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M. V. Zakhartsev · B. De Wachter · F. J. Sartoris
H. O. Pörtner · R. Blust

Thermal physiology of the common eelpout (*Zoarces viviparus*)

Abstract We investigated the temperature dependence of some physiological parameters of common eelpout (*Zoarces viviparus*) from different locations (North Sea, Baltic Sea and Norwegian Sea) on acclimation temperature (3 °C and 12 °C) and acute temperature variation. The lethal limit of 12 °C-acclimated eelpout was determined as the critical thermal maximum [loss of equilibrium (LE) and onset of muscular spasms (OS)] and it was found to be 26.6 °C for LE and 28.8 °C for OS for all populations. However, these parameters do not have any relevant ecological interpretation. We therefore investigated the effect of gradually increased water temperature on standard metabolic rate (measured as resting oxygen consumption Mo_2) and critical oxygen concentration ($[O_2]_c$) of eelpouts. Acclimation to low temperature (3 °C) resulted in partial compensation of Mo_2, paralleled by a decrease of activation energy for Mo_2 (from 82 kJ mol^{-1} at 12 °C to about 50 kJ mol^{-1} at 3 °C) in North Sea and Baltic Sea eelpouts. At the same time, Norwegian eelpout showed no acclimation of oxygen demand to warm temperature (12 °C) at all. The scope for eelpout aerobic metabolism shrank considerably with increased acclimation temperature, as $[O_2]_c$ approached water oxygen concentrations. At 22.5 ± 1 °C the $[O_2]_c$ reached air saturation, which is equivalent to the upper critical temperature (Tc_{II}) and at this temperature the aerobic scope for the metabolism completely disappeared. In line with previous insight, the comparative analysis of the temperature dependence of Mo$_2$ of *Z. viviparus* from different populations suggests that a pejus (sub-critical) temperature for this species is about 13–15 °C. In conclusion, the capacity to adjust aerobic metabolism relates to thermal tolerance and the bio-geographical distribution of the species. Global warming would thus be likely to cause a shift in the distribution of this species to the North.

M. V. Zakhartsev (✉) · B. De Wachter · R. Blust
Department of Biology, University of Antwerp,
University of Antwerp—RUCA, Groenenborgerlaan 171,
2020 Antwerp, Belgium
E-mail: Maxim.Zakhartsev@ua.ac.be
Tel.: +32-3-2180347
Fax: +32-3-2180497

F. J. Sartoris · H. O. Pörtner
Marine Biology/Ecological Physiology,
Alfred-Wegener-Institute, Bremerhaven, Germany

Introduction

Aquatic ectotherms with a wide latitudinal distribution are interesting animal models to study the mechanisms of temperature adaptation. A wide latitudinal distribution implies that the species is exposed to significantly different temperature regimes in various sections of its natural habitat. Usually, such species are also exposed to considerable seasonal temperature variations, and are called eurythermal (Precht et al. 1973). Comparative study between populations or species from various climates should allow us to identify the physiological parameters crucial for the bio-geographical distribution of a species.

There are several criteria to evaluate the thermal limits of a species: (1) lethal temperatures and the time period of exposure, when mortality reaches 50% (LT_{50}), (2) the critical thermal maximum (CTMax), which is the temperature at which locomotory activity becomes

disorganized and the animal loses the ability to escape from the lethal conditions, (3) incipient lethal temperature (ILT), which is the temperature at which median LT_{50} is no longer dependent on exposure time (for review of these criteria see (Cossins and Bowler 1987). However, these criteria have only descriptive character and cannot provide us with a clear mechanistic understanding of limited thermal tolerance (Portner et al. 1998; Portner 2001).

There is a definite correlation between adaptation temperature and both the denaturation temperature of enzymes (Johnston 1985; Johnston and Walesby 1977; Sokolova and Portner 2001) and the temperature of membrane phase transitions (O'Brien et al. 1991). However, these temperatures do not explain the limitation of thermal tolerance. It is obvious that thermal death happens at temperatures that are much lower than the denaturation temperatures of any proteins or membrane phase transitions.

According to a recent concept of thermal adaptation and limitation (Portner et al. 1998, 2001; Portner 2002a) thermal tolerance is limited by oxygen availability to mitochondria. Accordingly, the capacity of oxygen delivery systems (oxygen uptake and distribution by ventilation and circulation) sets the limits of thermal tolerance (Portner 2002a). At critical temperatures (Tc) aerobic scope disappears and transition to an anaerobic mode of mitochondrial metabolism and progressive insufficiency of cellular energy levels occurs (Van Dijk et al. 1999). These processes define the reasons for thermal death and the critical temperature limits for survival.

Recently, the Tc-concept has been improved by the introduction of the concept of pejus temperatures into thermal physiology (Tp; pejus—getting worse) (Frederich and Portner 2000; Portner 2002a). The term pejus temperature is based on Shelford's law of tolerance (Shelford 1913) and it represents a sub-critical temperature, where thermal limitation already sets in at the transition from optimal to sub-critical (pejus) range, due to a limitation in aerobic scope. This limitation can be characterized by the onset of a decrease in aerobic scope for metabolism and, in a crustacean, was reflected by a drop of arterial Po_2 due to capacity limited ventilatory and cardiac performance (Frederich and Portner 2000).

Zoarces viviparus is one of the species with a latitudinal distribution ranging from the English Channel to the Russian White Sea, including the British Isles, the North Sea, the Baltic Sea, and the Norwegian coastline. Due to its wide range of latitudinal distribution, ubiquity and simple aquarium maintenance, *Z. viviparus* became one of the most convenient model species for environmental biomonitoring programs (BEEP[1]; ESB[2])

and also for studies of the mechanisms of temperature adaptation in eurythermal marine ectothermic animals (CLICOFI[3]). The goal of this article is to describe the thermal tolerance of different populations of *Z. viviparus* adopting the recent developments in the concepts of thermal tolerance.

Materials and methods

Eelpout

Common eelpouts (*Z. viviparus* Linnaeus, 1758) were collected at different locations: North Sea (island Helgoland, Germany); Baltic Sea (Kiel Bight, Germany); Norwegian Sea (Stavanger, Norway). The typical environmental conditions for those locations are listed in Table 1.

Acclimation

Upon delivery from the field, the fishes were allowed to recover in flow-through aquaria for at least 3 weeks under conditions equal to those in the field (temperature, salinity and light regime). Then fishes were divided into two groups and each was acclimated to 3 °C or 12 °C for at least 4 weeks. During recovery or acclimation, feeding was ad libitum with meat of cut shrimp (*Pandalus borealis*) or fish (*Gobius gobius*, 0.62 ± 0.21 g) once a week.

Critical thermal maximum

CTMax is the thermal point at which locomotory activity becomes disorganized and animals lose the ability to escape from lethal conditions. Either loss of equilibrium (LE) or onset of spasms (OS) defines the CTMax. According to common terminology applied by Lutterschmidt and Hutchison (1997a), the LE is the thermal point at which fish show inverted swimming; the OS is the thermal point at which fish shows muscular spasms, which are disorganized and high-frequency muscular movements, rigidity of the pectoral fins and, especially, high-frequency quivering of the opercula. In fact, rapid quivering of the opercula serves as a clear and reliable marker for OS. Body shuddering, gill distension and mouth gaping usually accompany these muscular spasms in fish.

The sequence of events for fish during an acute temperature increase is: agitated behaviour → loss of equilibrium → onset of spasms → heat rigor → coma → death. The difference (in degrees) between the temperatures of OS and death is very short, moreover, the tolerance to being exposed to temperatures above OS is very limited (several seconds), therefore, the animal must be returned to the acclimation temperature as fast as possible (Lutterschmidt and Hutchison 1997a).

