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Oxygen and capacity limited thermal tolerance of the lugworm *Arenicola marina*: A seasonal comparison

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ABSTRACT

Lugworms *Arenicola marina* were collected from Arcachon Bay in two summers and winters of consecutive years. The worms were acclimated to different temperatures (5 and 10 °C for winter animals and 15 °C for summer animals). Each group was investigated over an experimental temperature range concerning its optimum in exercise performance, acute growth rate as well as respiration and ventilation activities to reveal seasonal acclimatisation effects, potential inter-annual differences and the influence of laboratory acclimation temperatures on the parameters of interest. The groups investigated at the two consecutive summers yielded nearly identical results for ventilation and respiration activities. A clear seasonal difference developed in exercise performance, with an optimum at lower temperatures in winter than in summer, irrespective of acclimation temperature. Respiration and ventilation activities showed no significant differences between winter specimens acclimated to 10 °C and summer specimens acclimated to 15 °C. However, an acclimation temperature of 5 °C for winter animals caused noticeable differences to those acclimated at 10 °C. Acute growth rates differed seasonally as well as between acclimation temperatures with the highest rates found around 10 °C in summer and around 15 °C in winter. The lowest rates were recorded in winter worms acclimated to 5 °C. These acute patterns may reflect high thermal limits in warm acclimated winter worms and temperature dependent shifts in energy demand in summer animals.

1. Introduction

Arenicola marina is one of the most eminent secondary producers in the intertidal habitat. It is important for other epibenthic and infaunal animals aerating the habitat by sediment dwelling. The fresh weight of individuals may rise up to more than 30 g. Field studies in northern France showed that up to 346.6 kg lugworm faeces were produced per m^2 and year, which corresponds to a sediment layer of 21.5 cm height (Pollack, 1979). *A. marina* feeds on ciliates, microalgae and bacteria in the sediment and the overlying water. Bacterial biomass and chlorophyll a concentration in the sediment show a clear seasonal variability with a maximum occurring between April and October. In contrast, coastal surface waters show the highest bacterial biomass and concentrations of chlorophyll a in March to July (Hubas et al., 2007). Ciliates and other mesopsammon-organisms also show the highest density in summer (Pollack, 1979). The main food uptake by *A. marina* occurs during high tide, when the sediment is covered by surface water. *A. marina* not only exploits the nutrients enclosed in the upper sand layer by consuming the sand caving in from the surface like a funnel, but also extracts those from the surface water (Pollack, 1979) by generating a headward directed water current through its burrow (Wells, 1945). Suspended substances and planctonic organisms are trapped in the sand of the burrow headshaft, which acts as a filter (Krüger, 1957). The worm emits mucus to fill the interstices so that small colloidal particles are also retained. This way, the food region is enriched of organic material, which the lugworm ingests together with the sand.

A. marina is very abundant in Arcachon Bay. It is found at any beach (Boisseau, 1962) in densities up to 20 or sometimes 30 to 40 individuals per m^2, mostly in the intertidal zone and around the island Ile aux Oiseaux even in direct neighbourhood of oyster and eelgrass beds (Amoureux, 1966). The studied population is located at La Hume, a sheltered beach at the southern coast of Arcachon Bay. The surface inhabited by lugworms is a band of approximately 300 m width, bordered by a *Zostera nana* bed at the lower margin and a salt marsh vegetation at the upper margin (Cazaux, 1966). The population consists of at least three generations. The youngest generation is found from the end of March onward in the highest zone of the intertidal, close to the sandy beach. The older individuals with a body weight of up to 6 g inhabit the lower intertidal (Cazaux, 1966). At La Hume lugworms start the production of gametes around the end of April, spawning occurs between the end of August and the beginning of October. After reproduction, the oldest generation disappears and the younger ones resume growing until spawning of the next year (Cazaux, 1966).

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This strong seasonal pattern mirrors the temperature changes between seasons which are accompanied by a shift in the widths and positioning of thermal tolerance windows on a temperature scale. Seasonal changes in *A. marina* thermal tolerance have already been investigated in a North Sea population (Sommer, 2001; Sommer and Pörtner, 2004; Sommer et al., 1997; Wittmann, 2005; Wittmann et al., 2008). According to the concept of oxygen and capacity limited thermal tolerance, critical temperatures represent the threshold beyond which anaerobic metabolism is necessary for survival because the oxygen demand can no longer be met by aerobic processes (Frederich and Pörtner, 2000; Pörtner, 2001). A more or less parallel shift of both low and high critical temperature values was found with seasonal acclimatisation, characterised by anaerobic end product accumulation (Sommer et al., 1997). Investigations at the mitochondria level revealed that seasonal cold acclimatisation in North Sea lugworms involved a drop in mitochondrial density below summer values, combined with an increasing efficiency of aerobic energy production of each individual mitochondrion (Sommer, 2001; Sommer and Pörtner, 2004). Wittmann et al. (2008) compared thermal tolerance windows of North Sea lugworms during winter to those during summer and demonstrated a widening of the window, accompanied by a shift of critical temperatures towards higher values.

The present study was designed to assess the physiological responses accompanying seasonal acclimatisation and the biochemical changes reported above. Effects of temperature acclimation and inter-annual variation were investigated in a Southern population of the lugworm. Methods were chosen to investigate exercise performance capacity, metabolic energy demand and supply as well as somatic growth. Recordings of digging periods have already been established as a measure for muscular performance and were successfully applied to show differences in performance levels of lugworm populations from various latitudes (Schröer et al., 2009). The water volume which the lugworm pumps through its burrow gives some information about oxygen demand, as oxygen is extracted by gills and body surface from the bypassing water. Pumping frequency is changed to enhance or reduce the water volume flow through the burrow in order to adjust oxygen availability to the respective demand. The experimental setup simultaneously recorded water volume flow and oxygen content and also provided data for pumping frequency and oxygen extraction efficiency determination. Pumping frequency has already been used as a measure for performance depending on salinity (Shumway and Davenport, 1977) and temperature (Schröer et al., 2009; Wittmann, 2005; Wittmann et al., 2008). The mechanical aspects of the lugworm pump (Riisgård et al., 1996) and the biogeochemical consequences for the vented burrow fluids (Davey et al., 1990) have also been of interest. For the determination of acute growth optima, the incorporation of ^{13}C labelled phenylalanine was tracked by ^{13}C NMR spectroscopy as introduced by Wittmann et al. (2008).

2. Materials and methods

2.1. Animals

Specimens of the polychaete *A. marina* (L.) were collected in the intertidal zone at the sampling site in La Hume (44.65° N, 1.17° W) near Arcachon at the French Atlantic coast. Lugworms collected in August 2005 were used for investigations of respiration, ventilation and protein syntheses. For the same experiments during winter, animals were collected in January/February 2006. For yearly comparison the collection of animals was repeated in August 2006 and reinvestigated for potential annual changes. Digging performance was studied on animals from August 2006 and February 2007, for summer and winter, respectively. All worms were maintained in basins filled with natural sediment in a natural seawater flow-through aquarium system at the Alfred Wegener Institute until experimental use. The specimens collected in winter were divided between incubation temperatures of 5 °C and 10 °C, those collected in summer were kept at 15 °C. All animals were exposed to a salinity of 32‰ and a 12 h/12 h light/dark cycle in the aquaria and fed with ground and soaked Tetramin® flakes every other week.

