Parallel evolution of lake whitefish dwarf ecotypes in association with limnological features of their adaptive landscape

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Abstract

Sympatric fish populations observed in many north temperate lakes are among the best models to study the processes of population divergence and adaptive radiation. Despite considerable research on such systems, little is known about the associations between ecological conditions and the extent of ecotypic divergence. In this study, we examined the biotic and abiotic properties of postglacial lakes in which lake whitefish, *Coregonus clupeaformis*, occur as a derived dwarf ecotype in sympatry with an ancestral normal ecotype. We compared 19 limnological variables between two groups of lakes known from previous studies to harbour sympatric dwarf and normal ecotypes with high and low levels of phenotypic and genetic differentiation respectively. We found clear environmental differences between the two lake groups. Namely, oxygen was the most discriminant variable, where lakes harbouring the most divergent populations were characterized by the greatest hypolimnetic oxygen depletion. These lakes also had lower zooplankton densities and a narrower distribution of zooplantonic prey length. These results suggest that the highest differentiation between sympatric ecotypes occurs in lakes with reduced habitat and prey availability that could increase competition for resources. This in turns supports the hypothesis that parallelism in the extent of phenotypic divergence among sympatric whitefish ecotypes is associated with parallelism in adaptive landscape in terms of differences in limnological characteristics, as well as availability and structure of the zooplanktonic community.

Introduction

Divergent natural selection for the exploitation of different resources and habitats is considered a key process in population divergence and adaptive radiation (Smith & Skùlason, 1996; Schluter, 2000). This general hypothesis has been supported by many empirical studies that have documented parallel phenotypic evolution in independent lineages under similar ecological conditions (Schluter & McPhail, 1993; Smith & Skùlason, 1996; Ruzzante et al., 2003; Chiba, 2004). Divergent natural selection can only arise if there is an alternate ecological opportunity

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(*sensu* Simpson, 1953) that can fulfil the requirements for a species to complete its life cycle (Schluter, 2000). Although chance and history also play a role (Travisiano et al., 1995), biotic and abiotic properties of the environment are thought to be the primary forces shaping patterns of phenotypic divergence (Travisiano et al., 1995; Losos et al., 1998; MacLean & Bell, 2003; Langerhans & Dewitt, 2004). These niche characteristics will likely determine the strength of selection acting on phenotypic evolution (Kawecki & Ebert, 2004). Progress in understanding divergent phenotypic evolution is best achieved by the study of young lineages as conditions for their phenotypic peculiarities may still prevail and habitat-phenotype correlations are less likely to be affected by differences in ancestral phenotypes (Schluter, 2000). Such ideal conditions for rapid divergence likely prevailed in north temperate lakes that have been

cyclically affected by glacial expansions and retreats during the Pleistocene, perhaps as many as 20 times (Martinson *et al.*, 1987). Members of the north temperate freshwater and anadromous fish fauna share several attributes that corroborate the predicted effect of post-glacial ecological opportunity in priming rapid divergence, and make them putative candidates for studying the process of population divergence and adaptive radiation. Of particular interest is the occurrence of sympatric forms that predominate in salmonid fishes, but that also exist in phylogenetically remote families, including Gasterosteidae, Osmeridae, Centrarchidae and Catostomidae (reviewed in Robinson & Wilson, 1994; Smith & Skùlason, 1996; Bell & Andrews, 1997; Taylor, 1999; Robinson & Schluter, 2000). Despite the considerable interest that has been devoted to studying such systems, little attention has been paid to documenting the 'limnological landscape' (selective physical, chemical and biotic features of the lacustrine environment) and its possible role in explaining patterns of ecological and morphological divergence between sympatric forms of freshwater fishes.

Lake whitefish, *Coregonus clupeaformis* (Mitchill.), is particularly well-suited for studying the role of ecological opportunity in population divergence. In the St John River watershed (Maine, USA), a dwarf ecotype derived from an ancestral normal ecotype evolved in parallel and independently in at least six postglacial lakes (Bernatchez & Dodson, 1991; Campbell & Bernatchez, 2004). In these lakes, dwarf whitefish are found in sympatry with a normal population, from which they remain genetically distinct and partially reproductively isolated (Chouinard *et al.*, 1996; Pigeon *et al.*, 1997; Lu & Bernatchez, 1999; Lu *et al.*, 2001). Dwarf and normal sympatric ecotypes mainly use pelagic and epibenthic habitat, respectively (Bernatchez *et al.*, 1999), and these forms also exhibit phenotype-environment associations with their respective niches (Bernatchez *et al.*, 1999; Rogers *et al.*, 2002; Rogers & Bernatchez, 2005; Derome *et al.*, 2006). At the morphological level, the most discriminating trait between ecotypes is the gill-raker apparatus (Lu & Bernatchez, 1999), a highly heritable trait in whitefish (reviewed in Bernatchez, 2004). Here, the dwarf ecotype typically has more numerous and less separate gill-rakers than the normal population, probably to facilitate retention of smaller, planktonic prey (Robinson & Wilson, 1994; Sanderson *et al.*, 2001; Kahilainen *et al.*, 2004; Budy *et al.*, 2005). It has also been showed that divergent selection has played an important role in driving divergence in gill-raker characteristics (Rogers *et al.*, 2002), and that selection has acted more strongly on the dwarf relative to normal ecotype (Bernatchez, 2004). Also the dwarf ecotype is characterized by a smaller size at maturity, slower growth rate and an earlier age at maturation relative to normal ecotype (Rogers *et al.*, 2002; Rogers & Bernatchez, 2005), as is typical for planktivorous fishes in general (Robinson &

Wilson, 1994). Both ecotypes also differ in their swimming behaviour, whereby the dwarf ecotype typically swims more actively and prefers higher positioning in the water column in experimental laboratory conditions (Rogers *et al.*, 2002). A genetic basis for all of the above traits have been documented either by heritability estimates (Rogers *et al.*, 2002), gene mapping (Rogers & Bernatchez, 2005, Rogers *et al.*, 2007) or gene transcription (Derome *et al.*, 2006).

Most North American temperate lakes where cases of parallel phenotypic evolution have been reported in fishes typically show poor fish species diversity (review by Taylor, 1999; Schluter, 2000). For example, dwarf whitefish strictly occur in sympatry with the normal ecotype, and in the absence of the lake cisco, *Coregonus artedii*, a direct competitor species that may limit exploitation of the planktonic niche (Davis & Todd, 1998; Trudel *et al.*, 2001; Bernatchez, 2004; Derome & Bernatchez, 2006). However, the absence of lake cisco cannot explain in itself the among-lake variation in niche utilization and level of divergence between dwarf and normal whitefish ecotypes. Previous studies documented a variable degree of planktonic/benthic diet overlap as well as variable level of morphological specialization between ecotypes in different lakes (Bernatchez *et al.*, 1999; Lu & Bernatchez, 1999). These observations raised the hypothesis that different lakes represent different degrees of, or simply different, ecological opportunity, because of variation in lake-specific physico-chemical characteristics (Hindar & Jonsson, 1982; Griffiths, 1994; reviewed by Taylor, 1999), resource (trophic and spatial) availability (reviewed by Smith & Skùlason, 1996) and stability (reviewed by Kawecki & Ebert, 2004). All of these factors could affect the strength and direction of the selective forces (e.g. Robinson *et al.*, 2000; Nosil & Reimchen, 2005) acting on the dwarf ecotype.

