Phenotype-environment correlations in a putative whitefish adaptive radiation

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Summary

1. The adaptive radiation of fishes into benthic (littoral) and pelagic (lentic) morphs in post-glacial lakes has become an important model system for speciation. Although these systems are well studied, there is little evidence of the existence of morphs that have diverged to utilize resources in the remaining principal lake habitat, the profundal zone.

2. Here, we tested phenotype-environment correlations of three whitefish (*Coregonus lavaretus*) morphs that have radiated into littoral, pelagic and profundal niches in northern Scandinavian lakes. We hypothesized that morphs in such trimorphic systems would have a morphology adapted to one of the principal lake habitats (littoral, pelagic or profundal niches). Most whitefish populations in the study area are formed by a single (monomorphic) whitefish morph, and we further hypothesized that these populations should display intermediate morphotypes and niche utilization. We used a combination of traditional (stomach content, habitat use, gill raker counts) and more recently developed (stable isotopes, geometric morphometrics) techniques to evaluate phenotype-environment correlations in two lakes with trimorphic and two lakes with monomorphic whitefish.

3. Distinct phenotype-environment correlations were evident for each principal niche in whitefish morphs inhabiting trimorphic lakes. Monomorphic whitefish exploited multiple habitats, had intermediate morphology, displayed increased variance in gillraker-counts, and relied significantly on zooplankton, most likely due to relaxed resource competition.

4. We suggest that the ecological processes acting in the trimorphic lakes are similar to each other, and are driving the adaptive evolution of whitefish morphs, possibly leading to the formation of new species.

Key-words: ecological speciation, ecomorphology, gill rakers, niche, stable isotope analysis

Introduction

Adaptive radiation describes the rapid evolution of a common ancestor into an array of species that exploit different ecological niches (Grant & Grant 2008). Understanding what processes drive and maintain adaptive radiation is a central question in evolutionary ecology. The association between a particular morphology and a specific niche, referred to as phenotype-environment (PE) correlation, is recognized as an important factor in adaptive radiation (Schluter 2000). A general pattern has been reported from a series of adaptive radiations where divergence in the feeding apparatus is correlated with a specific trophic niche (Price 2008). This is especially evident in the divergence of beak morphology in Darwin's ground-finches *Geospiza* spp., where beak size and shape facilitates increased efficiency in foraging on specific sized seeds (Grant 1999). In adaptive radiations in fish, PE-correlations are generally related to foraging traits, i.e. associations between particular head, jaw and gillraker configurations or shapes and specific trophic niches (Schluter 1996; Clabaut *et al*. 2007), but few studies have provided new insights regarding PE-correlations in fishes.

Fishes of various evolutionary lineages inhabiting post-glacial lakes are renowned for their ecological variability (Robinson & Parsons 2002), and the adaptive radiation of these fishes has generally occurred along the pelagic-littoral

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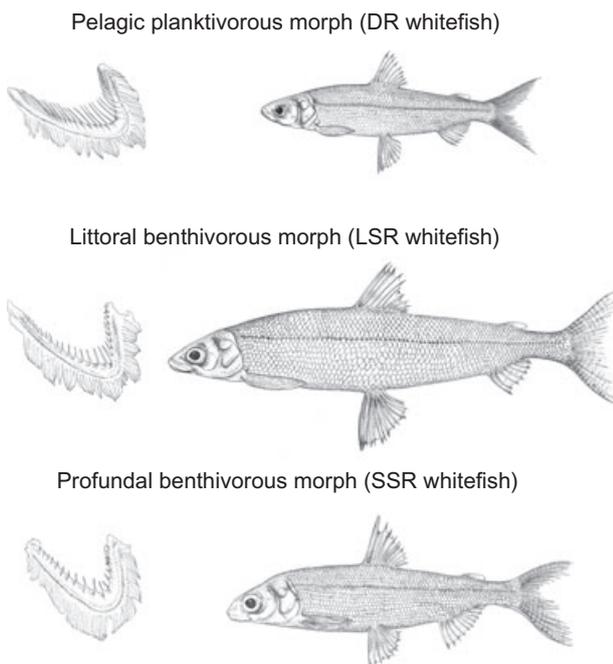
habitat axis, where pelagic morphs are morphologically adapted to forage on zooplankton and littoral morphs are adapted to feed on larger benthic macroinvertebrate prey (Robinson & Parsons 2002). Some Arctic charr *Salvelinus alpinus* L. lakes also support sympatric littoral and profundal morphs (Knudsen *et al.* 2006; Power *et al.* 2009), but divergence to all three principal lake habitats has only been described in coregonid fishes (Lindsey 1981; Kahilainen *et al.* 2004). The European whitefish *Coregonus lavaretus* L. (whitefish) is the most divergent of all coregonid fishes (Svärdson 1979; Bernatchez 2004; Hudson *et al.* 2007) and in contrast to other northern polymorphic fish lineages, exhibits distinct morphs (Fig. 1) exploiting three major lake niches *i.e.* littoral, profundal and pelagic (Kahilainen, Lehtonen & Könönen 2003; Kahilainen *et al.* 2004; Kahilainen, Alajärvi & Lehtonen 2005). Bernatchez (2004) noted that the whitefish species complex potentially fulfils the four detection criteria of adaptive radiation, i.e. common ancestry, rapid speciation, phenotype-environment correlation and trait utility (Schluter 2000). The common ancestry criterion is supported by phylogeographic studies indicating that whitefish populations in northern Fennoscandian (i.e. the continental part of Scandinavia) lakes have originated from a single northern lineage (Østbye *et al.* 2005). Furthermore, whitefish have rapidly radiated into pelagic and littoral morphs independently in many lakes and watercourses (Østbye *et al.* 2006). In this study, we evaluate PE-correlations in whitefish morphs using multiple ecomorphological methods, with particular consideration to examining patterns beyond the littoral-pelagic

resource axis. Here, use of the third major niche in lakes i.e. profundal habitats has not been extensively studied, and ecomorphological adaptations to this niche are generally poorly understood.