2.2. Field measurements

In parallel to animal collection, biotic and abiotic field parameters were recorded at the sampling site as described by Schröer et al. (2009). In particular, temperatures in air, tidal puddles and sediment were recorded with a thermometer (Testo 925, Testo, Lenzkirch, Germany) using a special temperature-receiving element (6 mm diameter, 500 mm length, TC Direct, Mönchengladbach, Germany). Salinity was measured in tidal puddles by use of a multiple parameter pocket measurement device (Multi 340i, WTW, Weilheim, Germany). The length of the tail shaft of the worm's burrow was taken as a measure of burrow depth by using a scaled metal stick inserted into the opening of the burrow. For abundance recordings, a 1 m × 1 m wooden frame was placed onto the intertidal sediment at haphazard and the number of faecal piles therein was counted. Bodyweight was measured using a scale (EMB 220-I, Kern, Balingen-Frommern, Deutschland).

2.3. Digging performance

Experimental temperatures were chosen at 4 °C steps, between −1 and 19 °C for the winter worms. Specimens sampled in summer were measured at 7 to 27 °C. For initial short-term acclimation animals were transferred to a plastic container placed into a temperature controlled aerated seawater bath. Temperature was changed at 2 °C h^{-1} starting from maintenance conditions and kept constant for at least 12 h at the new experimental temperature. After acclimation for at least 12 h, animals were transferred into the experimental setup 1 h prior to measurements.

The experimental setup and procedure were the same as described by Schröer et al. (2009). Briefly, the animals were positioned on the sediment surface and the duration of each digging period was recorded using a stopwatch. For an analysis of burrowing capacity during a limited time window this routine was repeated for 90 min and the number of digging periods was recorded. In total, five specimens of each group were examined at each temperature ($n = 5$). Summer (15 °C acclimated) data were published in our previous work recently (Schröer et al., 2009).

2.4. Respiration and ventilation experiment

Analyses of respiration and ventilatory activity were carried out as described previously (Wittmann et al., 2008). Briefly, measurements were performed in the dark using artificial burrows consisting of straight Perspex tubes with a rough inner surface. As in their natural burrows, animals generated a water current to provide themselves with oxygen. Air saturation of incurrent and excurrent water was monitored continuously with oxygen micro-optodes (PreSens, Regensburg, Germany). The volume flow produced by the worms was measured using an electromagnetic flowmeter (inner diameter of probe head: 3 mm, RT-500, Hugo Sachs Elektronik, March-Hugstetten, Germany).

Experiments started at a temperature of 4.5 °C for winter animals kept at 5 °C and at 10.7 °C for those kept at 10 °C. Summer animals were tested beginning at 15 °C. Temperature was changed at a rate of 1 °C h^{-1} (first lowered) by steps of 3 °C and kept constant for 6 h. Lugworms collected in winter were exposed to a temperature range from −0.0 to 22.8 °C, summer worms experienced a range from 2.8 to 26.1 °C. Mean oxygen partial pressure of incurrent and excurrent water (P_{IO_2} and P_{EO_2}, kPa) and weight specific volume flow (V_w, ml h^{-1} g^{-1}) were calculated for the last 3 h of each incubation

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period. From these data oxygen consumption (M_{O_2}, µmol O_2 h^{-1} g^{-1}) and the extraction coefficient (%) were determined. The analysis also included the mean volume flow during the phases of active ventilation (active volume flow, ml min^{-1}) and the number of contraction waves of the body wall (pumping frequency, min^{-1}). The water volume transported per peristaltic wave of the body wall musculature (wave volume, ml, see Wittmann et al., 2008) was calculated from volume flow recordings during active ventilation periods. Results from summer 2006 animals were published before (Schröer et al., 2009).

2.5. Protein biosynthesis

Analyses of protein biosynthesis were carried out as described previously (Wittmann et al., 2008) and following the principles outlined by Langenbuch et al. (2006). Briefly, uniformly labelled ^{13}C-L-phenylalanine, dissolved in filtered seawater (75 mmol l^{-1}), was injected at 40 µl g^{-1} body weight into the coelomic cavity of the lugworms. The animals were then inserted into their artificial burrows and incubated at temperatures of −0.9, 3.5, 6.9, 11, 15.2 and 19.2 °C for winter animals and 7.4, 10.2, 14.3, 17.8 and 22.8 °C for summer animals while they were actively ventilating their burrows. For animals collected in summer the experiment was carried out with 8 animals at each temperature. Animals were exposed to incubation temperatures for 24 h, then injected and incubated for another 30, 60, 90, 120, 180, 240, 300 and 360 min, respectively. For winter animals, 12 specimens were used at each temperature and 2 of them pooled after 30, 60, 120, 180, 240 and 360 min, respectively.

After the respective incubation time, the cuticulo-muscular tube was frozen in liquid nitrogen. The frozen tissue was ground under liquid nitrogen and extracted with TCA (trichloroacetic acid). The homogenate was centrifuged and supernatant and pellet were treated differently. The supernatant representing the cytosolic fraction and containing low molecular weight constituents was neutralised, dried and dissolved in D_2O (deuterium oxide).

The pellet was washed, suspended in distilled water and neutralised. After centrifugation, the supernatant containing water-soluble proteins was removed and stored while the pellet was boiled in a water bath with 1 M NaOH. The water-insoluble proteins were then added to the water-soluble protein fraction and both were dried. The total protein was dissolved in D_2O.

Both cytosolic and protein extracts were measured in a NMR Spectrometer (9.4 T Avance, Bruker, Rheinstetten, Germany) at frequencies of 100.6 MHz for ^{13}C spectra. The same parameters of the NMR recordings were used as described in Wittmann et al. (2008).

The protein contents of the protein extracts were quantified according to Bradford (1976). The amount of incorporated ^{13}C-L-phenylalanine was obtained as described by Wittmann et al. (2008). The calculated amount of incorporated ^{13}C-L-phenylalanine was related to the respective protein content of the sample and plotted against incubation temperature for incubation time. Due to the insensitivity of the NMR technique and the fact that only one sample per individuum could be analysed we could not track the time-dependent increase of incorporated ^{13}C-L-phenylalanine in sufficiently high resolution. Inter-individual differences exceeded those determined over time, which is why data points were pooled into two time frames: short incubation period (30 to 120 min) and long incubation period (180 to 360 min) (see also Wittmann et al., 2008).

