

Temperature-related physiological adaptations promote ecological divergence in a sympatric species pair of temperate freshwater fish, *Coregonus* spp.

J. Ohlberger^{1,*}, T. Mehner¹, G. Staaks¹ and F. Hölker^{1,2}

¹Leibniz-Institute of Freshwater Ecology and Inland Fisheries, 12587 Berlin, Germany; and ²European Commission, DG Joint Research Centre, Institute for the Protection and Security of the Citizen, Maritime Affairs, 21020 Ispra, Italy

Summary

1. Understanding the ecological forces that are responsible for the evolution and coexistence of closely related sympatric species is a major interest in evolutionary ecology. In a freshwater system context, flocks or pairs of sympatrically occurring fishes are commonly studied to identify causes and mechanisms of ecological divergence and speciation.

2. Whereas habitat and diet segregation along the pelagic–benthic axis is frequent in many species pairs in temperate freshwaters, two pelagic planktivores coexist in the post-glacial Lake Stechlin, Germany (common vendace, *Coregonus albula* and the endemic Fontane cisco, *C. fontanae*). Accordingly, disruptive selection on traits not related to feeding may have contributed to divergence. Since both species differ slightly in their vertical distribution, species-specific physiological adaptations related to temperature are likely.

3. We investigated standard and active metabolic rates (AMR) over the range of environmental temperature, which both species experience naturally (4–15 °C). Eighty-four specimens of 7–22 cm length were exposed to five relative flow velocities between 0.5 and 2.0 body lengths per second (BL s⁻¹) in swim tunnel respirometers.

4. Fontane cisco showed a reduced standard metabolic rate (SMR) compared to vendace over the entire temperature range. Further, activity metabolism was associated with lower energetic costs in Fontane cisco at 4 °C, but higher costs than in vendace at 8 °C and 15 °C. Total metabolic costs when swimming at 2 BL s⁻¹ were higher for vendace at < 8 °C, but higher for Fontane cisco if temperature exceeds 8 °C.

5. The results indicate that species-specific physiological adaptations contribute to ecological divergence, thus preventing competitive exclusion between the pelagic coregonids of Lake Stechlin. The existence of a vertical gradient in water temperature was probably a crucial factor for the evolution of both competing temperature-related physiological strategies.

Key-words: ecological divergence, metabolic adaptation, sympatric speciation, temperature

Introduction

Divergent or disruptive natural selection arising from differential resource use, competitive intraspecific interaction or ecological opportunity causes phenotypic divergence and is hypothesized to be a major cause of speciation (Schluter 2000; Coyne & Orr 2004; Dieckmann *et al.* 2004). Divergent natural selection leads to local adaptation of subpopulations

by evolving traits that provide an advantage under specific environmental conditions. Therefore, local adaptation plays a crucial role in generating phenotypic and population divergence in sympatry (Turelli, Barton & Coyne 2001), thus providing a mechanistic ecological understanding of the process of sympatric speciation (reviewed in Orr & Smith 1998; Bolnick & Fitzpatrick 2007). However, since the speciation history cannot be directly studied, it is fundamental to investigate which traits may be exposed to divergent natural selection and which ecological conditions promote diversification of these traits.

Sympatric species pairs have been described in several groups of teleost fishes from post-glacial temperate freshwater

*Correspondence author. Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department of Biology and Ecology of Fishes, P.O.B. 850 119, D-12561 Berlin, Germany. E-mail: ohlberger@igb-berlin.de

lakes, for example, in sticklebacks (*Gasterosteus* spp.), smelts (*Osmerus* spp.), charrs (*Salvelinus* spp.), and whitefish and ciscoes (*Coregonus* spp.) (see Taylor 1999). Some of these species pairs provide evidence for the process of sympatric speciation (e.g. Gislason *et al.* 1999; Knudsen *et al.* 2006), driven by competition for resources or habitats (Robinson & Wilson 1994; Schluter 1996). The common situation is the occurrence of limnetic–benthic species pairs that diverged into distinct ecotypes or species by exploiting either benthic food in profundal or littoral habitats, or planktonic food in pelagic habitats (e.g. Lu & Bernatchez 1999; McKinnon & Rundle 2002; Knudsen *et al.* 2006). The ecological segregation is supported by differences in morphological features, mainly related to the feeding process (Skúlason & Smith 1995). In contrast, in a few systems such as the North American Lake Superior, multiple pelagic planktivores coexist in sympatry without further benthic–pelagic segregation (Smith & Todd 1984). The ecological forces that are responsible for the evolution and coexistence of these competing populations remain unclear.

The co-occurrence of two pelagic planktivorous coregonid species is also described in the deep and oligotrophic, post-glacial Lake Stechlin (Germany), where the common European vendace (*Coregonus albula* (L.)) lives in sympatry with the endemic dwarf-sized Fontane cisco (*Coregonus fontanae* (Schulz & Freyhof)). The two species are easily distinguished by their differential spawning times, with vendace spawning in winter and Fontane cisco spawning from spring to summer. Both species exhibit distinct morphological characteristics, for instance, in the number of scales in the lateral line, body depth at dorsal-fin origin or gill raker length of the first gill arch (Schulz & Freyhof 2003). The evolution of this species pair by sympatric speciation has been suggested from mtDNA and microsatellite analyses (Schulz *et al.* 2006). Both species perform regular diel vertical migrations by ascending from their deepwater daytime habitat into shallower water layers during night-time (Helland *et al.* 2007, Mehner, Kasprzak & Hölker 2007). However, Helland *et al.* (2007) found that the average night-time population depths differed between the species, with Fontane cisco being found about 6 m deeper in the water column than vendace all over the year. Despite the fact that the weak depth segregation within the pelagic area may facilitate a depth-related feeding divergence, diet compositions of both species were rather similar with a clear dominance of planktonic food (Helland *et al.* 2008). Therefore, exploitative competition between the coregonids can be assumed to be high, and habitat segregation does not contribute substantially to competition avoidance. Rather, the strong correspondence between vertical fish distribution and the seasonally varying temperature gradient in Lake Stechlin (Mehner *et al.* 2007) suggests that metabolic adaptations exist that foster the coexistence of the coregonids in slightly differing thermal habitats.

