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ORIGINAL PAPER

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How does the cold stenothermal gadoid *Lota lota* survive high water temperatures during summer?

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Abstract The cold-stenothermal freshwater gadid *Lota lota* inhabiting the potamic regions of lowland rivers in central Europe, is exposed to summer temperatures up to 25 °C, which is far above the thermal preferendum of this species. Oxygen consumption rates, determined in field catches sampled at different times of the year, revealed that the basal metabolic rate is depressed during summer when water temperatures are high (152 ± 16 μmol O_2 100 g^{-1} h^{-1}at 22 °C in July compared to 250 ± 33 μmol O_2 100 g^{-1} h^{-1} at 6 °C in November). This observation led us to investigate whether the observed depression of the metabolic rate is caused by oxygen limitation due to thermal impairment of the ventilatory system, as has been observed in other species. Determination of anaerobic end products (lactate and succinate) in the liver tissue of fish caught at different sampling dates did not show an accumulation of anaerobic end products during the summer, indicating no oxygen limitation. Measurements of enzyme activities in the white musculature and liver suggest that enzymes involved in aerobic metabolism were down-regulated during summer, which may have contributed to the observed reduction of metabolic rate.

Keywords Fish · Temperature stress · Season · Oxygen consumption · Enzyme activity

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Abbreviations *CS* citric synthase · *LDH* lactate dehydrogenase · *PK* pyruvate kinase · *TCA* trichloroacetic acid

Introduction

Temperature is one of the most important abiotic factors, given that it affects the velocity of all biochemical processes. While homeotherms maintain stable body temperatures despite temperature fluctuations in the ambient medium, poikilothermic organisms may be subjected to large seasonal changes of body temperature. Although aquatic habitats generally show smaller thermal variations than air, the amplitude of seasonal temperature changes varies between different aquatic habitats. Generally, large water bodies are less affected by seasonal changes in air temperature. Therefore, marine organisms experience relatively small temperature variations compared to freshwater inhabitants.

The burbot *Lota lota* is the only member of the predominantly marine and cold stenothermal family Gadidae, that has invaded the fresh water habitat. Thus, this species has had to cope with considerably larger temperature changes than its marine ancestors and must have developed an effective survival strategy during evolutionary adaptation to the freshwater habitat. *L. lota* is mostly found in cool rivers and streams in North America and Northern Europe southward down to 40°N (Lelek 1987) and is considered a cold stenothermal species (Hofmann and Fischer 2002; Tiitu and Vornanen 2002). Especially along the southern distribution limit of *L. lota*, however, this species may experience summer peak temperatures of up to 25 °C (Hofmann and Fischer 2002). Our investigations on a population of *L. lota* in the river Oder (Germany) revealed that these animals reduce feeding rates during summer, when water temperatures are high, so that they have to rely on body reserves to fulfill their energetic needs (I. Hardewig, S. Volkmann, C. Wolter, P.L.M. van Dijk, F. Hölker, unpublished data). This suggests

that high water temperatures during summer may be stressful for *L. lota*. It has been shown for a variety of organisms, including fish, that at stressfully high temperatures oxygen supply becomes limiting. Firstly, aerobic scope falls until, finally, certain tissues become oxygen deficient and temperature induced anaerobiosis sets in (van Dijk et al. 1999; Frederich and Pörtner 2000; Pörtner 2001, 2002; Pörtner et al. 2000; Peck et al. 2002). This must be a time-limited situation and will eventually lead to death if temperatures do not return to tolerable levels.

In the present study, we investigated whether water temperatures above 20 °C, as they are experienced on a regular basis during the summer by populations of *L. lota* at our latitudes, induce oxygen deficiency or whether *L. lota* endures the high temperatures in a kind of summer quiescence by down regulation of the metabolic rate. To this end, we determined oxygen consumption rates of *L. lota* caught in the field at different times of the year. The quantification of anaerobic end products in the liver tissue of field catches should reveal whether a possible decrease of oxygen consumption during the summer is linked to oxygen limitation. Alternatively, a decrease of oxygen consumption could be induced by a regulatory reduction of energy turnover. In this case we would not expect an accumulation of anaerobic end products.

Material and methods

Animals

Fish were caught by electric fishing in the River Oder in the vicinity of the town Schwedt (stream-km 685–697). At seven sampling dates between June 2000 and July 2001 (Table 1), ten specimens of similar size were selected randomly from the entire catch for further laboratory analysis (in February 2001 only six specimens were caught). Juvenile fish were chosen to exclude an effect of maturation and spawning on the investigated parameters. We tried to select fish of the same year class (1999) during the whole investigation, so that we sampled mainly 1+ fish in 2000 and 2+ fish in 2001, which was confirmed by otolith analysis. The burbots were killed by a blow on their head shortly after capture. The liver and a sample of the anterior part of the white muscle were excised and freeze clamped in liquid nitrogen. Tissue samples were transported to the laboratory in liquid nitrogen and were then stored at −80 °C until analysis. Live fish caught in July 2002, September 2002 and November 2002 were taken to our holding facilities in Berlin in aerated plastic tanks. Fish were kept in aquaria for 5 days at their habitat temperature and under simulated natural light–dark cycles to allow them to recover from catching and transportation before they were used in respirometric experiments. Acute effects of temperature changes were determined in burbot that had been kept at an acclimation temperature of 16 °C and a light:dark cycle of 12 h:12 h for several months.

