

## Colonization of the freshwater environment by a marine invader: how to cope with warm summer temperatures?

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### ABSTRACT

The burbot *Lota lota* (L.) is the only member of the cold-stenothermal marine family Gadidae that invaded freshwater. To evaluate the evolutionary strategy allowing the invasion of freshwater habitats with high temperature amplitudes, we determined seasonal variations of the energy budget for juvenile burbot from the River Oder, Germany. Food consumption during summer was not sufficient to meet the animal's energetic requirements. During winter and spring, energy uptake surpassed the metabolic energy flux and left surplus energy for growth. Burbot growth is maintained throughout the year by two mechanisms: (1) growth that is reflected predominantly in length throughout the summer, when energy-rich liver tissue must be metabolized to provide energy, and (2) a synthesis of liver reserves during winter, when energy uptake exceeds energy dissipation. In contrast to most freshwater fish, which reduce growth rates during the winter months, the evolutionary burden of being cold-adapted, inherited from its marine ancestors, enables *L. lota* to maintain somatic growth throughout the year.

*Keywords:* bioenergetics, cold-stenotherm, Gadidae, growth, *Lota lota*, temperature tolerance.

### INTRODUCTION

Evolutionary temperature adaptations and the complex relationships between temperature, evolution and ecology are comparatively well studied at the level of cellular processes. The exploration of potential links between physiology and macroecology has, however, just begun (Clarke, 2003). One of the first macroecological hypotheses, for example, predicted a global pattern of biological diversity based on a mechanistic temperature dependence of metabolic rate (Allen *et al.*, 2002). Clarke (2003), however, argued that physiology and ecology cannot be integrated at the community scale, because species distribution borders are influenced by thermal physiology but set by biological interactions and resource availability. Nevertheless, we have evidence that the investigation of a burbot (*Lota lota* (L.))

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population in a temperate, large lowland river provides insights into temperature adaptation at the fish assemblage level.

The burbot is the only freshwater representative of the marine family Gadidae. Its freshwater lineage evolved approximately 5–15 million years ago in Europe in an enclosed marine environment, which became freshwater over time due to river inflows (Van Houdt, 2003; Van Houdt *et al.*, 2003). Burbot began to invade brackish waters as well as freshwater lakes and streams of the northern hemisphere down to a southern distribution limit at about 40°N (Lelek, 1987). Its marine relatives, such as Atlantic cod *Gadus morhua* (L.) and Polar cod *Boreogadus saida* (Lepechin), mainly inhabit polar and boreal habitats and are considered to be cold-stenotherm.

While large marine water bodies vary very little in water temperature, salinity and dissolved oxygen over many years (Eldredge, 1991; Pörtner, 2002a), large rivers often exhibit up to 50-fold annual discharge variations between low and high water levels, temperature variation of more than 20°C during the year and much variability in flow velocity (Puckridge *et al.*, 1998). Besides the change from hyper- to hypo-osmotic medium, which requires distinct osmoregulatory adaptations, the varying temperature regime provides a strong physiological barrier for marine fish to invade freshwater habitats. When switching from a very stable to a much more fluctuating environment, an invader has to cope with these variations and to develop an adaptive strategy to survive despite high summer temperatures. There are several evolutionary strategies for an originally cold-stenothermal marine species to invade freshwater habitats with high temperature amplitudes:

1. The species maintains its stenothermality and chooses water areas with lower summer peak temperatures.
2. The species adapts to its thermal environment by a shift of its temperature preferences in an evolutionary process. In this case, the freshwater form prefers higher water temperatures than its marine relatives.
3. The species remains cold-active, and restricts its main activities (e.g. spawning) to the winter season when water temperatures are low. The summer months are tolerated in some kind of quiescent state.

Strategy 1 can be realized especially in stratified lakes or along the river continuum in the summer-cold headwaters. For ancestral cold-stenothermal species following this strategy, seasonal migrations have to be expected to colder habitats or a distribution restricted to summer-cold waters. Species following strategy 2 should exhibit growth and activity patterns similar to primary freshwater fish species of the region, indicating their adaptation to the environmental conditions of temperate waters. If there are no cooler water bodies available and the species is still cold-active (strategy 3), significant signs of environmental stress have to be expected, such as restricted movements, starvation and reduced growth.

In temperate regions, sticklebacks (Gasterosteidae) and salmonids (Salmonidae) are the most recently evolved freshwater fish species. Both families comprise many diadromous species, which regularly migrate between salt- and freshwater. Salmonids follow the first strategy and preferentially inhabit the upper, cooler region of tributaries to avoid stressfully high water temperatures. Hodgson and Quinn (2002) found that populations of anadromous sockeye salmon *Oncorhynchus nerka* (Walbaum) timed their spawning migrations in a way that avoided water temperatures above 19°C – that is, populations in

warmer areas migrate in early summer or during fall, when water temperatures do not reach peak values.

Sticklebacks, however, inhabit waters of contrasting thermal regimes: *Gasterosteus aculeatus* L. from the Isle Verte in Quebec, Canada, for instance, migrate from the cold St. Lawrence Estuary (5°C) to high fluctuating temperatures (up to 30°C) in the intertidal salt marsh pools (Guderley and Leroy, 2001). Sticklebacks apparently show very high thermal plasticity. The capacity for thermal adaptation and the range of preferred temperatures vary between different stickleback species according to the thermal regime of their habitats (Wootton, 1984; Guderley and Leroy, 2001), suggesting that sticklebacks follow strategy 2 in coping with high summer temperatures. This suggestion is underlined by a strong activity peak of all sticklebacks during the vegetation period (Snyder, 1991) and their rest period in winter.

In contrast to salmonids and sticklebacks, the burbot is an example of complementary or secondary freshwater fish (*sensu* Myers, 1951), which are confined to the freshwater habitat. Like sticklebacks, burbots colonize waters of widely varying temperature regimes; however, in contrast to sticklebacks, they are known as winter-active fish with a spawning period between December and January (Bailey, 1972). This suggests that burbot potentially follow strategy 3.

We investigated a population of *L. lota* in the lowland River Oder, Germany, where water temperatures in summer commonly reach 24–25°C. Because in the lower Oder, as well as in the other lowland rivers and shallow lakes of the region, the complete waterbody is nearly homothermal, burbots colonizing these waters are not able to select areas of lower temperatures, and thus are unable to follow strategy 1. Therefore, we focused our study on the remaining strategies: Has the burbot evolved a preference for higher temperatures (strategy 2) or, the alternative hypothesis, adapted its behaviour to survive stressfully high temperatures during summer by reducing its activity and feeding levels, as suggested by strategy 3. According to this strategy, there are consequences of reduced feeding during summer in terms of growth maximization. Growing fast and large should be advantageous when juvenile fish are most threatened by predation at a body length that is about 10% of that of predatory fish (Paradis *et al.*, 1996). The evolutionary challenge to be maintained is to maximize growth at high temperatures. We investigated seasonal effects on energy allocation in juvenile *L. lota* from the River Oder. Consumption rates were calculated based on stomach content and compared with estimated energy dissipation rates due to metabolism, activity and growth. An apparent discrepancy between energy uptake and dissipation could be explained by an unusual seasonal pattern of degradation and resynthesis of body reserves.