Every single fish (Baltic $n = 7$; North Sea $n = 5$) was placed into individual flow-through aquaria (3 l) with water conditions according to acclimation parameters. The fish were isolated from visual contact. Fishes were exposed to an acute, highly precise increase in water temperature (starting from acclimation temperatures) by 1.0 °C min^{-1}. The water temperature increment was elicited by adding heated water at 60 °C. The water was thoroughly mixed by intensive air bubbling in order to secure uniform warming of the water mass. At OS, the temperature was immediately (within 20–30 s) decreased back to acclimation temperature by replacing "hot" with "cold" water, then the fishes were allowed to recover until the next morning. During the determination of CTMax, water

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Table 1 Average levels of salinity and water temperature at different locations (averaged for the water column between 0 m and 10 m)

| Area	Water temperature (°C)		Salinity (‰)		Source
	Season	Range	Season	Range	
Wadden Sea (North Sea)	Spring	6–7	Spring	27	(Brodte 2001)
	Summer	17–18	Summer	31	
Kiel Bight (Baltic Sea)	Spring*	2–4	–	14	(Böhnecke and Dietrich 1951);
	Summer	13	–	–	*(Fischer et al. 1992)
Gulf of Finland (Baltic Sea)	Spring**	0–4	–	6	(Kristoffersson and Oikari 1975);
	Summer**	15	–	–	**water surface
					(Böhnecke and Dietrich 1951)
Norwegian Sea, Hafrsfjord	Spring***	3–5	–	34	(Brodte 2001); ***(Böhnecke and
	Summer***	14–15	–	–	Dietrich 1951)
White Sea	Winter	−1	–	19–24****	(Babkov 1982); ****D. Lajus
	Spring	0	–	–	(Zoologisches Institut St. Petersburg),
	Summer	10	–	–	personal communication
	Autumn	5	–	–	
	Annual	4	–	–	

Fig. 1 A Schematic drawing of the closed respirometer: *1* respiration chamber; *2* cell of oxygen probe (WTW CellOx325); *3* cell for water sampling; *4* volume compensator with variable inside volume; V_1 and V_2 three-position valves [to connect (loop) or disconnect the respirometer]; *O₂-meter* oxygen meter (WTW Oxi325); *T °C-controller* external thermostat; *PC* personal computer for data collection; → direction of water flow. The drawing is depicting the respirometer in the "disconnected" state. **B** Schematic drawing of the flow-through respirometer: *1* respiration chamber; *2* fast connector (makes the setup either in connected or disconnected state); *3* cell of fibre-optic oxygen microsensor (PreSens, Germany); *4* peristaltic pump; *O₂-meter* oxygen meter MICROX8 (PreSens, Germany); *T °C-controller* external thermostat; *PC* personal computer for data collection; → direction of the water flow. Combination of the aquarium pump and the peristaltic pump provides accurate control of the flow rate through the respirometer. The drawing is depicting the system in the "connected" state

oxygenation at all times was no less than 90–95% of full saturation. Measurements were repeated for 5 consecutive days. There was no feeding during the experiment.

Respirometry

Closed respirometry

For an analysis of critical oxygen tensions (carried out at RUCA in Antwerp) the fishes were placed into closed respirometers, which could be in two states: "connected" and "disconnected" (Fig. 1A). Prior to handling the fishes were treated with 40 mg l⁻¹ MS222 (Ross and Ross 1999) for 3–5 min to initiate soft sedation (Sto-skopf 1992) and to prevent handling stress. Fishes were allowed to recover for the next 24 h. The inside volume of the respirometer was evaluated for each individual fish using the factor of inside volume (Fiv; Eqn 1).
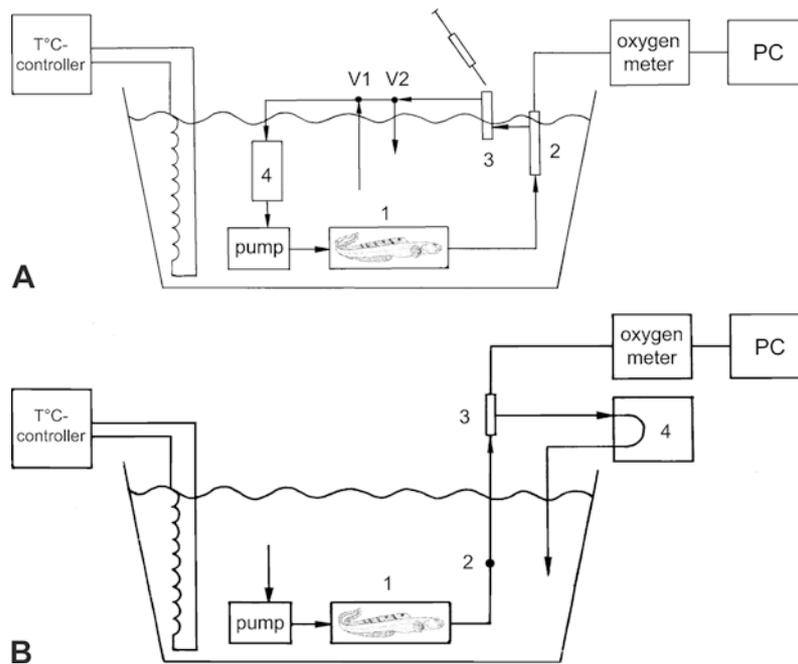
$$F_{iv} = \frac{V_{in} - w_f}{w_f} \tag{1}$$

where: *Fiv* factor of inside volume, V_{in} overall inside volume of the respirometer (ml) and w_f weight of fish (g). For eelpout the optimum Fiv was 14–16 at 12 °C, in this case, eelpouts reached extreme hypoxia (10–25 µM oxygen) within 3–3.5 h upon closure of the respirometer. The Fiv had to be increased with any temperature increment (ΔT) by 2.5% per degree.

$$V_{in} = (\Delta T) = V_{in}^{\circ} \exp(0.025\Delta T)$$
$$V_{in}^{\circ} = 15w_f \text{ (at } 12°C) \tag{2}$$

Increases of the inside volume of the respirometer by the volume compensator (No. 4 in Fig. 1A) allowed the offsetting of the temperature effect on the time taken to reach extreme hypoxia.

The water temperature was raised by 1 °C per 2 days in the temperature range between 12 °C and 18.5 °C. The measurements were carried out only during daytime. The measurement procedure consisted of two phases: measurement (for 3–3.5 h) and recovery (for 44.5–45 h), the latter being accompanied by the temperature increment (1 °C 2 days^{-1}). This protocol for the rate of temperature change was chosen to avoid the overlap/combination of two life-limiting factors: regular exposure to hypoxia and acute temperature change.

Eight oxygen meters Oxi325 with probes CellOx325 (WTW, Germany) were calibrated with solutions containing oxygen at 0% (saturated solution of Na_2SO_3) and 100% air saturation (aquarium water). All calibration solutions were temperature equilibrated in the experimental tank. The calibration procedure was carried out each time right before the onset of measurements.

To start the measurements the respirometers were "connected" (Fig. 1A). We used eight respirometers (seven with fishes and the eighth as a blank). The internal concentration of oxygen was monitored as a function of time (with a recording interval of once per minute, Windmill Logger V4.06, Windmill Software). The measurements were continued until the fish had reached extremely hypoxic conditions ([O_2]≈10–25 µM), then the respirometers were "disconnected" to allow normoxic water from the ambient aquarium to flush the respirometer, and the fishes were allowed to recover until the next measurement.

The rate of oxygen consumption (Mo_2), evaluated from 10-min intervals, was calculated according to formula:

$$(Mo_2)_i = \frac{([O_2]_i - [O_2]_{i+10}) \times (V_{in} - w_f) \times 10^{-3}}{(t_{i+10} - t_i) \times w_f} \tag{3}$$

[µmoles min^{-1} g^{-1}]; where: ([O_2]$_{i+10}$?[O_2]$_i$) change in oxygen concentration inside the respirometer within 10 min (µM); V_{in} overall inside volume of the respirometer (ml); ($t_{i+10}-t_i$) time interval (min), here 10 min; w_f weight of the fish (g). Mo_2 was calculated for

