellular pH, possibly related to the increased mitochondrial contents (Pörtner et al. 1990).

Latitudinal and seasonal adaptation

In North Sea animals there were no seasonal differences in the rate of acetate accumulation at −1.7 °C. It reached 0.074 ± 0.032 $\mu mol \cdot g^{-1}$ fresh $wt \cdot h^{-1}$ in summer animals and 0.054 ± 0.015 $\mu mol \cdot g^{-1}$ fresh $wt \cdot h^{-1}$ in winter. But the low Tc, beyond which acetate accumulated, seemed to be lower in lugworms collected in winter. In White Sea specimens the rate of acetate accumulation was 0.027 ± 0.017 $\mu mol \cdot g^{-1}$ fresh $wt \cdot h^{-1}$ and not significantly lower than in North Sea worms in both summer and winter. Rates of acetate accumulation at temperatures above the high Tc were also not significantly different in summer animals from the North Sea (0.088 ± 0.029 $\mu mol \cdot g^{-1}$ fresh $wt \cdot h^{-1}$ at 25 °C) and the White Sea (0.051 ± 0.007 $\mu mol \cdot g^{-1}$ fresh $wt \cdot h^{-1}$ at 21 °C) indicating similar capacities of acetate formation. From our data it can be concluded that a seasonal or latitudinal adjustment of the low or high Tc had no significant influence on the capacity of acetate formation.

The high threshold for the onset of anaerobic metabolism was found above 20 °C in summer animals from the North Sea. This temperature threshold is higher than in animals from the White Sea, where the Tc is close to 17 °C. Together with the finding that acetate concentrations reached significantly lower values in White Sea than in North Sea specimens at low temperatures, it seems that both low and high thresholds are shifted to lower temperatures in subpolar worms. The mechanism of the shift is still unknown, but the pattern of changes in succinate and propionate levels could support a general hypothesis as described below.

Concentrations of propionate and succinate were higher in White Sea than in North Sea animals after 10 h of incubation of high temperatures with a similar rate of

acetate formation. This means an increased flux through the succinate-propionate pathway and a higher rate of mitochondrial ATP production in subpolar compared to North Sea lugworms. Obviously, White Sea animals have a higher energy demand at high temperatures, which might be related to the smaller size of the subpolar worms (1–3 g compared to 5–8 g of North Sea animals). On the other hand, the number of mitochondria was demonstrated to increase during cold acclimation in fish muscle (Sisson and Sidell 1987; Eggington and Sidell 1989). The following hypothesis arises from the combined observations of lower Tc_I s and higher mitochondrial ATP production in White Sea as compared to North Sea animals. Mitochondrial proliferation and the cost of maintenance of a higher number of mitochondria (e.g. maintenance of ion (proton) gradients) could lead to a rise (a higher "idling") of basal metabolism. This would explain elevated rates of succinate and propionate formation at high temperatures in White Sea animals, which are living at lower annual mean temperatures than North Sea worms. The rise in aerobic capacity associated with an acclimation to cold may be required to shift Tc_I to lower values, but owing to increased mitochondrial energy turnover, may cause Tc_{II} to fall as well. Further study is required to investigate whether these predictions really hold true.

Long term incubations demonstrated that, within 1 week, North Sea animals were not able to adapt to temperatures beyond both high or low *T*c. Rising concentrations of acetate, propionate and succinate were caused by a temperature change from 12 to 25 or −1.7 °C. The onset of anaerobic metabolism and the pH decrease as well as the death of animals incubated at temperatures above the high *T*c indicate a time-limited situation, but the exact mechanisms limiting survival are not known. Death after cold stress linked to the onset of anaerobic metabolism, acidosis and the decrease in energy status below a possibly fatal level could be observed in *Sipunculus nudus* (Zielinski and Pörtner 1996).

In the White Sea mean monthly surface temperatures in the open parts of the Kandalaksha Guba increase from −1.5 °C in March to 13.5 °C within 5 months and decrease again to 0 °C during the subsequent 4 months (Zenkevitch 1963). The surface temperature in the southern North Sea changes from February to August from about 5 to 16 °C (Wolff 1983; Prandle and Lane 1995). The temperature change near the coast is even larger in both areas. Therefore, the fluctuations which White Sea animals are exposed to, are larger than the ones experienced by North Sea worms. This is probably the reason for a higher capacity to adapt to temperature changes in the subpolar compared to the boreal worms. A correlation between geographical distribution and zonation on the shore and the tolerance to temperature changes was also found in intertidal barnacles and limpets (Southward 1958).

After elevating the temperature to a value 4 °C above Tc_{II}, White Sea animals not only survived, but were actually able to acclimate as indicated by the reversal of an initial transition to anaerobiosis (Fig. 6). The concentrations of volatile fatty acids in the body wall tissue were highest after 4 h and declined again after 3 days. pH_i also rose again after an initial drop. The mechanisms of recovery remain unexplained. In the context of the above discussion it appears possible that the elevated mitochondrial content hypothesized above to prevail in White Sea specimens as a consequence of cold adaptation, may be reduced during long-term acclimation to high temperatures. Thereby, the cost of mitochondrial maintenance ("idling") is reduced, compensating for the Q_{10} effect. In consequence, Tc_{II} would be shifted to higher values. The limited ability to acclimate to temperature changes in North Sea specimens may therefore be linked to a limited capacity to adjust mitochondrial density. However, this hypothesis also requires further investigations.

Conclusions

A low and a high *T*c can be defined, both of which are characterised by the onset of anaerobic metabolism. Beyond each *T*c energy demand cannot be met by adequate O_2 supply owing to an insufficient capacity of ventilatory and circulatory mechanisms even under normoxia. Additional anaerobic energy production is required. Acetate is a main end product of temperature induced anaerobiosis in *Arenicola marina* both at low and high temperatures. Latitudinal or seasonal temperature adaptation lead to a more or less parallel shift of both *T*c values.

Between critical temperatures intracellular pH in the body wall musculature of *A. marina* changes in accordance with the α-stat hypothesis but slopes are no longer linear beyond the Tc_I s. The deviation from linearity is caused by metabolic acidification or may be due to a failure of active pH_i adjustment.

The ability of North Sea animals to adapt to temperatures beyond the *T*c is poor when compared to White Sea specimens. The larger range of temperature fluctuations at the White Sea is seen as a reason for the higher adaptational capacity of the subpolar animals. However, the exact mechanisms enabling survival under extreme temperature conditions and causing an adaptive shift of the *T*c are unknown. A hypothesis is developed that, among other mechanisms, *T*c is set by an adjustment of mitochondrial density and thus, aerobic capacity. The downward shift of the low *T*c would require a rise in mitochondrial content, whereas an upward shift of the high *T*c would call for a downward adjustment of mitochondrial density. With a more or less constant "distance" between the two *T*c values, setting the adequate *T*c requires a trade-off between cold or heat resistance and the associated rise or drop in energy turnover.

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