## METHODS

### Animals

Fish were sampled by electric fishing in the vicinity of the town of Schwedt, Germany (53°04'N, 14°17'E) during daytime. While mass and length determinations were carried out over more than 2 years (between May 1999 and November 2001), sampling for biochemical determinations was restricted to seven sampling dates between June 2000 and July 2001 (see Table 2). Ten specimens of similar size were selected randomly from the catch for further laboratory analyses (in February 2001, only six specimens were caught). Juvenile fish were

chosen to exclude an effect of maturation and spawning on the studied parameters. Throughout the investigation, we tried to select fish of the same year class (1999), sampling mainly 1+ fish in 2000 and 2+ fish in 2001, which was confirmed by otolith analysis. The burbot were killed by a blow to the head shortly after capture. After length and mass determination, the liver and a sample of the anterior part of the white muscle were excised and freeze-clamped in liquid nitrogen. The digestive tract was removed and stored in formalin. The otoliths were taken for age determination. Tissue samples were transported to the laboratory in liquid nitrogen and were then stored at  $-80^{\circ}\text{C}$  until analysis. The water temperature in the sampling area is shown in Fig. 1. The given temperatures can be regarded as the temperature the fish were exposed to prior to sampling, since the River Oder is a thermally homogeneous water body.

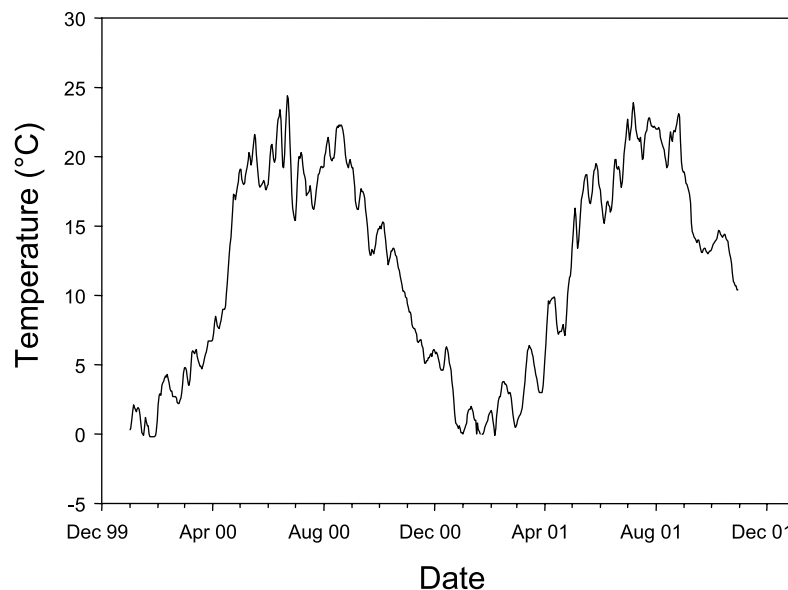
### Stomach and gut content

Stomachs were analysed to estimate the amount of food consumed, to determine its quality and to be able to calculate food consumption rates. Both occurrence and numerical methods (reviewed in Hyslop, 1980) have been used to record the frequency of full stomachs, to determine the filling index and to estimate the proportion of each food category.

The entire intestinal tract was emptied and the stomach contents weighed separately. The relative stomach content (SC) was determined as:

$$\text{SC } (\%M) = \text{stomach content} \cdot 100 \cdot M^{-1}$$

where  $M$  = total wet body mass.



**Fig. 1.** Water temperature in the River Oder in the vicinity of the town of Schwedt (stream km 685–697) between November 1999 and July 2001. Data were provided by the Landesumweltamt Brandenburg.

While stomach content represents a snapshot of the food intake immediately before catch, which could be subjected to diel variations, the intestine index provides an indication of long-term variations in feeding activity, since low feeding rates result in a degeneration of the gut tissue (Love, 1970; Pulliainen and Korhonen, 1990). The intestine index ( $K_i$ ) was calculated as:

$$K_i = M_i \cdot 100 \cdot M_c^{-1}$$

where  $M_i$  = mass of the empty intestinal tract and  $M_c$  = mass of the carcass (total mass – mass of liver and intestinal tract). Stomach content and gut content of the first third of the intestine were analysed separately. Prey items were identified to broad taxonomic groups, mainly to family.

### Hepatosomatic index

The relative mass of the liver was expressed as the hepatosomatic index (HSI):

$$HSI = M_l \cdot 100 \cdot M_c^{-1}$$

where  $M_l$  = liver mass.

### Determination of length, mass and condition factor

The length and mass distributions of juvenile burbot were monitored 12 times between May 1999 and November 2001. Total length was measured from the snout to the longest tip of the caudal fin. Mass was determined by weighing shortly after catching. The condition factor (CF) was calculated as:

$$CF = M \cdot 100 \cdot L_t^{-3}$$

where  $L_t$  is the total length of the fish.

Age groups were determined according to the length frequency distribution of the total catch (4601 specimens), which was validated by exact ageing, using otoliths.

### Biochemical determinations

For the determination of glycogen content, liver tissue was pulverized under liquid nitrogen and extracted with 0.6 M PCA (extract A). A 100- $\mu$ l aliquot of the homogenate was neutralized and amylase was added to hydrolyse glycogen to glucose (extract B). After incubation at 37°C, the reaction was stopped by addition of 0.2 M PCA. Both extracts were neutralized and centrifuged (10 min, 16,000 g). Glucose concentration was determined in both extracts as described by Bergmeyer (1988). Glycogen concentration was calculated as the difference in glucose concentration between extract B (total glucose) and extract A (free glucose). The glycogen content of the white muscle was not determined, since handling stress due to catching and preparation of the fish leads to a depletion of muscle glycogen stores (Wang *et al.*, 1994). Therefore, the muscle glycogen content of wild catches provides no indication of the nutritional status of the animal.

Protein and RNA were extracted as described by Munro and Fleck (1966). Protein concentrations were determined with a Biorad protein assay (Biorad). RNA was quantified spectrophotometrically at  $\lambda = 260$  nm. Total lipids were recovered from frozen tissue by

chloroform/methanol extraction as described by Saborowski and Buchholz (1996). Pulverized liver tissue (20–50 mg) or pulverized muscle tissue (50–100 mg) was added to 1 ml chloroform/methanol (1:2 v/v) and sonicated in a water bath at 20°C for 25 min. Samples were incubated at room temperature for 30 min (liver samples) or 16 h (muscle samples) to ensure complete extraction of total lipids. After centrifugation (10 min, 16,000 g), 50 µl (liver) or 500 µl (white muscle) of the supernatant were transferred to a glass vial. After complete evaporation of the chloroform/methanol solution, 2 ml concentrated H<sub>2</sub>SO<sub>4</sub> were added to each vial and boiled for 10 min in a water bath. The total fat content was determined by the sulphophospho-vanillin assay with a commercial test kit (Merck, 3321).

### Bioenergetics model calculations

#### Food consumption

The daily food consumption rate ( $C$ ) was calculated according to Eggers (1977):

$$C = S \cdot R \cdot 24$$

where  $S$  is the mean stomach content (mg dry mass) over a 24-h period and  $R$  is the instantaneous gastric evacuation rate (see Table 2 and the Appendix). Pääkkönen and Marjomäki (1997) described an exponential relationship between  $R$  and temperature ( $T$ ) at experimental temperatures between 1.3 and 12.6°C for *L. lota*:

$$R = 0.009e^{(0.109T)}$$

An extrapolation of this relationship to temperatures as high as 24°C must, therefore, be regarded with caution. A comprehensive laboratory study of temperature effects on *L. lota* showed maximal feeding rates at 16°C, while feeding rates dropped dramatically at temperatures above 20°C (Shodjai, 1980). In the same study, the optimum temperature for growth was determined to be 15–16°C. These data indicate that the gastric evacuation rates of *L. lota* might be maximal at 15–16°C. Consequently, we adapted the gastric evacuation model of Pääkkönen and Marjomäki (1997) to a temperature optimum model for gastric evacuation developed by Temming (1995) for *G. morhua*, which has similar temperature optima (Table 4):

$$R = 0.0085e^{0.12T} (1 - (1/(1 + e^{-0.427(T - 17.775)})))$$

The importance of different diet items varied seasonally. We converted food consumption into energy flux using energy equivalents for each food type given in Table 1.

