

Physiological specialization by thermal adaptation drives ecological divergence in a sympatric fish species pair

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ABSTRACT

Hypothesis: Divergence in thermal preference, in combination with temperature-related metabolic adaptations, promotes ecological specialization and co-existence of a sympatric species pair of coregonid fish.

Background: The coregonids show substantially lower divergence in diet composition than other sympatric fish pairs in temperate freshwater lakes, but segregate vertically within the pelagic area and differ in physiology with respect to metabolism.

Organism: Sympatric European vendace (*Coregonus albula*) and endemic dwarf-sized Fontane cisco (*C. fontanae*) from Lake Stechlin, Germany.

Methods: We determined the final thermal preferendum (FTP) of both species previously hatched and raised under identical laboratory conditions by using a shuttle-box system that allowed the fish to control body temperature through behavioural thermoselection.

Results: The species showed clearly different temperature selection behaviours. Vendace had a significantly higher FTP (9.0°C) than Fontane cisco (4.2°C). The FTPs comply with temperatures of minimum net swimming costs, suggesting that performance is optimized at the respective thermal preference of both species.

Conclusions: The correspondence of thermal preference, thermal performance functions, and thermal ecology shows that the species are adapted to different temperature regimes.

Keywords: *Coregonus* spp., divergence, sympatric species, temperature preference.

INTRODUCTION

Sympatric species pairs of temperate freshwater fish occupying postglacial lakes are commonly used as model systems to study the ecology of adaptive diversification. The adaptive

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speciation and radiation theory states that phenotypic divergence and speciation are ultimately the outcome of disruptive or divergent natural selection arising from differential resource use, competitive intraspecific interaction or ecological opportunity (Schluter, 2000; Coyne and Orr, 2004). Ecological opportunity is often found in newly colonized habitats like postglacial lakes, where sympatric species pairs are common (Skúlason and Smith, 1995; Day and Young, 2004). Many of these closely related sibling species show a clear ecological divergence (Schluter, 1996, 2000), which is required to avoid competitive exclusion and to enable co-existence (Coyne and Orr, 2004). Hence, much of the recent research in this field has focused on the role of ecological specialization in driving and maintaining divergence in sympatry.

Coregonines are among the most diverse freshwater fishes in temperate regions. Multiple forms or sympatric pairs within one genus are common in this group and some of the species have produced a considerable diversity in postglacial northern lakes (reviewed by Hudson *et al.*, 2007). Although indications for sympatric or allopatric diversification can hardly be teased apart, potential cases of ecologically based sympatric speciation from a single lineage have been identified (e.g. Lu and Bernatchez, 1999; Knudsen *et al.*, 2006; Schulz *et al.*, 2006). However, the speciation history cannot be directly studied by means of ecological field observations. Therefore, it is fundamental to identify trait differences and environmental conditions that promote a divergence in traits under disruptive selection, which might ultimately lead to evolutionary diversification.

Environmental temperature is a critically important factor for all ectothermic vertebrates. It has profound effects on almost all aspects of ectotherm behaviour and physiology, including individual performance, foraging ability, and fitness (Bennett, 1980; Huey, 1982; Videler, 1993; Huey and Berrigan, 2001; Bicego *et al.*, 2007). Hence, ectothermic vertebrates generally attempt to control body temperature within a species-specific range to optimize physiological processes and minimize disadvantageous temperature effects (Freidenburg and Skelly, 2004). Responses to changes or heterogeneity in environmental temperature occur on different time-scales (Hertz, 1981). Acute temperature adjustments are primarily achieved via behavioural thermoregulation (Cowles and Bogert, 1944; Bogert, 1949; reviewed in Casey, 1981). Individual acclimation on longer time-scales occurs via changes in enzyme expression, cell membrane modifications or alteration of the intracellular milieu (Hochachka and Somero, 1968; reviewed in Somero, 2004). Finally, on the time-scale of generations, evolutionary thermal adaptation may occur via changes in thermal performance functions (Angilletta *et al.*, 2006; Pörtner *et al.*, 2006). It is under debate, however, whether optimum temperatures of performance are subjected to strong selection pressures or are evolutionarily conservative (see Angilletta *et al.*, 2002). This may depend to a large extent on the abilities of an organism to acclimate to various temperatures and to control body temperature via thermoregulation, which varies considerably between different taxa and depends on the environmental conditions of the habitat.