Traditionally, variation in gill raker traits have been compared with habitat use and stomach content data: however, the use of stable isotope analysis (SIA) has recently increased throughout ecology (Grey 2006) including studies of putative radiations (Helland *et al.* 2008; Wolf *et al.* 2008) and has considerable potential in studies of evolutionary ecology. This partly reflects the ability of stable isotopes to reveal not only what a consumer has assimilated (Post 2002), but also due to isotopic differences between habitats, where it has been feeding (Harrod *et al.* 2005). A further utility is that SIA allows researchers to examine the existence of individual specialization and ecological variation in apparently homogenous consumer populations (Matthews & Mazumder 2004; Harrod *et al.* 2005; Araújo *et al.* 2007). As such, the exploitation of the principal lake habitats (littoral, pelagic and profundal) is likely to be reflected in isotopic values of different individuals. Due to the close association between morphology and trophic ecology, it is likely that any trophic specialism would also be reflected through general morphological adaptations to a particular niche.

Recent developments in how biologists describe variation in shape using geometric morphometrics (Parsons, Robinson & Hrbek 2003; Adams, Rohlf & Slice 2004) have had considerable success in identifying different groups of consumers based on shape variation (Zimmerman *et al.* 2009) and the adaptive character of shape variation (Schluter *et al.* 2004; Clabaut *et al.* 2007). The combination of geometric morphometrics and SIA potentially provides a useful means to examine PE-correlations.

Here, we examined ecological variation and PE-correlations in whitefish through a multidisciplinary approach, using both gillrakers and body shape (geometric morphometrics) for phenotype and both short- (stomach contents analysis (SIC)/habitat use) and long-term (SIA) measures of resource use. We compared two polymorphic lakes (named here as trimorphic lakes) inhabited by three whitefish morphs which were named according to body size and gillraker-counts as: small sparsely rakered whitefish (SSR), large sparsely rakered whitefish (LSR) and densely rakered (DR) whitefish (Fig. 1), with two monomorphic lakes that only supported monomorphic populations of the LSR whitefish. We suggest that the DR and SSR forms represent specialist whitefish morphs and therefore expected to see repeated patterns of resource segregation between morphs in trimorphic lakes. Furthermore, we expected that in monomorphic lakes, where LSR whitefish not subject to putative competition from more specialist whitefish morphs, LSR whitefish would show evidence of increased zooplanktivory, as zooplankton typically represents a preferred prey for lacustrine whitefish, as indicated in long-term field experiment (Heikinheimo, Miinalainen & Peltonen 2000). Here, planktivory represented an advantageous feeding strategy in terms of both increased consumption and growth rates in several different whitefish

Pelagic planktivorous morph (DR whitefish)

Littoral benthivorous morph (LSR whitefish)

Profundal benthivorous morph (SSR whitefish)

Fig. 1. Whitefish morphs and gill raker shape in trimorphic lakes. Typical first gill arch (left), body shape (right) and niche utilization of whitefish morphs (DR = densely rakered, LSR = large sparsely rakered and SSR = small sparsely rakered). Relative body size in the figure reflects the average size differences observed in study lakes.

populations. The increased growth rate associated with zoo-planktivory may reflect the greater energetic return of zoo-plankton relative to alternative, e.g. benthic prey (Cummins & Wuycheck 1971) which may also incur greater costs associated with handling or digestion (Werner & Mittelbach 1981). We also expected to find evidence of reduced ecological and morphological variation in LSR whitefish from trimorphic lakes, due to ecological character displacement due to competitive interactions with the specialist whitefish morphs.

If variation in whitefish morphology reflects adaption to particular environments, we expected to find a general pattern of resource-correlated morphology in all lakes (Schluter & McPhail 1992; Araújo *et al.* 2008; Bolnick & Lau 2008). We made the following predictions: (1) In trimorphic lakes, we hypothesized there would be clear correlation between habitat use and gill raker number; (2) In trimorphic lakes, we expected that measures of the short (stomach contents) and long-term (stable isotope values) diet of each morph would reflect their gill raker morphology and shape; and (3) Fish from monomorphic lakes should exhibit both an intermediate gill raker morphology and shape, but rely heavily on zoo-plankton due to their status as preferred prey; and (4) Consistent with reduced resource competition in the absence of specialist morphs, we expected monomorphic populations to display greater variance in gill raker morphology, body shape and niche width relative to LSR whitefish from trimorphic lakes.

Materials and methods

STUDY AREAS AND SAMPLING

Whitefish were collected from four oligotrophic subarctic lakes in Finnish Lapland (Table S1). Lakes Vuontisjärvi (Vuontis) and Kilpisjärvi (Kilpis), have monomorphic populations consisting of a large sparsely rakered (LSR) whitefish. Lakes Muddusjärvi (Muddus) and Paadarjärvi (Paadar) have trimorphic whitefish populations, including densely rakered (DR), small sparsely rakered (SSR) and LSR whitefish (Fig. 1, Table S1). Kilpis is situated in the north-west of Finland, whilst the remaining lakes are situated in a single watercourse in the north-east. Kilpis is also located at a higher altitude than the other lakes. Lakes are ice-free from June to November. Temperature and oxygen conditions in the three principal habitats, i.e. littoral and pelagic (where light levels are > 1% of that at the surface) and profundal (< 1% surface light levels) are suitable for whitefish year round (Table S1). The abundance and diversity of other fishes is low in each habitat and whitefish dominate (85–96% by abundance of survey catch) the fish community in each lake (Table S1).