The relation of the standard metabolism to temperature has been studied extensively and it was found earlier that temperature is the most important environmental factor if the standard metabolism in fish is considered (Jobling 1994).

Furthermore, temperature-related swimming capacity is considered as a main characteristic determining survival in many species due to its impact on food capture, reproduction, predator avoidance and habitat shifts (Videler 1993; Plaut 2001). The swimming physiology is thus of high ecological importance and presumed to be subjected to selection pressures that enhance evolutionary fitness (Reidy, Kerr & Nelson 2000; Arnott, Chiba & Conover 2006). Thus, when fish are exposed to long-term temperature changes they can obtain optimal performance by altering their swimming physiology either by acclimation or adaptation (see Guderley & Blier 1988) and it has been suggested that metabolic rate differences between populations of one species reflect adaptations to specific environmental conditions (Álvarez, Cano & Nicieza 2006). Accordingly, we tested whether the swimming metabolism of the coexisting coregonid species differed over the temperature range to which the species are naturally exposed. By measuring standard and active metabolic rates (AMR), the costs of living in differential thermal habitats could directly be compared between the species. We assumed that a temperature threshold for similar-sized specimens should exist, at which the metabolic advantage switches from one species to the other, thus predicting the species-specific depth distribution along a temperature–depth gradient. Such a correspondence between field observations and experimental results would bridge the gap in our understanding on how divergent natural selection conditions directly influence the physiology-based competitive strengths of fish populations and thus may contribute to the evolution of two competing temperature-related physiological strategies.

Material and methods

FISH HATCHING AND MAINTENANCE

Fontane cisco and vendace were caught in Lake Stechlin (53°10'N, 13°02'E), Germany, by gill netting (15 mm mesh size) during their spawning times from April to June and from December to January, respectively. Ripe adults were striped and propagated artificially. Both species were raised under identical laboratory conditions. Larvae were hatched in glass aquaria at 4 °C and subsequently raised in circular basins (80 L). They were fed with rotifers, brine shrimp and commercially available dry food for fish larvae. Juveniles and adult fish were maintained in 500–1000 L basins at about 6–7 °C and fed with salmon dry food. Before experiments started, individuals of both species were held at the respective temperatures of 4 °C and 8 °C for at least 2 weeks and at 15 °C for at least 1 week after a preceding steady increase for 1 week of 1 °C per day. Feeding was interrupted 48 h prior to experiments in order to avoid elevated oxygen consumption rates due to specific dynamic action. Specimens of the investigated dwarf-sized Fontane cisco ($n = 42$) and vendace ($n = 42$) were measured as 3.6–37.4 g (7–16 cm) and 10.2–75.0 g (10–22 cm), respectively.

EXPERIMENTAL DESIGN

The respirometer design and data analysis are described in detail by Ohlberger, Staaks & Hölker (2007). Experiments were conducted with two differently sized Brett-type tunnel respirometers. The small

Table 1. Statistical results for the regression analysis of the standard metabolic rate (J h^{-1}) to body mass (g) relationship according to $\text{SMR} = \alpha \times M^\beta$ for Fontane cisco and vendace at 4 °C, 8 °C and 15 °C. Given are the estimates and standard errors (SE) for parameters α and β as well as the P -values of the ANCOVA test for significance between the species

Temperature (°C)	Fontane cisco		Vendace		ANCOVA (P -values)	
	α (\pm SE)	β (\pm SE)	α (\pm SE)	β (\pm SE)	Slope	Intercept
4	1.12 (\pm 0.14)	0.85 (\pm 0.04)	1.13 (\pm 0.25)	0.94 (\pm 0.06)	0.001	–
8	2.58 (\pm 0.49)	0.71 (\pm 0.06)	2.27 (\pm 0.84)	0.84 (\pm 0.10)	0.105	0.018
15	6.97 (\pm 1.03)	0.61 (\pm 0.05)	6.18 (\pm 1.08)	0.71 (\pm 0.05)	0.886	0.002

and the large swim tunnel consisted of a measuring recirculation loop (25 L, 5 L), with a swimming section included (15 \times 40 cm, 10 \times 30 cm), an ambient tank surrounding the swimming section and a cooled reservoir (100 L, 60 L) for supply with aerated fresh water. Oxygen consumption rates of the fish during swimming were measured by automated and computerized intermittent-flow systems, where each phase of closed respirometry is followed by a ventilation phase to re-establish the preset oxygen level in the swimming section (see Ohlberger *et al.* 2005, 2007). The respirometers were placed in a climatic chamber and equipped with a chilling unit for fine-tuning of the temperature.

Swimming trials were conducted at three environmental temperatures (4 °C, 8 °C and 15 °C) with each fish swimming at five different relative speeds (0.5, 0.75, 1.0, 1.5 and 2.0 body lengths per second (BL s^{-1})). To allow adaptation to experimental conditions, a velocity of 0.5 BL s^{-1} was run for at least 24 h. Subsequently, velocities of 0.75, 1.0, 1.5 and 2.0 BL s^{-1} were run for 8–16 h each, day and overnight. After the experiment, fish were removed from the respirometer, weighed immediately and a blank value, that is, microbial background respiration was determined. It accounted for up to 21% and 33% of the total respiration in the small and large respirometer, respectively. The fish cross-sectional area did never exceed 10% of the area of the swimming chamber.

DATA ANALYSIS

The acute oxygen consumption rate ($\text{mg O}_2 \text{ h}^{-1}$) of the fish was computed by fitting a linear regression to the decrease in oxygen saturation against time for each phase of closed respirometry. These values were corrected for microbial respiration and converted into energy consumption rates (J h^{-1}) using an oxycaloric value of 14.2 J mg O_2^{-1} . Data from the adaptation phase at the beginning of each experiment, in which the fish showed elevated oxygen consumption rates due to handling stress, were excluded from further calculation. The AMR for every single swimming velocity per individual was computed from the lowest 10% of all oxygen consumption rates. This was done to determine the minimum AMR without elevated consumption rates due to spontaneous activity or stress phases (see Ohlberger *et al.* 2007). A power function was used to describe the metabolic rate to speed relationship: $\text{AMR} = a + b \times U^c$, where AMR is the total AMR (J h^{-1}), U is swimming speed (BL s^{-1}), and a , b and c are constants. Constant a represents the estimated standard metabolic rate (SMR), that is, metabolic rate at zero speed, and c is the speed exponent. By differentiation of this equation with respect to U and subsequent zero-setting, the swimming speed associated with minimum energetic costs per unit distance, also referred to as optimum speed, was determined (Videler 1993). Calculated optimum speeds may exceed the range of investigated speeds due to this procedure. The estimated SMR values were

plotted against body mass according to an allometric function: $\text{SMR} = \alpha \times M^\beta$, where SMR is metabolic rate at rest (J h^{-1}), M is body mass (g) and β is the mass exponent. The SMR regressions were compared by analysis of covariance (ANCOVA) for differences in slopes and intercepts and by the Johnson–Neyman technique (J–N Tech) to identify regions of non-significance (see White 2003).