#### Oxygen consumption

Shodjai (1980) measured the oxygen consumption of unfed burbot (average body mass = 27 g) at temperatures between 5 and 26°C. The data from these experiments were used to fit a polynomial equation of temperature-dependent oxygen consumption rates,  $\dot{V}O_2$  (mg · h<sup>-1</sup>). The equation yields a maximum at approximately 22°C:

$$\dot{V}O_2 \text{ (mg} \cdot \text{h}^{-1}) = -0.014T^2 + 0.642T - 2.0056 \quad (n = 7, r^2 = 0.98)$$

We consider this equation to represent routine metabolism and assume that the routine respiration rate includes the costs for activity of the rather sluggish, inactive *L. lota* (activity factor = 1.0; see Appendix).

The classical procedure of Beamish (1964) was applied for converting metabolic rates in fish of different size to the metabolism of 100-g individuals. For this, we used the allometric relationship between oxygen consumption rates  $\dot{V}O_2$  ( $\text{mg} \cdot \text{h}^{-1}$ ) and fish mass  $M$  (g) measured for burbot at 20°C by Shodjai (1980):

$$\dot{V}O_2 (\text{mg} \cdot \text{h}^{-1}) = 0.539 \cdot M^{0.676}$$

We used a value of 14% of consumed energy for specific dynamic action (SDA), the energy expenditure associated with digesting food (Brett and Groves, 1979). The appropriate oxycaloric equivalent for use in estimating energy metabolism in carnivorous fish has been suggested to be approximately 13.59 kJ per gram of oxygen consumed (Elliott and Davidson, 1975).

#### *Egestion and excretion*

Shodjai (1980) measured energy losses due to defecation of burbot at temperatures between 5 and 26°C. The data from these experiments were used to fit a polynomial equation to calculate relative losses ( $F$ ) of ingested energy dependent on temperature ( $T$ ). The equation yields the highest absorption of energy at approximately 17°C:

$$F(\%) = 0.0851T^2 + -2.8939T + 30.972 \quad (n = 14, r^2 = 0.79)$$

Rudstam *et al.* (1994) suggested that the energy lost by excretion might be modelled as a constant fraction of 6.6% of ingested energy.

#### *Growth rates from field data ( $G_{\text{field}}$ )*

The mean total length  $L_t$  was calculated, by age group, for all sampling dates. These mean values were used to calculate the length increment of burbot of similar age between sampling dates. Mass increments were converted into energy flux ( $\text{kJ} \cdot \text{day}^{-1}$ ) using a conversion factor of  $3.9 \text{ kJ} \cdot \text{g}^{-1}$  wet mass (Johnson *et al.*, 1999).

### Statistical analysis

Unless stated differently, comparison of means was performed by one-way analysis of variance with a *post-hoc* Tukey test. In the case of significant deviations from homogeneity of variances (Levene test,  $P < 0.05$ ), Dunnett-T3 was used as the *post-hoc* test. Statistical tests of significance were evaluated at the 95% level of confidence. All data are reported as the mean  $\pm$  standard error of the mean.

## RESULTS

### Gut content and bioenergetics model calculations

Burbots fed mainly on the larvae of insects and crustaceans, which represented more than 80% of the diet (Table 1). Dominant food items were Corophiidae (September–November) and Gammaridae (June; data not shown). In April and July 2001, however, food composition differed from the other sampling dates. During rising water levels in the River Oder in April, terrestrial food became available and burbot consumed substantial amounts of earthworms (Annelidae) and *Lepidurus apus* (Notostraca). Only in July 2001 was fish the

**Table 1.** Percentage of food organisms in the gut of *Lota lota* at different sampling dates (expressed as percentage of total gut content) and their energy and water content according to (1) Arzbach (1997), (2) Hephher (1988) and (3) Hölker *et al.* (2002)

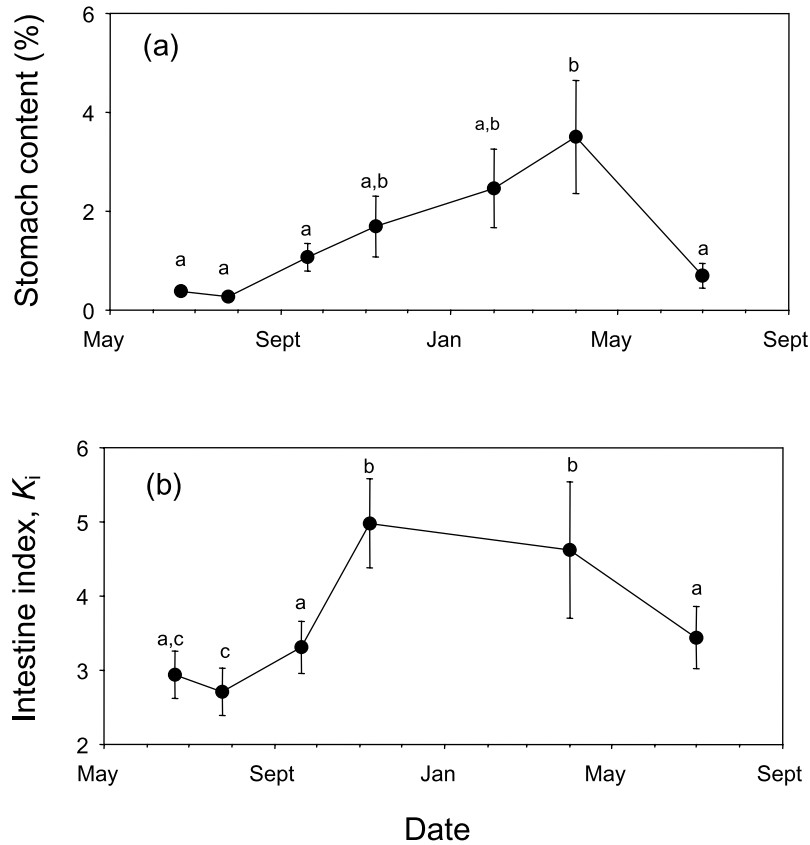
|                                                    | Fish               | Crustacea          | Insect larvae      | Mollusca           | Annelids           | Detritus          |
|----------------------------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-------------------|
| June 2000                                          | 2                  | 51                 | 45                 | 2                  | 0                  | 0                 |
| July 2000                                          | 10                 | 20                 | 70                 | 0                  | 0                  | 0                 |
| September 2000                                     | 3                  | 80                 | 10                 | 2                  | 2                  | 3                 |
| November 2000                                      | 10                 | 43                 | 5                  | 0                  | 2                  | 40                |
| February 2001                                      | 8                  | 30                 | 52                 | 0                  | 10                 | 0                 |
| April 2001                                         | 2                  | 2                  | 65                 | 3                  | 20                 | 8                 |
| July 2001                                          | 68                 | 20                 | 5                  | 7                  | 0                  | 0                 |
| Water content (%)                                  | 73.7 <sup>1</sup>  | 79.1 <sup>1</sup>  | 81.0 <sup>2</sup>  | 67.8 <sup>2</sup>  | 84.4 <sup>2</sup>  | 77.0 <sup>3</sup> |
| Energy content<br>(kJ · g dry mass <sup>-1</sup> ) | 20.12 <sup>1</sup> | 16.96 <sup>1</sup> | 21.08 <sup>2</sup> | 16.28 <sup>2</sup> | 23.03 <sup>2</sup> | 7.95 <sup>3</sup> |

dominant food item, while at the other sampling dates piscivory was of minor importance. In general, burbot of age 1+ behaved as benthivores.