Fig. 2 A Typical data set obtained during measurements of the critical oxygen concentration ($[O_2]_c$) and the rate of oxygen consumption (Mo_2) in closed respirometers for Baltic eelpout acclimated to 12 °C. Conditions of the measurements were: different water temperatures, the rate of temperature change was 1 °C per 2 days; water salinity was 17‰; measurements always lasted about 3–3.5 h; the body weight of the fish was 41.4 g. **B** Typical data set obtained during Mo_2 measurements using flow-through respirometry. The measurements consist of four phases: *1* baseline recordings; *2* adjustment of the flow rate; *3* recording of the oxygen concentration in excurrent water; *4* recording of the baseline. Connecting or disconnecting the respirometer occurred with the fast connector (*2*, in Fig. 1b). The oxygen concentration at the respirometer outlet was always set to within 100–75% (by means of adjusting the flow rate in phase *2* in order to avoid hypoxia exposures)
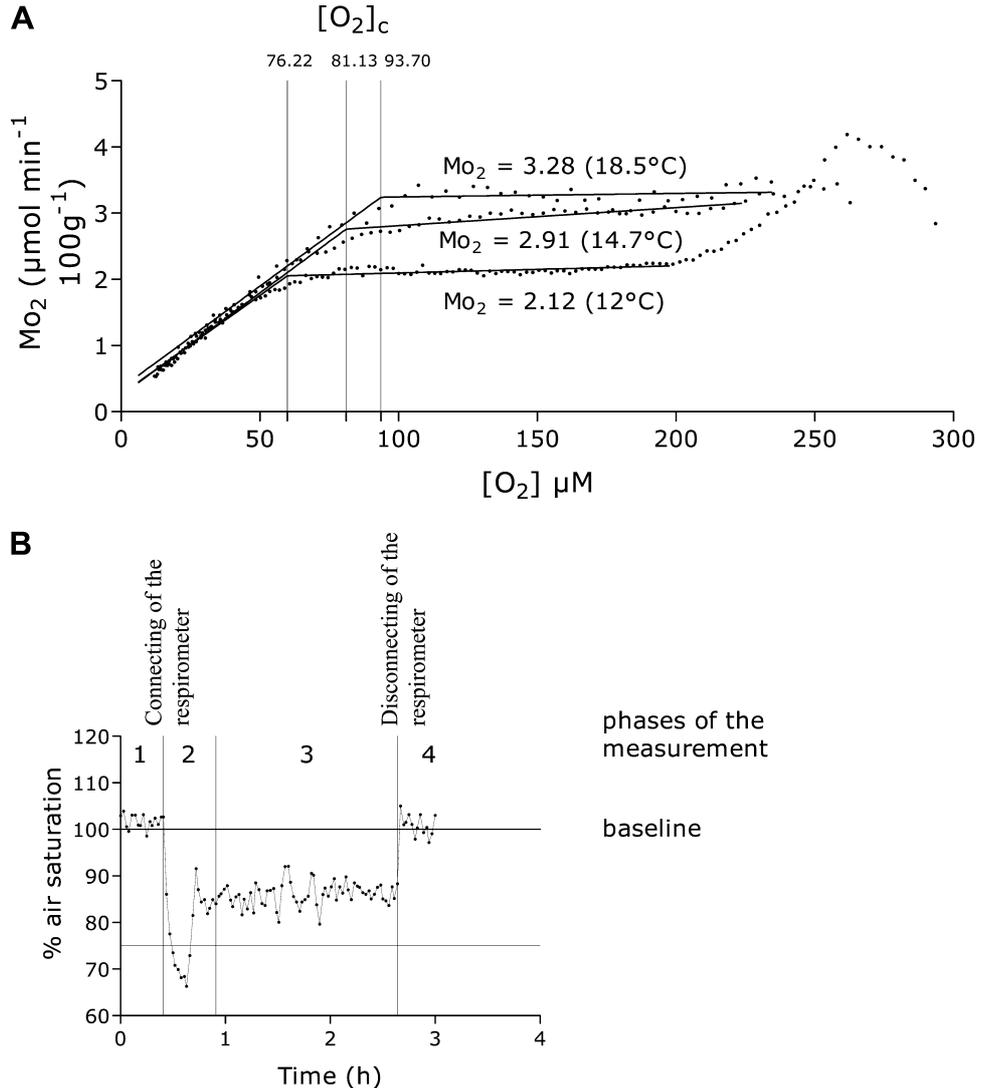
any moment i [$(Mo_2)_i$] from i = 1 to i = max. We used 1-min intervals to record the data, but Mo_2 was calculated for 10-min intervals. The blank Mo_2 was subtracted from the fish's Mo_2. $(Mo_2)_i$ was plotted against the averaged $[O_2]$ for the corresponding 10 min interval $[O_2]_i$ (Fig. 2A). Mo_2 was expressed in terms of micromoles of oxygen consumed within a minute per gram of fish body and normalized to the rate per 100 g fish body [μmoles min^{-1} 100 g^{-1}]. The basal metabolic rate was calculated as the averaged Mo_2 from the horizontal part of the graph (Fig. 2A) and expressed in the same units.

Critical oxygen concentration and basal metabolic rate

The critical oxygen concentration ($[O_2]_c$) not only depicts the transition from oxyregulation to oxyconformity during progressive hypoxia, but also the point of transition to anaerobic metabolism (Portner and Grieshaber 1993). Therefore, the T_c should be reached when the rise in $[O_2]_c$ during warming reaches oxygen concentrations of air-saturated water. $[O_2]_c$ was calculated as the break-point of the graph depicting the Mo_2–$[O_2]$ dependence (Fig. 2A) by means of two-phase linear regression analysis (GraphPad Prism V3.02, GraphPad Software) (Motulsky 1999; Yeager and Ultsch 1989). The $[O_2]_c$ was expressed in micromoles O_2 per litre of ambient water.

Flow-through respirometry

For an analysis of temperature-dependent changes in oxygen consumption (carried out at the AWI, Bremerhaven) fishes ($n = 8$) were placed into individual flow-through respiration chambers and were allowed to recover from the handling procedure for the next 24 h. The set-up allowed the respirometer to be in two states: "disconnected" and "connected" (Fig. 1B).

We used optical sensors (fibre-optic oxygen microsensors) to record the oxygen partial pressure in the water (Klimant et al. 1997). Optical oxygen sensors (also called oxygen optodes) have important advantages over common Clark type electrodes: they are extremely small (the tip size range from < 30 μm to 2 mm); they do not consume oxygen; their signal is not influenced by the flow rate, salinity, and pH of the water; they are insensitive to electrical interferences and magnetic fields. Therefore, they are perfect oxygen sensors to work with in flow-through respirometers. The oxygen meters MICROX8 (PreSens, Germany) with eight fibre-optic oxygen microsensors (PreSens, Germany) were calibrated in advance of the measurements with solutions containing oxygen at 0% (saturated solution of Na_2SO_3) and at 100% air saturation (aquarium water). All calibration solutions were temperature equilibrated in the experimental tank. The calibration procedure has been carried out right before the measurements took place.

The water temperature was raised by 3 °C each night and oxygen uptake was measured during daytime in the temperature range between 3 °C and 24 °C. Measurements with warm-acclimated animals were started at 12 °C and additional analyses with an additional set of warm-acclimated fishes were carried out at temperatures below 12 °C.

An aquarium pump and a peristaltic pump (Fig. 1B) were used to create water flow through the respirometer. After baseline oxygen levels had been monitored the respirometer was connected and water flow was adjusted (by means of the peristaltic pump) to a level that prevented oxygen partial pressure at the outlet of the respirometer from falling below 75% of incoming values (Fig. 2B). This precaution was taken in order to avoid hypoxic exposure of *Z. viviparus*. Oxygen concentration in the excurrent water of the respirometer was monitored as a function of time (once in 30 s; PreSens Software) during 1.5–2 h (phase 3, Fig. 2B). Then the respirometer was disconnected and the baseline recorded again to check for potential drift of the optodes (Fig. 2B).

The rate of oxygen consumption was calculated according to Eqs. 4, 5, 6, a combination of Dalton's (for moist air) and Henry's laws (Ultsch et al. 1980).

$$Po_2 = 0.2095(P_b - P_{H_2O}) \ [kPa] \tag{4}$$

$$[O_2]_i = Po_2 \times \alpha_{O_2} \ \left[\mu\text{moles litre}^{-1}\right] \tag{5}$$

$$Mo_2 = \frac{([O_2]_i - [O_2]_e) \times fr}{w_f} \ \left[\mu\text{moles min}^{-1}\text{g}^{-1}\right] \tag{6}$$

where: Po_2 partial oxygen pressure in air (kPa); 0.2095 fraction of oxygen in air; P_b air barometric pressure on the day of measurement (kPa); P_{H_2O} partial pressure of water vapour in air (kPa), considering temperature; $[O_2]_i$ oxygen concentration in incurrent water (μmoles litre^{-1}) (phase 1 or 4 "baseline" in Fig. 2B); α_{O_2} solubility coefficient for oxygen in water (μmoles litre^{-1} kPa^{-1}) as it depends on temperature and water salinity; $[O_2]_e$ averaged (for the period of measurement, phase 3 in Fig. 2B) oxygen concentration in excurrent water (μmoles l^{-1}), calculated as the percent fraction of incurrent (baseline) oxygen concentration; fr rate of the water flow through the respirometer (l min^{-1}); w_f weight of the fish (g). Mo_2 was expressed as μmoles of oxygen consumed within a minute per gram of fish body and normalized for 100 g fish body (μmoles min^{-1} 100g^{-1}).