Table 1 Sampling date, water temperature, and ranges of fish size for different catches of *L. lota*

Date	Water temperature (°C)	Mass (g)	Determination
21.06.2000	23.4	21.5–80.0	Biochemical
25.07.2000	18.3	20.7–56.9	Biochemical
20.09.2000	13.3	41.0–102.0	Biochemical
08.11.2000	7.6	52.0–110.0	Biochemical
27.02.2001	0.5	47.4–294.7	Biochemical
25.04.2001	8.9	39.5–147.5	Biochemical
04.07.2001	23	49.0–158.2	Biochemical
11.07.2002	22	4.9–9.5	Respirometric
25.09.2002	16	13.7–31.2	Respirometric
20.11.2002	6	20.7–34.3	Respirometric

Tissue extractions and analysis

For the determination of lactate, liver samples were extracted with perchloric acid (PCA) as described by Hardewig et al. (1998). Concentrations of lactate were determined according to Bergmeyer (1985). Succinate was analyzed by capillary electrophoresis according to a modified method from Agilent Technologies (Organic acid analysis kit P/N 5063–6510) (T. Hirse and H.O. Pörtner, unpublished data) after tissues were extracted with trichloroacetic acid (TCA). In brief, trichloric acid extracts were prepared using TCA (15%) and 0.12 g l^{-1} tatrate as an internal standard. Frozen tissue powder (100 mg) was suspended in the 3.5-fold volume of cold TCA and homogenized with an Ultra-Turrax for 10 s. After centrifugation (3 min, 16,000×*g*, 0 °C) the supernatant was pH neutralized by the 3-fold volume of 1:4 n-Octylamine:1,1,2-Trichlortrifluorethan (Freon), mixed and centrifuged (1 min, 16,000×*g*, 0 °C). The upper phase was stored at −80 °C. It was diluted 1:4 and filtered through a 0.2-µm injection filter prior to analyses. Succinate and malate were separated with an "eCap Capillary Tubing" (75 µm, 120 cm, Beckman 338473) at 27 kV and 15 °C, and detected (PDA) at 214 nm. The separating buffer consisted of 1/5/20 Brij 35 (Fluka)/Acetonitril (Riedel, Chromasolv)/organic acid buffer (Agilent).

Enzyme activities were measured in fresh tissue extracts. Tissues were pulverized under liquid nitrogen and about 100 mg ground tissue powder were added to 9 vol 50 mM Tris pH 7.4, 1 mM EDTA, 2 mM $MgCl_2$ for lactate dehydrogenase (LDH) and pyruvate kinase (PK). The tissue was homogenized briefly with an Ultra-Turrax and centrifuged for 5 min at 10,000×*g*. Activities were determined in the supernatant with a plate reader (Spectra Flour Plus, Tecan) at a wavelength of 340 nm and 25 °C. Previous measurements have shown that enzyme activity was not inhibited by this temperature.

The reaction mix contained 50 mM imidazole, 0.2 mM pyruvate, and 0.15 mM NADH for LDH. PK was determined in 50 mM imidazole, 5 mM ADP, 100 mM KCl, 10 mM $MgCl_2$, 0.15 mM NADH, 0.1 mM fructose1,6P, 5 mM phosphoenolpyruvate, 25 U/ml LDH.

For citric synthase (CS) 75 mM Tris pH 7.4, 1 mM EDTA was used as extraction buffer. CS activity was determined by the reduction of DTNB, which was monitored at $\lambda = 412$ nm. The reaction mix contained 75 mM Tris-HCl pH 8, 0.1 mM DTNB, 0.4 mM AcetylCoA, and 0.5 mM oxaloacetate. 10 ml DTNB stock solution (1 mM) was prepared freshly on a daily basis. DTNB was dissolved in water by sonification. Solubility was increased by addition of 50 µl 1 M KOH. Since alkaline pH decreases the stability of DTNB, addition of KOH was minimized.

On the samples from fish caught in April 2000 only lactate analysis was performed, since the tissue was defrosted during storage due to a failure of the freezer, before all extractions were carried out.