For the estimation of feeding rates, we quantified stomach content in relation to body mass (Fig. 2a). The stomach content amounted to about 0.3% body mass in June and July 2000, but began to increase in September and reached a maximum in April 2001 of 3.5% body mass. In July 2001, gut content was as low as in the previous year. These data suggest that *L. lota* reduced its feeding rate during the summer months, when water temperatures were around 20°C (see Fig. 1). This was supported by the intestinal index  $K_i$ , which increased significantly between November and April, indicating a higher feeding activity during winter (Fig. 2b). We calculated daily consumption rates by multiplying the mean stomach content adjusted for a 100-g fish by stomach evacuation rates (Table 2 and dashed line in Fig. 3). Consumption rates were lowest in July 2000 and July 2001 and showed peak values in September 2000 and especially in April 2001. To estimate whether energy uptake matches the energy requirement of the animal, we calculated the energy dissipated by respiration, excretion and egestion based on data reported in the literature (see Methods and Table 2). The comparison of energy consumption (dashed line in Fig. 3) and energy dissipation (solid line) revealed that consumption rates were not sufficient to meet the energetic needs of the animal during summer (hatched area in Fig. 3). During winter, however, energy uptake exceeded energy dissipation and left surplus energy for growth in the order of 1.08–3.46 kJ · day<sup>-1</sup>, normalized for a 100-g fish (grey area in Fig. 3).

### Length and mass increments

To compare calculated energy for growth with growth rates observed in the field, we analysed data pertaining to the length and mass distribution of the burbot population between May 1999 and November 2001 to estimate growth rates. Burbot of all three year classes studied showed a continuous increase in total length and mass during this period (Figs. 4a,b). However, the analysis of condition factors indicated that an increase in length



**Fig. 2.** Seasonal variations in (a) stomach content and (b) intestine index,  $K_i$ , of wild catches of *Lota lota* from the River Oder. Letters signify significant differences between groups.

was not always accompanied by a corresponding increase in body mass. Values of the condition factor decreased during the summer between April/May and November and showed a drastic increase during winter (Fig. 4c).

#### Hepatosomatic index and liver composition

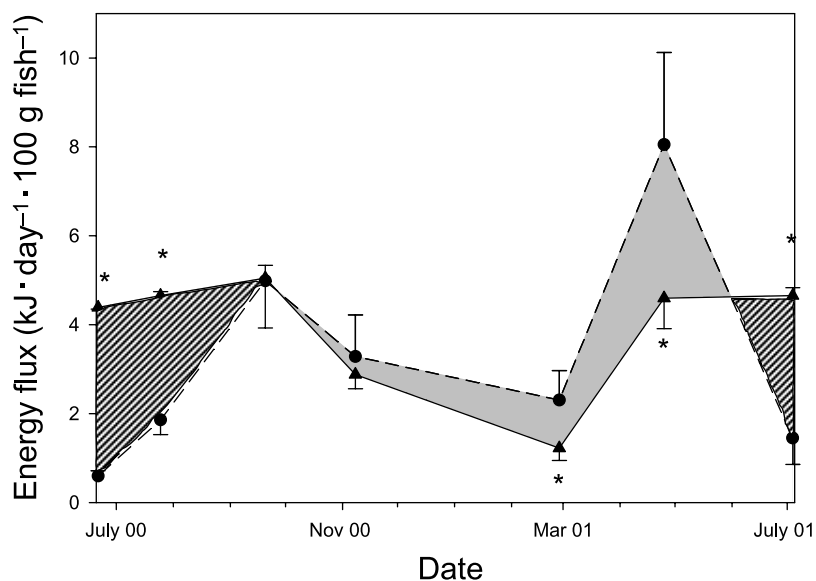
The hepatosomatic index, which is a measure of the nutritional status of the animal, showed a pattern similar to the condition factor (Fig. 5a). It declined during the summer, reaching a minimum of  $3.02 \pm 0.33$  in November 2001. In the subsequent winter months, the hepatosomatic index increased to a maximum of  $8.46 \pm 0.66$  in April 2001.

The liver tissue consisted mainly of fat (15–40%) and water (50–75%; Fig. 5b). Since the liver is a storage organ, providing substrates to the whole body, liver reserves are expressed as total amount normalized for a 100-g fish. The fat content showed strong seasonal variations with a maximal value of  $2.92 \pm 0.20 \text{ g} \cdot 100 \text{ g fish}^{-1}$  in June 2000. During the summer months, liver fat content declined to  $0.55 \text{ g} \cdot 100 \text{ g fish}^{-1}$  in November and increased again during winter. Liver glycogen stores were also depleted during summer. Glycogen content decreased earlier than lipid content (Fig. 5c). In July 2001, glycogen was already low while

**Table 2.** Ranges of fish sizes and main bioenergetic parameters calculated for the different sampling dates

|                                                      | Sampling date |               |               |               |               |               |               |
|------------------------------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                                                      | 21/06/2000    | 25/07/2000    | 20/09/2000    | 08/11/2000    | 27/02/2001    | 25/04/2001    | 04/07/2001    |
| $T$ (°C)                                             | 23.4          | 18.3          | 13.3          | 7.6           | 0.5           | 8.9           | 23            |
| $T_{\text{mean of 50 days}}$ (°C)                    | 19.8          | 19.3          | 19.2          | 12.3          | 1.6           | 6.2           | 18.4          |
| Mass (g)                                             | 21.5–80.0     | 20.7–56.9     | 41.0–102.0    | 52.0–110.0    | 47.4–294.7    | 39.5–147.5    | 49.0–158.2    |
| Length (cm)                                          | 13.6–23.0     | 14.7–19.5     | 15.5–25.0     | 19.6–24.4     | 18.5–32.5     | 17.3–27.2     | 19.0–26.5     |
| $R$ (h <sup>-1</sup> )                               | 0.0079        | 0.0261        | 0.0395        | 0.0219        | 0.0137        | 0.0231        | 0.0148        |
| $S^*$ (g dry weight)                                 | 0.167 ± 0.033 | 0.147 ± 0.026 | 0.304 ± 0.065 | 0.447 ± 0.127 | 0.351 ± 0.101 | 0.719 ± 0.185 | 0.211 ± 0.086 |
| $C^*$ (kJ·day <sup>-1</sup> )                        | 0.60 ± 0.12   | 1.86 ± 0.33   | 4.98 ± 1.06   | 3.28 ± 0.94   | 2.30 ± 0.66   | 8.05 ± 2.07   | 1.45 ± 0.59   |
| $\Sigma E_{\text{losses}}^*$ (kJ·day <sup>-1</sup> ) | 4.38 ± 0.04   | 4.65 ± 0.09   | 5.05 ± 0.29   | 2.88 ± 0.32   | 1.22 ± 0.27   | 4.60 ± 0.69   | 4.66 ± 0.17   |
| $G^*$ (kJ·day <sup>-1</sup> )                        | -3.79 ± 0.08  | -2.80 ± 0.24  | -0.07 ± 0.77  | 0.41 ± 0.62   | 1.08 ± 0.39   | 3.46 ± 1.39   | -3.21 ± 0.42  |
| $G_{\text{field}}^*$ (kJ·day <sup>-1</sup> )         |               | 1.16          | 0.94          | 0.94          | 0.65          | 0.52          | 0.04          |
| $G^*-G_{\text{field}}^*$ (kJ·day <sup>-1</sup> )     |               | -3.95         | -1.01         | -0.53         | 0.43          | 2.94          | -3.25         |

*Note:* Water temperature ( $T$ ) is reported as the actual temperature on the day of sampling and as the mean of the 50 days immediately before sampling.  $R$  = instantaneous gastric evacuation rate calculated for the respective water temperature (see Methods),  $S$  = mean stomach content,  $C$  = consumption,  $\Sigma E_{\text{losses}}$  = respiration + excretion + defecation =  $C - \Sigma E_{\text{losses}}$ ,  $G_{\text{field}}$  = growth calculated from field data,  $G - G_{\text{field}}$  = apparent energy gap as illustrated by the grey line in Fig. 8. \* Converted to 100-g individuals.