Small poikilothermic fish are thermoconforming animals that are highly dependent on environmental temperature due to the high specific heat and thermal conductance of water as well as the efficient heat exchange between body and water (Stevens and Sutterlin, 1976). Consequently, in thermally heterogeneous environments, most fishes can only control body temperature and thus performance abilities by selecting the thermal microhabitat where physiological function is relatively efficient (Beitinger and Fitzpatrick, 1979; Angilletta *et al.*, 2006). Accordingly, the thermal preference of fish has been correlated with optima in various aspects of physiological performance (e.g. Brett, 1971; Kellogg and Gift, 1983; Hölker *et al.*, 2004) and fishes are generally believed to be well adapted to the environmental temperature of their natural habitat (Magnuson *et al.*, 1979; Johnson and Kelsch, 1998; Pörtner, 2002).

In the deep postglacial Lake Stechlin (Germany), two coregonid species co-exist in sympatry within the pelagic habitat, common vendace [*Coregonus albula* (L.)] and the endemic dwarf-sized Fontane cisco [*Coregonus fontanae* (Schulz and Freyhof, 2003)]. They are easily distinguished by spawning time and have been described by distinct morphological characteristics (Schulz and Freyhof, 2003). Based on genetic analyses, it was suggested that this species pair has evolved via sympatric speciation (Schulz *et al.*, 2006). Both species are pelagic planktivores that show only weak divergence with respect to diet composition (Helland *et al.*, 2008). This contrasts the commonly observed limnetic–benthic divergence of sympatric fish pairs in northern temperate lakes, which is based on the exploitation of different food resources (e.g. Lu and Bernatchez, 1999; Knudsen *et al.*, 2006). In Lake Stechlin, it has been found that the average night-time population depths differ between the species, with Fontane cisco being found a few metres deeper in the water column than vendace throughout the year (Helland *et al.*, 2007). This divergence in vertical distribution is associated with a difference in average experienced water temperature. Moreover, in an earlier study on the species' metabolism, we found temperature-related differences in standard and active metabolic rates, which showed that swimming performance is optimized at different temperature regimes in these two sister species (Ohlberger *et al.*, 2008).

In this study, we wished to determine whether the species pair from Lake Stechlin diverged with respect to the two species' temperature preference. Our hypothesis was that the thermal preferences of the species would correspond to the divergence in thermal ecology and thermal physiological performance. This was based on the 'co-adaptation hypothesis', which states that the sensitivity of thermal performance and thermal preference are closely co-adapted in ectotherms (Angilletta *et al.*, 2006). This assumption holds if the fitness of an organism is positively related to performance – that is, if the selection of temperatures that maximize performance compared with other temperatures implies fitness gains. Accordingly, a shift in the temperature dependence of metabolic costs should provide selective pressures for a corresponding shift in thermal preference. We thus hypothesized that the species would show differences in their temperature selection behaviour as a consequence of adaptation to different thermal microhabitats, with the deeper living Fontane cisco preferring lower temperatures than vendace. We investigated the thermal selection behaviour in a free-choice experiment in the laboratory using individuals of both species that were previously hatched and raised under identical conditions. To elucidate which metabolic characteristics (i.e. thermal performance functions) are optimized at the preferred temperatures, we analysed the temperature dependence of standard and activity costs as well as derived metabolic characteristics like total and net costs of transport. This analysis was carried out based on metabolic rates data from previous studies on the two species (Ohlberger *et al.*, 2007, 2008).