Measurement of oxygen consumption

Oxygen consumption rates were determined in a flow-through respirometer consisting of six chambers arranged around a centrally placed oxygen sensor (Eschweiler, Kiel). The outflow of each chamber was alternately connected to the sensor during measurements. The temperature of the respirometer was controlled by a large bath of aerated water, which also served as a reservoir for inflowing water. The oxygen electrode was calibrated each morning

with water saturated with air (100% saturation) or N_2 (0% saturation). The calibration of the 100% value was repeated after each measurement. The 0% calibration was omitted between measurements because it was stable throughout the day. Blank oxygen depletion was determined in an empty chamber and did not exceed 5% of total oxygen consumption. O_2 consumption rates were normalized for a 100-g fish by using a mass exponent of 0.676 (Shodjai 1980).

For the determination of direct effects of temperature on oxygen consumption, five fish of body mass 35.2–57.2 g were placed into the respirometer chambers at 16 °C. Since digestion processes cause an increase of oxygen consumption (specific dynamic action), fish were deprived of food for 72 h before they were inserted into the respirometer. At 16 °C, ca. 94% of the last meal is evacuated from the digestive tract during this time (Pääkönen et al. 1999). Fish were allowed to acclimate to the experimental setup and to recover from handling stress for at least 24 h before oxygen consumption measurements were carried out. After water oxygenation had been recorded from all chambers (for at least 1.5 h per chamber) the temperature was raised overnight by 2 °C. Once 24 °C was reached, temperature was kept constant and oxygen consumption was determined over several subsequent days. To exclude a possible effect of starvation on oxygen consumption rates, the fish were taken out of their chambers after 5 days at 24 °C (i.e., after 10 days in the respirometer) and fed ad libitum for 4 days. After another 72 h food deprivation, fish were brought back into the respirometer and oxygen consumption measurements were repeated.

Freshly caught animals were allowed to recover from the catching procedure for 5 days in an aquarium before they were placed into the respirometer. Measurements were carried out at habitat water temperature at the time of catch and under a simulated natural light–dark cycle.

Statistical analysis

Statistical significance was tested at the $P \leq 0.05$ level using one factor ANOVA (temperature = factor) and Tukey's post-hoc test if the variances of the groups were homogeneous. In the case of non-homogeneous variances, the Dunett-T3-test was used. All data are given as means ± SE.

Results

Seasonal effects on oxygen consumption

Oxygen consumption was determined in field catches of *L. lota* in July, September, and November 2002 at the respective water temperature (Fig. 1). Oxygen consumption was lowest in July with 152 ± 16 µmol 100 g^{-1} h^{-1}at 22 °C and increased in September to 288 ± 26 µmol 100 g^{-1} h^{-1} (at 16 °C), despite decreasing temperatures. Between September and November, water temperature dropped drastically from 16 °C to 6 °C, but oxygen consumption rates of fish caught in November (250 ± 33 µmol 100 g^{-1} h^{-1} at 6 °C) were not significantly different from values determined in the September catch.

Temperature effects on oxygen consumption

In laboratory acclimated fish, oxygen consumption rates remained largely unaffected by short-term temperature

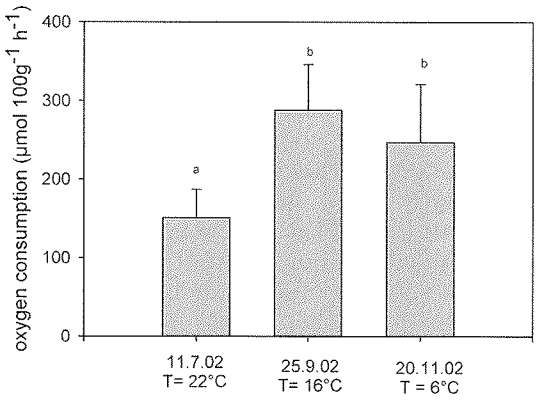

Fig. 1 Oxygen consumption rates of field catches of *Lota lota* at different times of the year (mean + SE, $N = 4$). The given date is the sampling date, T = temperature at which measurements were carried out. Groups with *different letters* are significantly different ($P \leq 0.05$)