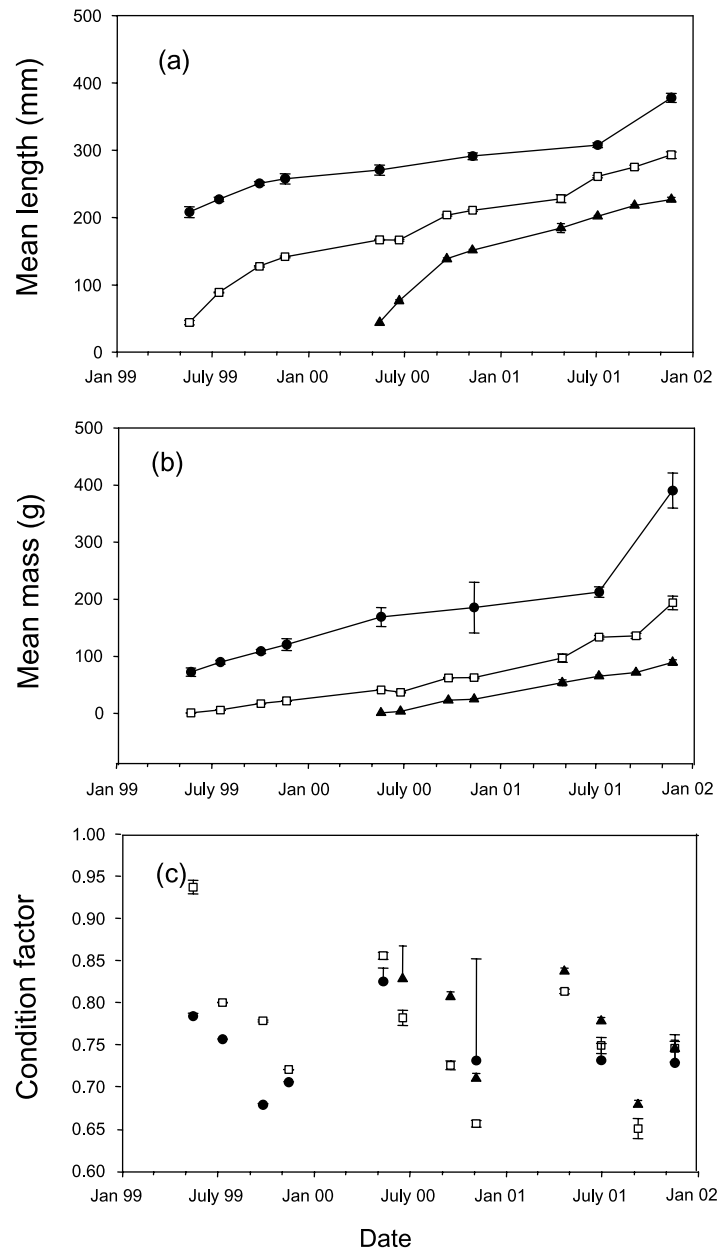


**Fig. 3.** Comparison of consumption rates (calculated from stomach contents, dashed line) and energy dissipation (as a sum of energy for routine metabolism and energy loss due to excretion and egestion, solid line). Hatched area expresses an energetic gap between consumption and energy dissipation. Grey area signifies surplus energy available for growth. All values were normalized for a 100-g fish. Asterisks indicate a significant difference between energy consumption and dissipated energy (Mann-Whitney *U*-test).

fat stores were still maintained. Glycogen content was lowest in November 2000 at  $43.9 \pm 18.0 \text{ mg} \cdot 100 \text{ g fish}^{-1}$  and increased about 20-fold to a maximum value of  $827.8 \pm 121.2 \text{ mg} \cdot 100 \text{ g fish}^{-1}$  in April 2001. Total liver protein content showed quite a different picture. It increased from  $128.2 \pm 11.1 \text{ mg} \cdot 100 \text{ g fish}^{-1}$  in June 2000 to  $289.0 \pm 60.0 \text{ mg} \cdot 100 \text{ g fish}^{-1}$  in February 2001 (Fig. 5d). Hence, there is a *de novo* protein synthesis in the liver of *L. lota* during summer. This is also supported by liver RNA content, which increased in a similar fashion as protein content, but the factorial increase was higher for RNA than for protein. This becomes evident when RNA content is expressed in  $\mu\text{g} \cdot \text{mg protein}^{-1}$ , which is an indicator for the translational capacity of a tissue (Houlihan *et al.*, 1993). This parameter was enhanced in the liver tissue of *L. lota* between November and April (Fig. 6).

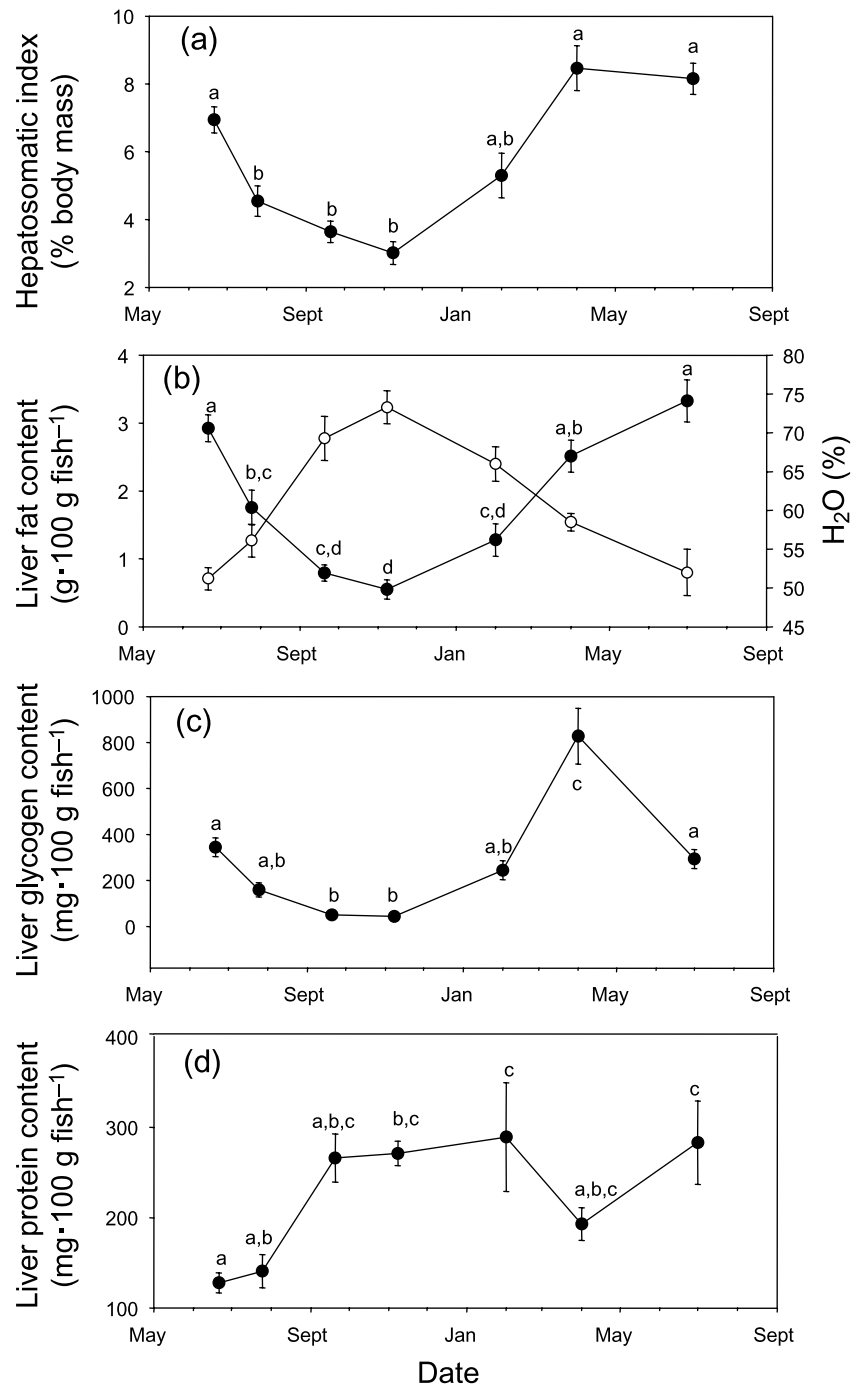
### Composition of the white musculature