The temperature-specific β value from the allometric function was subsequently used to standardize the total AMR values for body mass to evaluate the mass-corrected AMR to speed relationship. Moreover, subtracting the SMR from these AMR values delivered the mass-corrected net AMR at a specific temperature. The net AMR to speed relationship for all individual data points at one temperature was then fitted using the previously described power function without parameter a : $\text{net AMR} = b \times U^c$. The power fits were tested for overall significance between the data sets of the two species using F -statistics (Zar 1996). This test was performed by comparing the pooled residuals of both discrete regressions to the regression residuals of the combined data set.

To compare the total energy expenditure of both species with respect to temperature, we calculated the gross AMR per unit time at 4 °C, 8 °C and 15 °C based on the derived SMR and net AMR functions: $\text{gross AMR} = \alpha \times M^\beta + (b \times U^c) \times M$. The gross AMR was then plotted as a function of swimming speed, covering not only the measured but also the range of calculated optimum speeds, and as a function of mass, covering the investigated mass overlap between the species at all three temperatures.

Results

Fontane cisco had a significantly lower SMR compared to vendace at all three temperatures (Fig. 1; Table 1). At 4 °C the regressions differed in slope (ANCOVA type III SS: $F_{3,24} = 13.67$, $P = 0.001$) with a range of non-significance below 10.7 g (J–N Tech). At 8 °C and 15 °C, there were no significant differences in slopes but in intercepts (ANCOVA, 8 °C: $F_{2,25} = 6.43$, $P = 0.018$; 15 °C: $F_{2,25} = 12.13$, $P = 0.002$), showing no regions of non-significance (J–N Tech). Further, the scaling relationship of the SMR with body mass (slope, b) tended to be lower in Fontane cisco at all three temperatures (Table 1). The scaling exponent increased with decreasing temperature in both species, ranging from 0.61 to 0.85, and from 0.71 to 0.94 in Fontane cisco and vendace, respectively.

Optimum swimming speeds for all three temperatures, calculated from the individual AMR to speed relationships, ranged from 2.0 to 2.9 (mean \pm SD = 2.6 \pm 0.5) and from 2.0 to 2.8 (mean \pm SD = 2.4 \pm 0.4) BL s^{-1} in Fontane cisco and vendace, respectively.

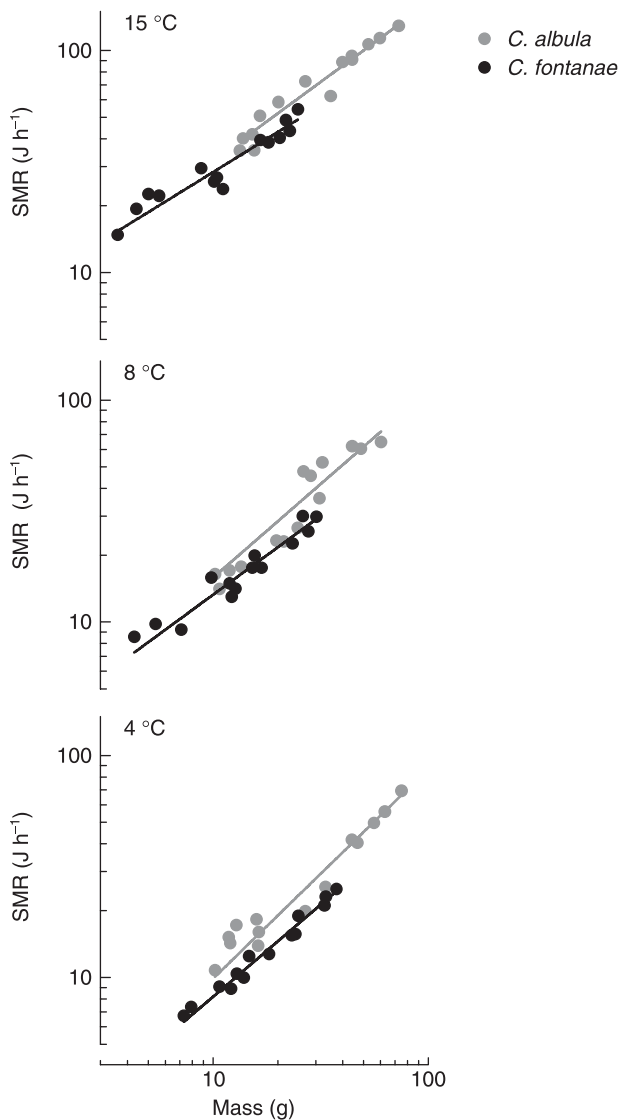


Fig. 1. Allometric relationships for the standard metabolic rate ($SMR = \alpha \times M^\beta$) of Fontane cisco (black) and vendace (grey) at 4 °C, 8 °C and 15 °C. The standard metabolic rate is lower in Fontane cisco at all three temperatures. At 4 °C the regressions differ significantly in slope, whereas at 8 °C and 15 °C there is a significant difference in intercepts. The slope of the scaling relationship (β) also tends to be lower in Fontane cisco at all three temperatures. The respective values for α and β as well as the ANCOVA tests are given in Table 1. Note: double-logarithmic plots.

The net AMR ($J h^{-1} g^{-1}$) of the Fontane cisco was higher at 8 °C and 15 °C, but lower at 4 °C compared to vendace (Fig. 2; Table 2). The differences between the data sets of the two species were significant at all three temperatures (F -test: $P < 0.001$). The speed exponents (c), that is, the rates of increase in metabolic costs with increasing speed, varied with temperature in both species, but the calculated overall mean value was the same in both species ($c = 2.3$).