In the present study, we tested the hypothesis that differences in patterns of divergence observed in the gill-raker apparatus between sympatric dwarf and normal ecotypes are associated with lake-to-lake variation in limnological features and planktonic community structure (size distribution and taxonomic composition) that could influence ecological opportunity or competitive interactions. We address this hypothesis by comparing 19 biotic and abiotic characteristics between two groups of lakes known from previous studies to harbour sympatric dwarf and normal ecotypes with high and low levels of phenotypic and genetic differentiation respectively (Bernatchez, 2004).

Materials and methods

Study sites

All six lakes in the St John River watershed that are known to harbour sympatric whitefish ecotypes were used for this study (Fig. 1). As previously documented
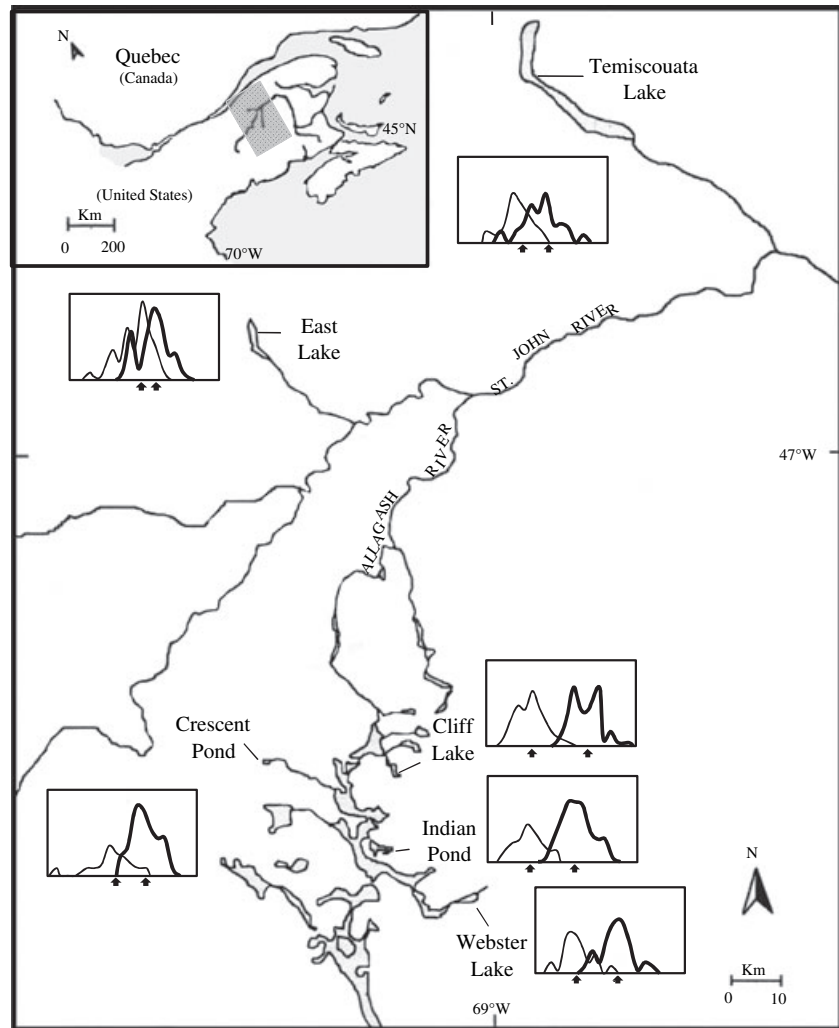
Fig. 1 Location map of sampling sites in the St John river watershed in southeastern Quebec (Canada) and northeastern Maine (USA). Group A lakes (harbouring highly differentiated whitefish ecotypes: Cliff Lake (CL), Indian Pond (IP) and Webster Lake (WL); Group B lakes (low differentiation): Temiscouata Lake (TL), East Lake (EL), Crescent Pond (CP). The graphs illustrate phenotypic differences between dwarf (bold lines) and normal (thin lines) obtained from the discriminant function analysis of eight meristic traits (modified from Lu & Bernatchez, 1999). Most of the variation was explained by gill-raker number. Arrows represent the centroids of the discriminant analysis in each lake.

(reviewed in Bernatchez, 2004), a first group of three lakes (hereafter called group A) harbours phenotypically and genetically divergent sympatric ecotypes [Cliff Lake (CL), Indian Pond (IP) and Webster Lake (WL)], whereas the second group (hereafter called group B) comprises three lakes harbouring sympatric ecotypes with a low level of differentiation [Crescent Pond (CP), Témiscouata Lake (TL) and East Lake (EL)]. The mean eigenvalue obtained from a discriminant function analysis was significantly higher for group A than for group B lakes (2,176 vs. 0,662; *t*-test, $P = 0,014$) (details in Lu & Bernatchez, 1999). Similarly, the mean *F*st value was significantly higher for group A than for group B lakes (0,160 vs. 0,039; *t*-test, $P = 0,047$) (details in Lu & Bernatchez, 1999). Lake cisco (*C. artedii*) is absent from all six lakes, but all harbour healthy populations of two whitefish predators: lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*; except in CP).

Five sampling stations were randomly selected in each lake between the depths of 5 and 25 m, where both whitefish ecotypes are most commonly found. Both forms are known to avoid warm waters of the littoral zone (< 5 m) during the growing season (Bernatchez, unpubl. obs.). Sampling stations were located by using a geographic positioning system and bathymetric maps and were sampled at two different times of the year (16–26 June, 20–28 August) in 2003.