2.6. Statistics

Statistical analysis was performed using GraphPad Prism version 4.0c for Macintosh (GraphPad Software, San Diego, California, USA). Nonlinear regression curves were fitted to temperature dependent burrowing activities, using the equation $EP(T) = F_1(T) + F_2(T) = (A_1e^{B1T} + C_1) + (A_2e^{B2T} + C_2)$ according to Pörtner and Knust (2007). For weight specific volume flow data the equation $V_w(T) = Ae^{BT} + C$ was used as described in Wittmann et al. (2008). Significant deviations of V_w values from the hypothetical value extrapolated from the exponential relationship were identified by one-sample Student's t-test. One-way ANOVA was performed to detect significant changes in pumping frequency, extraction coefficient and protein synthesis values over the temperature range within each group (F-test). Two-way ANOVA combined with a Bonferroni posthoc test was applied to detect significant differences in pumping frequency between the groups at each temperature. Q_{10} values were calculated for specific temperature ranges using standard procedures as in Sommer and Pörtner (2002). One-way ANOVA and Tukey's posthoc test were used to identify differences in field data between the groups. Statistical significance was identified at the $p \leq 0.05$ level. All data are given as means ± SE if not stated otherwise.

3. Results

3.1. Field measurements

Biotic and abiotic field parameters (Table 1) were recorded to characterise the natural habitat conditions of the population at the time of collection during the respective season. Salinity was significantly ($p<0.001$) lower in winter than in summer, whereas variability between the two consecutive years was small. Temperatures in air, tidal puddles and sediment (20 cm depth) differed significantly ($p<0.001$) between winter and summer. Differences between the two winters or the two summers, respectively, were much smaller, although some significant deviations could still be found (air temperature in winter 2006 vs. winter 2007: $p<0.001$; temperature in tidal puddles summer 2005 vs. summer 2006: $p<0.001$; sediment temperature in 20 cm depth: $p<0.001$ between all groups), as data were only available for the week of animal collection. Burrow depth (length of the tail shaft) did not show any seasonal differences. Abundance also did not vary between winter and summer. A seasonal variation in animal weight could be detected; specimens collected in summer 2006 were significantly heavier than animals collected in winter (vs. winter 2007: $p<0.001$; vs. winter 2006: $p<0.05$). The value for summer 2005 is not representative, as juveniles were comprised in the measurement.

3.2. Digging performance

Fig. 1 shows the temperature dependent number of digging periods performed during 90 minutes experimental intervals. Lugworms collected in winter and acclimated to 5 °C showed up to 8.60 ± 0.24 digging periods 90 min^{-1} at 15.4 °C. Digging performance decreased rapidly towards higher temperatures and more slowly towards lower temperatures. At −0.8, 3.0 and 7.0 °C, digging activity stayed on the same level of about 4 periods 90 min^{-1}. The regression analysis of performance exhibited a maximum of 7.35 digging periods 90 min^{-1} at 14.2 °C.

Winter animals acclimated to 10 °C showed similar digging performances. They displayed the highest number of digging periods (7.80 ± 0.37 90 min^{-1}) at 15.3 °C. As seen in 5 °C acclimated worms, digging performance decreased rapidly towards higher temperatures and more slowly towards lower temperatures. In contrast to the latter, values at −1.1, 3.0 and 6.9 °C did not stay at the same level but increased steadily. The performance model indicated a maximum of 7.48 periods 90 min^{-1} at 13.3 °C.

Lugworms collected in summer and acclimated to 15 °C performed the highest number of digging periods (5.80 ± 0.80 90 min^{-1}) at 23.2 °C, while the model predicted a maximum of 5.26 periods 90 min^{-1} at 19.6 °C. As observed in winter worms, performance capacity decreased more rapidly towards higher than towards lower

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t1.1 **Table 1**
Abiotic and biotic field parameters at the time of collection of *Arenicola marina* specimens at the sampling site in La Hume near Arcachon (Atlantic, France), mean values ± SD.

t1.2
t1.3

Sampling month	Salinity (‰)	T (°C) air	T (°C) tidal puddles	T (°C) in 20 cm depth	Length of tail shaft (cm)	Abundance (m^{-2})	Weight (g)
t1.4 August 2005	35.8 ± 1.2	20.2 ± 2.4	20.7 ± 2.4	20.3 ± 0.6	8.0 ± 2.4	22.7 ± 5.0	4.1 ± 1.4
	n = 17	n = 13	n = 13	n = 13	n = 6	n = 6	n = 29
t1.5 January 2006	26.7 ± 0.5	7.6 ± 2.4	9.5 ± 2.3	7.1 ± 0.8	13.0 ± 2.4	23.0 ± 9.1	4.7 ± 1.6
	n = 25	n = 23	n = 23	n = 23	n = 10	n = 20	n = 72
t1.6 August 2006	34.1 ± 0.6	22.1 ± 2.4	25.8 ± 4.1	22.2 ± 0.8	12.9 ± 2.9	28.4 ± 11.2	5.7 ± 1.8
	n = 20	n = 20	n = 20	n = 20	n = 20	n = 20	n = 30
t1.7 February 2007	25.1 ± 4.2	12.5 ± 0.4	12.1 ± 0.8	9.0 ± 0.3	12.8 ± 2.0	8.2 ± 3.2	4.1 ± 1.3
	n = 10	n = 10	n = 10	n = 10	n = 10	n = 13	n = 30