The typical data set of the Mo_2 measurement and the sequence of operational steps are shown in Fig. 2B. The fishes were exposed to a temperature change of 3 °C per night starting from acclimation temperatures. They stayed in the respirometers during the whole experimental period and were not fed during the analyses.

Statistical analysis

Prior to any statistical analysis the rate specific results were normalized to 100 g fish weight in each experimental group according to Sokal and Rohlf (1995).

Prior to any statistical tests, normal distribution and homogeneity of variance of the data were estimated using a Kolmogorov-Smirnov test from GraphPad InStat V3.05. Statistical treatments were performed using standard one-way ANOVA (GraphPad InStat V3.05) and repeated-measures two-way ANOVA (Statistica V5.1) tests. We used Tukey's post-hoc comparisons to compare all pairs of variance with unequal n. Differences were considered significant when probability levels were less than 0.05.

Both correlation and multiple linear regression analyses were performed using GraphPad InStat V3.05. Two-phase linear regression analyses were performed according to recommendations of Motulsky (1999), the regression model was introduced into GraphPad Prism V3.0 for analysis. Non-linear regression analyses to fit oxygen consumption data were performed using an exponential growth model (GraphPad Prism V3.0). Highest R^2, smallest absolute sum of squares (ASS) and smaller numbers of equation components were main criteria of the quality of the fit by the various regression models (Motulsky 1999).

We used an F-test to compare entire curves of temperature-dependent rates of oxygen consumption according to Motulsky (1999) (GraphPad Prism V3.0) and an unpaired t-test was used to compare best-fit values of non-linear regression variables.

Results

Morphological data

We did not find significant differences (one-way ANOVA, $P > 0.05$) in morphological data (weight, length) between populations (North Sea, Baltic Sea, Norwegian Sea), ($n = 132$), therefore, the morphological data from all studied populations were pooled and presented in Fig. 3.
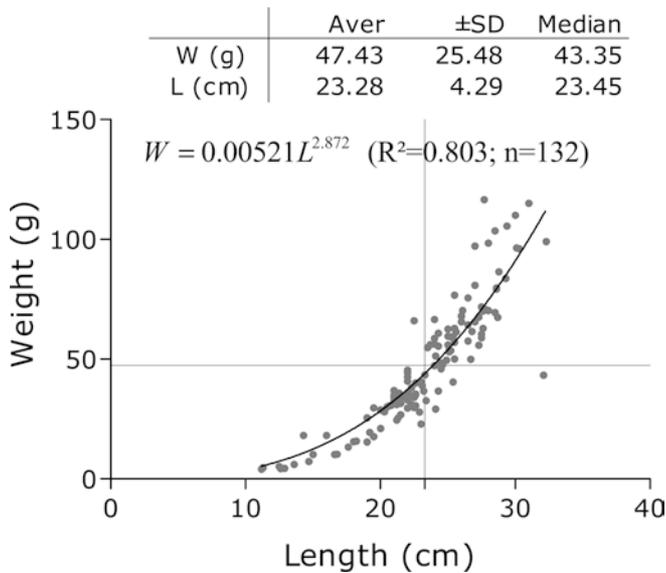
	Aver	±SD	Median
W (g)	47.43	25.48	43.35
L (cm)	23.28	4.29	23.45

$W = 0.00521L^{2.872}$ ($R^2 = 0.803$; $n = 132$)

Fig. 3 Morphological data of common eelpout (*Zoarces viviparus*). The *broken lines* indicate the means of the corresponding parameters

Critical thermal maxima

LE is a less reliable parameter to record CTMax than OS in the case of eelpout, because of the bottom-dwelling lifestyle of the fish (visual observation). Normally, eelpouts are lying on the bottom and use pectoral fins and body bending to maintain balance; therefore, LE was not well expressed in some cases even if it took place. In contrast, OS was always very clear. There was a very significant (repeated measures two-way ANOVA, $P < 0.01$) difference between these parameters (LE and OS) in both populations. Repeated measures two-way ANOVA analysis (Table 2) also showed that there was no significant difference ($P > 0.05$) in LE and OS between the two populations from North and Baltic Seas. We did not find a significant ($P > 0.05$) heat hardening effect in both parameters for the two populations when comparing the consecutive CTMax measurements during 5 days (repeated measures two-way ANOVA). The only exception was in the case of OS of Baltic eelpout on the second day of measurements (Table 2), however, this

significant increase could not be verified on the consecutive days. Therefore, we have discarded this data point as it probably represents a fluctuation. There was no correlation between CTMax and fish weight ($P > 0.05$). Thus, there was no significant difference in CTMax between North Sea and Baltic Sea populations.

Progressive changes in the behavioural response to an acute temperature increment (1 °C min^{-1}) are compiled in Table 3. Typically, behavioural changes in the eelpout consist of several quite clear steps: (1) anxiety: the fish starts to show agitated behaviour, (2) fin up: the fish erects the pectoral fin, (3) active swimming: the fish starts to move actively, (4) attempt to escape: the fish tries to jump out of the aquarium, (5) LE, and (6) OS. Data are pooled from both populations (Baltic and North Sea) and all measurements, since no heat hardening and no significant differences between Baltic and North Sea populations were observed.

Critical oxygen concentration

For maintained experimental conditions we checked the relationship between Fiv of the closed respirometer and time to extreme hypoxia at 12 °C. It is very important to maintain the time to extreme hypoxia to be able to clearly detect the transition from oxyregulation to oxyconformity. There was a very significant positive correlation between Fiv and time to extreme hypoxia ($r = 0.856$, $P < 0.01$, $n = 27$), which is described by a linear relationship: Fiv $= 0.904 + 0.0734$time ($R^2 = 0.823$; $n = 27$), where *time* is the time to extreme hypoxia (min). Using Fiv values of 14–16 (at 12 °C) and a temperature correction factor of $+ 2.5\%$ of the initial volume of the respirometer per 1 °C temperature increment the inside volume of the closed respirometer was successfully adjusted such that each individual fish reached extremely hypoxic conditions within 3–3.5 hours at any temperature.

The $[O_2]_c$ was measured between 12 °C and 18.5 °C only for eelpout from the Baltic population acclimated to 12 °C. We could not go beyond 18.5 °C, as the eelpouts died at higher temperatures.

It is necessary to point out that eelpouts recovered extremely well after exposure to extremely hypoxic conditions. Right after the measurements the fish were

Table 2 Critical thermal maxima (CTMax) measured as loss of equilibrium (*LE*) and/or onset of spasms (*OS*) for Baltic Sea (m $= 44.0 \pm 12.2$ g) and North Sea (m $= 9.9 \pm 3.1$ g) eelpouts, which were acclimated to 12 °C

Time of measurement (h)	Baltic				North Sea			
	LE ± SD (°C)	n	OS (°C) ± SD	n	LE ± SD (°C)	n	OS ± SD (°C)	n
0	25.7 ± 1.2	7	28.0 ± 1.0	7	27.1 ± 0.9	5	29.1 ± 0.2	5
24	27.1 ± 1.0	6	29.1 ± 0.6**	6	26.4 ± 0.2	2	28.8 ± 1.1	5
48	27.2 ± 0.5	4	28.8 ± 0.9	6	25.4 ± 0.8	3	28.4 ± 1.1	4
72	27.4 ± 0.5	6	28.8 ± 0.8	6	26.3 ± 1.5	4	28.5 ± 1.4	4
96	27.0 ± 0.9	5	28.6 ± 1.1	6	27.5 ± 0.6	3	29.1 ± 0.5	4
Average	26.8 ± 1.1	–	28.7 ± 0.9	–	26.6 ± 1.1	–	28.8 ± 0.9	–

**Difference is significant from control (0 h; $P < 0.01$)

Table 3 Progressive changes in the behavioural responses of *Zoareces viviparus* to a progressive rapid temperature increase (1 °C min⁻¹). Data from both populations (Baltic and North Sea) and all measurements are pooled, for reference see Table 2. Values are means ± SD (*n*). *Anxiety*: the fish starts to show agitated behaviour; *Fin up*: the fish erects the pectoral fin; *Active swimming*: the fish starts to move actively; *Attempt to escape*: the fish tries to jump out of the aquarium

	Temperature (°C)
	12
Acclimation temperature	
Anxiety	18.8 ± 2.2 (42)
Fin up	20.1 ± 2.0 (44)
Active swimming	22.1 ± 2.0 (31)
Attempt to escape	23.6 ± 2.0 (17)
LE	26.7 ± 1.1 (45)
OS	28.7 ± 0.9 (56)

very quiescent; however, after 2–3 h their usual behaviour was restored. To ensure that post-hypoxic recovery was more or less complete the fish were allowed to rest for 48 h after each $[O_2]_c$ measurement.