MATERIALS AND METHODS

Fish

Fontane cisco and vendace were caught by gill netting (15-mm mesh size) during their respective spawning times in Lake Stechlin (53°10'N, 13°02'E), Germany. Ripe adults were striped and propagated artificially. Larvae were hatched in 50-litre glass aquaria and subsequently maintained in 80-litre circular basins. They were fed with brine shrimp, rotifers, and commercially available dry food for fish larvae. Juveniles were transferred to

rectangular basins of 500–1000 litres and fed with salmon dry food. The fish were acclimated under a 12:12 h photoperiod to a temperature of 4°C for at least one month before the experiment began. Specimens of the investigated Fontane cisco and vendace measured 9–14 cm in total length.

Thermal preference determination

The thermal preferendum is operationally defined as the range of environmental temperatures in which an animal aggregates in a free-choice situation. In contrast to the acute thermal preferendum, which is selected as a short-term response and which is affected by previous acclimation temperature, the final thermal preferendum (FTP) is the temperature selected as a long-term response, which is unaffected by previous acclimation (Reynolds and Casterlin, 1979; Kelsch and Neill, 1990).

Experimental design

The experimental set-up we used was an electronically controlled shuttle-box system, which allowed the fish to control their body temperature by moving between two aquarium sections with a slight difference in water temperature (Neill *et al.*, 1972). The shuttle-box consisted of two interconnected compartments (50 × 50 × 250 cm and 600 litres in total) large enough to ensure normal swimming behaviour. A plastic panel with a centred hole of 10 cm diameter separated the compartments so that the fish could move freely between them. The aquarium was placed under a dark curtain to reduce disturbance of the fish and with light sources above it to control illumination. A constant temperature difference of 2 K (Kelvin) was established between the sections by using cooling coils and electric heaters (thermostat: Lauda UKT 1000). A computer controlled the measuring and regulating units with software for temperature regulation (Staaks, 1996). Thirty-two pairs of infrared transmitters and photocells continuously monitored the activity of the fish within the sections. Fish distribution was registered by the count of impulses at the photocells. The system was automatically cooled down or heated up in a dynamic manner according to the distribution of the fish between the sections. When fish preferred the warmer section, temperature in both compartments was increased and vice versa until the fish distributed evenly between the sections and the system's temperature stabilized.

Experimental procedure

Five experiments with six individuals each were performed on both species. Before an experiment, fish were maintained in the shuttle-box aquarium at the acclimation temperature of 4°C for one week to allow habituation to the system. Three fish were placed in each of the two compartments one week before the experiments began. Because coregonids are schooling fish, we used groups of six individuals and treated each of these groups statistically as one unit. After the start of an experiment, the fish usually controlled water temperature within one day. During the tests, fish were fed once a day with dry food at a random schedule. To avoid an accidental runaway of the system before fish had learned to behaviourally control the temperature, maximum speed of temperature change was limited to 2 K · h⁻¹, maximum water temperature was set to 22°C, and minimum water temperature was limited by the cooling capacity to approximately 3°C. According to previous

experimental experiences (Staaks, 1996) and after the first test with Fontane cisco (group 8), an experimental period of 15 days was considered sufficient. After testing the first group of vendace (group 1), the experimental period for vendace was adjusted to about 25 days. Unfortunately, the controlling computer collapsed during two experiments so that test periods were a few days shorter in one vendace and one Fontane cisco group (groups 4 and 6), which nevertheless seemed to have gravitated to their final preference and were therefore included in the data analysis.