Limnological variables

For the lakes in Maine USA (Cliff, Indian, Crescent, Webster), altitude, fetch, volume (*V*), surface area, mean depth (Z_{moy}), maximal depth (Z_{max}) (Wetzel, 2001) were obtained from topographical and bathymetric maps and from information available on the PEARL website (http://pearl.spatial.maine.edu). For the lakes in Québec (Témiscouata and East), altitude and Z_{max} were interpreted from maps, whereas volumes, surfaces and Z_{moy} were obtained from bathymetric maps imported as images in a vector-based Geographic Information System

(GIS) software package Arc GIS 8.3 and georeferenced on a 1 : 50 000 Arc GIS map (National Topographic Data Base, Natural Resources Canada). Using the perimeter and area function of this software, data were obtained for each 6.1-m (20 ft) depth interval. Lake volume was then estimated by the method of summation of the frusta of a series of truncated cones of the strata (Wetzel, 2001). Shoreline development (DL) of each lake was calculated following Wetzel (2001) and with data obtained from perimeter values calculated in Arc GIS software. Chemical and physical characteristics of all lakes were measured at the deeper station of the lake, when the Z_{max} was < 25 m, for both June and August with a 5-L Go-flow bottle. For total phosphorus (TP), coloured dissolved organic matter (CDOM), Chlorophyll *a* (Chl *a*) and dissolved inorganic carbon (DIC), bottles were washed and samples were preserved following the NLET (National, Laboratory for Environmental Testing, Burlington, ON, Canada) standard protocols. Water transparency, or Secchi depth (Z-Secchi), was measured by using a 20-cm Secchi disk (Wetzel & Likens, 1979). Temperature and oxygen were measured every 0.5 m with an oxygen meter (YSI model 58). Oxygen concentrations were measured in mg L^{-1} and transformed to percent saturation (%) following Wetzel & Likens (1979). Temperature and oxygen profiles were obtained at the same stations in 2002 and 2003. Oxygen saturation (O_2-hypolimnetic) and water temperature values (T_{max}-*z*) at the bottom of the lakes in August were the two variables considered for more detailed statistical analyses. The epilimnion and metalimnion of each lake were defined as in Hutchinson (1957). Two water samples were taken in each stratum for Chl *a*, CDOM, DIC and TP analyses. Following sampling, Chl *a* samples were filtered onto 25-mm Whatman glass fiber filters (grade GF/F) and preserved in cryogenic vials in liquid nitrogen until laboratory analysis. In the laboratory, Chl *a* analysis were performed following Nusch (1980) with a Cary Eclipse spectrofluorometer. Prior to sample analyses, a standard curve was generated by using a standard Chl *a* solution (*Anacystis nidulans*; Sigma-Aldrich GmbH, Seelze, Germany). Concentrations were calculated by using Jeffrey & Welschmeyer's (1997) equation. CID samples were analysed by Gran titration on the evening of the date of sampling with the method of Wetzel & Likens (1979); pH was measured with a Hanna instruments pH-meter (HI 8424 Microcomputer). TP samples were acidified to pH 2 with 1 M H_2SO_4 the same day of sampling and were analysed at the NLET laboratory (Environment Canada, Burlington, ON, Canada). CDOM samples were stored in the dark at 4 °C and then filtered through 0.2-μm cellulose acetate membrane prior to absorbance measurements at 320 nm with an Ultrospec 2000 (Pharmacia Biotech, Piscataway, NJ, USA) spectrophotometer (Williamson *et al.*, 1999; Belzile *et al.*, 2002). Mean values of these limnological variables were calculated for each lake from all data collected throughout the summer, for both the epilimnion and metalimnion.

Zooplanktonic Variables

Zooplankton were collected at each station, in June and August of 2003, using a 12-cm Wisconsin plankton net with an 80-μm mesh size. Vertical hauls, at approximately 1 m s^{-1}, were made starting at 0.5 m from the bottom to the surface. Samples were preserved in 95% ethanol until laboratory analyses (Rodriguez *et al.*, 1993). The depth of the vertical hauls was used to calculate the total water volume filtered at each lake. In the laboratory, samples were filtered onto a 300-μm sieve. All individuals were counted and identified to genus or family except for copepods, which were assigned as calanoid, cyclopoid, harparticoid or nauplii categories (Thorp & Covich, 1991). For each sample, individuals of a given taxonomic group were pooled in an Eppendorf tube and preserved in 95% ethanol until length measurements were made. Twenty individuals of each taxon in each sample were measured following Culver *et al.* (1985) to the nearest 0.025 mm using either a dissecting microscope with an ocular micrometer. These samples were then divided into 0.1-mm length classes spanning a range from 0.3 to 2.5 mm. The organisms that measured 2.5 mm or more were put in the same length class because they represented < 1% of all the biomass and density in a sampling station. Absolute numbers of individuals of each length class were transformed to density (mean number per class) by multiplying the frequency of individuals of a given taxon in a length class by the number of individuals per liter of this taxon at a given station.

Dry weight (DW) of each measured individual was calculated by using the following length–weight regressions: Lacasse & Magnan (1992) for Bosminidae (named as taxon 5), *Ceriodaphnia* spp. (taxon 17), *Daphnia* spp. (taxon 6), Sididae (taxon 7), *Polyphemus pediculus* (taxon 9), *Leptodora kindii* (taxon 13) and *Holopedium* spp. (taxon 4); Dumont *et al.* (1975) for calanoids (taxon 1), cyclopoids (taxon 2) and Rotifera (taxon 11); Culver *et al.* (1985) for copepoda nauplii (taxon 3), Johnston & Cunjak (1999) for Chaoboridae (taxon 10) and Chironomidae (taxon 19); Meyer (1989) for Ephemeroptera (taxon 15), Coleoptera (taxon 12) and Hydracarina (taxon 8); and Rahkola *et al.* (1998) for *Asplanchna* spp. (taxon 16). The total biomass (g DW L^{-1}) was estimated following the same procedure as for density. For Annelida (taxon 14), biomass was not estimated and so not included in the biomass estimation.

Statistical analyses

Limnological differences between lake groups

All limnological variables were included in the analyses to avoid the loss of information (Rodriguez *et al.*, 1993). Logarithmic transformations log (*x* + 1) were applied to

all environmental variables except pH to reduce skew distribution (Weckstrom *et al.*, 1997) and to better approximate assumptions of normality and homoscedasticity. Selection of the most informative variables for discriminating lake groups A and B was made by using a stepwise ANOVA (STEPDISC procedure, α = 0,05). Statistical differences between the two lake groups for each selected variable were further assessed by Student's *t*-tests. All of these statistical analyses were completed by using SAS (v. 8.2; SAS Institute, Cary, NC, USA). Principal component analysis (PCA) was then performed by using PRIMER-E (v. 5, PRIMER-E Ltd., Plymouth, UK) to evaluate similarities and differences among lakes and *a posteriori* differences between the two lake groups, after removal of correlations between variables (Weckstrom *et al.*, 1997).

Prey availability and variation in density and biomass

Lake group differences in resource availability, both in terms of density and biomass, were analysed by using a two factor (month and lake group) mixed procedure (Littell *et al.*, 2004). Individual lakes represented the basic sampling unit for this statistical test. Mean density and biomass values were calculated over the five stations surveyed in each lake, which was nested in its respective lake group and then considered as a random factor.