The total energy expenditure per unit time during swimming (gross AMR) increased with speed, body mass and temperature. The relative total costs of the species compared to each other varied depending on temperature (Fig. 3). At 4 °C

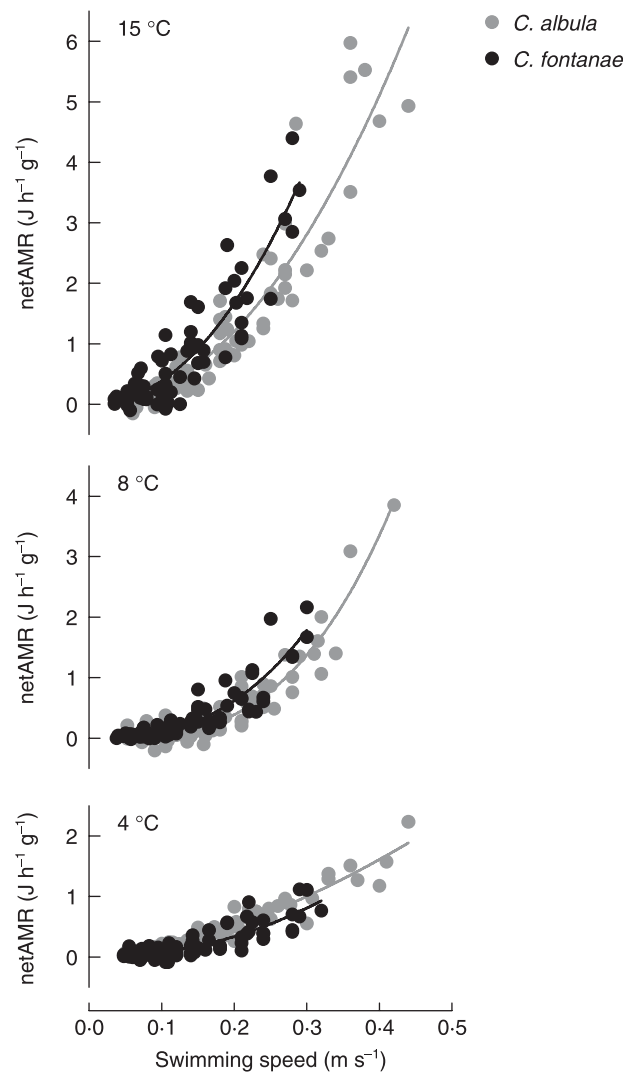


Fig. 2. Relationships between mass-corrected net active metabolic rate and absolute swimming speed ($netAMR = b \times U^c$) for Fontane cisco (black) and vendace (grey) at 4 °C, 8 °C and 15 °C. The net active metabolic rate ($J h^{-1} g^{-1}$) is significantly higher in Fontane cisco at 8 °C and 15 °C, but it is significantly lower at 4 °C. The respective values for b and c as well as for the F -statistics are given in Table 2.

Fontane cisco had lower total costs than vendace over the entire speed range. At 8 °C and 15 °C Fontane cisco had lower costs at speeds up to 2 and 1.5 BL s^{-1} , respectively, but experienced higher energetic costs compared to vendace at swimming speeds above these values (Fig. 3).

Discussion

DIFFERENCES IN METABOLIC COSTS

Our study reports significant physiological differences in standard and swimming metabolism between two sympatric coregonid fishes. In the dwarf-sized Fontane cisco, standard metabolism was lower than in vendace over the whole temperature range tested. However, the net AMR in Fontane

Table 2. Statistical results for the regression analysis of the net active metabolic rate ($\text{J h}^{-1} \text{g}^{-1}$) to swimming speed (m s^{-1}) relationship according to net $\text{AMR} = b \times U^c$ for Fontane cisco and vendace at 4 °C, 8 °C and 15 °C. Given are the estimates and standard errors (SE) for parameters b and c as well as the F - and P -values of the F -test for significance between the species

Temperature (°C)	Fontane cisco		Vendace		F -test	
	b (\pm SE)	c (\pm SE)	b (\pm SE)	c (\pm SE)	F -value	P -value
4	10.70 (\pm 3.43)	2.15 (\pm 0.23)	7.345 (\pm 0.64)	1.66 (\pm 0.07)	17.19	< 0.001
8	41.61 (\pm 11.98)	2.62 (\pm 0.20)	57.76 (\pm 10.70)	3.11 (\pm 0.17)	11.99	< 0.001
15	49.91 (\pm 11.99)	2.11 (\pm 0.16)	34.35 (\pm 5.12)	2.08 (\pm 0.13)	11.72	< 0.001

cisco was lower than in vendace only at 4 °C, but meant higher costs at 8 °C and 15 °C. This diversification was corroborated by the estimates of total costs. At a temperature of 4 °C, Fontane cisco had lower total costs over the whole mass and swimming speed range due to low standard and active metabolic costs. At 8 °C and 15 °C, swimming was associated with higher total costs in Fontane cisco than in vendace at speeds above 2 and 1.5 BL s^{-1} , respectively. This means that the slope of the relationship between metabolic rate and temperature differs between the species pointing to the influence of temperature itself in dictating this slope through energetic trade-offs and evolutionary temperature adaptation. Consequently, these two diverging trends in standard and swimming metabolism with temperature suggest that Fontane cisco is better adapted to lower environmental temperatures compared to the co-occurring vendace.