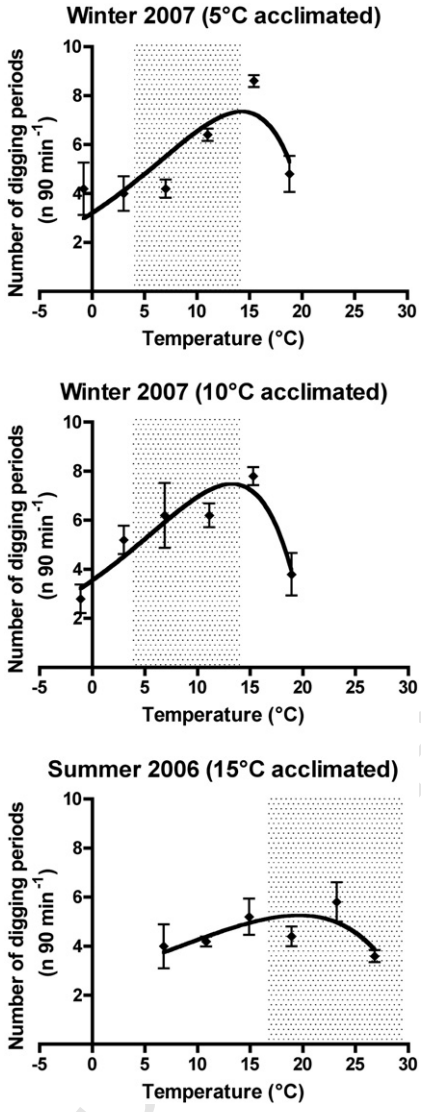

Fig. 1. Temperature dependent burrowing capacity in *Arenicola marina*. Mean values ± SE for animals collected in summer 2006 and in winter 2007 at the French Atlantic coast, n = 5. Winter animals were acclimated to 5 and 10 °C and tested between −1.1 and 18.9 °C. Summer animals were acclimated to 15 °C and investigated between 6.8 and 26.8 °C (depicted from Schröer et al., 2009). Data were fitted to the equation $EP(T) = F_1(T) + F_2(T) = (A_1e^{B1T} + C_1) + (A_2e^{B2T} + C_2)$ with EP(T) = temperature dependent muscle exercise performance capacity. The first term, $F_1(T) = A_1e^{B1T} + C_1$, represents the temperature dependence of aerobic processes supporting exercise performance. The second term, $F_2(T) = A_2e^{B2T} + C_2$, represents the parallel exponential rise in processes limiting aerobic scope and thus exercise performance capacity. For the winter data (5 °C acclimated): $A_1 = -12.02$, $B_1 = 0.1385$, $C_1 = 1.800$, $A_2 = 15.23$, $B_2 = 0.1276$, $C_2 = -1.807$, $r = 0.6472$. For the winter data (10 °C acclimated): $A_1 = 7.403$, $B_1 = 0.1173$, $C_1 = 0.9182$, $A_2 = -3.791$, $B_2 = 0.1494$, $C_2 = -0.9866$, $r = 0.6810$. For the summer data: $A_1 = 4.083$, $B_1 = 0.06221$, $C_1 = -0.4552$, $A_2 = -1.081$, $B_2 = 0.1038$, $C_2 = 0.1652$, $r = 0.3723$. Shaded area: naturally experienced habitat temperatures in the respective season.

temperatures. Temperature dependence was less pronounced and lower maximum values were reached in summer animals, all values stayed between 3.6 and 5.8 digging periods 90 min^{-1}.

3.3. Respiration and ventilation experiment

Fig. 2 depicts temperature dependent weight specific volume flow (ml g^{-1} h^{-1}) in winter animals acclimated to 5 and 10 °C and in summer worms acclimated to 15 °C from two years. Specimens collected in winter and acclimated to 5 °C showed an exponential increase in weight specific volume flow over the experimental temperature range with a Q_{10} value of 5.57 ± 0.63 and remarkably larger error bars at temperatures ≥17.2 °C.

Winter animals acclimated to 10 °C also exhibited an exponential increase over the experimental temperature range, but the Q_{10} of 2.38 ± 0.38 was significantly lower than in 5 °C acclimated specimens.

Animals collected in summer 2005 and acclimated to 15 °C displayed a Q_{10} of 2.36 ± 0.12 within the temperature range of exponential increase, similar to 10 °C acclimated winter specimens. At 2.8 °C only one value was available, as the other worms stopped ventilation activity during cooling. No statistics could be applied here. The value recorded at 2.8 °C was lower than all values at 5.4 °C, therefore, the one at 2.8 °C was excluded from the exponential range.

Summer lugworms from 2006 (also acclimated to 15 °C) displayed the same exponential range as those from the preceding year, the value at 2.9 °C differed significantly from the hypothetical value predicted by the regression curve. The Q_{10} of 2.24 ± 0.23 was comparable to the one evaluated in 2005.

In general, weight specific volume flow curves were similar in winter and summer animals acclimated to 10 °C and 15 °C, respectively. Only the data collected in winter animals acclimated to 5 °C differed remarkably with around two times higher values in the temperature range above 15 °C (Fig. 2).

Fig. 3 shows temperature dependent pumping frequencies (min^{-1}) during the phases of active ventilation. Winter specimens that were acclimated at 5 °C showed a significant increase (F-test) over the experimental temperature range. Values at 19.7 and 22.6 °C displayed large variability with means significantly higher than in all other groups. Animals acclimated to 10 °C in winter and to 15 °C in summer both displayed a significant increase (F-test) with warming. Pumping frequency in lugworms collected in summer 2005 did differed significantly neither from the frequency recorded in winter animals acclimated to 10 °C nor from data collected in summer 2006. The only significant differences observed were the values at 10.9 and 23.2 °C from summer 2006 animals in comparison to both winter groups.

Fig. 4 depicts the temperature dependent extraction coefficient (%), i.e. the proportion of oxygen that the animals were able to withdraw from the inflowing water. Lugworms sampled in winter and acclimated to 5 °C showed a more or less stable extraction coefficient in the temperature range between 0 and 5.1 °C while a slight decrease was observed upon warming. In contrast, animals acclimated at 10 °C displayed a significant rise in the extraction coefficient from 0 to 17.7 °C followed by a slight decline. Summer specimens

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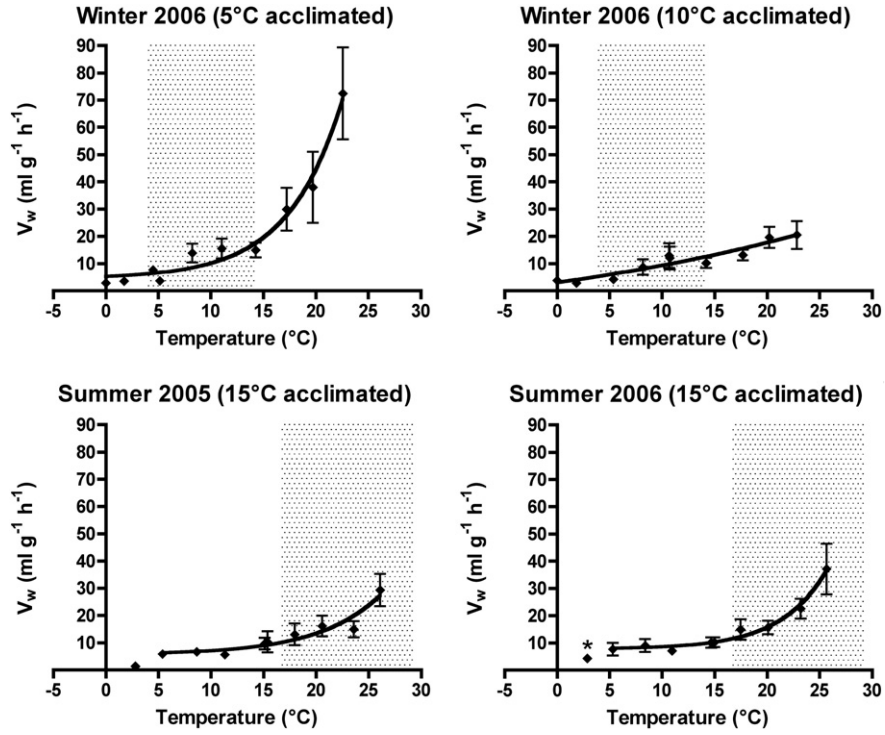