Data analysis

Temperature data were recorded at 10-min intervals and subsequently aggregated to daily mean values. To identify qualitative features of the individual recordings, an interval sequence approach was applied (Konstantinov and Yoshida, 1992). As a first step, kernel smoothing was applied using a loess smoother (Cleveland *et al.*, 1992) to remove noise from the time-series (Fig. 1). We used a Gaussian kernel, a smoothing window of 7 days, and an output step size of one hour. After this, segments with a temperature increment of less than $1 \text{ K} \cdot \text{day}^{-1}$ and a minimum duration of one day were regarded as stable periods, or if not as increasing or decreasing segments respectively. Variances were compared using the *F*-test and mean values using the Welch *t*-test, since homogeneity of variance was violated ($F = 9.64$; $df_1 = df_2 = 4$; $P = 0.0497$). Approximate normality of the samples was ensured graphically. The computations were performed using the data analysis system R (R Development Core Team, 2007) and the add-on package qualV (Jachner *et al.*, 2007).

Additionally, we analysed the temperature dependence of several metabolic characteristics (data in Ohlberger *et al.*, 2008) to detect physiological traits that are optimized at the respective final thermal preferences of the species. Standard and active metabolic rates, total swimming costs, and net costs of transport were plotted, for the three temperatures 4, 8, and 15°C , as a function of body mass and subsequently fitted according to a two-parameter power function ($a \cdot M^b$) (see Ohlberger *et al.*, 2007). These linear regressions were tested for overall significance of the data sets using *F*-statistics.

RESULTS

The two species differed clearly in their temperature selection behaviour (Fig. 1). Vendace generally showed multiple-phase behaviour. At the beginning of an experiment, these fish rapidly increased water temperature within a few days to $13\text{--}21^\circ\text{C}$ and maintained these temperatures for 5–12 days. Only group 1 had a short intermittent period at lower temperatures before returning to about 15°C . Subsequently, vendace groups decreased water temperature to approximately $5\text{--}6^\circ\text{C}$ and maintained these for a few days. Finally, the fish gravitated to an intermediate-temperature range between 7 and 11°C . Only group 2 immediately gravitated to a temperature range of $9\text{--}10^\circ\text{C}$ after the initial high-temperature phase. All inter-phase periods were characterized by rapid temperature increases or decreases of up to $9 \text{ K} \cdot \text{day}^{-1}$. In contrast to vendace, Fontane cisco showed no distinct phases of temperature selection behaviour in most cases. All Fontane cisco groups usually selected water temperatures of about $4\text{--}5^\circ\text{C}$ (i.e. similar to their acclimation temperature) over the entire course of the experiments. Only small variations were observed in two of the groups. Group 7 peaked to 9.9°C after a few days, but rapidly returned to the $4\text{--}5^\circ\text{C}$ temperature range. Group 8 showed an intermediate increase to about 6°C , but returned to