Muscle fat concentration was about 50 times lower than that of the liver, emphasizing the function of liver as a storage organ for body reserves. Even when considering the relatively small size of the liver (about 5–7% of body mass, as opposed to approximately 50% for white muscle), the total fat depot of the liver ( $2.9 \text{ g} \cdot 100 \text{ g fish}^{-1}$  in July 2000) is about seven times larger than the fat depot of the white musculature ( $432 \text{ mg} \cdot 100 \text{ g fish}^{-1}$  in July 2000). Accordingly, the fat depot of the white musculature did not change significantly over the course of the year (Fig. 7a). Only in August 2000 and July 2001 was a significant decrease of white muscle fat reserves observed. The protein content of the white muscle was not affected by season (Fig. 7b). Only in July 2001 was protein content significantly

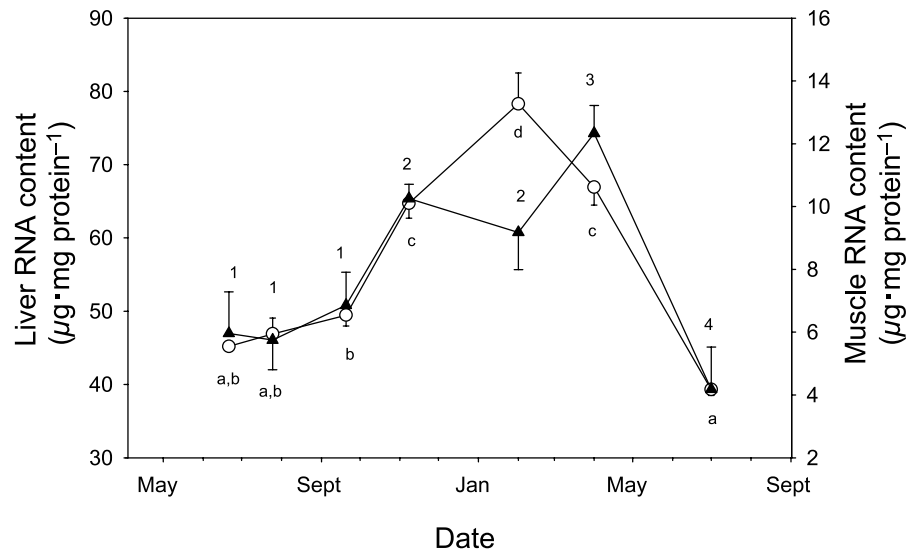


**Fig. 4.** Mean length (a), mass (b) and condition factor (c) of *Lota lota* from the River Oder. ●, year class 1998; □, year class 1999; ▲, year class 2000.

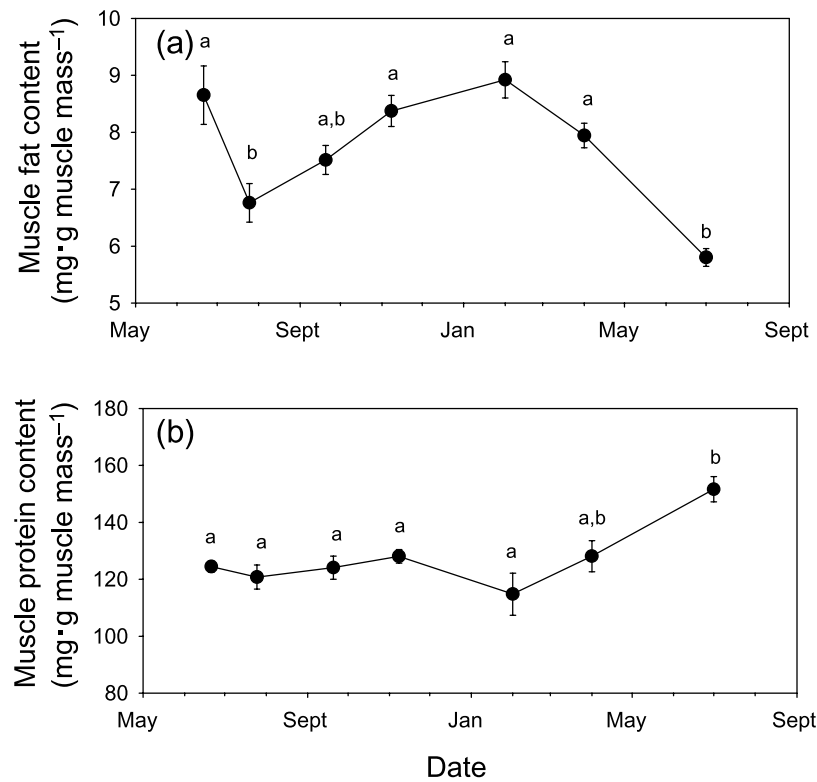
increased. This trend was not observed in the previous year, however. The white muscle RNA content was significantly increased during winter (Fig. 6). The RNA content of white muscle and liver showed concomitant changes. However, the factorial increase was higher in muscle than in liver (2.5-fold vs 1.4-fold; Fig. 6). Apparently, protein synthesis processes are



**Fig. 5.** Seasonal variations in liver composition of wild catches of *Lota lota*: (a) hepatosomatic index; (b) water content (○) and fat content (●); (c) glycogen content; (d) protein content. All concentrations are for total liver content in a 100-g fish. Letters signify significant differences between groups.



**Fig. 6.** Seasonal changes in RNA content in  $\mu\text{g} \cdot \text{mg protein}^{-1}$  as a measure of biosynthetic capacity of the white muscle ( $\circ$ ) and the liver ( $\blacktriangle$ ) of *Lota lota*. Significant differences are indicated by different letters (for muscle) or numbers (for liver).



**Fig. 7.** Seasonal variations in the composition of the white muscle of *Lota lota*: (a) fat content, (b) protein content.

more strongly enhanced in white muscle than in liver. The water content of the white musculature did not change significantly over the year and remained at  $81.9 \pm 2.5\%$  (results not shown).

## DISCUSSION

### Seasonal variations in the energy budget

Our data on stomach content and gut index suggest that *L. lota* reduces its food consumption during the summer when water temperatures are high. This is supported by several studies on the feeding behaviour of *L. lota*. In a burbot population at Lake Superior (Canada), the stomach content was highest in January and lowest between July and September (Bailey, 1972). Pulliainen and Korhonen (1990) reported a significant fall in the intestine index between June and August in wild catches of *L. lota* from the brackish water region of the northwestern Bothnian Bay in Finland.