The relatively high mass-scaling exponents found in both species deviate from the three-fourth power law (West, Brown & Enquist 1997), which might be explained by generally steeper metabolic scaling relationships in ectothermic compared to endothermic animals (McNab 2002). The finding that the exponent itself is temperature dependent is in accordance with many other experimental studies where temperature was the major extrinsic factor altering the exponent of scaling relationships, but the reason for such a temperature-dependence is not known (Clarke 2004). Since differences in mass exponents were significant between temperatures but not significant between species, this does not relate to the observed species-specific divergence in metabolism. The optimum swimming speeds (lowest costs) of Fontane cisco and vendace were found in the range of 2–3 BL s^{-1} . Dabrowski, Takashima & Law (1989) developed a bioenergetics model on coregonid fish growth and predicted optimum speeds for juvenile vendace of 1.8 to 4 BL s^{-1} . Generally, free-ranging fish swim close to optimum speed during routine movements like foraging (Videler 1993; Webb 1993). In subarctic Norwegian lakes, vendace activity was recorded by *in situ* stationary hydroacoustics, and swimming speeds of up to 3 and rarely even 4 BL s^{-1} were estimated during crepuscular or stronger light conditions (Gjelland *et al.* 2004). All these studies suggest that swimming speeds above 2 BL s^{-1} are common under natural conditions. Accordingly, the advantage of having lower total metabolic costs during foraging switches for similar-sized fish from Fontane cisco to vendace at a temperature threshold of *c.* 8 °C.

TEMPERATURE AS ECOLOGICAL RESOURCE

The prediction of a temperature threshold in relative metabolic advantage at *c.* 8 °C, which is based on the experimental results, can be compared with the experienced environmental temperatures at the average population depths of the fishes in Lake Stechlin over the seasonal course. According to catches with a midwater trawl in several water depths, the Fontane cisco population was never found in water layers with a temperature above 7.7 °C (Helland *et al.* 2007). In contrast, the average population depth of vendace was continuously in shallower water layers, suggesting that vendace experienced on average higher water temperatures. Accordingly, there is strong empirical evidence that the differences in vertical distribution between the two closely related coregonids are correlated to species-specific metabolic adaptations to slightly differing temperature ranges.

Early studies treated temperature as an ecological resource, and grouped fish into thermal guilds according to their preferred temperature (Magnuson, Crowder & Medvick 1979). Differences in depth distribution between fishes belonging to different thermal guilds corroborated that temperature gradients create spatial structures in vertical fish distribution (Rudstam & Magnuson 1985). If temperature and food availability were experimentally modified, the habitat choice of fish followed primarily by their temperature preference, and food was ranked second in the decision hierarchy (Krause, Staaks & Mehner 1998). Accordingly, the existence of vertical gradients in water temperature directly influences the physiology-based competitive strengths of fish populations (Jensen *et al.* 2006), and thus may contribute to the co-occurrence of competing species such as the two planktivorous coregonids in Lake Stechlin.

TEMPERATURE-RELATED METABOLIC ADAPTATIONS

The metabolic adaptation of Fontane cisco comprises two different physiological mechanisms. Standard costs are reduced over the whole temperature range. The standard metabolism includes the energy demand for many different processes thus representing the cost of living a particular lifestyle at a given temperature (Clarke 2003). In contrast to the SMR reductions, activity costs in Fontane cisco are also reduced at lower temperatures, but elevated at higher temperatures, as compared to vendace. This pattern might be explained by a

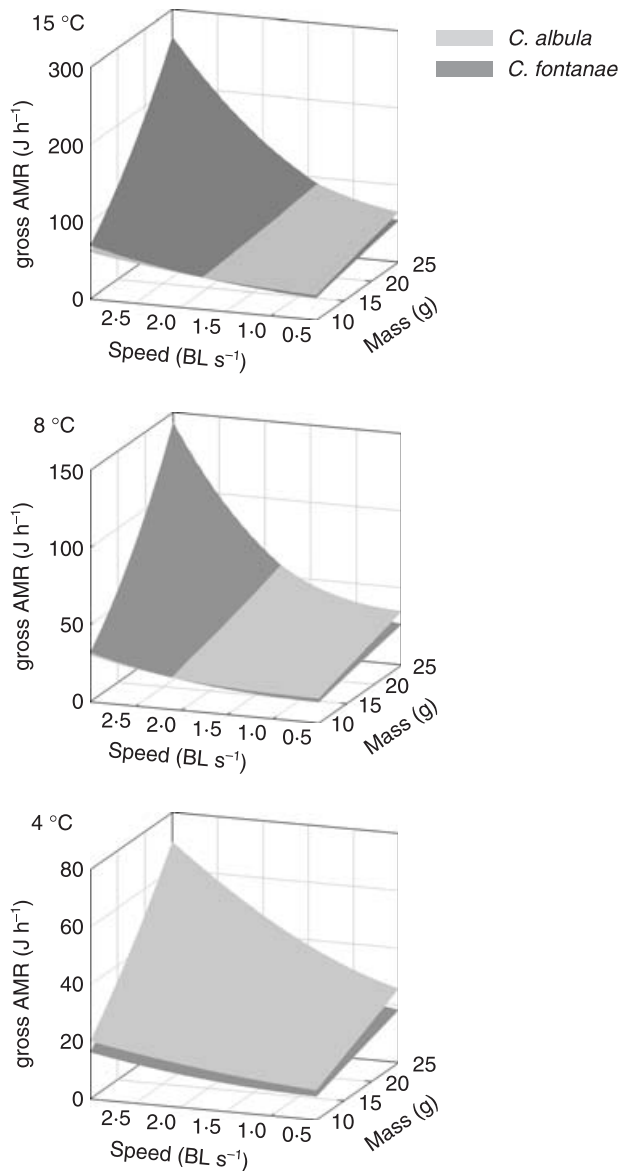


Fig. 3. Relationships of the total energetic costs (gross AMR) over swimming speed and body mass for Fontane cisco (dark grey) and vendace (light grey) at 4 °C, 8 °C and 15 °C. Gross AMR was calculated according to the previously derived functions for the SMR and the mass-corrected net AMR (gross AMR = $\alpha \times M^\beta + (b \times U^c) \times M$). All plots are presented on the same x and y scales, covering the measured plus the range of calculated optimum speeds, and the overlapping mass range of the species at all three temperatures. At 4 °C swimming costs are lower in Fontane cisco over the whole speed and mass range, whereas at 8 °C and 15 °C this energetic advantage is restricted to low swimming speeds. At the higher temperatures vendace is the stronger competitor during foraging at speeds above 2 BL s⁻¹ due to the lower energetic costs.

trade-off mechanism in the physiological characteristics of the swimming musculature. For instance, thermal specialization optimizing performance at a particular temperature may be accompanied by a trade-off reducing the ability to respond to changing environmental temperature (Huey & Hertz 1984). In general, ectothermic organisms acclimate by adjusting

physiological rates as well as their biochemical composition, thereby favouring the maintenance of function and capacity at a new acclimation temperature, but not necessarily at other temperatures (Guderley 2004). Important evolutionary responses to temperature change include modifications to membrane properties and intracellular milieu (Clarke 2003), temperature-dependent gene expression of key aerobic enzymes (Lucassen *et al.* 2006) and changes of the amount of mitochondrial enzyme or total mitochondrial density, which in turn affect standard metabolism and aerobic exercise capacity (see Pörtner *et al.* 2006). The consideration that temperature adaptation enhances evolutionary fitness in a new environment is based on the 'beneficial acclimation hypothesis' (Huey & Berrigan 1996). However, knowledge about the evolutionary significance of temperature acclimation and adaptation is still incomplete (see Clarke 2003).