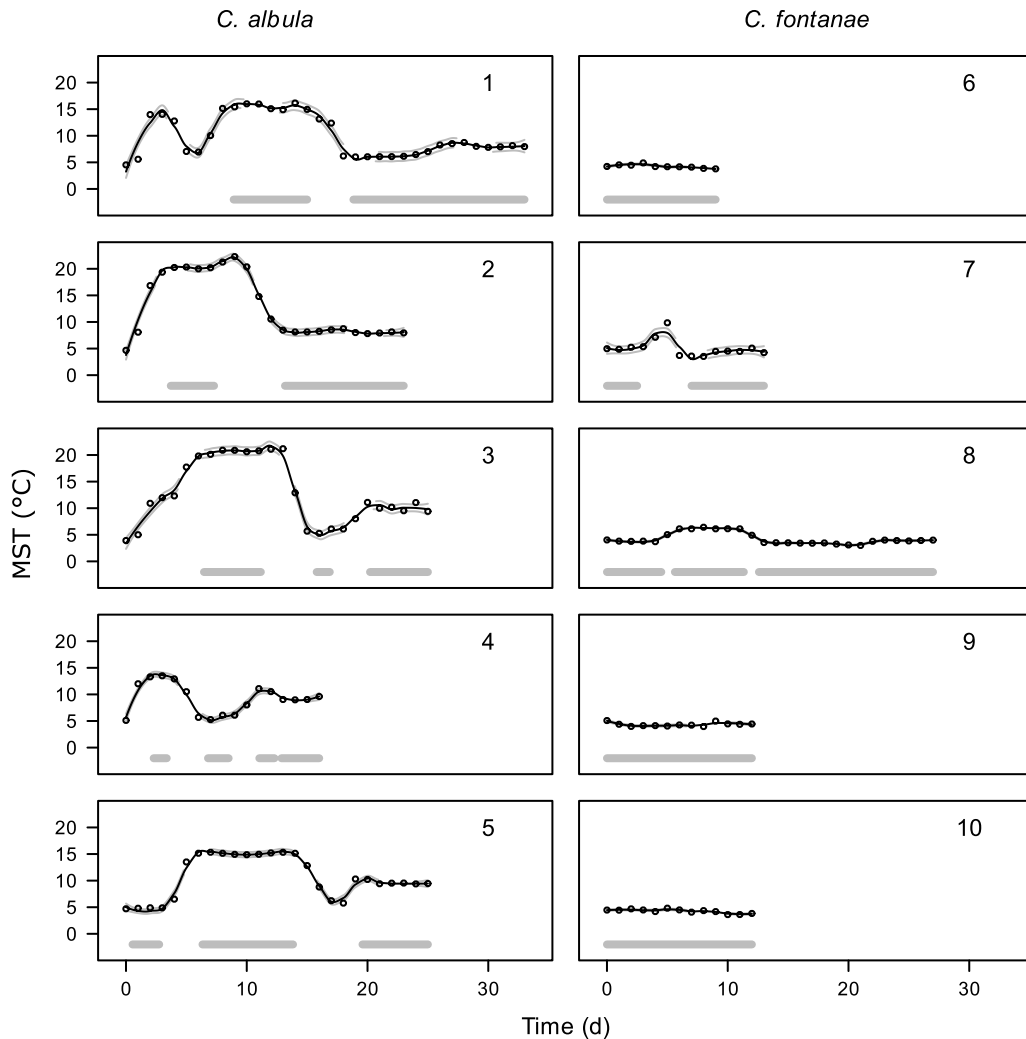


Fig. 1. Time-series of mean selected temperatures (MST, °C) during shuttle-box tests for all groups of vendace (*C. albula*, 1–5) and Fontane cisco (*C. fontanae*, 6–10). Daily mean values (circles) were fitted using a loess smoother (solid lines) and its standard error (dashed lines). An interval sequence approach was used to identify qualitatively similar periods of each time-series. This was done to compare the constant periods of the time-series, as indicated at the bottom of each graph (grey bars), within and between species.

below 5°C after a few days. Qualitatively, a significantly higher proportion of the vendace groups (5 vs. 1) showed clear indications of overcompensation – that is, a rapid temperature increase at the beginning of the experiment (Fisher’s exact test, $P = 0.048$).

The final thermal preferendum (FTP) was calculated as the mean value of the last 5 days of behavioural thermoregulation, since these days were determined as the minimal constant final period in all groups tested. For Fontane cisco, the FTP for all groups ranged from 3.9 to 4.6°C with a species average of 4.2°C, whereas for vendace, the FTP for all groups varied