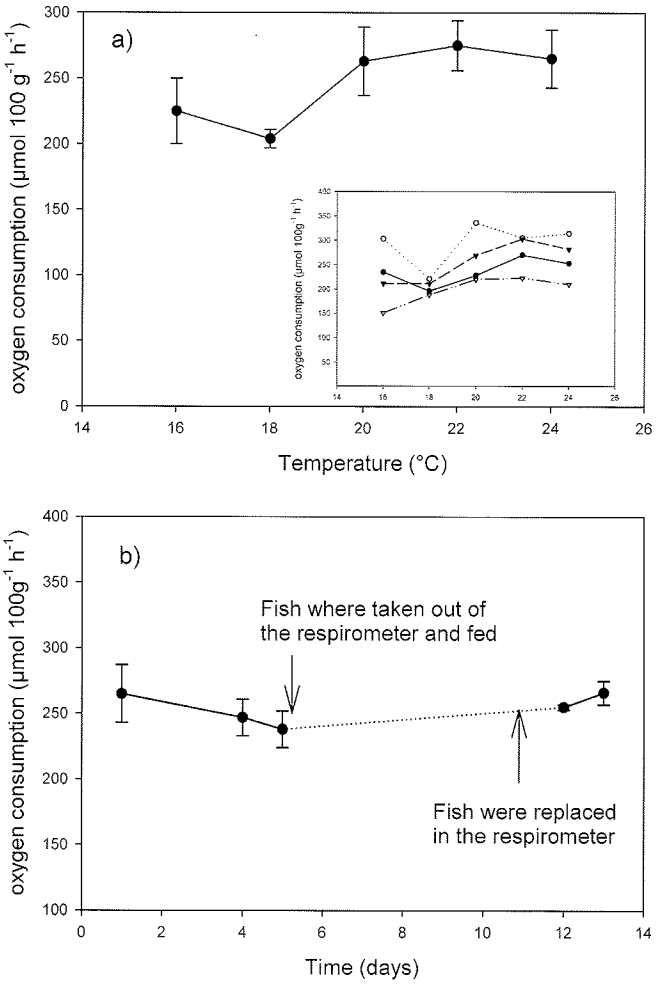

Fig. 2a, b **a** Acute effect of temperature on oxygen consumption of *L. lota* acclimated to 16 °C (mean + SE, $N = 4$). *Inset*: data of individual fish. **b** Oxygen consumption during long-term exposure to 24 °C. Fish were fed between day 5 and day 11

increments of 2 °C between 16 °C and 24 °C (Fig. 2a). However, the data collected in the individual fish suggest that oxygen consumption increased slightly between

16 °C and 20–22 °C but remained constant thereafter (Fig. 2a, inset). Mean Q_{10} values ranged between 1.49 and 3.08, between 16 °C and 22 °C, respectively, while a Q_{10} of 0.81 was determined between 22 °C and 24 °C. When fish were kept continuously at 24 °C, oxygen consumption rate decreased slightly but not significantly between day 1 and day 5, from 265 ± 22 µmol 100 g^{-1} h^{-1} to 238 ± 14 µmol 100 g^{-1} h^{-1}, respectively (Fig. 2b). It should be noted that at this time point the fish had been deprived of food for 12 days. In order to exclude the possibility that the low rates of oxygen consumption at temperatures above 20–22 °C were due to starvation effects (Wieser et al. 1992), fish were refed after day 5 at 24 °C. After refeeding, oxygen consumption increased only slightly to 266 ± 9 µmol 100 g^{-1} h^{-1}.

Metabolite concentrations in the liver tissue

In order to determine whether high water temperatures lead to an accumulation of anaerobic end products, we determined the concentrations of lactate, succinate, and malate in the liver tissue in field catches of burbot (Fig. 3). White muscle tissue was not investigated because the levels of anaerobic end products in this tissue are likely to have been affected by stress incurred during the catching procedure.

Lactate levels in the liver were not correlated with water temperatures. They were slightly elevated in animals caught in July, September, and November 2000. In February 2001, lactate levels were lowest, but increased again in April, when water temperatures were only around 9 °C. The concentrations of succinate and malate, both intermediates of the citric acid cycle and possible anaerobic intermediate (malate) or end products (succinate), were increased between September and February. This increase was significant for malate but not for succinate.

Enzyme activities in the liver and white muscle

CS as a representative of oxidative metabolism showed a significant increase in activity during the winter months in the white musculature which is an indication of cold compensation of the aerobic metabolism of *L. lota* (Fig. 4). CS activity increased significantly from 1.45 ± 0.11 U g fresh $mass^{-1}$ in July 2000 to 2.09 ± 0.15 U g fresh $mass^{-1}$ in November 2000 and fell back to 1.27 ± 0.06 U g fresh $mass^{-1}$ in July 2001. The glycolytic enzyme PK displayed opposite seasonal changes: highest activities were determined in June and July 2000/2001 with 203.8 ± 7.7 U g fresh $mass^{-1}$ and 151.6 ± 8.35 U g fresh $mass^{-1}$, respectively, compared to the lowest value of 95.0 ± 4.7 U g fresh $mass^{-1}$ determined in February 2001. Similarly, LDH activity was increased in white muscle during July in 2000 and 2001 (110.6 ± 4.0 U g fresh $mass^{-1}$ and 107.0 ± 7.7 U g fresh $mass^{-1}$, respectively) compared to the cooler period between September and February.