Whitefish were sampled from all habitat types during ice-free periods between 2002–2004 using multimesh gill nets, trawl and seine (details in Kahilainen & Lehtonen 2003; Kahilainen *et al.* 2004). Whitefish catches were field-identified to morph according to appearance and gillraker morphology (Kahilainen & Østbye 2006). Samples were immediately frozen (−20 °C) and transported to the laboratory. Putative prey samples were collected from each study lake for inclusion in stable isotope mixing models. Benthic macroinvertebrates were collected from littoral (depth 1–5 m), and profundal (15–40 m) areas using an Ekman grab (area 272 cm²). Profundal sampling was limited to 30 m in Vuontis (maximum depth = 31 m). Zooplankton were collected throughout the water column with a Limnos tube sampler (length 1 m, volume 7·1 L), and concentrated on a 50 μm mesh.

MEASUREMENTS

Whitefish were defrosted, and each fish was given identification code after which total length (± 1 mm) and blotted mass (± 0·1 g) were recorded. Gillrakers were counted from the first left arch under a binocular microscope and stomachs were removed for diet analysis. Prey items were identified and the relative contribution of each food category to the diet of individual whitefish was estimated using the points method (Hynes 1950). Due to cost and time considerations, SIA and geometric morphometrics (GM) were limited to *c.* 30 individuals per morph/lake combination representing the observed size range in the lake.

NICHE OVERLAP AND BREADTH

Resource overlap (diet and habitat use) was examined using Schoener's index (1968). Niche overlap values range from 0 (complete niche segregation) to 1 (complete niche overlap). We used a threshold of D ≥ 0·6 to represent biologically significant overlap following Wallace (1981). In order to compare trophic niche breadth, we calculated the Levins' index, B (Levins 1968) for each morph/lake combination. In this case, B can vary from 1 (a single food category) to 10 (equal representation of all 10 food categories). Levins' index was also calculated to examine niche breadth of the different morphs in terms of habitat use (i.e. use of profundal, littoral or pelagic habitats). Here B can vary between 1 (use of a single habitat type) to 3 (equal use of all three habitats).

STABLE ISOTOPE ANALYSES

A small sample of dorsal muscle tissue was excised from each whitefish. Samples were dried, ground, weighed into tin cups and analysed for carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope ratios and elemental C and N composition (see Harrod *et al.* 2005 for details). Repeated analyses of internal roach *Rutilus rutilus* (L.) standards inserted after every six samples resulted in typical isotopic precision of < 0·1 ‰ (carbon) and < 0·3 ‰ (nitrogen).

C:N ratios of muscle varied between the morphs (ANOVA: $F_{7,234}$ = 11·5, P < 0·001), suggesting variation in lipid concentrations (Kiljunen *et al.* 2006). As lipids are depleted in ^{13}C (DeNiro & Epstein 1977), any variation in lipid concentrations between morphs could influence our comparisons of δ^{13}C. Therefore all δ^{13}C data were arithmetically lipid-normalized according to Kiljunen *et al.* (2006). Whitefish SIA data (δ^{13}C and δ^{15}N) were often heteroscedasitic, and non-normally distributed. However, F_{max} ratios were < 10, and sample sizes were large, and effectively identical for each morph (n_{range} = 29–31). Hence, ANOVA-based comparisons should be robust (Tabachnick & Fidell 2001). In order to provide information on long-term habitat uses and to compare assimilation patterns from putative prey from different habitats between the different whitefish morphs we used SIAR (Parnell *et al.* 2010), a Bayesian mixing model that runs under R (R Development Core Team, 2009), and allows the inclusion of errors reflecting isotopic estimates of both putative prey resources and trophic fractionation (Panell *et al.*, 2010). Firstly, we used δ^{13}C and δ^{15}N values from individual whitefish and calculated mean (± SD) δ^{13}C and δ^{15}N values from putative prey from littoral, profundal and pelagic habitats of each study lake to estimate the mean (± 95% credibility intervals) proportion of prey assimilated by the different whitefish morphs from each of the three major habitats.

We then examined individual consumption patterns. In all cases we used standard trophic fractionation values based on mean ($\pm$SD) estimates for muscle tissue from McCutchan *et al.* (2003 : $\Delta^{13}C = 1\cdot3 \pm 0\cdot30, \Delta^{15}n = 2\cdot9 \pm 0\cdot32$).

GENERAL STATISTICS

We compared variation in mean length, gill raker (GR) counts, $\delta^{13}C$ and $\delta^{15}N$ using analysis of variance (ANOVA), and used Bonferroni-adjusted *post hoc* comparisons to indicate significant ($P < 0\cdot05$) variation between morphs. Levene's tests were used to compare variance values for GR counts and shape between LSR whitefish from monomorphic and trimorphic lakes. Dietary comparisons were complicated by differences in sample size among morphs, and bootstrapping (Efron & Tibshirani 1993) was used to generate (n bootstraps = 1000) robust estimates of mean prey proportions for sample size. All reported errors with means are 95% confidence intervals. Unless noted otherwise, statistics were produced through SYSTAT 12.02.00 (SYSTAT Software Inc, Chicago, IL, USA).

GEOMETRIC MORPHOMETRICS

We used a landmark-based geometric method to characterize shape variation (Adams, Rohlf & Slice 2004; Zelditch *et al.* 2004). Individual shape was assessed from the Cartesian coordinates of 20 two-dimensional landmarks from the right flank of each fish (Fig. S1) generated using tpsDig v 2·05 (Rohlf 2006). A generalized Procrustes analysis (GPA) was then conducted using MorphoJ v1.00k (Klingenberg 2008). We examined the allometric effect of size on shape by conducting a multivariate regression of shape on $\log_{10}$ centroid size, with whitefish morph identified as a sub-group. Size had a significant ($P = 0\cdot0002$) effect on shape and subsequent analyses were conducted on size-corrected Procrustes distances, removing any influence of allometry on shape. Principal Component scores were calculated from size-corrected data and examined through Discriminant Function Analysis (DFA) to estimate shape differences among morphs. To demonstrate shape variation along the first discriminant function axis (DFA1), we produced thin-plate deformation grids in MorphoJ. In addition, we calculated individual Procrustes distances from group means in tpsSMALL (Rohlf 2003) to compared shape

variance among morphs (details in Cardini, Thorington & Polly 2007).