Our model calculations revealed that food consumption during summer is not sufficient to fuel the energetic needs of the animal. During winter, however, energy consumption exceeds energy requirements, providing surplus energy for growth. Insufficient energy uptake during the summer is very unusual for freshwater fish because of high food densities during this season. Most fish species use the summer months to boost their energy stores to approach winter in a well-fed condition (Cunjak, 1988). The insufficient feeding activity of *L. lota* during summer is probably a reaction to high water temperatures, since reduced feeding activity was also observed in laboratory experiments at temperatures above 20°C (Shodjai, 1980). Apparently, temperatures around 20°C are stressful for *L. lota*: Tiitu and Vornanen (2002) detected an impairment of heart function by inducing an atrio-ventricular block in isolated hearts of burbot when temperatures reached 18°C. Several studies on fish have shown that stressfully high temperatures lead to an impairment of the ventilatory and respiratory systems, resulting in decreased scope for aerobic activity (Farrell, 1996; Pörtner, 2002b). Therefore, decreased swimming and hunting activity may have a protective effect for fish approaching their thermal tolerance limits. Svetovidov (1962, as cited by Pulliainen and Korhonen, 1990) described that *L. lota* spends the summer in a comatose state with low feeding and swimming activity. These data suggest that *L. lota* follows strategy 3 mentioned in the Introduction – that is, it remains a cold-active species despite the strong temperature variations in its environment and it survives high water temperatures in a state of reduced activity. This is further supported by the fact that *L. lota* is a winter spawning species (Bailey, 1972), so that it can afford to reduce its activity during the summer.

However, a reduction in feeding rates during summer appears to have a negative effect on growth rates. Our calculations (Fig. 3) suggested that growth of *L. lota* is restricted to winter and spring, whereas a slight loss in body mass should occur during the summer. But our field data painted a different picture. *Lota lota* exhibited a length increment throughout the year between May 1999 and November 2001 (Fig. 4). Growth was not isometric, however, but growth reflected in length exceeded gain in mass so that the condition factor decreased during the summer. This is advantageous in terms of growth maximization at low energetic costs – a gain in length without a corresponding weight gain is energetically less costly.

### How to grow without surplus energy

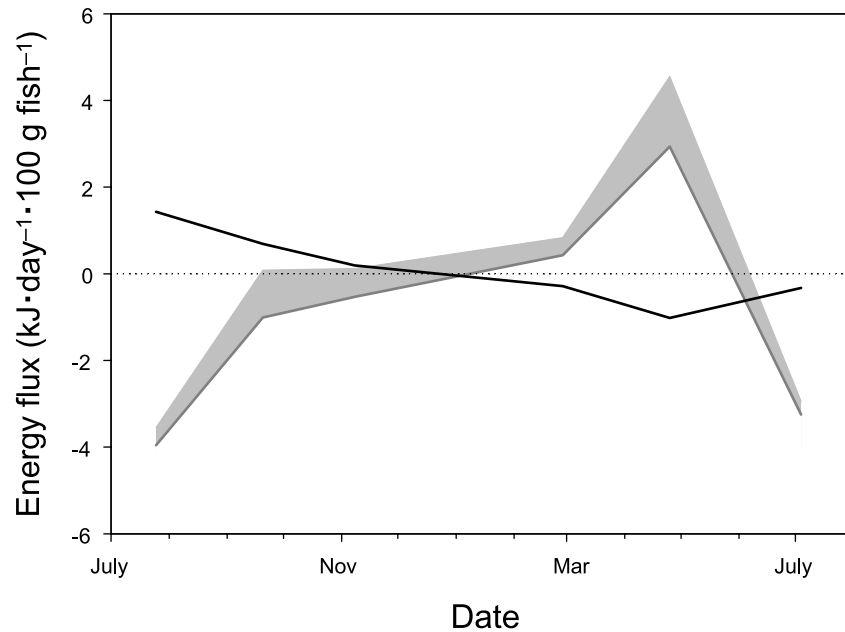
Where does the energy for growth come from when our model calculations show no surplus energy during the summer? To address this question, we analysed seasonal changes in the hepatosomatic index and the composition of muscle and liver. The liver index of *L. lota* reached values of 8.5, which is comparably high. The hepatosomatic index of fish normally ranges between 1.5 and 3 (channel catfish *Ictalurus punctatus* (Rafinesque): Kent *et al.*, 1992; red drum *Sciaenops ocellatus* (L.): Craig *et al.*, 2000) and even between 0.5 and 1.5 in cyprinids (Segner and Braunbeck, 1990). Gadidae generally have large livers with a hepatosomatic index of between 2 and 6 in wild catches (Shul'man, 1974), indicating the prominent role of the liver as a storage organ in this family. In *L. lota*, liver fat as well as liver glycogen stores were depleted between June and November and were restored during the winter months. This is unusual among freshwater fish, because in most investigated freshwater species fat and energy content of the tissues increase during spring and summer but decrease during fall and winter due to low food availability or reduced hunting capacity at low temperatures (e.g. *Salmo salar* L. and *Salmo trutta* L.: Berg and Bremset, 1998; *Leuciscus idus* (L.): Segner and Braunbeck, 1990).

Our data suggest that *L. lota* is not able to ingest sufficient energy between June and November and additional energy must be obtained by the depletion of glycogen and fat stores in the liver. This is in good agreement with our model calculations (Fig. 3). We calculated the energy flux due to degradation of liver reserves based on an energy content of 39 kJ·g fat<sup>-1</sup> and 17 kJ·g glycogen<sup>-1</sup> (Brett and Groves, 1979; see Table 3). In Fig. 8 we summarize the data on seasonal changes of energy fluxes in *L. lota*. The grey line represents the discrepancy between energy consumption and energy dissipation as a sum of metabolism, egestion, excretion and growth (values taken from Table 2). Negative values express an energy deficit; positive values signify excess energy. The black line shows the energy provision (positive values) or energy uptake (negative values) by the liver. Figure 8 shows that the energetic gap between consumption and energy dissipation can be widely accounted for by the liver energy flux.

**Table 3.** Changes in fat and glycogen reserves and the energy content of the liver of a 100-g fish

| Period                    | Δ fat<br>(mg) | Δ energy<br>content (kJ) | Δ glycogen<br>(mg 100) | Δ energy<br>content (kJ) | Total energy<br>derived from<br>fat and<br>glycogen<br>(kJ·day <sup>-1</sup> ) |
|---------------------------|---------------|--------------------------|------------------------|--------------------------|--------------------------------------------------------------------------------|
| 06/00 to 07/00 (34 days)  | -1167         | -45.513                  | -185                   | -3.145                   | +1.43                                                                          |
| 07/00 to 09/00 (57 days)  | -964          | -37.596                  | -109                   | -1.853                   | +0.69                                                                          |
| 09/00 to 11/00 (49 days)  | -244          | -9.516                   | -7                     | -0.119                   | +0.20                                                                          |
| 11/00 to 02/01 (111 days) | +729          | +28.431                  | +201                   | +3.417                   | -0.29                                                                          |
| 02/01 to 04/01 (57 days)  | +1237         | +48.243                  | +583                   | +9.911                   | -1.02                                                                          |
| 04/01 to 07/01 (70 days)  | +814          | +31.746                  | -534                   | -9.078                   | -0.32                                                                          |

*Note:* The last column displays the energy flux (kJ·day<sup>-1</sup>) resulting from changes in liver composition. A positive sign signifies energy provision due to mobilization of liver reserves.



**Fig. 8.** Seasonal variation in the energy budget of *Lota lota*. The grey line shows the energy gap between consumption and energy dissipation by metabolism, egestion, excretion and growth (values from Table 2). The shaded area represents the maximal error due to underestimation of food consumption (for further explanation, see text). The solid black line shows the energy flux from liver reserves (values from Table 3). All values were normalized for a 100-g fish.