Spatial variability in biomass and density were measured as the coefficient of variation [CV = 100 *(SD/mean)] for each lake, where stations were the sampling units (see for temporal stability; Caldeira *et al.*, 2005). A mixed procedure with CV of biomass and density as response variables was then performed as above to test for differences between the two lake groups. Biomass and density values were log-transformed to satisfy the assumptions of normality and homocedasticity of error terms.

Availability and spatio-temporal variation in prey diversity and size

A repeated measure ANOVA using a mixed procedure was applied to evaluate lake group differences for different classes of prey length and between months. Although repeated measures models are more typically used for a response variable measured repeatedly over time in an experimental unit, they can also be applied to variables distributed through space (Littell *et al.*, 2004). In this analysis, the response variables were density and biomass, the experimental unit was lake (random components) nested within lake group (treatments that are fixed component) and subject was the lake by month interaction. For each lake, individuals of all taxa were summed for a given month within a size class. The 23 response measurements were taken at each of the five stations and were averaged prior to analysis. As the response measurements for the 23 size classes were measured on the same community in each lake, the independence assumption on the error terms for the

ANOVA was likely violated (Littell *et al.*, 2004). Consequently, the error term was assumed as normally distributed with constant variance, and the dependence expressed with a covariance structure of size class. Log-transformed values of biomass and density were analysed to satisfy the normal distribution and homocedasticity of error terms assumption. The appropriate covariance structure was determined to be first order auto regressive [AR (1)] for density and first order AR heterogeneous [ARH (1)] for biomass over all covariance structures examined using the AIC and SBC (Littell *et al.*, 2004). All analyses were performed by using procedures available in SAS.

Spatial variability in community biomass was measured as the CV of biomass per size class, measured over all five stations in each lake. The CV of community biomass can be expressed as: $\sqrt{[(\sum variance+\sum covariance)/\sum biomass]}$ where the denominator is the average community biomass of the five stations (Caldeira *et al.*, 2005). Here, total variance ($\sum$ variance) was the sum of the variances of community biomass for all size class over the five stations of each lake. Total covariance ($\sum$ covariance) was the sum of covariances between pairs of classes over the five stations of each lake. The above equation provided a tool for understanding how these three components (total variance, total covariance and total biomass) affect the spatial variability of biomass production of the communities and how spatial variability varies with size class. High total variance and covariance indicate high spatial variability (Caldeira *et al.*, 2005). The same mixed procedure was applied for measuring the CV of biomass and density as response variables.

A similarity matrix was used, ANOSIM, to test for differences between zooplanktonic assemblages in terms of species composition relative to biomass and density. The resulting *R* statistic provided the estimated differences of mean rank between and within assemblages. These assemblages were considered to be different when similarities were greater within than those observed between. *R*-values were interpreted as showing a strong difference at $R > 0.75$, an intermediate difference at $R > 0.5$, a small difference at $R > 0.25$, and a random grouping at $R = 0$. A similarity of percentages routine, SIMPER, was used to assess the contributions of different taxa to observed assemblage dissimilarities. ANOSIM and SIMPER were performed by using the PRIMER-E v. 5 program. Calculation of dominant taxa of each lake group and by month for biomass and density response variable were performed to provide global estimates of community structure (Winkler *et al.*, 2005).

Results

Limnological differences between lake groups

The limnological characteristics of the six study lakes are summarized in Table 1. The stepwise ANOVA revealed

Table 1 Characteristics or the six study lakes with high (A) or low (B) differentiation or dwarf and normal lake whitensn ecotypes. Abiotic variables describe limnological characteristics of each lake (see Materials and methods for details on abbreviations). Biotic variables relate to planktonic invertebrates communities in each lake. Numbers identify taxa present in each lake as well as the dominant taxon in terms of biomass and density, (see Materials and methods for details on taxonomic identification by numbers).

| Data type	Variable	Month	Lake group A			Lake group B		
			CL	IP	WL	CP	TL	EL
Limnological	O_2-hypolimnetic (%)		8.04	7.83	9.17	54.24	86.04	76.05
	TP (μg L^{-1})		3.99	21.94	6.23	5.53	6.88	4.93
	Chl *a* (μg L^{-1})		1.32	1.44	1.26	1.03	1.76	0.84
	PH		6.65	6.48	6.29	6.47	7.45	6.81
	CID (mg CL^{-1})		17.44	9.02	9.91	6.62	17.76	5.81
	CDOM (m^{-1})		17.61	9.37	23.71	10.18	11.28	20.94
	Z-Secchi (m)		6.00	6.00	3.22	4.75	5.25	4.25
	Volume (10^6m^3)		11.15	28.51	13.19	9.25	2511.26	132.88
	Surface (10^6m^2)		2.09	4.89	2.00	1.25	79.84	8.85
	Fetch (10^3m)		10.68	7.81	5.63	5.94	14.06	6.36
	T_{max}-z (°C)		8.30	12.10	8.00	7.30	12.40	6.60
	Z_{max} (m)		19.81	15.85	13.41	20.73	74.68	30.48
	Z_{moy} (m)		5.33	5.83	6.59	7.38	38.93	16.92
	DL (m)		2.05	2.00	2.00	1.84	3.35	2.17
	Altitude (m)		294.13	286.51	271.58	370.64	148.80	320.10
	Latitude (°)		46.40	46.26	46.16	46.42	47.60	47.18
	Longitude (°)		69.25	69.29	69.08	69.61	68.75	69.55
Invertebrate	Taxa		1–8;10–12;16;17	1–10;15–18	1–7;9–11;13; 16;17;	1–10;16;17	1–3;5–10;13 16;17	1–7;13;14
	Dominant taxa, taxon (%)							
	Biomass	June	1 (54.6)	1 (81.0)	1 (51.4)	1 (83.9)	1 (59.5)	1 (58.6)
		August	1 (78.2)	6 (36.0)	1 (39.2)	6 (39.2)	1 (59.1)	1 (57.4)
	Density	June	16 (42.6)	1 (83.6)	1 (50.0)	1 (79.1)	1 (43.1)	1 (58.5)
		August	1 (53.8)	6 (42.8)	1 (43.0)	1 (27.8)	1 (41.2)	1 (45.7)
	Range size, mm	0.3–3.6	0.3–2.3	0.3–3.6	0.3–5.3	0.3–5.8	0.3–3.4	
	Mean size, mm (std)	June	0.75 (0.03)	0.63 (0.04)	0.68 (0.10)	0.65 (0.06)	0.96 (0.08)	0.85 (0.04)
		August	0.79 (0.04)	0.86 (0.09)	0.67 (0.02)	0.81 (0.10)	0.88 (0.05)	1.02 (0.14)
	Total							
	Biomass,	June	63.5 (16.0)	18.3 (6.8)	12.6 (4.8)	17.9 (4.9)	26.5 (8.3)	18.6 (10.6)
	mg DW m^{-3} (std)	August	10.1 (3.1)	10.2 (4.0)	17.7 (6.6)	35.7 (16.1)	39.8 (7.8)	37.2 (15.9)
	Density, 10^3	June	15.0 (2.8)	6.4 (2.9)	3.3 (1.1)	5.2 (1.6)	3.4 (1.2)	3.6 (1.5)
	ind m^{-3} (std)	August	2.9 (1.0)	3.8 (1.5)	6.1 (2.0)	8.0 (3.0)	6.7 (1.3)	5.4 (3.2)