Fig. 2. Temperature (°C) dependent weight specific volume flow (ml g^{-1} h^{-1}, mean values ± SE) in *Arenicola marina* dwelling in an artificial burrow. Animals were collected in summer 2005, in winter 2006 and in summer 2006 at the French Atlantic coast. n = 5 except for winter (5 °C acclimated) at 22.6 °C, winter (10 °C acclimated) at 10.7 °C as well as summer 2005 at 15.4 and 5.4 °C with n = 4 and summer 2005 at 2.8 °C with n = 1. Winter worms were acclimated to 5 and 10 °C one half each, summer animals to 15 °C. The winter specimens were investigated from −0.0 to 22.8 °C, both summer groups were exposed to a temperature range from 2.8 to 26.1 °C. (Summer 2006 data depicted from Schröer et al., 2009). Data were fitted to $V_W(T) = Ae^{BT} + C$ (Wittmann et al., 2008). For the winter 5 °C acclimated group: A = 0.8083, B = 0.1952, C = 4.497, r = 0.8118. For the winter 10 °C acclimated group: A = 19.41, B = 0.02816, C = −16.36, r = 0.6654. For the summer 2005 group: A = 0.2620, B = 0.1694, C = 5.714, r = 0.6989. For the summer 2006 group: A = 0.08428, B = 0.2273, C = 7.832, r = 0.7495. Asterisks (*) designate data points, which are significantly different from the exponential regression curve (p = 0.0234 for summer 2006 at 2.9 °C). Shaded area: naturally experienced habitat temperatures in the respective season.

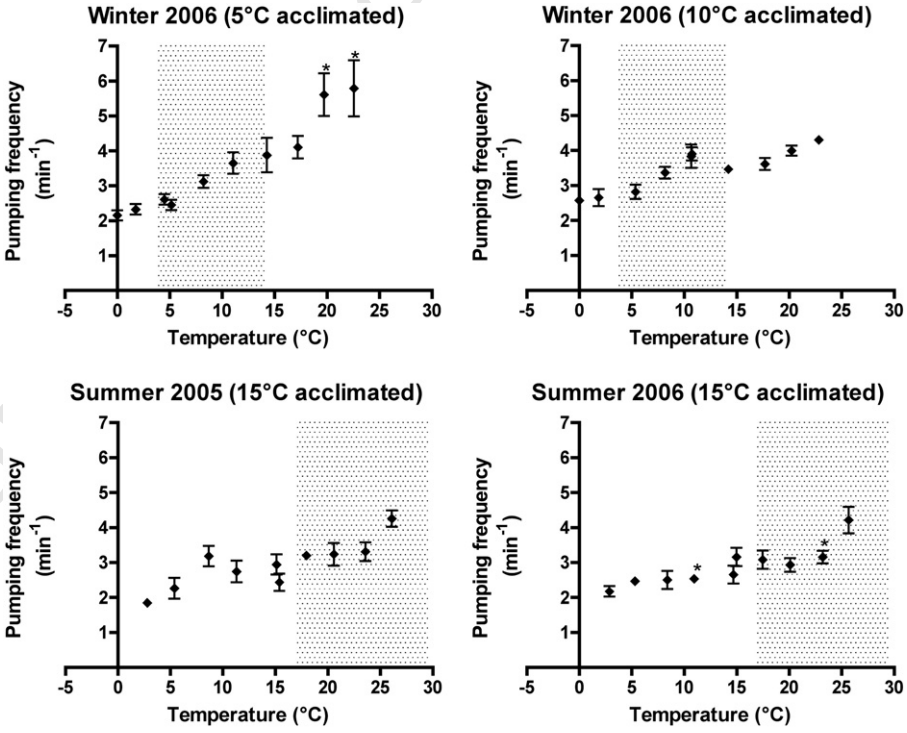

Fig. 3. Ventilatory performance of *Arenicola marina* dwelling in an artificial burrow. For each group (winter 5 °C acclimated, winter 10 °C acclimated, summer 2005 and summer 2006 (after Schröer et al., 2009)) pumping frequency (min^{-1}) is plotted against incubation temperature (°C). Mean values ± SE; n = 5 except for winter (5 °C acclimated) at 19.7 and 22.6 °C, winter (10 °C acclimated) at 10.7 °C as well as summer 2005 at 15.4 and 5.4 °C with n = 4 and summer 2005 at 2.8 °C with n = 1. Sampling time and acclimation see legend Fig. 2. Incubation temperatures ranged from 2.8 to 26.1 °C for summer worms and from −0.0 to 22.8 °C for winter animals. Shaded area: naturally experienced habitat temperatures in the respective season.

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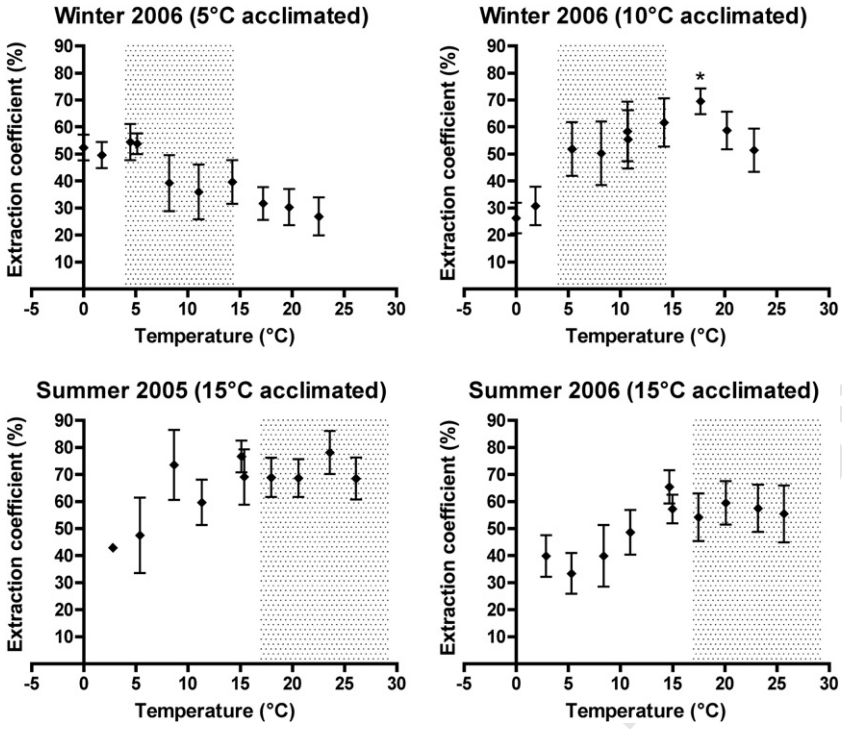

Fig. 4. Oxygen extraction by *Arenicola marina* dwelling in an artificial burrow. The oxygen extraction coefficient (%) versus incubation temperature (°C) is shown for the four groups (winter 5 °C acclimated, winter 10 °C acclimated, summer 2005, summer 2006 (from Schröer et al., 2009), mean values ± SE, for further information see Fig. 2. * = significantly higher than at −0.0 °C.

from both years showed a slight increase between 2.8 and 14.7 °C. With further warming, values stayed more or less constant. Again, the extraction coefficient in warm acclimated winter lugworms and summer specimens shows similar trends and differed from data obtained in winter animals acclimated at 5 °C.