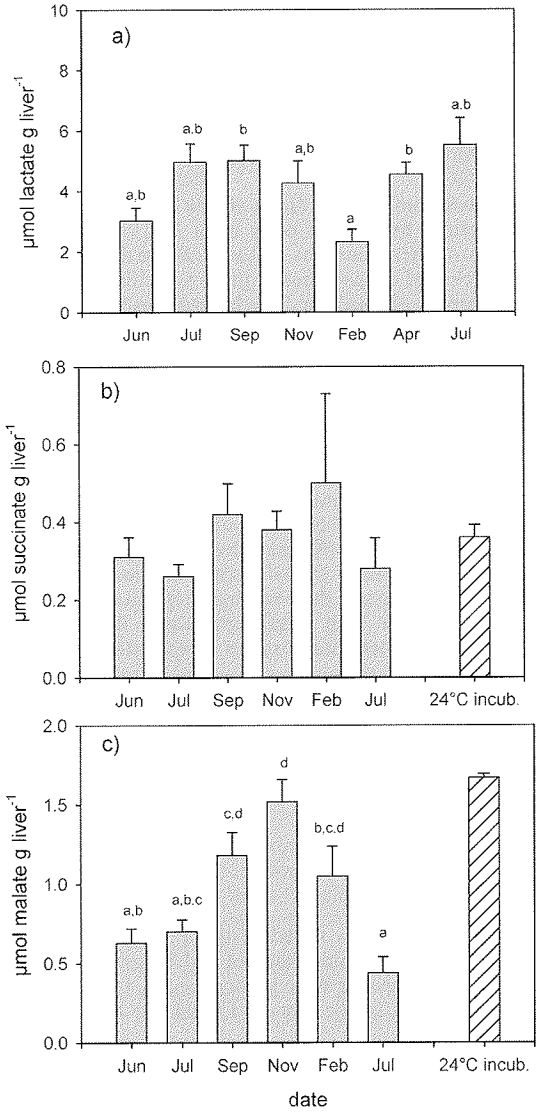

Fig. 3a–c Concentration of: **a** lactate, **b** succinate, and **c** malate in the liver of *L. lota*. *Grey bars*: fish caught at different times of the year; *hatched bars*: fish taken from the respirometer after 13 days at 24 °C. Groups with *different letters* are significantly different ($P \leq 0.05$)

In liver tissue, specific LDH activity remained more or less constant throughout the year (Fig. 5a). When the total activity per liver of a 100-g fish was calculated, however, LDH activity decreased between June and November from 560.0 ± 31.85 U $liver^{-1}$ to 266.2 ± 24.5 U $liver^{-1}$ and increased again thereafter (Fig. 5b). These differences are induced by seasonal changes in liver size from a minimum of $3.02 \pm 0.33\%$ body mass in November to a maximum of $8.46 \pm 0.66\%$ body mass in April due to the mobilization of liver reserves observed during the summer (I. Hardewig, S. Volkmann, C. Wolter, P.L.M. van Dijk, F. Hölker, unpublished data).

Glucose-6-phosphate dehydrogenase, an enzyme that provides NADPH for anabolic processes, decreased during summer from 53.1 ± 6.3 U $liver^{-1}$ in July to