PE-CORRELATIONS

PE-correlations were tested both with established (gill rakers, diet, habitat) and more recently developed (morphological shape and isotopes) methods. For PE-correlations at the individual-level, we used the Partial Least Squares function of MorphoJ to compare shape with the the proportion of pelagic prey in the diet (arcs$\sqrt{}$x transformed) from SIAR. To examine PE-correlations at the population level, we compared morphology using mean morphological shape scores from DFA1 and mean gill raker counts to environment indicators i.e. mean proportions of pelagic prey (SCA and SIA) and habitat use (all arcs$\sqrt{}$x transformed) using Spearman's rank correlation. As sample sizes were small (n pairs = 8) we produced Bootstrapped estimates (n bootstraps = 1000) for the estimates of correlation coefficients. We used one-tailed probability values, as we predicted that whitefish phenotype would be predictably shift along a resource gradient (e.g. increased use of pelagic resources).

Results

GILL RAKERS AND BODY SIZE

Mean gill raker counts (Figs 1 and 2) differed among the morphs (Fig. 2a: ANOVA $F_{7,2058} = 4253\cdot8$, $P < 0\cdot001$). Gill raker counts were lowest in SSR whitefish ($17 \pm 0\cdot2$), intermediate in LSR whitefish ($25 \pm 0\cdot3$) and highest in DR whitefish ($34 \pm 0\cdot1$). LSR whitefish showed greater variance ($F_{447,308} = 2\cdot43$, $P < 0\cdot001$) in gill raker counts from monomorphic ($\sigma = 9\cdot2$) than trimorphic ($\sigma = 3\cdot77$) populations. There were no significant correlations between gill raker count and body size (total length) in any of the whitefish morphs (all $P > 0\cdot05$). Length comparisons were conducted using $\log_{10}$ transformed data, but untransformed lengths are given here for clarity. Morphs differed in mean length (ANOVA: $F_{7,2364} = 113\cdot7$, $P < 0\cdot001$). There was a consistent size
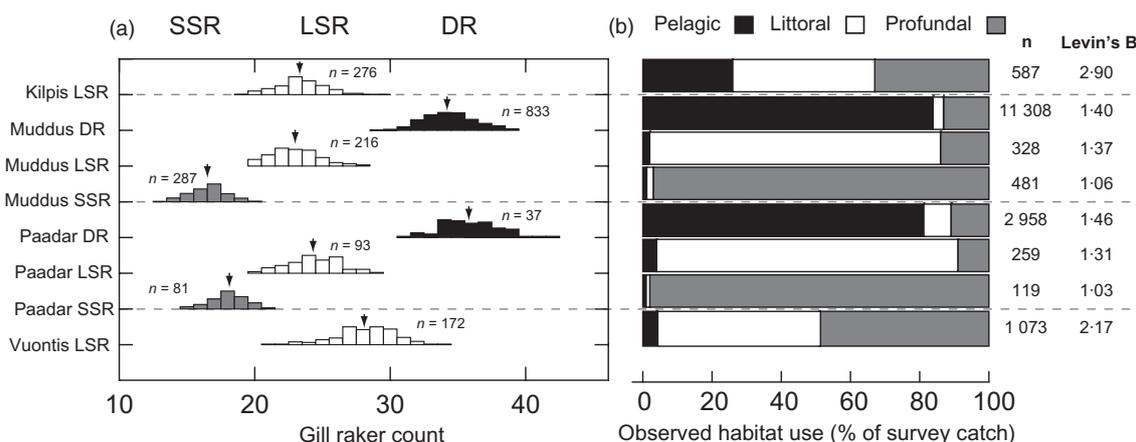
Fig. 2. Phenotypic traits of whitefish morphs and respective foraging environments. (a) Gill raker frequency distributions and (b) habitat use from whitefish morphs collected from the four study lakes (DR = densely rakered, LSR = large sparsely rakered and SSR = small sparsely rakered). Arrows denote mean gillraker values, habitat is based on percentage contribution to survey catch (n) and Levin's B indicates niche breadth (1 = single habitat, 3 = all habitats used in equal proportions).

Table 1 Variation in niche overlap among whitefish morphs in the study lakes. Pairwise comparisons of Schoener's niche overlap index (D) based on: (A) diet (above diagonal) and (B) habitat use (below diagonal) and between whitefish morphs

		(A) Diet (stomach contents)							
		Kilpis	Muddus			Paadar			Vuontis
		LSR	DR	LSR	SSR	DR	LSR	SSR	LSR
(B) Habitat use (catch data)	Kilpis LSR	–	0·52	0·47	0·27	0·35	**0·67**	0·34	**0·79**
	DR	0·43	–	0·11	0·18	0·56	0·24	0·21	0·57
	Muddus LSR	0·57	0·18	–	0·53	0·05	**0·60**	0·34	0·35
	SSR	0·36	0·16	0·17	–	0·11	0·37	**0·64**	0·26
	DR	0·44	**0·95**	0·20	0·14	–	0·19	0·17	0·37
	Paadar LSR	0·54	0·16	**0·95**	0·12	0·21	–	0·41	0·59
	SSR	0·35	0·15	0·16	**0·99**	0·12	0·11	–	0·30
	Vuontis LSR	**0·78**	0·21	**0·63**	0·52	0·22	**0·60**	0·51	–

SSR = small sparsely rakered, LSR = large sparsely rakered, DR = densely rakered. Values of 0 indicate no overlap and values of 1 complete overlap. Biologically significant values (≥0·6) are shown in bold.