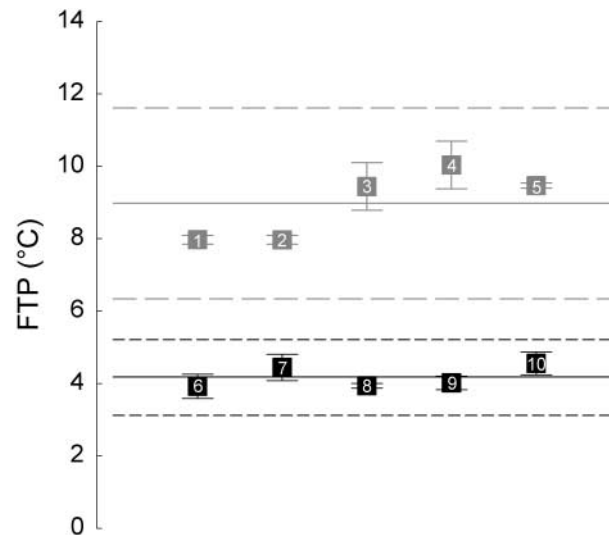


Fig. 2. Final thermal preferenda (FTP, °C) of vendace (*C. albula*, grey) and Fontane cisco (*C. fontanae*, black). Shown are all five groups (numbers according to Fig. 1) from each species (squares) with standard deviation (error bars) as well as the species' grand means (solid lines) with 95% confidence intervals (dashed lines).

from 8.0 to 10.0°C with a species average of 9.0°C (Fig. 2). The difference between the species was significant according to the Welch *t*-test ($t = 10.78$, $df = 4.8$; $P = 0.0002$). Furthermore, the variance of the mean FTP values was significantly lower in Fontane cisco than in vendace groups ($F = 9.87$, $df_1 = df_2 = 4$; $P = 0.048$).

The analysis of metabolic characteristics (data in Ohlberger *et al.*, 2008) revealed different patterns of temperature dependence. Standard and active metabolic rates as well as total costs of transport increased continuously with increasing temperature in both species. In contrast, the net cost of transport, which is the minimum amount of energy needed by a fish to swim one unit distance, was lowest in Fontane cisco at 4°C but lowest in vendace at 8°C (Fig. 3). Temperature effects were significant in both species according to *F*-statistics ($F > 8$, $df = 26$; $P < 0.01$).

DISCUSSION

The two coregonids from Lake Stechlin showed clearly different temperature selection behaviours during the course of the experiments. Vendace initially preferred relatively high temperatures and finally gravitated to intermediate temperatures around 8–10°C, whereas Fontane cisco generally selected low temperatures around 4°C. Consequently, the species had significantly different final preferenda with an average species-specific FTP of 9.0°C for vendace and 4.2°C for Fontane cisco. The determined FTPs correspond to optimum temperatures of net swimming costs in both species.

The temperature preference experiments in this study were performed for at least 2 weeks, although it is generally believed that fish gravitate to their final thermal preferenda in electronic shuttle-boxes within 1–2 days (McCaughey, 1977). Vendace selected significantly higher temperatures during the first days than they finally preferred. We thus have to conclude that