A typical data set of $[O_2]_c$ and Mo_2 obtained by closed respirometry is presented in Fig. 2A. The correlation analysis reveals that there is an extremely significant positive correlation ($P < 0.001$) between rate of oxygen consumption, critical oxygen concentration and temperature (Table 4). The relationship between Mo_2 and $[O_2]_c$ is linear (Fig. 6) and the following run test showed that there is no a significant departure from linearity ($P > 0.05$). The multiple linear regression analysis (GraphPad InStat V3.05) of data revealed that the relationship between $[O_2]_c$, Mo_2 and temperature is also described by linear equation:

$$[O_2]_c = 21.698 + 11.998 Mo_2 + 1.166T \tag{7}$$
$$\left(R^2 = 0.417, n = 29, P < 0.001\right)$$

where $[O_2]_c$ critical oxygen concentration (μM); Mo_2 rate of oxygen consumption (μmol min⁻¹ 100g⁻¹); T estimation temperature (°C).

The temperature dependencies of Mo_2 is described by exponential curve (Table 5); consequently the temperature dependence of $[O_2]_c$ is also describes by exponential curve (Fig. 8). These equations display a relatively low R^2 value, due to high scattering of the data over a relatively short temperature interval (12–18.5 °C). However, statistical comparison analyses (R^2; absolute sum

Table 4 Matrix of correlation coefficients for rate of oxygen consumption (Mo_2; μmol min⁻¹ 100g⁻¹), critical oxygen concentration ($[O_2]_c$; μM) and measuring temperature (between 12 °C and 18.5 °C)

	$[O_2]_c$	Mo_2	Measuring temperature
$[O_2]_c$	1.0000	0.6877*	0.6310*
Mo_2	0.6877*	1.0000	0.5367*
Measuring temperature	0.6310*	0.5367*	1.0000

*$P < 0.001$

Table 5 Rate of oxygen consumption (Mo_2; μmol min⁻¹ 100g⁻¹) and the Arrhenius activation energy (E_a; kJ mol⁻¹) for Mo_2 of common eelpout (Z. viviparus) from different locations and acclimation temperatures (AT). (k rate constant (°C⁻¹); Tc_{II} upper critical thermal limit, characterised by transition to anaerobic metabolism). Respirometer type and protocol of measuring temperature change: a "flow-through" and 3 °C per night, b "closed" and 1 °C per 2 days

Location	AT (°C)	Range of measuring temperature (°C)	Respirometer type	Mo_2 at AT (μmol min⁻¹ 100g⁻¹)	E_a ± SE (kJ mol⁻¹)	Equation $Mo_2 = Mo_2 \times exp(kT)$	Tc_{II} (°C)	Reference
Common eelpout (Z.viviparus)								
North Sea	3	0–24	a	1.01 ± 0.26	55.5 ± 1.03	$Mo_2 = 0.7644 \times exp(0.0887 \times T)$	21–24	(Van Dijk et al. 1999)
	12	0–24	a	1.53 ± 0.87	82.9 ± 1.41	$Mo_2 = 0.4691 \times exp(0.1096 \times T)$		
Baltic Sea	3	0–24	a	1.03 ± 0.24	41.83 ± 0.36	$Mo_2 = 0.9457 \times exp(0.0602 \times T)$	–	This study
	12	0–24	a	1.80 ± 0.83	82.44 ± 1.12	$Mo_2 = 0.4855 \times exp(0.1114 \times T)$		
	12	12–18.5	b	1.58 ± 0.51	75.61 ± 0.67	$Mo_2 = 0.4893 \times exp(0.1012 \times T)$	22.5 ± 1	
Norwegian Sea	3	3–21	a	0.79 ± 0.21	54.76 ± 0.89	$Mo_2 = 0.6753 \times exp(0.0723 \times T)$	–	This study
	12	3–21	a	1.50 ± 0.26	46.71 ± 1.10	$Mo_2 = 0.6565 \times exp(0.0783 \times T)$		

of square; standard deviation of the residuals) revealed that the chosen functions are the best to fit the data.

Rate of oxygen consumption

The comparison of methodological approaches to measure Mo_2 and its temperature dependence (closed respirometry with a temperature change by 1 °C per 2 days versus flow-through respirometry with a temperature change by 3 °C per night) did not reveal significant differences (F-test, $P < 0.05$; Table 5 and Fig. 4).

Oxygen consumption rates of cold-acclimated (3 °C) and warm-acclimated (12 °C) Z. viviparus from different locations are presented in Fig. 5 and Table 5. The analysis includes data for North Sea eelpout obtained from Van Dijk et al. (1999; Fig. 1a). At low temperatures Mo_2 of cold-acclimated Z. viviparus from North Sea and Baltic Sea were significantly (50–60%) higher than those of warm-acclimated eelpout acutely exposed to low temperature (Fig. 5A, B). Oxygen consumption of warm-acclimated eelpout from North Sea and Baltic Sea showed a more rapid increase with temperature than Mo_2 of cold-acclimated eelpout from the same location. Accordingly, the Arrhenius activation energy for oxygen consumption was higher for warm- than for cold-acclimated eelpout (Table 5). However, we did not observe such effects in Norwegian eelpout (Fig. 5C), where no difference between acclimation groups was visible. Moreover, the levels of Mo_2 of both cold- and warm-acclimated Norwegian eelpouts were similar to those of Mo_2 of cold-acclimated Baltic Sea eelpout (Table 5). The comparison of Mo_2 levels among all cold-acclimated eelpouts from the different locations revealed no difference between populations at temperatures less than 13–15 °C; however, at high temperatures (>13-15 °C)
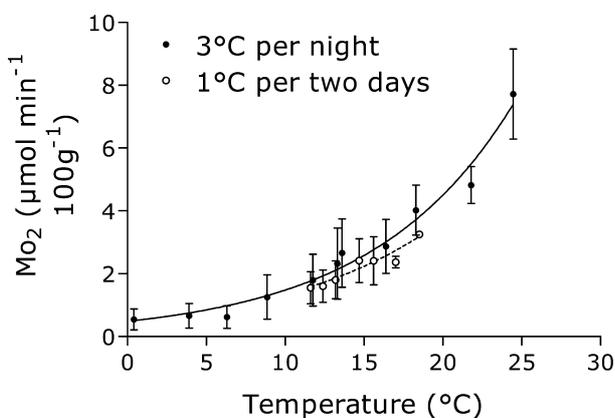
cold-acclimated North Sea specimens displayed significantly higher levels of Mo_2 than those from the Baltic and Norwegian Seas. The comparison of Mo_2 among all warm-acclimated eelpouts from different locations, again, showed no difference between populations at temperatures less than 13–15 °C; however, at high temperatures (>13–15 °C) Norwegian eelpout displayed significantly lower Mo_2 values than their North Sea and
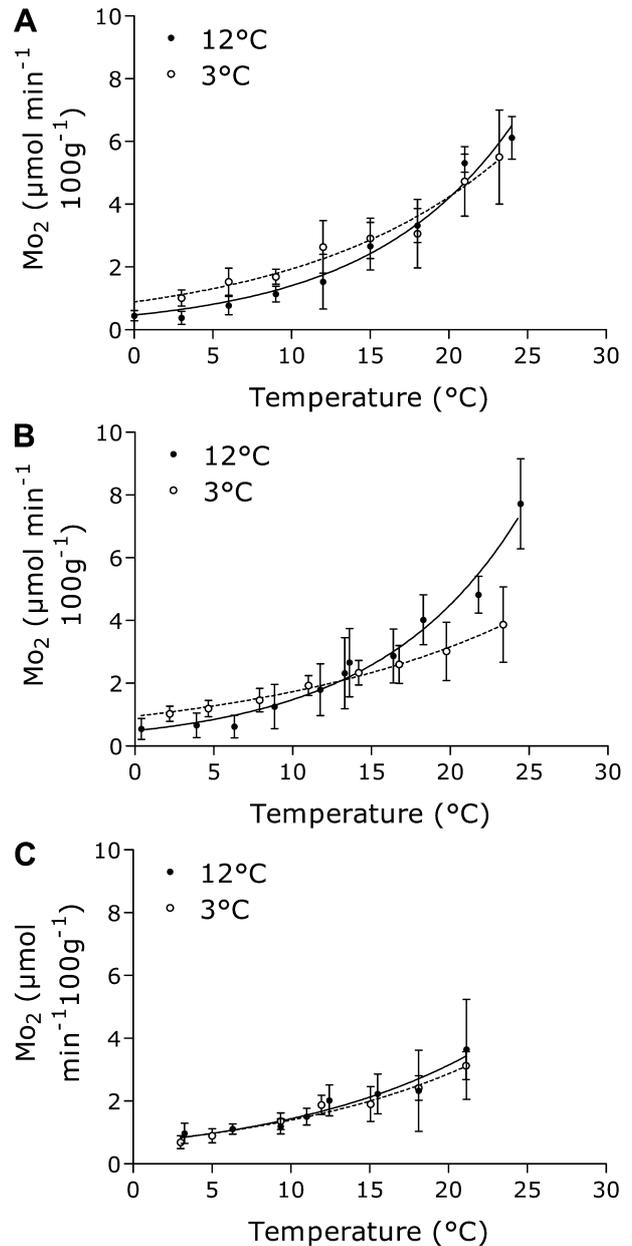