ENERGETIC CONSEQUENCES

The standard and swimming costs of a fish are of high ecological importance, since they account for a great deal of the fish's energy budget (Jobling 1994; Hölker & Breckling 2002). Therefore, standard metabolism and swimming capacity are seen as a target of energy-reducing strategies and are presumed to be subjected to selection pressures that enhance evolutionary fitness (Reidy *et al.* 2000). Physiological adaptations of these metabolic rates might therefore be an adequate adaptive strategy to decrease energy losses for fishes in environments with low resource levels and high exploitative competition (Mueller & Diamond 2001). Correspondingly, the reduced metabolic costs at low temperature in Fontane cisco presumably compensate for energetic disadvantages associated with lower food densities in deeper water layers. In Lake Stechlin, the majority of zooplankton is concentrated in water layers close to the thermocline, and zooplankton biomass substantially declines with increasing water depths (Mehner, Hölker & Kasprzak 2005a). Accordingly, the occurrence of the dwarf-sized Fontane cisco in deeper layers than vendace has the consequence that the overall diet availability is reduced for this species. The lower energetic needs of this species in cooler waters may compensate for these disadvantages. If we calculate the energy expenditure for a 20-g fish swimming close to optimum speed at 2.5 BL s⁻¹, Fontane cisco need 19% less energy than vendace at a water temperature of 4 °C, but 16% more energy when swimming at 15 °C. The demand for energy intake, that is, for foraging, is reduced or enhanced accordingly. Svärdsön (1979), based on his broad study of *Coregonus* spp. in Swedish lakes, hypothesized that being dwarf-sized might be an adaptation to living in deep layers with its associated cold environments and reduced food densities. The main purpose of such adaptations might be to save energy primarily for the production of large and relatively numerous eggs, a life-history feature typical for the order Salmoniformes to which the coregonids belong (Vila-Gispert, Moreno-Amich & Garcia-Berthou 2002).

IMPORTANCE FOR ECOLOGICAL DIVERGENCE AND SPECIATION

In freshwater fishes, there is ample evidence that phenotypic diversification and ultimately sympatric speciation are the outcome of divergent natural selection, most often driven by resource competition (Robinson & Wilson 1994; Schluter 1996). In the majority of sympatric freshwater species pairs, the common pattern of resource partitioning is found along the limnetic–benthic axes (e.g. Gislason *et al.* 1999; Knudsen *et al.* 2006). However, the process of diversification and adaptive radiation might also be facilitated by ecological opportunity (Schluter 2000), which is often found in newly colonized habitats (Skúlason & Smith 1995). Recent studies suggest that ecological opportunity in species-poor post-glacial lakes promotes adaptive diversification in fishes via disruptive selection mediated by intra-specific competition (Bolnick 2004; McKinnon *et al.* 2004).

In the post-glacial Lake Stechlin, genetics based on microsatellites, mtDNA and AFLP data suggest that the evolution of vendace and Fontane cisco has occurred in sympatry (Schulz *et al.* 2006; K. Pohlmann, T. Mehner, unpublished data). However, in contrast to the benthic–limnetic diversification as found in other *Coregonus* or *Salvelinus* systems, the coregonids in Lake Stechlin are both pelagic, planktivorous feeders with weak diversification in feeding morphology (Schulz & Freyhof 2003). Accordingly, exploitative competition between the species is assumed to be intense. The metabolic adaptations as indicated in this study may facilitate coexistence since the two coregonid species can partition the pelagic zone into different thermal habitats to achieve energetic advantages over the competitor. This is corroborated by diet analyses of fishes from Lake Stechlin which demonstrated weak absolute differences between Fontane cisco and vendace, but a strong influence of depth of occurrence on the prey species composition (Helland *et al.* 2008). Thus, the metabolism-driven depth diversification is an ecological mechanism promoting reduced interspecific competition. The Fontane cisco strategy of lowered metabolism and dwarfism compensates for energetic disadvantages in deeper water layers compared to the pre-existing vendace strategy. Thus, we suggest that the existence of a vertical gradient in water temperature directly influences the competitive strengths of these fish populations and thereby contributed to the evolution of two competing temperature-related physiological strategies.

The reported divergence in metabolism may have contributed to speciation. Adaptations to different temperature regimes have been reported for sibling species in some taxa, for instance, scorpionflies (see Sauer, Vermeulen & Aumann 2003) and polychaetes (Kruse, Strasser & Thiermann 2004). Smith & Todd (1984) formulated the idea that the divergence and speciation in the Great Lakes ciscoes might be driven by steep environmental gradients of the lacustrine environment. Moreover, theoretical studies suggest that speciation along gradients may be a common process (Doebeli & Dieckmann 2003). It is interesting to note that sympatric species pairs of *Coregonus* occur in just two lakes in Germany, Lakes Stechlin

and Breiter Luzin (Schulz *et al.* 2006), both of them being the deepest lakes in that region (Mehner *et al.* 2005b). Thus, environmental gradients may be steepest in these lakes, suggesting that species diversifications along the gradients are likely.