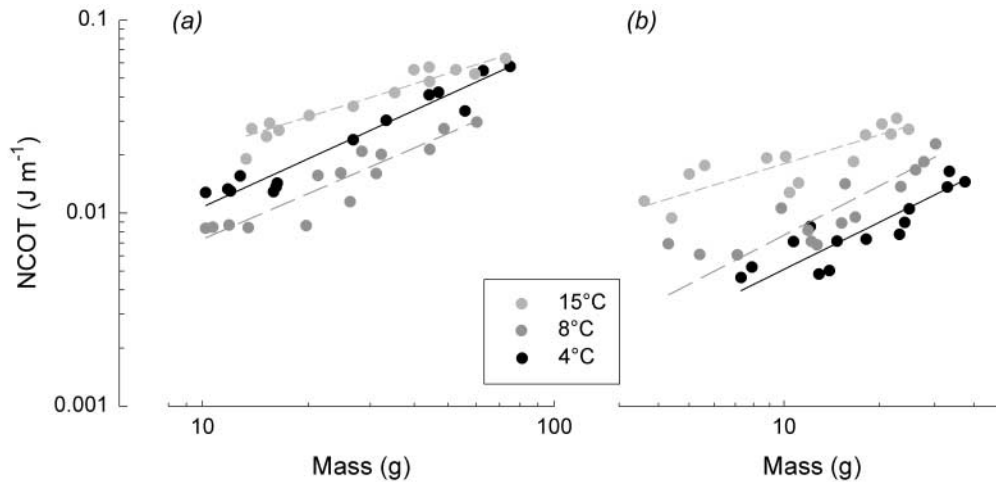


Fig. 3. Net costs of transport (NCOT, $\text{J}\cdot\text{m}^{-1}$) for (a) vendace (*C. albula*) and (b) Fontane cisco (*C. fontanae*) at 4°C (black, solid line), 8°C (dark grey, long-dashed line), and 15°C (light grey, short-dashed line) as a function of body mass (g) according to a two-parameter power function ($\text{NCOT} = a \cdot M^b$). NCOT represents the minimum energy needed by a fish to swim one unit distance. The mass range differed according to the size difference of the Fontane cisco and vendace populations. The original data are available in Ohlberger *et al.* (2008) and methodological details can be found in Ohlberger *et al.* (2007). Note: double-logarithmic plots.

previous preference tests may have failed to determine FTPs in fish due to short test periods. The initially high-temperature preference of vendace may be explained by over-regulation as an adaptive strategy after acclimation to sub-optimal temperatures (Huey and Berrigan, 1996). Ectotherms generally experience a decelerating effect of cold temperature on cellular processes. In fishes, cold acclimation increases muscle aerobic capacity through an increase in mitochondrial volume density or changes in mitochondrial oxidative capacity (Sänger, 1993; Guderley and Johnston, 1996). Thus, as opposed to evolutionary cold adaptation, short-term acclimation to lower temperatures causes a rise in mitochondrial density or enzyme concentration, which increases maintenance costs (Pörtner, 2002; Guderley, 2004). This cellular mechanism may cause the initial shift of vendace to above-optimum temperatures after cold acclimation to re-establish lower mitochondrial maintenance costs via a reduced mitochondrial density. Fontane cisco, in contrast, may not experience elevated energy costs due to evolutionary cold adaptation. However, our explanation for this compensatory mechanism remains speculative and demands further research.

Our conclusion is supported by the fact that the observed difference in final thermal preference is generally consistent with a previous study on the species' metabolic rates (Ohlberger *et al.*, 2008). Respiration analyses revealed significant differences in temperature-dependent metabolic costs indicating that Fontane cisco are competitively superior at lower temperatures in terms of metabolism (i.e. they experience lower metabolic costs), whereas vendace experience lower energy costs at higher temperatures. Additionally, our recent analysis on swimming costs indicates that the species' net costs of transport are lowest in Fontane cisco at 4°C and in vendace at 8°C, which is in good accordance with the species-specific final thermal preferences of 4.2 and 9°C respectively. Hence, swimming performance is optimized at the respective thermal preference of both species.

The simultaneous evolution of divergent temperature-dependent swimming costs and the divergence in thermal preferences that correspond to temperatures maximizing swimming efficiency (Figs. 2 and 3) indicate that thermal preference and at least some thermal performance functions, like the net costs of transport, are co-adapted in these fish. The close correspondence is in line with the results of other studies on the physiology of ectotherms that concluded that temperature preference is adaptive (e.g. Johnson and Kelsch, 1998) and usually corresponds to body temperatures that maximize performance (Angilletta *et al.*, 2002, 2006; Beitinger and Fitzpatrick, 1979). However, it has to be mentioned that the thermal preference of fishes is influenced by various factors such as season (Tapaninen *et al.*, 1998), photoperiod (Staaks, 1996), ontogeny (Edsall, 1999), salinity (Stauffer and Boltz, 1994), oxygen concentration (Schurmann *et al.*, 1991), and nutritional status (van Dijk *et al.*, 2002). Hence, preferred temperatures may reflect species-specific values only to some degree, and the concept may not hold for comparisons when studying species with highly variable thermal preferences, such as those observed for instance in anuran amphibians (reviewed in Navas *et al.*, 2008). Nevertheless, the concept has been confirmed repeatedly in fish studies and thermal gradients are widely used as a comparative method to detect interspecific differences between species (Coutant, 1977; Konecki *et al.*, 1995; Larsson, 2005).