Fig. 5A–C Mo_2 of common eelpout (Z. viviparus) from the North Sea (**A**), Baltic Sea (**B**) and Norwegian Sea (**C**) measured in a flow-through respirometer. The rate of temperature increase was 3 °C per night, salinity 33‰ for North and Norwegian Seas and 17‰ for Baltic Sea animals. *Filled circles* fitted with a *solid curve* were obtained from 12 °C-acclimated fishes, whereas *unfilled circles* fitted with a *broken curve* originate from 3 °C-acclimated fishes. Values are means ± SD (n = 7–8). Data for North Sea eelpout were adopted from Van Dijk et al. (1999). See also Table 5

Fig. 4 Comparison of the protocols of temperature change and experimental set-ups exemplified in 12 °C-acclimated common eelpout (Z. viviparus) from the Baltic Sea. The Mo_2 in the flow-through respirometer (*filled circles, solid curve*) was measured at a temperature change of 3 °C per night (n = 8), whereas the Mo_2 in the closed respirometer (*empty circles, broken curve*) was measured at a temperature change of 1 °C per 2 days (n = 7). Salinity was 17‰. Values are means ± SD. See also Table 5

Baltic conspecifics. Arrhenius plots of M_{O_2} of all experimental groups revealed linear slopes and no break over the investigated temperature range (runs test; $P > 0.05$).

An unpaired t-test of non-linear regression variables [a and b for $y = a \times \exp(bx)$] revealed a general trend for variables to change over the course of temperature acclimation (Table 5). In the case where the temperature profile of M_{O_2} changed after acclimation to low temperature (3 °C), the quantitative variable (a) of the regression equation became significantly higher ($P < 0.05$), but, at the same time, the qualitative variable (b) of the regression equation became significantly lower ($P < 0.05$). This observation applied to eelpout from North and Baltic Seas, but not to Norwegian eelpout.

Discussion

Acclimation temperature

Acclimation temperatures of 3 °C and 12 °C were chosen in order to cover the natural temperature range of the species. 3 °C reflects the winter period, 12 °C is quite close to the optimal temperature for somatic growth of eelpout, which is 13–14 °C (recalculated from data in Fonds et al. 1989). The optimal temperature for somatic growth was determined on the basis of growth experiments: daily growth in body length (mm day^{-1}), daily weight increments in ash-free dry weight (mg AFD day^{-1}) and daily weight increment in energy (J day^{-1}) (Fonds et al. 1989). We did not use higher acclimation temperatures (above 18 °C), because eelpout have a lower survival rate during long-term acclimation at temperatures above 18 °C (M. Zakhartsev, personal observation); moreover, eelpout have an upper lethal temperature of about 22–23 °C (Fonds et al. 1989).

Critical thermal maximum

The analysis of CTMax is classified as a dynamic method to evaluate thermal tolerance limits, unlike the determination of LT$_{50}$, which is a static method (Lutterschmidt and Hutchison 1997b). Therefore, the value of CTMax depends on the experimental protocol (rate of temperature increment and acclimation temperature). The heating rate of 1 °C min^{-1} was chosen according to Lutterschmidt and Hutchison (1997b), because with a faster protocol (> 1 °C min^{-1}), lag-periods between changes in body and water temperature may interfere. On the other hand, a slower protocol (< 1 °C min^{-1}) might result in the interference of acclimation effects (for instance, changes in protein synthesis). Since the CTMax protocol does not relate to a direct ecological interpretation (Lutterschmidt and Hutchison 1997a, 1997b) it can be used only as a value for comparative physiological study. Our measurements did not reveal any differences between two eelpout populations (North Sea and Baltic Sea) despite quite significant differences in their environmental conditions (seasonal temperature range and salinity, Table 1). This means that the physiological mechanisms, which are setting the limits for acute temperature resistance (CTMax), have identical properties in both populations, despite of all environmental differences.

We did not observe any heat hardening effect despite daily heat exposure (for 5 consecutive days of CTMax measurements). This may indicate that in eelpout the rate of the expression of heat protective mechanisms (heat shock proteins, changes in protein synthesis etc) is quite slow or absent. These findings may suggest that eelpout prefer to live in a more or less stable environment with little daily temperature fluctuations. However, the behavioural response of eelpout to acute temperature increments (1 °C min^{-1}; Table 3) shows that 12-°C-acclimated fishes do not respond to acute warming until the temperature reaches 18 °C. This means that the warm-acclimated fish can tolerate such acute temperature elevations.

Comparison of respirometer types and protocols

We used two different respirometry methods, which led to similar results (Fig. 4 and Table 5), despite the different approaches to calculate M_{O_2} (Eqn. 3 versus Eqns. 4, 5, 6; Table 5). Data obtained by closed respirometry ([O_2]$_c$ and M_{O_2} for a narrower temperature range) perfectly match the data collected in the flow-through respirometer (M_{O_2} for a broader temperature range). However, there was a difference in mortality: when temperature was increased by 1 °C per 2 days all fish died at 18.5 °C, whilst with a temperature change of 3 °C per night they could survive up to 24 °C. The repeated exposure to extreme hypoxia and subsequent recovery possibly explains the enhanced mortality during the closed respirometry experiments. As animals likely exceed pejus temperatures and experience a loss in aerobic scope beyond 14–15 °C (due to capacity limitations of the circulatory system; C. Bock, F. Sartoris, H.O. Pörtner, unpublished data), increased mortality between pejus and critical temperatures may result from enhanced oxidative stress which is already enhanced when temperature-induced functional hypoxia sets in (Pörtner 2002a, 2002b) and which likely becomes aggravated when high temperature and hypoxic exposures are combined.

Rate of oxygen consumption

Oxygen consumption rates of eelpout from different locations were investigated in order to reveal adaptive differences in metabolic rates. As shown earlier (Van Dijk et al. 1999) the eelpout, as a carnivorous sit-and-wait predator, is mainly inactive with extremely little spontaneous activity. Therefore, the measured oxygen

consumption rate (Mo$_2$) can be considered equivalent to standard metabolic rate (SMR). For this particular reason, the eelpout is a perfect animal model for respiratory studies.

In all populations oxygen demand increased in an exponential way with rising temperature (Fig. 5), with no instantaneous metabolic rate compensation. Our oxygen uptake measurements revealed no difference in SMR between 12 °C-acclimated Baltic and North Sea populations (Table 5). A compensatory rise in the SMR of cold-acclimated (3 °C) Baltic and North Sea eelpouts occurred compared to warm-acclimated (12 °C) fish (Fig. 5A, B). In contrast, Norwegian eelpout did not at all display warm-acclimated metabolic rates (Fig. 5C) but kept their SMR (at both acclimation temperatures) at the level corresponding to cold-acclimated Baltic eelpout. This may indicate that the SMR of Norwegian eelpout is permanently adjusted to life in the cold. This population probably does not encounter large temperature variations during seasonal or daily cycles, due to life in deep and cold Norwegian fjords.