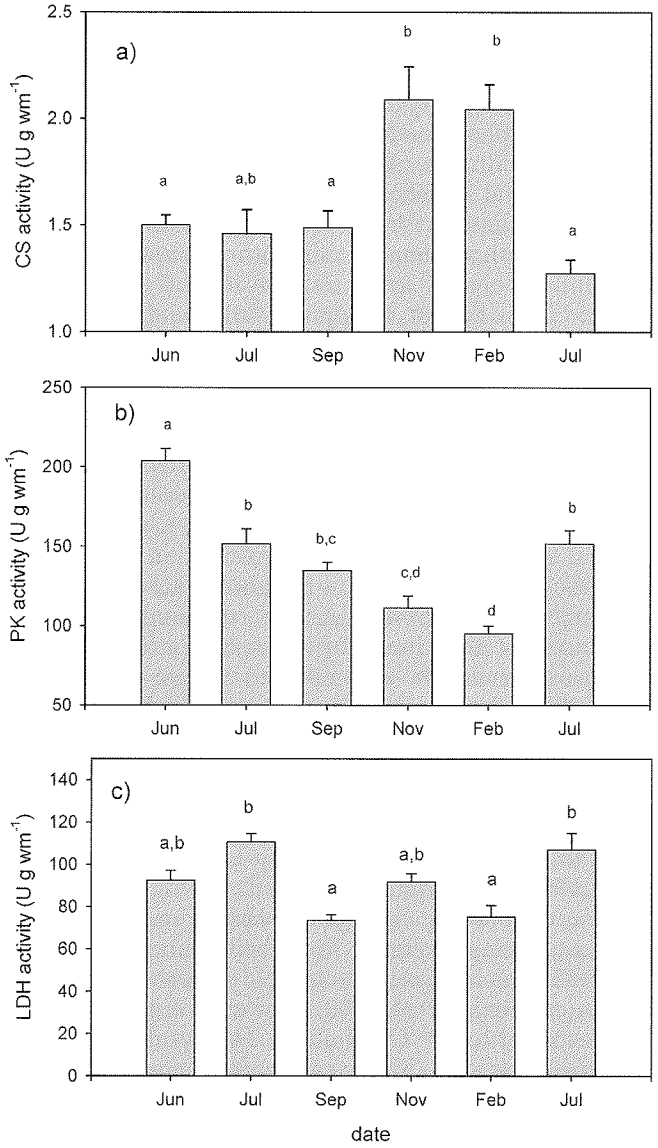

Fig. 4a–c Activity of: **a** citric synthase (*CS*), **b** pyruvate kinase (*PK*), and **c** lactate dehydrogenase (*LDH*) in the white musculature of *L. lota* caught at different times of the year (mean ± SE, $N = 10$ except for February: $N = 6$). Groups with *different letters* are significantly different ($P \leq 0.05$)

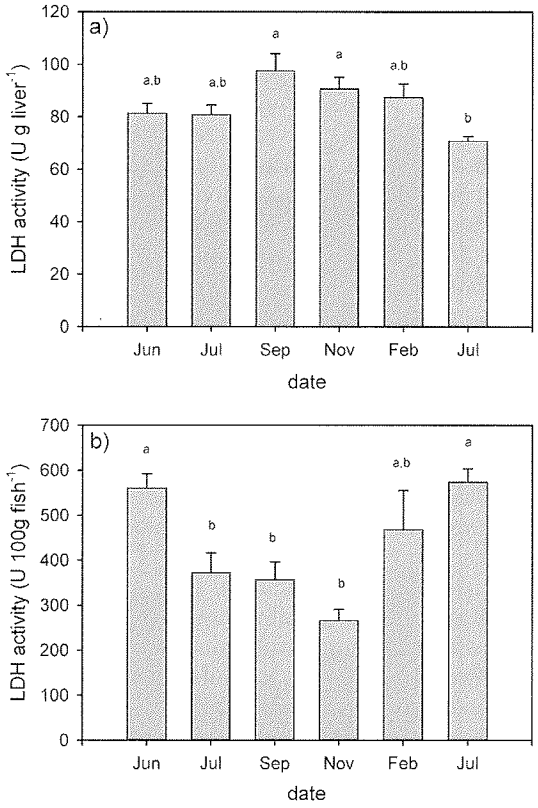

Fig. 5a, b LDH activity in the liver of *L. lota* caught at different times of the year (mean ± SE, $N = 10$ except for February: $N = 6$). Groups with *different letters* are significantly different ($P \leq 0.05$). **a** Specific enzyme activity per gram of liver. **b** Total enzyme activity in the liver of a 100-g fish

13.1 ± 1.9 U liver^{-1} in November 2000 and increased again thereafter (Fig. 6a). This pattern is even more pronounced when enzyme activity is expressed as total activity per liver of a 100-g fish (Fig. 6b).

Discussion

The oxygen consumption rates that were determined in the present study can be considered as standard metabolic rate since the animals did not move around but appeared unstressed and remained settled at the bottom of the respirometer chamber throughout the measurements. *L. lota* is strongly nocturnal (Lelek 1987) and respirometric measurements were carried out during the day, when these fish are inactive.

Specimens of *L. lota* caught during summer 2002 showed surprisingly low oxygen consumption rates when compared to winter animals. Apparently this species not only decreases swimming and hunting activity during summer (I. Hardewig S. Volkmann, C. Wolter, P.L.M. van Dijk, F. Hölker, unpublished data), but also reduces its basal metabolic rate when water temperatures are high. Is metabolic rate down regulated during summer or is the observed reduction in oxygen consumption caused by the inability to take up sufficient oxygen due to thermal stress? When we exposed 16 °C-acclimated fish to acute temperature changes (2 °C per day), oxygen consumption rates remained largely independent from temperature between 16 °C and 24 °C; similar results have also been found by Shodjai (1980) on *L. lota*, who even found a slight decrease of respiration rates above 20 °C ($Q_{10} = 0.8$ between 20 °C and 24 °C). Generally, oxygen consumption of fish displays a distinct temperature dependence with Q_{10} values between 1.5 and 3 (Beamish 1964; van Dijk et al. 1999). Beamish (1964) determined oxygen consumption rates in several fish species and found that oxygen consumption increased less steeply with temperature in the high temperature range; Q_{10} values decrease when animals