### Evolutionary aspects

The evolutionary advantage of maintaining growth during summer at the expense of liver reserves is an increase in individual fitness in two ways. First, faster growth allows an early switch during ontogeny to larger food items such as fish. Several studies have shown that the food composition of *L. lota* changes with size (e.g. Ryder and Pesendorfer, 1992). While small burbot rely exclusively on small food items like crustaceans and insect larvae, larger specimens switch to fish as the main food source. Since fish generally have a higher energy content than invertebrates, feeding on fish is energetically more efficient than feeding on invertebrates. Mittelbach and Persson (1998), in a study comparing a variety of freshwater species, revealed that an early switch to piscivory has a positive effect on the size-at-age in all subsequent years. Since the maturation and fecundity of fish is often size dependent, maximization of growth leads to higher rates of reproduction (Roff, 1982). Secondly, high growth rates reduce predation risk, since the period in which fish are available for gape-limited predators is shortened. Mainly eel (*Anguilla anguilla* (L.)), pikeperch (*Sander lucioperca* (L.)) and adult conspecifics feed on juvenile burbot, of which pikeperch in particular is strongly gape-limited. Mooij (1996) found a correlation between the vulnerability of cyprinids to predation by pikeperch and the ratio between length of prey and predator. In years when cyprinid length was high (88%) in relation to percoid length, mortality was lower than in years when cyprinid length was only 63% of percoid length.

The strategy of *L. lota*, going into energetic debt during summer and boosting it during winter, requires certain physiological adaptations. To be able to boost energy stores during winter, the metabolic activity of the animals must be maintained at a high level during winter, despite low water temperatures. A suite of acclimatory mechanisms to compensate for decelerating temperature effects have been described for various fish species. A main feature is the increase in the amounts of enzymes to maintain high enzymatic activities at low temperatures. In the white muscle, a compensatory increase in total enzyme concentration is masked by high amounts of contractile proteins and is, therefore, not detectable. Liver protein, however, consists mainly of enzymes and often shows an increase during cold acclimation (protein hypertrophy; Kent *et al.*, 1988). Our data clearly show an increase in total liver protein during winter. Future investigations will show whether this results from enhanced enzyme concentrations in the liver. Another indication for a stimulation of enzyme synthesis is the increase in specific RNA content in the liver. This trend is even more pronounced in the white muscle. In this tissue, change in specific RNA content is often correlated with change in growth rate, since somatic growth is mainly due to the synthesis of muscle proteins (Houlihan *et al.*, 1993).

An additional feature that supports the strategy of growing at the expense of liver reserves is the high energy content of the liver tissue compared to the energy content of the whole fish ( $17.4 \text{ kJ} \cdot \text{g}^{-1}$  calculated from our data for liver in June 2000 versus  $3.9 \text{ kJ} \cdot \text{g}^{-1}$  for the whole body; Johnson *et al.*, 1999). Due to this ratio, a small decrease in liver mass can fuel a large increase in body mass.

Interestingly, the above two features that allow *L. lota* its exceptional survival strategy are typical traits of the gadoid family. Gadidae are generally classified as lean fish, with low fat content in the muscle and localized fat stores, mainly in liver or intestines, resulting in a low overall fat content of the animal (Shul'man, 1974). It has been hypothesized by Shul'man (1974) that this low fat content of the body has an adaptive value for benthic or demersal fish because of a high density and, therefore, reduced buoyancy. Clearly, for *L. lota* this feature has an additional advantage because it allows an increase in muscle mass at low energetic cost.

The second physiological trait typical for gadoids is the adaptation to low water temperatures. Most data in the literature are for Atlantic cod (*Gadus morhua*), which we therefore use for comparison. Table 4 shows that temperature preference and the optimum temperatures for feeding and growth are very similar for *L. lota* and *G. morhua*. We propose that, although *L. lota* evolved early from its marine relatives (Van Houdt *et al.*, 2003), it maintained several typical physiological traits of the gadoid family, which allowed this species to develop a special lifestyle that is quite distinct from other freshwater species. Therefore, *L. lota* is an impressive example of a species following the strategy of remaining cold-active after invasion of a eurythermal environment (strategy 3; see Introduction). Maintaining high hunting and feeding activity during the winter is advantageous in terms of a reduction in interspecific competition for food resources and predator avoidance, since most freshwater species show low activities during winter. Furthermore, being winter-active, this species is able to maintain somatic growth throughout the year, while most freshwater species reduce growth rates during the winter months. However, approaching its thermal tolerance limits on a regular basis during the summer, *L. lota* may be extremely vulnerable to long-term temperature increases due to global warming (Beamish, 1995).

**Table 4.** Comparison of temperature preference ( $T_{\text{pref}}$ ), optimum temperature for feeding ( $T_{\text{opt(feed)}}$ ) and growth ( $T_{\text{opt(growth)}}$ ), and upper lethal temperature ( $T_{\text{lethal}}$ ) for *Lota lota* and the marine gadid *Gadus morhua*

|                                           | <i>Lota lota</i>         | <i>Gadus morhua</i>  |
|-------------------------------------------|--------------------------|----------------------|
| $T_{\text{pref}}$                         | 11.4–14.2°C <sup>a</sup> | 13.9°C <sup>b</sup>  |
| $T_{\text{opt(feed)}}$                    | 16°C <sup>c</sup>        | 16°C <sup>d</sup>    |
| $T_{\text{opt(gastric evacuation rate)}}$ |                          | 15°C <sup>e</sup>    |
| $T_{\text{opt(energy absorption)}}$       | 12–20°C <sup>c</sup>     |                      |
| $T_{\text{opt(growth)}}$                  | 15–16°C <sup>c</sup>     | 14°C <sup>f</sup>    |
| $T_{\text{lethal}}$                       | c. 28°C <sup>c</sup>     | 23–24°C <sup>d</sup> |

<sup>a</sup> P.L.M. van Dijk, G. Staaks and I. Hardewig (unpublished results), Hofmann and Fischer (2002); <sup>b</sup> Schurmann and Steffensen (1992); <sup>c</sup> Shodjai (1980); <sup>d</sup> McKenzie (1934); <sup>e</sup> Tyler (1970); <sup>f</sup> Jobling (1988).

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## APPENDIX: CALIBRATION OF THE BIOENERGETIC MODEL

The calculation of consumption rates from stomach content presumes that burbot feed continuously so that sampling time will not influence the results. Since burbot are nocturnal, feeding rates may certainly be higher at night, and thus the determination of the stomach content during the day may underestimate consumption rates. Shodjai (1980) determined a maximum factor of 1.7 between

feeding rates at night and day. Therefore, we multiplied consumption rates by a factor of 1.35 (mean between 1.7 for night and 1.0 for day) to correct for the possible underestimation of nocturnal feeding rates. This correction affects the energetic gap between energy consumption and dissipation (presented in Fig. 8) only slightly, as indicated by the shaded area.

The deviations between the energetic gap and liver energy flux (Fig. 8), especially at the extremes during July and April, could result from an over- and under-estimation of energetic costs of swimming activity during summer and winter, respectively. For our calculations, we assumed that the routine respiration rate includes the costs for activity of the rather sluggish, inactive *L. lota* (see Methods), which could be a source of uncertainty in bioenergetics models (Hölker and Breckling, 2002). During peak feeding time in April (8.5°C), for instance, increased swimming and hunting activity may result in higher energy expenditure. Multiplication of the respiration rate during April by a factor of 2 for increased activity would lead to an energy excess of only 1.01 kJ·day<sup>-1</sup> instead of 2.94 kJ·day<sup>-1</sup>, which would match perfectly the energy uptake of the liver of -1.02 kJ·day<sup>-1</sup>. On the other hand, decreasing this factor during July (20–23°C) would reduce the energy gap between consumption and energy dissipation, which can be filled by energy from the liver. This would support strategy 3, suggesting a decrease in activity at high water temperatures.