Table 2 Diet composition of whitefish morphs in study lakes. Stomach contents as bootstrapped mean % dietary composition (95% CI in parentheses) by morph

	Kilpis	Muddus			Paadar			Vuontis
Prey category	LSR	DR	LSR	SSR	DR	LSR	SSR	LSR
Copepods	4·7 (0·11)	25·6 (0·08)	1·7 (0·07)	11·0 (0·13)	70·0 (0·62)	7·8 (0·30)	17·4 (0·44)	7·4 (0·17)
Pelagic cladocerans	35·4 (0·26)	58·9 (0·08)	1·7 (0·08)	0·2 (0·02)	27·2 (0·57)	9·6 (0·33)	0 (–)	43·4 (0·54)
Eurycercus sp.	29·8 (0·24)	0·1 (0)	18·9 (0·25)	9·4 (0·12)	0 (–)	33·0 (0·57)	18·4 (0·59)	35·8 (0·48)
Chironomid larvae	6·9 (0·12)	3·8 (0·04)	23·3 (0·26)	30·8 (0·17)	0 (–)	9·7 (0·39)	6·5 (0·19)	1·3 (0·06)
Chironomid pupae	0·3 (0·02)	1·7 (0·03)	23·5 (0·29)	8·3 (0·11)	0·1 (0·02)	0·1 (0·02)	0 (–)	2·4 (0·13)
Bivalves	4·1 (0·07)	0 (–)	6·7 (0·10)	37·0 (0·17)	0 (–)	8·0 (0·29)	57·6 (0·76)	2·8 (0·09)
Gastropods	10·4 (0·17)	0 (–)	11·3 (0·20)	0 (–)	0 (–)	10·2 (0·43)	0 (–)	1·8 (0·11)
Large insect larvae	0 (–)	0·7 (0·02)	9·7 (0·15)	1·6 (0·05)	0·1 (0·01)	20·0 (0·54)	0·1 (0·02)	2·0 (0·14)
Surface insects	7·1 (0·16)	9·0 (0·06)	1·3 (0·07)	0 (–)	2·6 (0·27)	1·6 (0·22)	0 (–)	2·0 (0·13)
Others	1·2 (0·07)	0·2 (0·01)	1·7 (0·06)	1·7 (0·06)	0 (–)	0 (–)	0 (–)	1·3 (0·09)
% Pelagic prey	47·5 (0·33)	95·1 (0·13)	28·5 (0·31)	20·7 (0·18)	98·3 (0·51)	18·7 (0·49)	17·4 (0·44)	55·2 (0·61)
% Benthic prey	52·5 (0·33)	4·9 (0·13)	71·5 (0·31)	79·3 (0·18)	1·7 (0·51)	81·3 (0·49)	82·6 (0·44)	44·8 (0·61)
Levin's B	4·18	2·37	5·77	3·84	1·77	5·25	2·50	3·08
n	311	872	264	335	96	126	84	173

SSR = small sparsely rakered, LSR = large sparsely rakered, DR = densely rakered and lake (Kilpis and Vuontis = monomorphic, Muddus and Paadar = trimorphic). Levins' index of trophic niche width and the number of stomachs (*n*) examined are also shown. The % contribution of pelagic prey items was calculated as the sum of copepods, pelagic cladocerans, chironomid pupae and surface insects. All other prey categories are considered to be benthic.

hierarchy between the morphs (Fig. 1): LSR whitefish were largest (mean TL ± 95% CI = 201 ± 4 mm); SSR whitefish intermediate (167 ± 4 mm) and DR whitefish the smallest (142 ± 3).

HABITAT

Whitefish habitat use varied in the study lakes (Fig. 2b), and niche overlap between different morphs was low (mean Schoener's D = 0·39) (Table 1). In trimorphic lakes, the different morphs showed high levels of habitat fidelity: DR whitefish were mainly captured from pelagic, LSR whitefish from littoral, and SSR whitefish from profundal habitats (Fig. 2b). In monomorphic lakes, LSR fish were caught either in all habitats equally (Kilpis) or predominantly in littoral and profundal habitats (Vuontis). Parallel PE-correlations were

evident in trimorphic lakes: SSR whitefish with the lowest gill raker counts used profundal, intermediate gill rakered LSR whitefish used littoral, and DR whitefish with highest gill raker counts used pelagic (Fig. 2). In contrast, LSR whitefish from monomorphic lakes used all habitat types indicating the lack of any association between phenotype and environment, showing increased habitat niche width values (Fig. 2b: Kilpis = 2·9, Vuontis = 2·17) relative to LSR whitefish from trimorphic lakes (Muddus = 1·37, Paadar = 1·31).

DIET

Niche segregation was clear in polymorphic lakes: DR whitefish were pelagic planktivores, SSR whitefish were profundal benthivores and LSR whitefish were littoral benthivores (Fig. 2, Tables 1 and 2). In contrast, monomorphic LSR

whitefish consumed considerably more pelagic prey (51%) than their polymorphic counterparts (24%). LSR whitefish from trimorphic lakes were typically more benthivorous (combined mean for Muddus and Paadar = 76%) whereas pelagic zooplankton was more important for LSR whitefish from monomorphic lakes. DR whitefish consumed pelagic prey (combined mean = 97%), especially zooplankton (90% of diet). SSR whitefish were benthivorous (combined mean of benthic prey = 81%), consuming mainly benthic macroinvertebrates (ca. 65%).

In general, LSR whitefish generally fed on a greater range of prey taxa (mean niche width: B = 4·6) than the SSR (3·2) or DR (2·1) whitefish. Monomorphic LSR whitefish had reduced trophic niche width (Kilpis = 4·18, Vuontis = 3·08) relative to LSR whitefish from trimorphic lakes (Muddus = 5·77, Paadar = 5·25).