There was a general tendency for a decrease in Arrhenius activation energy (E$_a$) of SMR as a result of acclimation to low temperature (Table 5, from 80–90 kJ mol^{-1} to 40–47 kJ mol^{-1}). This drop was accompanied by a partial compensatory rise in SMR levels at low temperature. However, this was not the case for Norwegian eelpout. A change in E$_a$ suggests that the changes in oxygen uptake go hand in hand with changes in the kinetic properties of the oxygen consuming biochemical machinery. The fact that Norwegian eelpout did not show such changes may indicate their inability to adjust to warm temperatures above 13–15 °C.

According to Table 5, acclimation to low temperature results in an increased M$_{O2}$ at 0 °C (Mo$_2$°) and decreased rate constant (k). Change of Mo$_2$° indicates that the fish aerobic metabolism underwent quantitative changes. At the same, decrease of k and consequently E$_a$ indicated qualitative changes in the aerobic metabolism. The observation is in line with literature findings that the number of mitochondria that participate in oxygen consumption increases in the cold, but at the same time their kinetic properties remained suppressed by low temperature. Thus, in the course of acclimation to the cold, the aerobic metabolism undergoes only quantitative compensatory change.

Comparative analysis of temperature dependence of Mo$_2$ among populations showed that all populations are indistinguishable at temperatures below 13–15 °C. These temperatures coincide with the pejus temperature observed in a separate study (see above), but the cause and effect relationships remain obscure.

Very little is known about the genetic homogeneity of eelpout along the range of distribution. We found only one comprehensive research of eelpout population along Danish waters (there are a number of publications dedicated to population genetics of *Zoarces* by Hjorth, Simonsen, Frydenberg, Gyldenholm, Chrisitansen published in Hereditas over the period between 1971 and 1988). These authors (Frydenberg and Simonsen 1973) found that the most of the eelpouts' loci (28–31%) are polymorphic. The *Zoarces* population in the Danish belts is subdivided into smaller units with a restricted level of genetic exchange (Hjorth and Simonsen 1975). In Mariager Fjord (Denmark) even local populations 10 km apart have been found to be genetically distinct (Christiansen et al. 1981). Associated variations in morphological characters were kept virtually unchanged for more than 60 years (Christiansen et al. 1988). This genetic background in combination with the non-migratory behaviour of eelpout suggests that all studied populations (North Sea, Baltic Sea and Norwegian Sea) are to some extent genetically isolated, due to very limited gene exchange between "settlements". Such genetic differences may be linked to the functional differences (differences in oxygen consumption) observed in this study.

Critical oxygen concentration

A time span of 3–3.5 h has been chosen for [O$_2$]$_c$ measurements, because within this time we were able to get a clear hypoxia-induced hypometabolic response (transition from oxyregulation to oxyconformity which may include transition to anaerobic metabolism; Boutilier and St-Pierre 2000). A faster protocol did not provide us with a clear picture of transition from oxyregulation to oxyconformity, possibly owing to the depletion of internal oxygen stores. The slower protocol caused the fish to stay under hypoxia for quite a long time; therefore some fishes did not survive this protocol.

The eelpout is a clear metabolic regulator (oxyregulator), because Mo$_2$ is independent on oxygen concentration (Fig. 2A) within a certain range of ambient oxygen concentrations. The [O$_2$]$_c$ is the oxygen concentration in water where Mo$_2$ in an oxyregulator becomes dependent on ambient oxygen concentration (Herreid 1980; Ultsch et al. 1980) and a metabolic machine switches on anaerobic energy production; such transition is exemplified in many cases (Pörtner and Grieshaber 1993). Accordingly, [O$_2$]$_c$ is the oxygen concentration when diffusion of oxygen to mitochondria begins to limit oxidative phosphorylation. However, anaerobic ATP production can only temporarily meet the sustained energy demands of the various cellular ATP consuming processes. Shortage of substrates and accumulation of deleterious end products (Van Dijk et al. 1999; Zielinski et al. 2000) set temporal constraints on anaerobiosis as a long-term solution to severe oxygen limitation (Boutilier and St-Pierre 2000).

According to Herreid (1980), Mo$_2$ is a function of oxygen availability and total oxygen conductance throughout the body:

$$Mo_2 = G_T(P_i o_2 - P_m o_2) \tag{8}$$

$$\frac{1}{G_T} = \sum \left(\frac{1}{G_V} + \frac{1}{G_G} + \frac{1}{G_B} + \frac{1}{G_C} + ... \right) \tag{9}$$

where P_io_2 is incoming, environmental oxygen partial pressure (kPa); P_mo_2 is oxygen partial pressure in mitochondria (kPa); G_T is total oxygen conductance ($l \, min^{-1} \, 100g^{-1}$); G_V is oxygen conductance due to ventilation; G_G is oxygen conductance across the gill surface; G_B is oxygen conductance via the circulation including cardiac output and respiratory pigment affinities; G_C is oxygen conductance of the cellular membrane and cytoplasm. Thus, oxygen consumption can remain constant in a situation of declining P_io_2 (i.e. hypoxia) only if the conductance of oxygen flow increases and there is indeed a number of mechanisms to fulfil this precondition (increasing ventilation, increasing blood circulation, etc.; Frederich and Pörtner 2000). However, all of these mechanisms have certain limits of functional capacity. To support an increasing functioning of G_T an animal also needs adequate energy supply, which sooner or later will become limiting, last not least due to the limited use of anaerobic energy production (discussed above).

Mo_2 and $[O_2]_c$ can be considered as physiologically coupled parameters, because there is a very significant correlation between them ($r = 0.6877$, $P < 0.001$) that is described by linear equations (Fig. 6; Eqn. 7). The level of Mo_2 is the temperature-modulated variable (Table 5), which sets the level of $[O_2]_c$. It is obvious that the higher the oxygen demand, the higher the oxygen concentration at which the above-mentioned constraints for anaerobic energy production and total oxygen conductance will set in.

The $[O_2]_c$ was defined by Herreid (1980) as a function of two variables, Mo_2 and G_T:

$$[O_2]_c = \frac{Mo_2}{G_T} \tag{10}$$

Therefore, $[O_2]_c$ will rise whenever aerobic metabolism increases, and it will fall whenever oxygen conductance rises (Herreid 1980). Our data are in line with this consideration (Fig. 6; Eqn. 7). These relationships
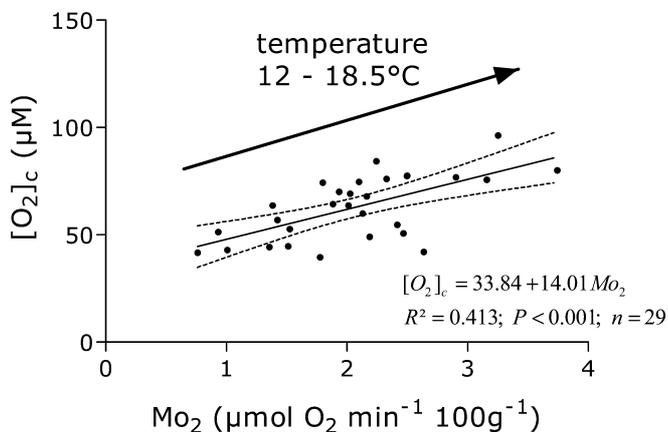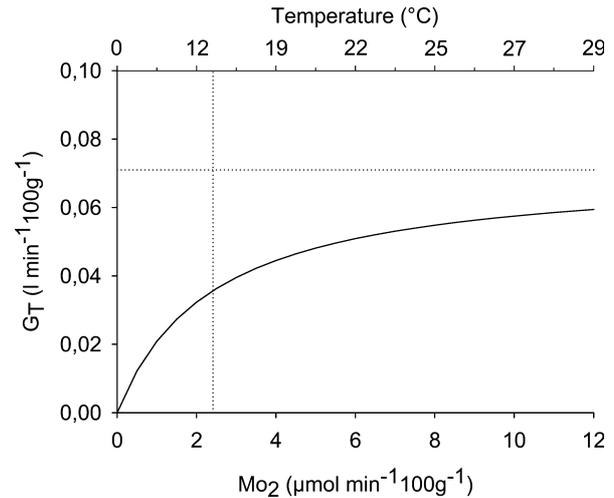
Fig. 7 Theoretical approximation of the relationship between total oxygen conductance (G_T; definition according to Eqn. 9) and Mo_2 for 12 °C-acclimated Baltic eelpout (for Mo_2 between 0 °C and 24 °C; Fig. 5B). The G_T reaches saturation level at 0.071 l min^{-1} $100g^{-1}$ and half-saturation at 2.36 μmol min^{-1} $100g^{-1}$ of Mo_2. Warm-acclimated eelpouts reach this level of Mo_2 at 14.2 °C. It means that at temperatures above 14–15 °C the metabolic capacity to shift $[O_2]_c$ to lower values by increasing G_T (Eqn. 10) will be restricted