3.4. Protein biosynthesis

Fig. 5 shows the temperature dependent incorporation of ^{13}C-phenylalanine into proteins of the body wall after short and long term incubation times. A comparison of the three graphs reveals an overall increase of the amount of incorporated ^{13}C-phenylalanine with acclimation temperature. In particular, winter animals acclimated to 5 °C displayed no increase in ^{13}C-phenylalanine content over time at −0.9 °C. Data points for short and long incubation periods resulted similarly at around 4.4 nmol mg^{-1} protein. At all other incubation temperatures, at least a slight increase between short and long incubation times was detectable with a significant difference at the highest incubation temperature (19.2 °C). In winter worms acclimated to 10 °C, the rise in the amount of incorporated amino acids over time was seen at all incubation temperatures. The maximum of 24.26 ± 1.55 nmol mg^{-1} protein was reached at 15.2 °C after long incubation times. Summer animals also showed an increasing incorporation of labelled phenylalanine over experimental time at all incubation temperatures. The largest amount of ^{13}C-phenylalanine was incorporated at 10.2 °C, reaching 32.48 ± 1.24 nmol mg^{-1} protein, a value significantly higher than at all other temperatures after short as well as long incubation periods.

4. Discussion

The aim of this study was to investigate potential seasonal acclimatisation effects in physiological performances of the lugworm *A. marina* from a southern population and to distinguish possible effects of acclimation temperature and inter-annual variability. Recently, we could show that animals from the southern-most population displayed a lower performance level than their counterparts from higher latitudes (Schröer et al., 2009).

Our results show clear seasonal differences in temperature tolerance and performance of *A. marina*. These are most likely due to seasonal temperature changes, however, other biotic and abiotic parameters also differ seasonally with potential influences on organismic performance. We observed a higher animal weight in summer 2006 compared to both winter samplings (Table 1), which might be explained by a higher food availability in summer and more importantly, the onset of gamete production. In summer 2005, juveniles were included in the samples and resulted in a lower average weight than in 2006 and not higher than in winter. Under field conditions growth in *A. marina* was only observed during spring and summer until October (Beukema and de Vlas, 1979; Newell, 1948; Smidt, 1951; Wolff and de Wolf, 1979) whereas during autumn and winter growth was absent. Moreover, the weight of adults tends to decrease in winter (Beukema and de Vlas, 1979; Newell, 1948). Pollack (1979) argued that the lower mean animal weight in winter is not necessarily due to individual weight loss but may also result from the immigration of smaller worms to the sampling area and/or from a higher mortality among older and thereby larger worms. Any effects of lower food availability in winter are exacerbated by a higher precipitation in this season, easily visible in the changing salinity of the surface water (Table 1). The lugworm shelters from the influence of freshwater by closing its burrow and reducing its pumping and feeding activities during rainfall (Pollack, 1979). The maximally tolerated daily salinity variation amounts to 4–6‰ (Amoureux, 1966).

Maximum food availability from April to October coincides perfectly with the period of reproductive growth from the end of April until the beginning of October (Cazaux, 1966). Although water temperature has been well correlated with reproductive cycles and has often been considered to be a major factor in their control, studies have also

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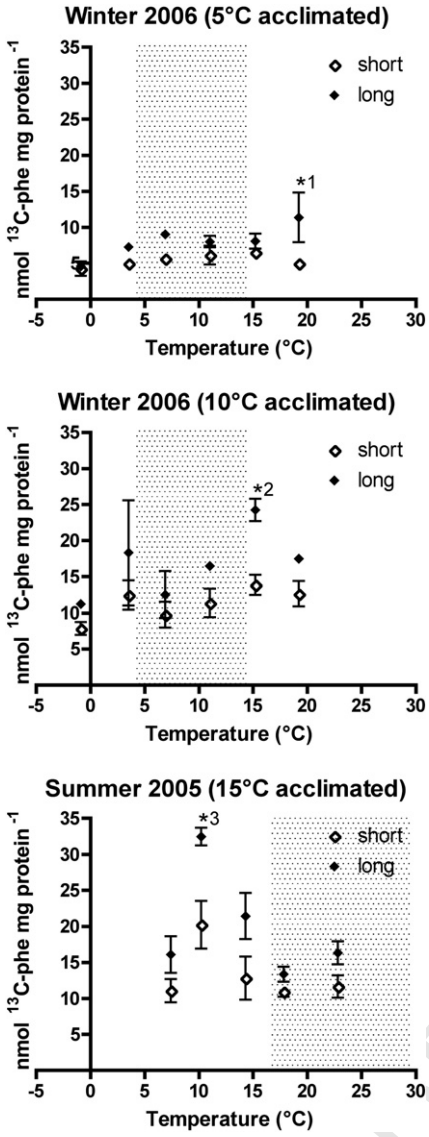

Fig. 5. Amount of incorporated ^{13}C-phenylalanine (nmol mg^{-1} protein, means ± SE) into protein of the cuticulo-muscular tube of lugworms, dependent on incubation temperature. Specimens were collected in August 2005 and January/February 2006. Winter worms were acclimated to 5 and 10 °C one half each and summer animals to 15 °C. Incubation temperatures ranged from −0.9 to 19.2 °C for the winter groups and from 7.4 to 22.8 °C for summer animals. Open diamonds: amount of incorporated ^{13}C-phenylalanine after 30 to 120 min of incubation; closed diamonds: amount after 180 to 360 min; n = 4 for summer data except for short incubation times at 22.8 °C with n = 3, n = 3 for winter data. *1 = significantly higher than the values after short incubation times at −0.9, 3.5 and 19.2 °C as well as at −0.9 °C after long incubation times. *2 = significantly higher than the values after short incubation times at −0.9 and 6.9 °C. *3 = significantly higher than all other values.

emphasised the importance of local food conditions (MacDonald and Thompson, 1986), as demonstrated by Newell et al. (1982) in *Mytilus edulis* populations, which experienced nearly identical temperature cycles but different regimes of food availability.

Our measurements of acute somatic growth rates by tracking the incorporation of phenylalanine into the body wall revealed an optimum temperature for growth without substrate limitation at 15.2 °C in winter animals and at 10.2 °C in summer animals (Fig. 5). Interestingly, neither 15.2 °C is experienced in winter nor 10.2 °C in summer in the natural habitat. A look at the sea surface temperature (Ifremer, 2007), which correlates well with the sediment temperature in 20 cm depth (Nießing, 2006), showed that temperatures between 10 and 16 °C are experienced in March, April and May as well as in October, November and December. Our winter animals which were acclimated to 10 °C showed maximum somatic growth at 15.2 °C and thereby, the capacity to tolerate higher temperatures. This might indicate an early shift to sustain spring conditions. Somatic growth is expected to take place in spring (March to May) when temperature is at its optimum and food is already available, as was the case in our aquarium system. Phytoplanctonic and therewith phytobenthic growth takes place a bit earlier in the year at Arcachon (Ifremer, 2007) compared to the more northern beaches, for which the food availability data are mentioned in the Introduction. The fact that *A. marina* juveniles are found already at the end of March (Cazaux, 1966) also argues for food availability already in March.