In trimorphic lakes, evidence for parallel dietary segregation was supported by extremely low within-lake dietary overlap among SSR, LSR and DR whitefish (Table 1: Muddus = 0·27, Paadar = 0·26). Comparisons of similar morphs across lakes showed niche overlap indices were either close to (DR: D = 0·56) or over (LSR: D = 0·60, SSR: D = 0·64) the threshold of biological significance. LSR whitefish diets overlapped across the lakes (range = 0·47–0·79; mean = 0·63). The greatest diet overlap (0·79) was between mono-

morphic LSR whitefish, whereas overlap between mono- and polymorphic LSR whitefish was lower (mean = 0·52).

STABLE ISOTOPE ANALYSIS

Similar whitefish morphs clustered together in isotopic space, whereas mean $\delta^{13}C$ values differed among morphs (Fig. 3a; ANOVA: $F_{7,234} = 46·9$, $P < 0·001$). *Post hoc* tests (Bonferroni) show that on average LSR whitefish from Paadar had enriched $\delta^{13}C$ values ($-23·8 \pm 0·61$ ‰) relative to the other three lakes where $\delta^{13}C$ values overlapped (Kilpis $-26·3 \pm 0·47$ ‰; Muddus, $-26·6 \pm 0·53$ ‰; Vuontis $-25·2 \pm 0·92$ ‰). Mean $\delta^{13}C$ values were slightly enriched in DR and SSR whitefish in Paadar (DR $-27·8 \pm 0·27$ ‰; SSR $-28·4 \pm 0·41$ ‰) relative to Muddus (DR $-28·7 \pm 0·31$‰; SSR $-28·7 \pm 0·34$‰), but this was not statistically significant (DR $P = 0·47$; SSR $P = 1$). Mean $\delta^{15}N$ values differed between morphs (ANOVA: $F_{7,234} = 113·17$, $P < 0·001$), but not within-morph (all comparisons $P = 1$, Fig. 3a).

Prey $\delta^{13}C$ and $\delta^{15}N$ values in pelagic, littoral and profundal habitats for each lake are provided in Table S2, but are shown pooled in Fig. 3 for purposes of clarity as they showed common patterns. $\delta^{13}C$ values of prey varied between different lake habitats, and benthic macroinvertebrates became increasingly ^{13}C depleted with depth,
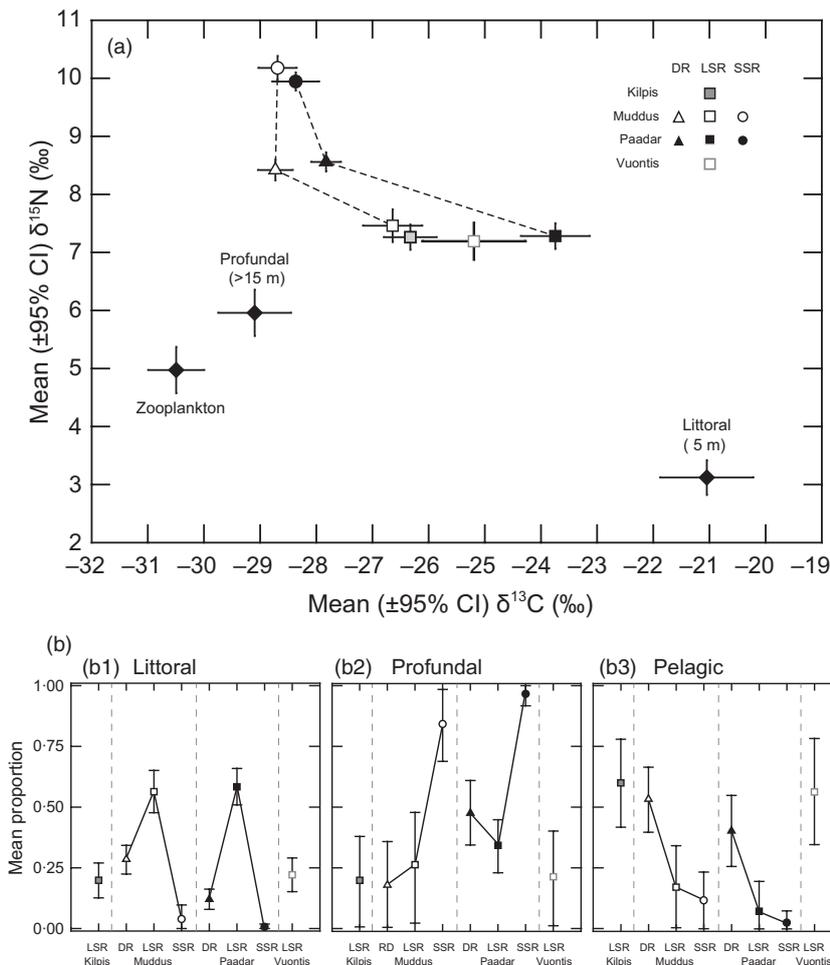
Fig. 3. Long-term niche segregation of whitefish morphs shown through variation in $\delta^{13}C$ and $\delta^{15}N$: (a). Lakes, whitefish morphs (DR = densely rakered, LSR = large sparsely rakered and SSR = small sparsely rakered) and prey resources (benthic macroinvertebrates from profundal and littoral, zooplankton = pelagic) are labelled in figure. Dashed lines join morphs found in the trimorphic lakes. Principal energy sources for whitefish morphs estimated with isotope mixing model SIAR (b). Comparison of mean (±95% Bayesian credibility intervals) estimates of dietary proportions of prey from littoral (b1), profundal (b2) and pelagic (b3) habitats is shown for each morph.
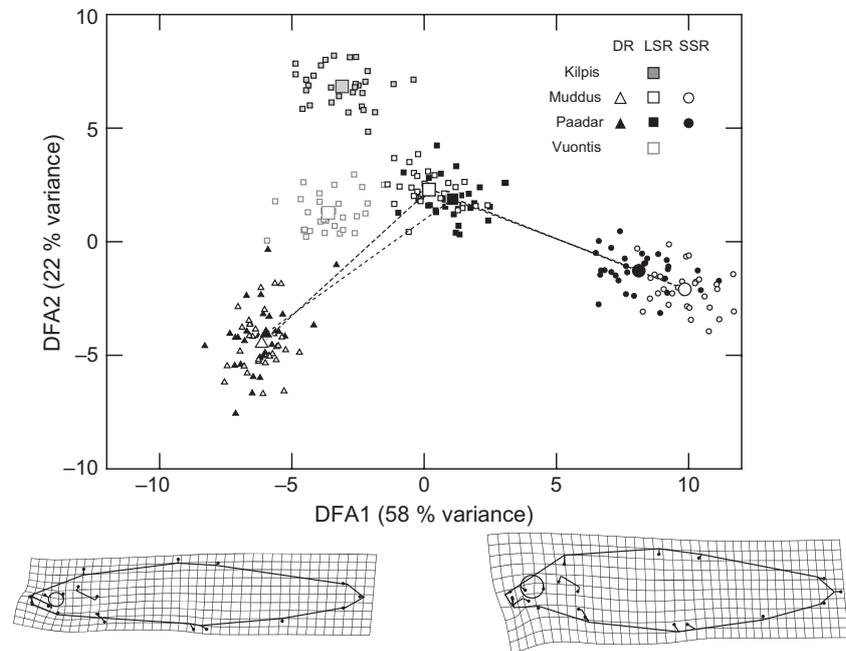
Fig. 4. Shape analysis of whitefish morphs (DR = densely rakered, LSR = large sparsely rakered and SSR = small sparsely rakered). Variation in whitefish phenotype (body shape) presented as discriminant function plot with group centroids for mono- and trimorphic lakes. Deformation grids show shape variation along the first DF axis exaggerated (×2) to highlight the differences between the morphs.