Previous ecological surveys on the Lake Stechlin coregonids have revealed a spatial segregation along the vertical gradient of the lake (Helland *et al.*, 2007). The Fontane cisco population inhabited deeper and cooler water layers than vendace and was never found in water layers with a temperature above 7.7°C (Helland *et al.*, 2007). However, the observed FTP of Fontane cisco is remarkably low, since the general thermal optimum for coregonines, including the genera *Stenodus*, *Prosopium*, and *Coregonus*, ranges from 8 to 15°C (Elliott, 1981). The FTPs of *Coregonus* spp. have rarely been analysed. The only final preferendum test on vendace comes from Tapaninen *et al.* (1998), who studied populations from deep Finnish lakes and reported a thermal preference of 9°C for immature fish after long-term acclimation to 2°C. Thus, the calculated FTP for vendace from this study is in good accordance with data in the literature, whereas the FTP for Fontane cisco is far below all values reported to date. The comparison further supports the conclusion that a character shift in thermal preference primarily occurred in Fontane cisco due to adaptation to cooler temperature rather than in vendace.

Comparisons between closely related sympatric species like that performed in the present study provide the most convincing evidence for the importance of temperature adaptation in ecological and possibly evolutionary diversification on small spatial scales. Ecologists have recognized that gradients in water temperature can create spatial structures in fish distribution (Rudstam and Magnuson, 1985) and the existence of temperature gradients is believed to directly influence the physiologically based competitive strengths of fish populations (Jensen *et al.*, 2006). The temperature regime of a deep temperate lake exerts especially strong selection pressures on pelagic fish populations due to the wide range of water temperatures. In other words, it offers the opportunity for an ecologically based physiological segregation along the temperature–depth gradient, which reduces interspecific competition and thereby facilitates co-existence. An example of adaptive differences in thermal physiology promoting ecological divergence is described for the fine-scale pattern of vertical zonation among closely related intertidal animals (Somero, 2002). Similarly, niche segregation between different salmonid species in Scandinavian lakes is facilitated by differentiation in thermal preference (Larsson, 2005). Ecological segregation by using different microhabitats along the depth gradient of a lake is further believed to facilitate co-existence

of native whitefish (*Coregonus lavaretus*) and invading vendace in the sub-arctic Pasvik hydrosystem (Bohn *et al.*, 2008).

The reported differences in thermal preference, in combination with temperature-related metabolic adaptations and the observed vertical segregation, suggest environmental temperature as a driving force during the evolutionary divergence of the Lake Stechlin coregonids. Similar processes of ecologically based speciation along steep biotic or abiotic gradients may have occurred also in other freshwater systems, like that suggested for the speciation of the Great Lakes ciscoes (Smith and Todd, 1984). This idea is corroborated by theoretical studies indicating that speciation along environmental gradients may be a common process in nature (e.g. Doebeli and Dieckmann, 2003). The conclusion that the depth of the lake and the corresponding temperature gradient were important prerequisites for the evolutionary diversification is further supported by biogeographical considerations. Sympatric pairs of *Coregonus* spp. occur in only two lakes in Germany, Lake Stechlin and Lake Breiter Luzin (Schulz *et al.*, 2006). These two lakes are the deepest in that region (Mehner *et al.*, 2005) and both are located at the southern border of the distribution range of vendace (Kottelat and Freyhof, 2007). Consequently, we can assume that these lakes have the broadest temperature ranges and the steepest gradients compared with other lakes where vendace occur. This supports our conclusion that the temperature–depth gradient has facilitated an ecological and finally evolutionary diversification.

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