Our summer animals which were acclimated to 15 °C showed maximum acute somatic growth at an exposure temperature of 10.2 °C. Following the same rationale as before, this might be seen as a preparation for autumn conditions. This might also involve constraints on energy budget in the warmth where cold exposure slows motor activity and supports a shift of resources to growth. After spawning in autumn, the worms are expected to replenish their glycogen reserves for winter survival (see below). So growth is controlled by exposure rather than acclimation temperature. Similarly complex interactions were previously shown for juveniles of the oyster *Ostrea edulis*, which displayed a maximum scope for growth at an acclimation temperature of approximately 17 °C and an exposure temperature of approximately 25 °C, a common condition found in shallow waters during the summer months (Buxton et al., 1981). In *A. marina*, all energy is invested into reproductive growth during summer, while somatic growth takes place in spring and autumn, suggesting a close relationship between energy available for growth, the reproductive cycle and thermal limits. In fact, Füßner (2009) observed a higher sensitivity to temperature changes in North Sea summer worms compared to North Sea spring animals, which might be due to the increased energy expenditure for reproductive growth.

Bayne and Newell (1983) suggested that scope for growth and growth efficiency are more dependent on food availability than on temperature. Both, a trade-off between growth and reproduction and a strong dependence on food availability, were found in the giant scallop *Placopecten magellanicus*: scope for growth was low or negative during winter, rapid gamete maturation was observed during the spring bloom and somatic weight declined during gamete development while it increased after spawning and during periods of low gametogenic activity (MacDonald and Thompson, 1986). Similarly, low or negative growth during the winter and generally higher values in the spring and/or summer are also described for *Chlamys islandica* (Vahl, 1980), *M. edulis* (Bayne and Widdows, 1978; Thompson, 1984) and *Mya arenaria* (Gilfillan et al., 1976). Whether somatic tissue weight declines during gamete development in *A. marina* has not been investigated so far and may depend on local temperature and food conditions. For example, *Chlamys varia* shows both, simultaneous reproductive and somatic growth as well as gamete development fuelled by somatic reserves, depending on environmental conditions (Shafee, 1980). In *Macoma balthica*, somatic growth becomes negative during gametogenesis when temperatures are high, but continues despite gamete development at low temperatures (DeWilde, 1975). High food availability combined with low temperatures and thereby a reduced metabolic demand results in high scope for growth. These conditions would allow for simultaneous somatic and reproductive growth and might be found at Arcachon in April and May. In these months, gamete development already begins (Cazaux, 1966) and temperature ranges around the optimum for somatic growth (see above).

Laboratory experiments on somatic growth in *A. marina* were already carried out by de Wilde and Berghuis (1979). Their study was performed on animals from the Netherlands with constant food supply and excluding the influence of reproduction by using juvenile worms. In that study, maximum growth rates in length and weight were observed at 20 °C with a small influence between 5 and 20 °C and a strong influence between 20 and 25 °C, as at 25 °C

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the growth rate was considerably lower than at 5 °C (de Wilde and Berghuis, 1979). It should be noted that these were acclimated, not acute growth rates. In natural populations of juvenile lugworms growth rates were usually found much lower than in the experiments. It may be assumed that the main limiting factor for growth in *Arenicola* is food limitation, caused either by food competition with other small mud flat inhabiting organisms or by a poor quantity and/or quality of the organic matter available (Boon and Haverkamp, 1979; de Wilde and Berghuis, 1979). Interestingly, the increase in biomass, i.e. the product of numerical density and mean weight, was highest at 10 °C in laboratory experiments, indicating a higher mortality with rising temperature, probably caused by permanently low oxygen concentrations induced by the combination of high amounts of organic matter and high temperatures (de Wilde and Berghuis, 1979). In field studies, the highest mortality rates were found from January to March, decreasing during spring and resulting in considerably lower values in summer than in winter (Pollack, 1979). This observation suggests once more that food availability is a limiting factor for growth in the field.

The only remarkable seasonal difference was found in exercise performance (Fig. 1). Corresponding to the concept of oxygen and capacity limited thermal tolerance (Pörtner and Knust, 2007; Pörtner et al., 2004), digging activity displays an asymmetric bell shaped curve, as already shown in a previous study (Schröer et al., 2009). This study demonstrated clear seasonal acclimatisation with a more pronounced optimum at lower temperatures in winter and a wider and less distinct performance curve with an optimum at higher temperatures in summer. The data suggest a trend for curve width to increase from winter to summer at the expense of a decrease in performance amplitude. Comparing cold- and warm-adapted lugworms from different latitudes (Schröer et al., 2009) a trade-off between the width and the amplitude of the performance curve also became apparent (cf. Angilletta et al., 2002; Huey and Hertz, 1984; Pörtner, 2006). For ectothermic vertebrates, it has already been shown that muscle twitch tension decreases with increasing temperature in fast-twitch muscles (Bennett, 1984). In addition, viscoelastic properties of cell membranes change with temperature, as seen for example in human red blood cells (Hochmuth et al., 1980). So higher temperatures in summer might result in a higher elasticity of cell membranes and change the worm's whole bodywall. For this reason, only a lower internal resting pressure (turgor) might be achieved in lugworms, causing lower amplitudes of body wall contraction pressure resulting in reduced digging performance. Seymour (1971) observed that a higher resting pressure resulted in a higher peak pressure in burrowing lugworms. Our observations of the internal pressure in burrowing lugworms by use of a catheter (data not shown) in fact exhibited a tendency of higher pressures at lower temperatures, but rising contraction frequencies with rising temperature. Therefore, the internal pressure might be the limiting factor at higher temperatures, while contraction frequency may be the limiting factor at lower temperatures.

Temperature fluctuation is higher in summer (16 to 29 °C) than in winter (4 to 14 °C), which might be one reason for a broader performance curve in summer. The lower performance amplitude in summer argues for a trade-off between reproductive growth and exercise performance. Performance curves of 5 and 10 °C acclimated specimens in winter were nearly identical leading to the conclusion that acclimation temperature alone has no effect on performance capacity. Consequently, seasonal acclimatisation does not only depend on temperature, but other seasonal changes (e.g. photoperiod, precipitation and food availability), as well as competing physiological processes like reproductive growth can also play an important role for the observed seasonal differences in muscular performance (i.e. digging activity).