with mean $\delta^{13}C$ values (pooled across lakes) ranging between $-21\cdot0$ ($\pm 0\cdot8$) ‰ in littoral, and $-29\cdot1$ ($\pm 0\cdot6$) ‰ in profundal samples. Zooplankton were the most ^{13}C depleted of prey ($-30\cdot5 \pm 0\cdot5$ ‰). Prey $\delta^{15}N$ values differed between lake habitats. Profundal benthic macroinvertebrates were most enriched in $\delta^{15}N$ ($6\cdot0 \pm 0\cdot7$‰), intermediate from zooplankton ($5\cdot0 \pm 0\cdot4$ ‰) and ^{15}N depleted from littoral habitats ($3\cdot1 \pm 0\cdot3$‰).

The results of the isotope mixing model (SIAR) revealed marked and repeated differences in assimilation patterns in LSR whitefish from monomorphic and trimorphic lakes (Fig. 3b). By comparing the relative contribution of littoral, profundal and pelagic prey, it was apparent that littoral prey were most important to LSR whitefish from trimorphic (Fig. 3b1 : mean estimated proportions Muddus = 0·56, Paadar = 0·58) but not monomorphic lakes (Kilpis = 0·20, Vuontis = 0·22). Profundal prey were most important to SSR whitefish (Fig. 3b2 : Muddus = 0·84, Paadar = 0·97). Pelagic prey (Fig. 3b3) were important to both DR whitefish (Muddus = 0·5, Paadar = 0·4) and LSR whitefish from monomorphic lakes (Kilpis = 0·60, Vuontis = 0·56). Comparison of the contribution (arcs√x transformed proportion) of the different putative prey resources to individual whitefish belonging to SSR, DR, LSR (monomorphic) or LSR (trimorphic) morphs indicated significant differences (e.g. ANOVA of pelagic resources: $F_{3,237} = 59\cdot5$, $P < 0\cdot001$). Post hoc comparisons showed that LSR whitefish from monomorphic lakes relied on a similar proportion of pelagic prey as zooplanktivorous DR whitefish (Fig. 3b3 : $P = 1\cdot0$).

SHAPE ANALYSIS

We examined fish shape as a means of distinguishing between whitefish morphs and to also compare these differences with

measures of niche utilization such as diet, habitat and SIA. DFA produced seven significant DFs differentiating between morphs, (Table S3 : Wilk's lambda < 0·001, d.f. = 252, 1375, $P < 0\cdot0001$), and the first two DFs accounted for 80% of shape variation (Fig. 4). Jack-knifed comparisons indicated that ≥97% of whitefish were classified to the correct lake/morph combination (Table S4). SSR whitefish were characteristically deep-bodied, with a large eye and subterminal mouth (Fig. 4 shows variation along DF1 in terms of a deformation grid). DR whitefish had a fusiform body, with eyes situated lower in the head relative to SSR or LSR whitefish and a superior mouth. LSR whitefish were intermediate in body shape to the DR and SSR whitefish, but had a terminal mouth. DFA2 (deformation grids not shown) largely described variation in head size, with SSR and DR whitefish having enlarged heads relative to LSR whitefish.