(Fig. 6; Eqn. 10) allow us to estimate roughly how big the range of variability is for G_T. First of all, according to Eqn. 10 and due to the linear relationship between Mo_2 and $[O_2]_c$ (Fig. 6), the relationship between Mo_2 and G_T is described by a hyperbolic equation which reaches saturation. For an Mo_2 between 0 μmol min^{-1} $100g^{-1}$ and 10 μmol min^{-1} $100g^{-1}$ (corresponding to a temperature range between 0 °C and 25 °C for 12 °C-acclimated Baltic eelpout) it yields a maximum, saturated level of G_T at 0.071 l min^{-1} $100g^{-1}$ and a level of half-saturation at 2.36 μmol min^{-1} $100g^{-1}$ of Mo_2 (Fig. 7). This is the level of Mo_2 12 °C-acclimated Baltic eelpout reaches approximately at 15 °C. This means that at 15 °C, 50% of the full capacity of the total gas conductance of Baltic eelpout is already used and at temperatures above 15 °C, the metabolic capacity to shift $[O_2]_c$ to low enough values by means of increasing G_T will become more limited. This theoretical approximation is in line with experimental observations and explained by a progressive limitation of aerobic scope and, thus, the oxygen limitation of thermal tolerance. In parallel with a warm-induced rise in oxygen demand, harsh environmental conditions, which may be combined with high temperature exposure (high salinity and low barometric pressure), will result in a further reduction of oxygen concentrations in water and will enhance oxygen limitation for the eelpout. Finally, the warm-induced rise in $[O_2]_c$ will approach the environmental oxygen concentration indicating complete loss of aerobic scope (Fig. 8). At this point, the $[O_2]_c$ corresponds, by definition, to the upper critical temperature (Tc_{II}), which is characterized by transition to anaerobic metabolism (see above, (Pörtner 2001, 2002a, 2002b;

Fig. 6 Correlated changes in the Mo_2 (μmoles min^{-1} $100g^{-1}$), $[O_2]_c$ (μM), and temperature (°C) for common eelpout (*Z. viviparus*) from the Baltic population (salinity = 17‰) acclimated to 12 °C ($n = 7$). The rate of temperature change was 1 °C per 2 days

In Fig. 6: temperature 12 - 18.5°C; $[O_2]_c = 33.84 + 14.01 Mo_2$; $R^2 = 0.413$; $P < 0.001$; $n = 29$
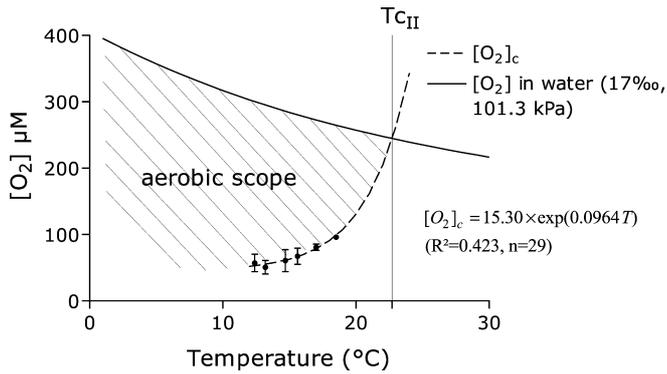
Fig. 8 The scope for aerobic metabolism (*shaded area*) is dependent on temperature, water salinity, and barometric pressure. The concentration of dissolved oxygen in water (*solid curves*) decreases with temperature, while the critical oxygen concentration ($[O_2]_c$) (*filled circles fitted by broken curve*) increases in exponential way. The upper critical temperature (Tc_{II}) is set at temperatures where two of these curves intersect. Consequently an animal is forced to switch over to anaerobic metabolism. This example is given for eelpout from Baltic Sea. Unfavourable combinations with high salinity and low barometric pressure (33‰, 90 kPa) will shift the Tc_{II} further down. Values of $[O_2]_c$ are means $\pm$ SD

Pörtner and Grieshaber 1993; Sommer et al. 1997; Van Dijk et al. 1999). According to an extrapolation from Fig. 8, the $[O_2]_c$ for Baltic eelpout reaches the natural oxygen level in Baltic sea water (17‰, 101.3 kPa) at a temperature of 22.5 ± 1 °C, corresponding to 250 µM of oxygen (Fig. 8). Low barometric pressure and high water salinity will shift this thermal limit to even lower temperatures.

Our calculations perfectly match the conclusions of Van Dijk et al. (1999) who found that, for *Z. viviparus*, the upper critical temperature limit ("Tc_{II}; characterised by the onset of anaerobic metabolism, evidenced by the accumulation of lactate and succinate") is located between 21 °C and 24 °C (Table 5). Fonds et al. (1989) also found that mortality of the eelpout was rather high at temperatures above 20 °C. They concluded that the upper lethal limit for *Z. viviparus* probably lies close to 22–23 °C. Our data are in line with these earlier observations and demonstrate that for water breathing ectothermic animals, the Tc_{II} is the same as the temperature where $[O_2]_c$ meets ambient oxygen concentration (Fig. 8). At the Tc_{II}, due to capacity limitation of the oxygen delivery system, the animals face a mismatch between oxygen demand and oxygen availability. This phenomenon results in transition into hypoxia and, possibly, a hypometabolic phase that is time-limited. Accordingly, behavioural avoidance of such temperatures should result, with the consequence that bio-geographical distribution of this species becomes limited.

Conclusions

We have summarised the key events in the thermal physiology of eelpout (*Z. viviparus*) in Table 6, considering both data from this study and the literature. The

Table 6 Key events in thermal physiology of common eelpout *Z. viviparus*

Temperature (°C)	Description	Rate of temperature change (ΔT°C/Δt)	Reference
< 0	Lower temperature limit for growth	Growth experiment (28–36 day at fixed temperature)	(Fonds et al. 1989)
4	Avoidance temperature during winter	Natural	Personal communication G. Naevdal (University of Bergen, Norway)
13–14	Optimal temperature for somatic growth	–	(Fonds et al. 1989)
> 13–15	The difference in Mo_2 among populations becomes expressed above this temperature	3°day^{-1}	This study
~15	Total gas conductance (G_T; l min^{-1} 100g^{-1}) through the eelpout body becomes used on 50% of full capacity and further increments of G_T will be restricted	Theoretical approximation	This study
18	Avoidance temperature during summer	Natural	Personal communication G. Naevdal (University of Bergen, Norway)
18.5	High mortality	1°C day^{-1}, combined with frequent hypoxia	This study
> 20	High mortality	Growth experiment (28–36 day at fixed temperature)	(Fonds et al. 1989)
21–24	Tc_{II} upper critical temperature (onset of anaerobic metabolism)	3°C day^{-1}	(Van Dijk et al. 1999)
22.5 ± 1	Tc_{II} (intersection of the $[O_2]_c$ with ambient $[O_2]$)	1°C day^{-1}	This study
22–23	Upper lethal temperature limit	Growth experiment (28–36 day at fixed temperature)	(Fonds et al. 1989)
> 24	High mortality	3°C day^{-1}	(Van Dijk et al. 1999)

traditional method of analysing the limits of thermal tolerance shows ecologically irrelevant temperatures (28.8 °C). With increasing temperature, standard metabolic rate rises in an exponential way. At the same time, the scope for aerobic metabolism becomes gradually decreased beyond pejus temperatures (13–15 °C) and is finally lost when the $[O_2]_c$ reaches water oxygen levels (at 22.5 ± 1 °C for Baltic eelpout), e.g. at critical temperatures, where animals are forced to switch over to anaerobic metabolism. At this point, energy demands no longer match energy production and as a result survival becomes time-limited and depends on the capacity of anaerobic metabolism.

The capacity limitation of the oxygen delivery system probably sets the first physiological limit for survival in the warm. The upper pejus temperatures for the eelpout (13–15 °C) are close to average temperature maxima in the natural habitat, thereby limiting latitudinal distribution of the species. The critical temperature likely correlates with species distribution, but will be relevant only at temperature extremes and is only tolerated passively. For short-term survival at more extreme temperatures, possibly experienced in the shallow water of the Wadden Sea, the elevated hypoxia tolerance of *Z. viviparus* may become important.

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