significant limnological differences differences between the two lake groups, whereby lakes in group A (high divergence) were characterized by a more pronounced oxygen depletion with increasing depth than lakes in group B (low divergence) during August, the summer period of maximum stratification ($F = 204.49$, $P = 0.0001$) (Fig. 2). Moreover, mean depth was significantly higher in group B lakes (Z_{moy}; $F = 15.55$, $P = 0.029$), whereas dissolved organic matter (CDOM; $F = 25.50$, $P = 0.037$) and TP ($F = 504.27$, $P = 0.028$) concentrations were lower in these lakes. The ANOVA with three factors (lake groups, months, years) showed no significant difference for oxygen saturation (O_2-hypolimnetic) between years 2002 and 2003 in the month-by-year-by-lake group interaction ($F = 0.06$, $P = 0.815$), where lakes was nested within the two lake groups. The difference between months was significant in the mixed model of month-by-lake group interaction ($F = 31.02$; $P = 0.0001$) (Fig. 2). PCA analysis also showed that

hypolimnetic oxygen saturation was the best variable explaining differences between lake groups (Fig. 3).

Resource structure and spatio-temporal stability

No significant difference was observed between lake groups, either in terms of variation of total biomass and density between months ($F = 5.45$, $P = 0.080$ and $F = 3.45$, $P = 0.137$ respectively) or when months were pooled ($F = 2.62$, $P = 0.181$; $F = 0.02$, $P = 0.897$). Also, there was no significant difference in spatial heterogeneity in terms of the coefficients of variation between the two lake groups, either for biomass ($F = 0.20$, $P = 0.676$) or density ($F = 0.0$, $P = 0.957$).

In contrast, a highly significant difference between the two lake groups was observed in size structure of planktonic prey communities for both biomass ($F = 2.97$, $P < 0.0001$) and density ($F = 1.96$, $P = 0.009$; Fig. 4). The lake groups differed for the size classes
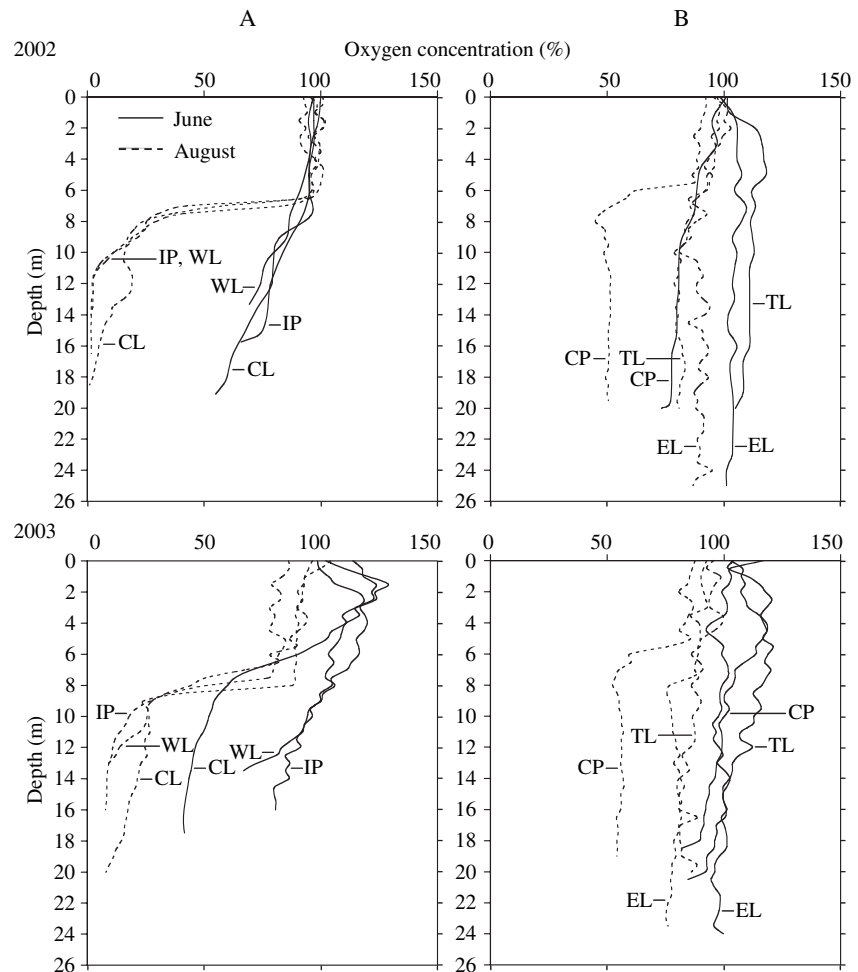
Fig. 2 Oxygen profiles of each lake nested by lake groups A and B in June and August 2002 and 2003. ANOVAS showed significant difference between lake groups by month interaction ($P = 0.0001$).

1.6–2.0 mm in terms of both biomass ($P < 0.05$) and density ($P < 0.001$). Overall, group B lakes were characterized both by a higher biomass and density of larger prey compared with group A lakes. Moreover, the range of prey size distribution was broader in B lakes relative to A. No significant temporal variation in size structure was observed. Similarly, no significant spatial heterogeneity in terms of coefficient of variation was observed in community size structure for both independent variables (total biomass, $F = 0.05$, $P = 0.835$ and total density, $F = 0.27$, $P = 0.632$).

Calanoid copepods were the dominant prey type in terms of total density and total biomass in both lake groups and at all times. For both years, the second most abundant taxa were cyclopoid in June and *Daphnia* sp. in August (Fig. 5). These taxa also predominated in size classes that differed significantly between lake groups (Fig. 5; Table 2). Species contribution to similarity, (using SIMPER and ANOSIM) was assessed in terms of within-assemblage and between assemblage similarity, both between lake groups and between months in each lake group. The calculated ANOSIM R (and P in%) were −0.078

(75.8%) and 0.096 (19.3%) for density and biomass, respectively, indicating the absence of significant difference of taxonomic diversity between lake groups. When comparing sampling months within lake group, ANOSIM R (and P in%) were −0.037 (80%) and 0.259 (20%) for density (A and B lake groups) and −0.111 (60%) and 0.63 (10%) for biomass (A and B lake groups). Thus, significant differences between month zooplanktonic assemblages for both density and biomass were observed for group B lakes only. All SIMPER analyses showed that calanoids accounted for all dissimilarities between assemblages as well as similarities within assemblage.