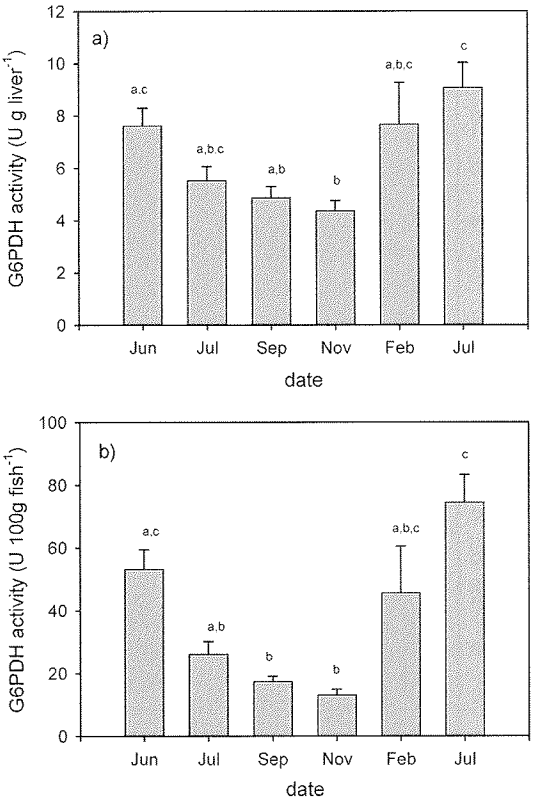

Fig. 6a, b Glucose-6-Phosphate-dehydrogenase (*GPDH*) activity in the liver of *L. lota* caught at different times of the year (mean ± S.E, $N = 10$ except for February: $N = 6$). Groups with *different letters* are significantly different ($P \leq 0.05$). **a** Specific enzyme activity per gram of liver. **b** Total enzyme activity in the liver of a 100-g fish

approach their thermal tolerance limits. The mechanism underlying this phenomenon is not quite clear.

Farrell (1996) investigated the effect of high temperatures on cardiovascular performance in fish, and suggested that at temperatures above the preferred temperature of a species, maximum cardiac performance does not continue to increase but begins to plateau. This leads to a decrease of cardiac scope and, therefore, to a reduced scope for activity. The exponential rise in resting oxygen consumption is not yet affected, however. At temperatures approaching the upper incipient thermal limit of the species, cardiac output starts to decrease and resting oxygen consumption is also impaired. In fact, hyperoxia was found to alleviate thermal effects on circulatory work, leading to lower oxygen consumption values and possibly higher thermal tolerance in hyperthermic Antarctic eelpout, *Pachycara brachycephalum* (Mark et al. 2002).

L. lota shows a temperature preference of 13.5 °C (P. van Dijk, G. Staaks, I. Hardewig, unpublished data). Hofmann and Fischer (2002) even determined a final temperature preferendum as low as 11.4 °C. According to Farrell's hypothesis (1996), *L. lota* inhabiting the River Oder has to deal with a reduced cardiac scope for about half of the year (April–October), when water temperatures reach values above the thermal preferendum of 11–13 °C (I. Hardewig, unpublished observations).

It is not clear, however, above which temperature threshold resting oxygen consumption is impaired and whether this phenomenon is responsible for the observed low metabolic rates of *L. lota* caught during summer. Hofmann and Fischer (2002) determined a critical thermal maximum (the temperature that an animal can tolerate no longer than 10 min without showing behavioral indications of stress such as loss of equilibrium) of 31.5 °C for burbot. Shodjai (1980) found that *L. lota* did not survive a temperature of 28 °C in laboratory acclimation experiments.

Impairment of physiological functions may set in at lower temperatures, however. In a study on isolated hearts of *L. lota*, temperatures above 18 °C caused an atricoventricular block and arrhythmic contractions (Tiitu and Vornanen 2002). This may suggest that the absence of thermal stimulation of oxygen consumption at temperatures above 20 °C and the low metabolic rates determined in summer-acclimatized *L. lota* may be caused by capacity limitations of the circulatory system. Extreme oxygen limitation would be accompanied by an increase in anaerobic end products, especially those which indicate mitochondrial hypoxia, such as succinate, as has been shown for other heat-stressed organisms (Pörtner et al. 2000). In the eelpout *Zoarces viviparus*, succinate levels in the liver increased two fold when the animals were exposed to temperatures close to their lethal limit (van Dijk et al. 1999). In invertebrates, such as the lugworm *Arenicola marina* and the clam *Laternula elliptica*, high environmental temperatures above their thermal tolerance limit lead to an increase of anaerobic end products in their tissues (cf. Pörtner et al. 2000). However, burbot that had been exposed to 24 °C for about 14 days did not show elevated levels of succinate or lactate in the liver tissue. Also field catches of *L. lota* did not display an accumulation of the investigated anaerobic end products when water temperatures approached 20 °C (in June and July 2000 and July 2001). On the contrary, both malate and succinate were elevated during the winter months between September and February. This may be an indication of enhanced flux rates of the citric acid cycle during winter (Newsholme and Crabtree 1979; Chih and Ellington 1986), which is in agreement with higher oxygen consumption rates (see Fig. 1). Thus, we do not believe that *L. lota* is strongly oxygen limited during the summer. It is more likely that metabolism is down regulated when water temperature increases above 20 °C. Animals may respond to the onset of heat-induced limitation in aerobic scope by metabolic depression below standard metabolism even before undergoing anaerobic metabolism.