We do not state that a correlation between physiological traits and environmental variables provides evidence for evolutionary change *per se*, but infer adaptation as the most likely explanation for the pattern we observe in these closely related sympatric species (but see Garland & Adolph 1991). Further, as illustrated by Garland & Adolph (1994), two-species comparisons are believed to provide only weak evidence for evolutionary correlations. However, in the case of sympatric species pairs, no equally useful alternative approach based on multiple-species comparisons or incorporation of phylogenetic information is possible. Moreover, the two species investigated in this study provide exceptionally strong evidence for the role of natural selection in phenotypic divergence, because they co-exist in sympatry, have a short evolutionary history since speciation and show a huge niche overlap in most environmental dimensions. Random genetic drift and genetic differentiation due to the process of speciation itself that might have affected phenotypic traits cannot be ruled out as a cause of differentiation, but the clear relation of trait differences to environmental conditions strongly suggests a phenotype–environment association in this case.

Acknowledgements

We thank Henrik Zwadlo for technical assistance and Karena Kuntze for feeding the study animals. Two anonymous referees provided valuable comments that improved the manuscript. The experiments comply with the German Guidelines for Animal Care.

References

- Álvarez, D., Cano, J.M. & Nicieza, A.G. (2006) Microgeographic variation in metabolic rate and energy storage of brown trout: countergradient selection or thermal sensitivity? *Evolutionary Ecology*, **20**, 345–363.
- Arnott, S.A., Chiba, S. & Conover, D.O. (2006) Evolution of intrinsic growth rate: metabolic costs drive trade-offs between growth and swimming performance in *Menidia menidia*. *Evolution*, **60**, 1269–1278.
- Bolnick, D.I. (2004) Can intraspecific competition drive disruptive selection? An experimental test in natural populations of sticklebacks. *Evolution*, **58**, 608–618.
- Bolnick, D.I. & Fitzpatrick, B.M. (2007) Sympatric speciation: models and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 459–487.
- Clarke, A. (2003) Costs and consequences of evolutionary temperature adaptation. *Trends in Ecology and Evolution*, **18**, 573–581.
- Clarke, A. (2004) Is there a universal temperature dependence of metabolism? *Functional Ecology*, **18**, 252–256.
- Coyne, J.A. & Orr, H.A. (2004) *Speciation*. Sinauer Associates, Sunderland, MA.
- Dabrowski, K., Takashima, F. & Law, Y.K. (1989) Bioenergetic model for the analysis of the ontogenetical aspects of coregonid fish growth. *Ecological Modelling*, **44**, 195–208.
- Dieckmann, U., Metz, J.A.J., Doebeli, M. & Tautz, D. (2004) *Adaptive Speciation*. Cambridge University Press, Cambridge.
- Doebeli, M. & Dieckmann, U. (2003) Speciation along environmental gradients. *Nature*, **421**, 259–264.
- Garland, T. & Adolph, S.C. (1991) Physiological differentiation of vertebrate populations. *Annual Review of Ecology and Systematics*, **22**, 193–228.
- Garland, T. & Adolph, S.C. (1994) Why not to do 2-species comparative studies—limitations on inferring adaptation. *Physiological Zoology*, **67**, 797–828.