Discussion

Competition has been considered as the key ecological determinant of population divergence (reviewed in Robinson & Wilson, 1994). Namely, interspecific competition between closely related species may impede differentiation. In *Coregonus*, there is strong indirect evidence that the presence of lake cisco, *C. artedii* hampered the evolution of the dwarf lake whitefish ecotype (Davis
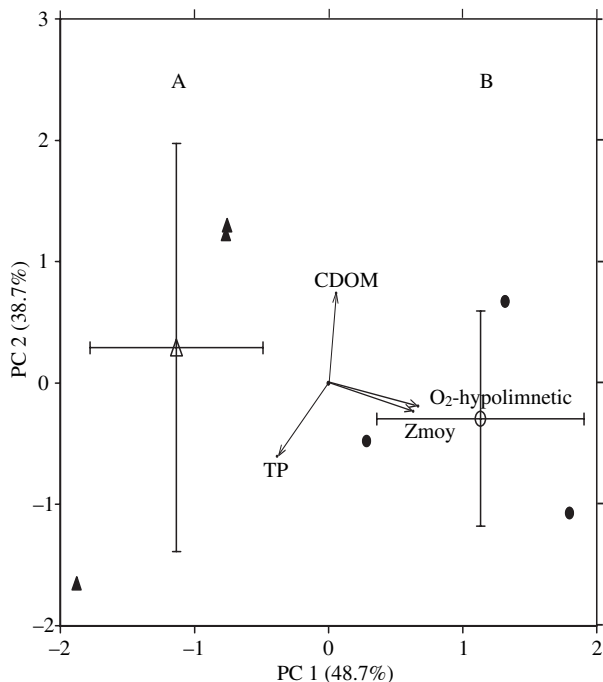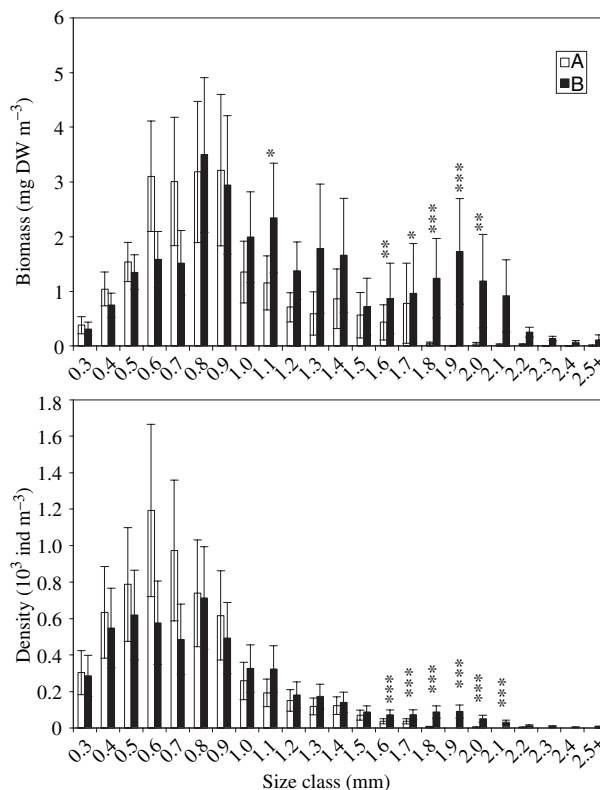
Fig. 3 Principal component analysis of abiotic factors explaining differences between lakes (group A: triangles, group B: circles). The proportions (in percent) of the variance explained by the principal component are given within parentheses. Open symbols represent mean and SD for each lake group.


Fig. 4 Size class (in mm) comparison of zooplankton communities in terms of biomass and density between lake groups. White bars; lake group A (high differentiation between whitefish ecotypes), black bars; lake group B (low differentiation). *$P \leq 0.01$, **$P \leq 0.005$ and ***$P \leq 0.001$ for mixed ANOVAS where the repeated factor was size class (mm) and the random factor was lake nested within lake group. Back transformations of the explanatory variables and their SE are given.

& Todd, 1998; Trudel *et al.*, 2001; Bernatchez, 2004; Derome & Bernatchez, 2006). Yet, the absence of this competitor has not systematically resulted in phenotypic differentiation and population divergence to occupy the pelagic niche by the dwarf ecotype (Bernatchez *et al.*, 1999). These latter observations suggested that factors other than a reduction of interspecific competition are involved in explaining patterns of ecotypic divergence in whitefish. Random processes are difficult to rule out as a possible explanation. However, a pattern of repeated parallel phenotypic evolution in many lakes suggests that divergence is unlikely to occur in the absence of deterministic processes, which could include intraspecific competition (Bolnick, 2001, 2004) and/or the presence of similar ecological pressures (Smith & Skùlason, 1996; Schluter, 2000). Below, we address the hypothesis that differences in abiotic and biotic characteristics we observed between lakes may translate into different ecological landscapes, which in turn may influence the potential for competitive interactions and the level of divergence observed between sympatric whitefish ecotypes.

Parallelism in abiotic features and levels of ecotypic differentiation

A given lake may vary in its physical and chemical gradients (Hindar & Jonsson, 1982; Taylor, 1999), and

also differ from other similarly structured lakes in terms of trophic levels, morphometric parameters and other limnological features (Griffiths, 1994; Wetzel, 2001). This study was based on a 'global variable searching' approach to identify environmental factors that may influence differentiation or the potential for competitive interactions between sympatric forms of freshwater fishes. Such a multivariate approach allows the identification of factors that discriminate lacustrine differences separately or together, with ecotypic differentiation, and the ability to illustrate the proportion of the effect that can be attributed to each factor (Rodriguez & Magnan, 1995).