Changes in the expression of enzymes involved in energy turnover may contribute to the observed decrease in metabolic rate. We found an increase of oxidative capacity during the winter, as indicated by about a 1.6-fold difference of citrate synthase activity in the white muscle of *L. lota* between winter and summer animals. A compensatory increase of the oxidative capacity of white

musculature is observed in many temperate fish species (Johnston and Dunn 1987). However, in most cases, the differences are more pronounced than the one we observed between winter- and summer-acclimatized burbot. For example, Guderley and Gawlicka (1992) found a 2.2-fold increase of CS activity in rainbow trout between acclimation temperatures of 18 °C and 4 °C. Guderley (1990) formulated the hypothesis that acclimatory changes in oxidative enzyme activities are reduced when the acclimation temperatures bracket the thermal preferendum of a species, because no compensation occurs above the preferred temperature. Whether this is correlated with the observed impairment of the oxygen provision at temperatures above the preferred temperature (Farrel 1996; see above) is not yet clear. Guderley's hypothesis is confirmed, for example, by data on female ninespine stickleback, *Pungitius pungitius*, which prefers temperatures of between 9 °C and 12 °C. Acclimation to 20 °C and 3 °C results in only a 1.5-fold difference in CS activity (Guderley and Foley 1990). The same explanation holds for *L. lota*: the thermal optimum of the species lies around 13 °C (see above), which is an intermediate temperature between the peak in summer (c. 25 °C) and the low during winter (c. 0.5 °C). According to Guderley's hypothesis (Guderley 1990), CS activity should be constant at water temperatures between 13 °C (September 2000) and 23 °C (June and July 2000/2001), which is confirmed by our data.

Although CS activity shows partial compensation with respect to acclimatization temperature, the observed changes in enzyme activity cannot fully explain the differences in oxygen consumption between summer and winter animals. The over compensation of metabolic rate resulting in lower rates during summer at high water temperatures is certainly a result of many metabolic changes including changes of enzyme expression, but also other factors such as hormonal influences, possibly triggered by temperature but also by the light–dark cycle. Since burbot shows a very distinct nocturnal activity pattern, extended day length during summer may induce an overall decrease in metabolic activity. Future research will investigate the effect of the light–dark cycle on the physiology of burbot.

LDH activities are generally low in the white musculature of *L. lota* (between 70 U g^{-1} and 110 U g^{-1}) compared to 260 U g^{-1} in goldfish and 2,723 U g^{-1} in perch (Moon and Foster 1995). This indicates a low glycolytic capacity, which is typical for demersal fish. Both glycolytic enzymes PK and LDH showed an activity pattern that was opposite to that of CS, and were increased during the warmest months of June and July. In many species, glycolytic enzymes remain largely unaffected by temperature acclimation or seasonal influences (e.g., chain pickerel, Kleckner and Sidell 1985; common carp, Johnston et al. 1985, ninespine stickleback, Guderley and Foley 1990; Atlantic cod, Pelletier et al. 1993). The unusual increase of glycolytic capacity during the summer in *L. lota* may indicate that the aerobic scope of the animal is decreased at this time of the year (see above) and the fish have to rely more strongly on anaerobic energy provision when intense muscle activity becomes unavoidable.

In the liver, the activity of Glu-6-PDH decreased during the summer, but increased again over the winter, closely following the course of the depletion and repletion of liver reserves observed in these animals (I. Hardewig S. Volkmann, C. Wolter, P.L.M. van Dijk, F. Hölker, unpublished data). The replenishment of liver fat stores, occurring between November and July (I. Hardewig S. Volkmann, C. Wolter, P.L.M. van Dijk, F. Hölker, unpublished data) requires NADPH, which is provided by the Glu-6-PDH-catalyzed dehydration of glucose-6-phosphate. The observed pattern of Glu-6-PDH is obviously not induced by a general increase in protein content in the liver tissue, since LDH activity remained unaffected by season. Liver enzymes seem to be differentially regulated during seasonal acclimatization, indicating a shift in metabolic functions.

In summary, our data indicate that *L. lota* shows relatively low metabolic rates during the summer when water temperatures reach values of 20 °C and above. This decrease is, however, not caused by insufficient oxygen supply due to an impairment of the circulatory system. The reduction of metabolic rate seems to be induced by regulatory control mechanisms involving a decrease in enzyme expression. These mechanisms allow *L. lota* to survive high summer temperatures in a state of metabolic depression, with low food intake and low energy expenditure. During winter, however, *L. lota* remains active due to a compensatory increase in metabolic activity. This strategy enables *L. lota* to refill its body reserves and spawn during winter, when competition for food resources is less and predation risk is low, as most freshwater fish show reduced activities during the winter season.

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