- Gislason, D., Ferguson, M., Skúlason, S. & Snorrason, S.S. (1999) Rapid and coupled phenotypic and genetic divergence in Icelandic Arctic char (*Salvelinus alpinus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 2229–2234.
- Gjelland, K.O., Bohn, T., Knudsen, F.R. & Amundsen, P.A. (2004) Influence of light on the swimming speed of coregonids in subarctic lakes. *Annales Zoologici Fennici*, **41**, 137–146.
- Guderley, H. (2004) Metabolic responses to low temperature in fish muscle. *Biological Reviews*, **79**(2), 409–427.
- Guderley, H. & Blier, P. (1988) Thermal acclimation in fish-conservative and labile properties of swimming muscle. *Canadian Journal of Zoology*, **66**, 1105–1115.
- Helland, I.P., Freyhof, J., Kasprzak, P. & Mehner, T. (2007) Temperature sensitivity of vertical distributions of zooplankton and planktivorous fish in a stratified lake. *Oecologia*, **151**, 322–330.
- Helland, I.P., Harrod, C., Freyhof, J. & Mehner, T. (2008) Lack of resource segregation in a sympatric pair of coregonid fish – did speciation predate ecological divergence? *Evolutionary Ecology Research*, <http://www.evolutionary-ecology.com/forthcoming.html>.
- Hölker, F. & Breckling, B. (2002) Influence of activity in a heterogeneous environment on the dynamics of fish growth: an individual-based model of roach. *Journal of Fish Biology*, **60**, 1170–1189.
- Huey, R.B. & Berrigan, D.A. (1996) Testing evolutionary hypotheses of acclimation. *Animal and Temperature: Phenotypic and Evolutionary Adaptation* (eds I.A. Johnston & A.F. Bennett), pp. 205–237. Cambridge University Press, Cambridge.
- Huey, R.B. & Hertz, P.E. (1984) Is a jack-of-all-temperatures a master of none. *Evolution*, **38**, 441–444.
- Jensen, O.P., Hrabik, T.R., Martell, S.J.D., Walters, C.J. & Kitchell, J.F. (2006) Diel vertical migration in the Lake Superior pelagic community. II. Modeling trade-offs at an intermediate trophic level. *Canadian Journal of Fisheries and Aquatic Sciences*, **63**, 2296–2307.
- Jobling, M. (1994) *Fish Bioenergetics*. Chapman & Hall, London.
- Knudsen, R., Klemetsen, A., Amundsen, P.A. & Hermansen, B. (2006) Incipient speciation through niche expansion: an example from the Arctic charr in a subarctic lake. *Proceedings of the Royal Society B*, **273**, 2291–2298.
- Krause, J., Staaks, G. & Mehner, T. (1998) Habitat choice in shoals of roach as a function of water temperature and feeding rate. *Journal of Fish Biology*, **53**, 377–386.
- Kruse, I., Strasser, M. & Thiermann, F. (2004) The role of ecological divergence in speciation between intertidal and subtidal *Scoloplos armiger* (Polychaeta, Orbiniidae). *Journal of Sea Research*, **51**, 53–62.
- Lu, G. & Bernatchez, L. (1999) Correlated trophic specialisation and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. *Evolution*, **53**, 1491–1505.
- Lucassen, M., Koschnick, N., Eckerle, L.G. & Pörtner, H.O. (2006) Mitochondrial mechanisms of cold adaptation in cod (*Gadus morhua* L.) populations from different climatic zones. *Journal of Experimental Biology*, **209**, 2462–2471.
- Magnuson, J.J., Crowder, L.B. & Medvick, P.A. (1979) Temperature as an ecological resource. *American Zoologist*, **19**, 331–343.
- McKinnon, J.S. & Rundle, H.D. (2002) Speciation in nature: the threespine stickleback model systems. *Trends in Ecology and Evolution*, **17**, 480–488.
- McKinnon, J.S., Mori, S., Blackman, B.K., David, L., Kingsley, D.M., Jamieson, L., Chou, J. & Schluter, D. (2004) Evidence for ecology's role in speciation. *Nature*, **429**, 294–298.
- McNab, B.K. (2002) *The Physiological Ecology of Vertebrates: A View from Energetics*. Cornell University Press, Ithaca, NY.
- Mehner, T., Hölker, F. & Kasprzak, P. (2005a) Spatial and temporal heterogeneity of trophic variables in a deep lake as reflected by repeated singular samplings. *Oikos*, **108**, 401–409.
- Mehner, T., Diekmann, M., Brämick, U. & Lemcke, R. (2005b) Composition of fish communities in German lakes as related to lake morphology, trophic state, shore structure and human use intensity. *Freshwater Biology*, **50**, 70–85.
- Mehner, T., Kasprzak, P. & Hölker, F. (2007) Exploring ultimate hypotheses to predict diel vertical migrations in coregonid fish. *Canadian Journal of Fisheries and Aquatic Sciences*, **64**, 874–886.
- Mueller, P. & Diamond, J. (2001) Metabolic rate and environmental productivity: well-provisioned animals evolved to run and idle fast. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 12550–12554.
- Ohlberger, J., Staaks, G., van Dijk, P.L.M. & Hölker, F. (2005) Modelling energetic costs of fish swimming. *Journal of Experimental Zoology*, **303A**, 657–664.
- Ohlberger, J., Staaks, G. & Hölker, F. (2007) Effects of temperature, swimming speed and body mass on standard and active metabolic rate in vendace (*Coregonus albula*). *Journal of Comparative Physiology B*, **177**, 905–916.
- Orr, M.R. & Smith, T.B. (1998) Ecology and speciation. *Trends in Ecology and Evolution*, **13**, 502–506.
- Plaut, I. (2001) Critical swimming speed: its ecological relevance. *Comparative Biochemistry and Physiology A*, **131**, 41–50.
- Pörtner, H.O., Bennett, A.F., Bozinovic, F., Clarke, A., Lardies, M.A., Lucassen, M., Pelster, B., Schiemer, F. & Stillman, J.H. (2006) Trade-offs in thermal adaptation: the need for a molecular to ecological integration. *Physiological and Biochemical Zoology*, **79**, 295–313.
- Reidy, S.P., Kerr, S.R. & Nelson, J.A. (2000) Aerobic and anaerobic swimming performance of individual Atlantic cod. *Journal of Experimental Biology*, **203**, 347–357.
- Robinson, B.W. & Wilson, D.S. (1994) Character release and displacement in fishes – a neglected literature. *American Naturalist*, **144**, 596–627.
- Rudstam, L.G. & Magnuson, J.J. (1985) Predicting the vertical distribution of fish populations-analysis of cisco, *Coregonus artedii*, and yellow perch, *Perca flavescens*. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 1178–1188.
- Sauer, K.P., Vermeulen, A. & Aumann, N. (2003) Temperature-dependent competition hierarchy: a mechanism stabilizing the phenological strategy in the scorpionfly *Panorpa communis* L. *Journal of Zoological Systematics and Evolutionary Research*, **41**, 109–117.
- Schluter, D. (1996) Ecological speciation in postglacial fishes. *Philosophical Transactions of the Royal Society of London B*, **351**, 807–814.
- Schluter, D. (2000) *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
- Schulz, M. & Freyhof, J. (2003) *Coregonus fontanae*, a new spring-spawning cisco from Lake Stechlin, northern Germany (Salmoniformes: Coregonidae). *Ichthyological Exploration of Freshwaters*, **14**, 209–214.
- Schulz, M., Freyhof, J., Saint-Laurent, R., Ostbye, K., Mehner, T. & Bernatchez, L. (2006) Evidence for independent origin of two spring-spawning ciscoes (Salmoniformes: Coregonidae) in Germany. *Journal of Fish Biology*, **68**, 119–135.
- Skúlason, S. & Smith, T.B. (1995) Resource polymorphisms in vertebrates. *Trends in Ecology and Evolution*, **10**, 366–370.
- Smith, G.R. & Todd, T.N. (1984) Evolution of species flocks of fishes in north temperate lakes. *Evolution of Fish Species Flocks* (eds A.A. Echelle & I. Kornfield), pp. 45–68. University of Maine Orono Press, Orono.
- Svärdson, G. (1979) Speciation in Scandinavian *Coregonus*. *Institute of Freshwater Research Drottningholm Report*, **57**, 1–95.
- Taylor, E.B. (1999) Species pairs of north temperate freshwater fishes: evolution, taxonomy, and conservation. *Reviews in Fish Biology and Fisheries*, **9**, 299–324.
- Turelli, M., Barton, N.H. & Coyne, J.A. (2001) Theory and speciation. *Trends in Ecology and Evolution*, **16**, 330–343.
- Videler, J.J. (1993) *Fish Swimming*. Chapman & Hall, London.
- Vila-Gispert, A., Moreno-Amich, R. & Garcia-Berthou, E. (2002) Gradients of life-history variation: an intercontinental comparison of fishes. *Reviews in Fish Biology and Fisheries*, **12**, 417–427.
- Webb, P.W. (1993) Swimming. *The Physiology of Fishes* (ed. D.H. Evans), pp. 47–73.
- West, G.B., Brown, J.H. & Enquist, B.J. (1997) A general model for the origin of allometric scaling laws in biology. *Science*, **276**, 122–126.
- White, C.R. (2003) Allometric analysis beyond heterogeneous regression slopes: use of the Johnson–Neyman technique in comparative biology. *Physiological and Biochemical Zoology*, **76**, 135–140.
- Zar, J.H. (1996) *Biostatistical Analysis*. Prentice-Hall, New Jersey.

Received 9 October 2007; accepted 17 January 2008

Handling Editor: John Speakman