Here, there was an association between the extent of phenotypic divergence and limnological features of the lakes. Namely, we identified several abiotic characteristics distinguishing two groups of lakes harbouring sympatric dwarf and normal whitefish ecotypes that evolved in parallel and showing high and low level of phenotypic/genetic divergence respectively (Lu & Bernatchez, 1999). The three lakes harbouring the most divergent
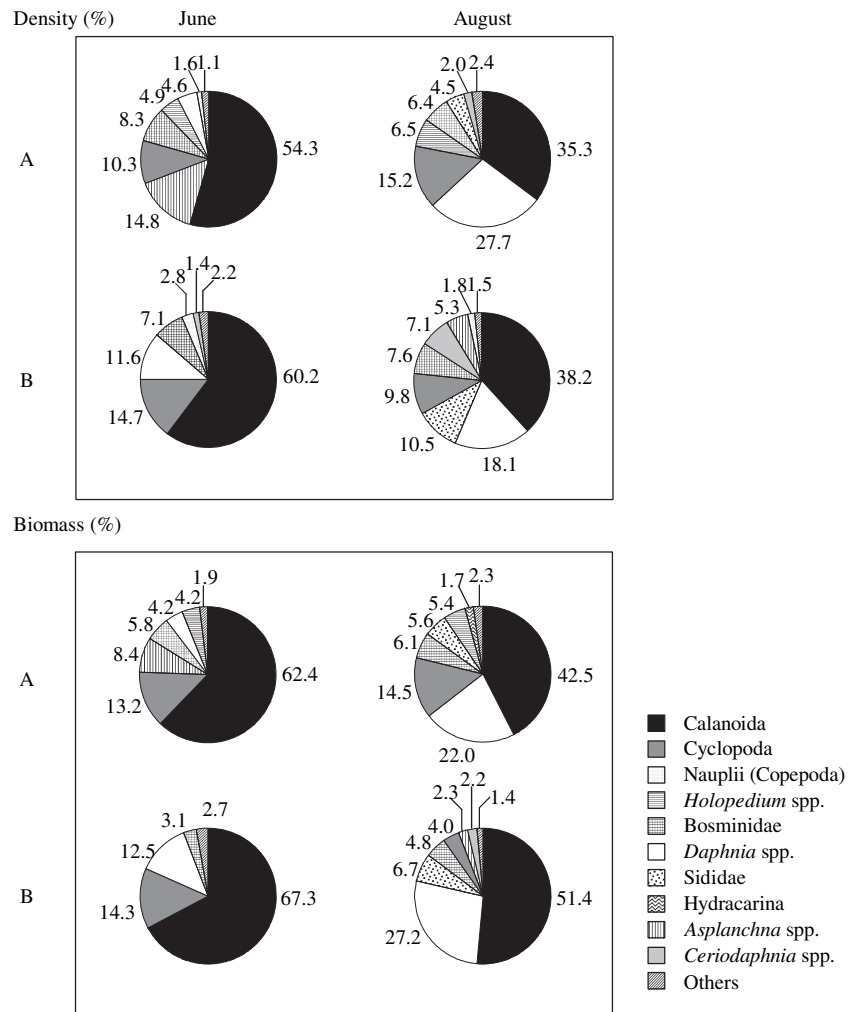
Fig. 5 Relative biomass and density of zooplankton by taxon in lake group A (high differentiation between whitefish ecotypes) and B (low differentiation) for each month. Values represent the percent contribution of each taxon for a month in a lake group.

sympatric populations (Cliff L., Webster L., Indian P.) were characterized by lower mean depth and higher dissolved organic matter (CDOM) and TP concentrations compared with those lakes harbouring less differentiated populations (Crescent P., East L., Témiscouata L.). However, the primary variable differentiating the two lake groups was oxygen: the lakes harbouring the most divergent populations were characterized by pronounced oxygen depletion in the hypolimnion relative to the other three lakes during the period of highest observed thermal stratification. The observed importance of hypo-limnetic oxygen depletion is particularly interesting as this is the most integrative variable that reflects primary production and microbial activities relative to lake morphometry throughout the growing season (Hutchinson, 1957). Differences in hypolimnetic oxygen depletion between lakes may have various causes. For instance, it may reflect differences in lake morphometry and spatial extent of the hypolimnion vis-à-vis thermal and oxygen diffusion to the hypolimnion. It might also result from a

top-down effect of the absence of large zooplankton in group A lakes, allowing more phytoplankton growth and thus more sedimentation of autochthonous organic carbon into the hypolimnion. Oxygen depletion may have major implications for habitat availability as it likely reduces the water volume available for whitefish populations as, as for any salmonid species, coregonines need well-oxygenated water (Scott & Crossmann, 1974). Here, total lake volume was not in itself a factor significantly linked to the observed level of phenotypic differentiation (one-way ANOVA, $P = 0.230$). However, *a posteriori* analysis revealed that the reduction in the proportion of available habitat between 5 and 25-m depth in August relative to June was significant only for lakes harbouring the most differentiated sympatric whitefish forms (one-way ANOVA, $P < 0.0001$). Also, limnetic ecotypes of the European whitefish (*C. lavaretus*) preferentially inhabit the lake bottom in daytime (Meyer, 1989; Kahilainen *et al.*, 2004 in Kahilainen *et al.*, 2005) to escape predators. Whereas this study does not allow the establishment of a

Table 2 Dominant taxa in each size class (0.3 mm to up to 2.5 mm) for lake groups A and B and for each month (June and August). Dominant taxa are given in terms of relative biomass (above) and relative density (below). Taxa explaining significant differences between lake groups in terms of size classes (see Fig. 5) are bolded. '.'; size class not represented.

| Length class (mm) | Lake group | | | | | | | |
| | A-June | | A-August | | B-June | | B-August | |
	Taxa	Biomass (%)	Taxa	Biomass (%)	Taxa	Biomass (%)	Taxa	Biomass (%)
0.3	5	88.96	5	52.74	5	52.80	5	77.19
0.4	5	64.02	5	45.46	1	66.76	5	50.74
0.5	16	42.60	1	45.19	1	69.95	5	40.74
0.6	1	56.05	1	61.68	1	81.78	5	28.19
0.7	1	51.93	1	61.00	1	83.87	1	31.55
0.8	1	79.72	1	74.48	1	80.89	1	86.22
0.9	1	79.05	1	48.70	1	77.53	1	87.08
1.0	1	41.52	2	27.13	1	47.86	1	66.20
1.1	2	46.64	2	50.04	1	59.42	1	47.38
1.2	1	46.46	6	67.56	1	73.62	6	48.97
1.3	1	59.83	6	94.48	1	70.21	1	73.52
1.4	1	69.09	6	83.45	1	74.76	1	61.06
1.5	6	50.60	6	67.33	6	59.35	6	80.90
1.6	1	79.41	6	99.62	1	61.79	6	61.29
1.7	1	92.33	6	97.77	6	49.93	6	72.33
1.8	.	.	6	98.49	6	55.01	6	81.77
1.9	.	.	6	92.49	1	72.50	6	66.32
2.0	6	100.00	6	98.25	6	51.81	1	53.26
2.1	12	100.00	10	100,0	1	73.47	1	70.18
2.2	19	100.00	6	91.72	6	100.00	6	99.29
2.3	.	.	10	100.00	6	100.00	6	98.80
2.4	.	.	.	.	.	.	6	98.90
2.5+	.	.	10	88.87	6	100.00	10	96.64
	Taxa	Density (%)	Taxa	Density (%)	Taxa	Density (%)	Taxa	Density (%)
0.3	5	73.65	5	40.79	3	58.05	5	49.17
0.4	5	43.69	1	34.89	1	77.84	5	26.87
0.5	16	40.42	1	43.53	1	78.14	2	23.60
0.6	1	51.29	1	57.90	1	79.18	17	24.87
0.7	1	47.16	1	53.55	1	79.77	17	30.14
0.8	1	69.49	1	56.92	1	75.40	1	81.55
0.9	1	69.28	6	42.55	1	65.80	1	80.80
1.0	1	33.84	6	60.52	2	45.36	1	48.43
1.1	2	38.57	6	62.85	1	47.92	6	47.24
1.2	1	47.52	6	90.38	1	60.63	6	72.90
1.3	1	66.64	6	97.32	1	57.99	6	49.28
1.4	1	65.77	6	95.51	1	56.16	6	60.33
1.5	6	79.93	6	88.91	6	76.41	6	91.91
1.6	**1**	**51.70**	*6*	*98.29*	**6**	**65.41**	*6*	*82.30*
1.7	**1**	**78.96**	*6*	*88.88*	**6**	**71.00**	*6*	*87.96*
1.8	**.**	**.**	*6*	*91.79*	6	78.52	*6*	*91.94*
1.9	**.**	**.**	*6*	*72.73*	*6*	*52.62*	*6*	*83.67*
2.0	*6*	*100.00*	*6*	*91.70*	*6*	*75.31*	*6*	*67.21*
2.1	12	100.00	10	100.00	1	51.88	6	53.71
2.2	19	100.00	6	74.49	6	100.00	6	97.54
2.3	.	.	10	100.00	6	100.00	6	94.10
2.4	.	.	.	.	.	.	6	93.62
2.5+	.	.	10	93.45	6	86.18	10	96.87