Thermal acclimatisation of muscle performance has already been shown before in other marine invertebrates as in the European queen scallop, *Aequipecten opercularis* (Bailey and Johnston, 2005) and the giant scallop, *P. magellanicus* (Guderley et al., 2008). In these studies, higher performance amplitudes also result in cold acclimated animals. Cold acclimated *A. opercularis* attained higher swimming velocities and accelerated faster at winter temperatures than warm acclimated animals at summer temperatures (Bailey and Johnston, 2005). A study by Guderley et al. (2008) reported that even handling stress had less impact on cold than on warm acclimatised animals of the species *P. magellanicus*. Guderley (2004) suggested that locomotor performance and reproduction are closely coupled, as muscle metabolic capacities fall in parallel with glycogen mobilisation for gametogenesis, and reproductive fitness will be favoured more than maintenance of performance. This interpretation might explain our observations on lugworms as well. In our study, winter acclimatised worms displayed the highest performance optima, as reproductive growth takes place in summer (from end of April to the beginning of October, Cazaux, 1966) and spawning occurs during autumn (October to November). So in summer animals the gonads take up most of the body mass, whereas winter animals do not invest any energy into reproduction. Also *A. opercularis* and *P. magellanicus* both have their spawning period until October and achieve performance maxima in winter after spawning. Füßner (2009) observed a much narrower thermal tolerance window in *A. marina* from the North Sea during the time of spawning than in specimens during the early stage of gamete production.

Consistent with previous findings (Schröer et al., 2009), performance optima were found within the naturally experienced temperature range during the respective season in this study. In winter, modelled performance maxima of 14.2 and 13.3 °C, respectively, were found close to the upper limit of naturally experienced habitat temperatures of around 14 °C. In contrast, the modelled performance maximum of 19.6 °C in summer was found close to the lower limit of habitat temperatures during the respective season (around 17 °C). This leads to the conclusion that performance, despite its seasonal acclimatisation, is well adapted to the yearly mean habitat temperature of approximately 16 °C ensuring constant performance during the whole year. By extrapolating the model curve, it also becomes obvious that the upper critical temperature in summer, which is predicted by the model at 31.7 °C, nearly falls into the naturally experienced range of habitat temperatures of up to 29 °C in summer. This suggests that the population is more sensitive to warming effects in summer than in winter.

Effects of temperature acclimation without the influence of reproduction have been investigated in tadpoles of *Limnodynastes peronii* as well as tadpoles and adults of *Xenopus laevis* (Wilson and Franklin, 1999; Wilson et al., 2000). Cold acclimated organisms reached a higher swimming velocity at low temperatures than warm acclimated specimens, while at high temperatures warm acclimated animals showed a higher swimming performance than cold acclimated ones. This simple relationship is overlaid with the effects of reproductive growth in our study when comparing digging activity in winter and summer acclimatised lugworms.

Despite a nearly identical performance curve, the group of winter animals acclimated to 5 °C showed a much higher volume flow than those acclimated to 10 °C. Differences became obvious especially at temperatures above 15 °C, resulting in a high Q_{10} value of 5.57 ± 0.63 compared to values between 2.24 and 2.38 in the other winter and summer groups. 5 °C acclimated winter specimens showed their highest extraction coefficients at temperatures from 0.0 to 5.1 °C, whereas 10 °C acclimated winter worms and both groups of summer worms exhibited their lowest extraction coefficient values in this temperature range. Cold compensation at the cellular level likely occurred at 5 °C. Sommer and Pörtner (2004) found changes in mitochondrial functions taking place in *A. marina* from the North Sea, which were acclimated to 0 °C in comparison to those acclimated to 5 and 11 °C. These findings suggest that cold acclimatisation occurs below a threshold temperature rather than progressively

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with falling environmental temperatures. The other way round, returning to warm acclimatisation also seems to occur stepwise, as Wittmann (2005) found different thermal tolerance windows in worms which experienced a warming pulse of 3 °C compared to those which were investigated before. Somatic growth (Fig. 5) was minimal in our 5 °C acclimated compared to 10 °C acclimated winter lugworms. These findings could be an indication for a temperature induced dormant condition as it is found in the common shallow-water and littoral bivalve *Cardium* (=*Cerastoderma*) *edule* L. in winter (Newell and Bayne, 1980). Dormancy has also been found in other marine polychaetes like *Lanice conchilega* (Cáceres, 1997). Dormant individual relies on carbohydrate reserves for maintenance energy requirements (Newell and Bayne, 1980). Lugworms also show a decrease in the glycogen content of the body wall between November and February (Nießing, 2006), which coincides with the time of low food availability (see above). Accordingly, winter specimens in our study displayed a significantly lower fresh weight than summer animals (Table 1). Field observations revealed a lower faeces production and hence food uptake from October to January compared to the spring and summer months (Pollack, 1979), possibly initiated by a drop in temperature and/or a reduced food availability. It was shown in laboratory experiments that faeces production and hence feeding activity is dependent on the food content of the sediment. Considerable differences between actual numbers of lugworms and number of faecal casts occurred, indicating part of the animals to be inactive at less favourable conditions in poor sediments (de Wilde and Berghuis, 1979).

Also the organic matter content of the water was shown to influence the pumping activity (Krüger, 1964). Our 5 °C cold-acclimated winter lugworms pursued the strategy of a "conserver", which lives under conditions of short food, feeds at a low rate because a higher rate might increase the costs of foraging more than the gains, and has a low growth rate in favour of longevity (Branch et al., 1988). In contrast to volume flow, pumping frequency, extraction coefficient and growth, the modelled exercise performance curves and maxima (Fig. 1) were nearly identical for both winter groups. This emphasises that maintenance of muscle exercise capacity seemed to be important for winter lugworms. An explanation might be the observation of active winter migrations in *A. marina*, initiated by temperatures close to critical values (Werner, 1956). When the limits of acclimatory adjustment are reached, the animals leave their habitat and rebury in a lower zone of the intertidal. De Wilde and Berghuis (1979) made a similar observation in a laboratory experiment, when lugworms fed and maintained at 5 °C left the sediment for migration.

5. Conclusions

Seasonal differences become obvious in exercise performance curves of *A. marina*. Winter animals exhibit an optimum at around 14 °C and a high performance amplitude. In summer, the optimum is shifted towards 19 °C, accompanied by a widening of the performance window and lower performance amplitudes. A trade-off between exercise capacity and reproductive growth seems to take place in summer. In addition to temperature food availability is likely an important factor controlling seasonal acclimatisation processes. Somatic growth may occur mostly in spring and autumn, when food is available, and outside the reproductive period. The experimentally determined optimum combination of acclimation and exposure temperatures for growth matches well with the temperature conditions found in spring and autumn in the worms' natural habitat. Altogether, the present results show that temperature determines the metabolic state of lugworms despite of the season. Furthermore, our data confirm that the investigated lugworm population from Arcachon lives at the upper level of its thermal limit, making it most susceptible for warming waters in the near future.

6. Uncited references

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