direct association between habitat size and the level of phenotypic differentiation, this has also been suggested in other studies. Robinson *et al.* (2000) reported that phenotypic polymorphism in the pumkinseed sunfish (*Leptomis gibbosus*) was more pronounced in lakes characterized by smaller volume of open water habitat. In summary, our observations and those of Robinson *et al.* (2000) suggest that a reduction of habitat availability at

certain times of the year may result in increased competition and selection driving phenotypic divergence. In the particular case of whitefish, our results indicate that oxygen depletion may be a key environmental factor responsible for explaining differences observed between lakes.

An alternative hypothesis could be that zooplankton community differences result from a differential, top-down effect of predation by dwarf whitefish. This, however, seems highly unlikely, given that such dramatic differences are typically associated with presence/absence of predators (e.g. Brooks & Dodson, 1965). Here, dwarf whitefish occurred in all lakes under study with no striking differences in density between the two lake classes, as deduced by catch per unit effort (L. Bernatch-ez, Université Laval, unpubl. data).

Parallelism in prey availability and ecotypic differentiation

Just as beak morphology in relation to seed size exploitation is a key feature in the phenotypic differentiation of finches (Boag & Grant, 1981; Smith, 1993; Grant & Grant, 2002; Benkman, 2003), studies on the adaptive divergence of northern temperate particulate feeding fishes have identified gill-raker characteristics as a key trait between diverging populations (Schluter & McPhail, 1993; Robinson & Wilson, 1994; Lu & Bernatchez, 1999). Gill-raker numbers and spacing is believed to influence the efficiency of the retention of small prey (Schluter & McPhail, 1993; Robinson & Wilson, 1994; Budy *et al.*, 2005) by potentially regulating fluid dynamics in the buccal cavity (Sanderson *et al.*, 2001). In whitefish, Kahilainen *et al.* (2004, 2005) reported a link between gill-raker numbers, the length distribution of taxa preyed upon, and the available taxa in one given lake. Others have also associated gill-raker characteristics and taxa found in stomach contents or in the environment (see Hindar & Jonsson, 1982; Malmquist *et al.*, 1992; Bernatchez *et al.*, 1999; Kahilainen *et al.*, 2003). To our knowledge, however, this study is the first to provide support to the hypothesis that parallelism in the extent of phenotypic divergence among sympatric fish population is paralleled by differences in availability and structure of the zooplanktonic community (but see Robinson *et al.*, 2000).

Variation in availability and size distribution of the planktonic prey community is important aspects of the selective landscape that could influence the observed level of divergence in gill rakers between sympatric whitefish ecotypes. Indeed, this study revealed that lakes harboring lake whitefish populations with the highest level of ecotypic differentiation were characterized by a narrower range of planktonic prey length potentially available to the dwarf ecotype. It is noteworthy that whereas stomach content analysis has not been conducted in this study, previous studies showed that the dwarf whitefish ecotype feeds on the same prey as those we documented here (Chouinard & Bernatchez, 1998; Bernatchez *et al.*, 1999; see also Bernatchez, 2004). A narrower and smaller length distribution of the planktonic invertebrate community have been shown to result from a higher predation rate by planktivorous species (Brooks & Dodson, 1965), such as the dwarf whitefish ecotype. Such a zooplanktonic structure has also previously been interpreted as evidence for resource limitation, and increased potential for competition, which could result in a higher relative fitness of individuals with a higher efficiency in planktonic prey retention. Thus, a narrower range of prey size could plausibly increase selective pressures for a more efficient exploitation of the zooplanktonic prey and therefore specialization characterized by a higher number of gill-raker numbers (Magnan, 1988; Svanbäck & Persson, 2004).

An evaluation of the size structure of the zoobenthic prey as well as a characterization of the overlap between the planktonic and the benthic prey over many years will be required required to define fully the selective landscape and its role in shaping patterns of differentiation observed in each lake between whitefish ecotypes (Benkman, 2003). However, this study provides one of the very few examples in studies of sympatric fish ecotypes of the usefulness of comparing ecological features across many environments to improve our understanding of the role of adaptive landscape on population divergence. Here, this approach revealed that parallelism in the extent of phenotypic divergence among sympatric fish population is associated with the adaptive landscape, both in terms of differences in limnological characteristics, as well as availability and structure of the zooplanktonic community. We propose that these differences have resulted in different niche opportunities that may have influenced the evolution of lake whitefish populations. Namely, our results support the hypothesis that lakes characterized by limnological conditions favouring increased potential for both intraspecific (intra-ecotypic) and/or interspecific (inter-ecotypic) competitive interactions will harbour genetically and phenotypically more differentiated sympatric forms.

As a closing remark, evolution is conspicuously lacking as a theme in limnological textbooks (Livingstone, 2002) yet there is a natural convergence between evolutionary and limnological perspectives on aquatic ecosystems. Studies of the type undertaken here on the adaptive limnological landscape may represent a first step towards bridging the gap between evolutionary (e.g. Schluter, 2000) and aquatic ecological theories (e.g. Carpenter *et al.*, 1985).

Acknowledgments

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