DF scores indicated clustering of similar morphs in trimorphic lakes, whereas LSR from monomorphic lakes clustered between LSR and DR whitefish from trimorphic lakes. Centroids of similar morphs from different lakes were compared with ANOVA: Muddus and Paadar LSR whitefish were similar ($P = 1$), as were DR whitefish ($P = 1$) in both DFA1 and DFA2. SSR whitefish DF1 scores differed slightly between lakes ($P = 0\cdot01$), but DF2 scores were similar ($P = 1$). DF1 scores of mono- and trimorphic LSR whitefish differed ($P < 0\cdot001$), with monomorphic LSR whitefish having DF1 scores closer to that of DR whitefish ($P = 0\cdot07$). DF2 scores of LSR whitefish were similar in all cases ($P = 1$) apart from Kilpis LSR ($P < 0\cdot001$). Shape variance was similar across the different whitefish morphs (Levene's test of Procrustes distances from group mean: $F_{7,233} = 1\cdot45$, $P = 0\cdot18$), and there was no evidence of increased shape variance in monomorphic LSR whitefish relative to their counterparts from trimorphic lakes (Levene's test: $F_{1,118} = 1\cdot22$, $P = 0\cdot27$).
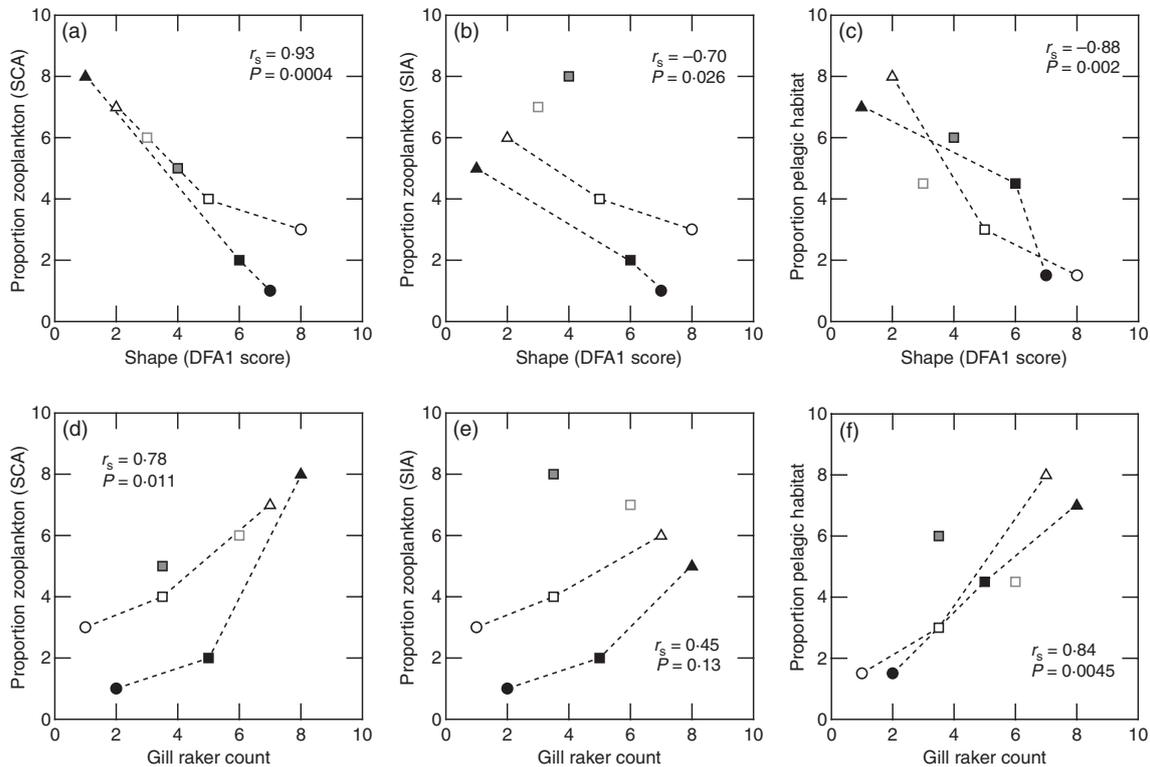
Fig. 5. Phenotype-environment correlations of whitefish at the population level. Upper row (5a–c) shows correlations (Spearman's rank, one-tailed *P*-values) between shape (mean DFA1 scores) and niche (mean proportion of pelagic diet, SIA and habitat proportions) across whitefish morphs. The lower row (5d–f) presents correlations between mean gill raker count and the same niche parameters. Dashed lines join morphs found sympatrically in the trimorphic lakes. The format of markers follows that of (Figs 3 and 4).

PE-CORRELATIONS

Shape and the proportion of pelagic prey in the diet (SIAR) of whitefish from all morph/lake combinations showed a significant individual-level PE-correlation (partial least squares analysis: Correlation = 0·213, *P* = 0·012). At the population level, PE-correlations were significant between both shape and gill raker counts and utilization of the pelagic niche (see Fig. 5 for details) in all but one case. The level of zooplanktivory in monomorphic LSR whitefish revealed by SIA (Fig. 3b3) reduced the strength of the association between zooplanktivory estimated by both SIA and shape (Fig. 5b), and gill raker count (Fig. 5e). When the monomorphic LSR morphs were discounted from these analyses, the strength of the relationship between the use of pelagic resources estimated through SIA and shape ($r_s = -0·83$, *P* = 0·02) and gill raker counts ($r_s = 0·71$, *P* = 0·057) both increased.

Discussion

We demonstrated a similar divergence by whitefish to the principal lake niches (littoral, profundal and pelagic) in both trimorphic lakes, whereas monomorphic whitefish displayed lake-specific and far less specialized morphology and niche utilization. The profundal morph was most distinct, both in terms of both ecology and morphology. Although we revealed an overall PE-correlation between whitefish pheno-type (shape) and the level of consumption of a preferred whitefish prey (zooplankton), PE-correlations were most marked in trimorphic lakes with distinct pelagic, profundal and littoral morphs (Fig. 1). Monomorphic LSR whitefish showed a marked reliance on zooplanktivory relative to LSR fish from trimorphic lakes. The differences in diet, morphology and habitats use of of LSR whitefish in the two lake types suggest that LSR whitefish show ecological character displacement when found sympatry with the more specialized whitefish morphs.

Fish radiations in post-glacial lakes typically follow the divergence of morphs along the pelagic-littoral habitat axis (Schluter & McPhail 1993; Robinson & Wilson 1994). However, only a few studies have demonstrated divergence beyond pelagic and littoral morphs within a single system (Lindsey 1981; Skúlason & Smith 1995). To our knowledge, this study provides the first evidence of divergence by a polymorphic fish to the major available habitats (i.e. profundal, littoral and pelagic areas) in multiple subarctic lakes. In our study, niche overlap in sympatric morphs was extremely low in terms of habitat and diet. Monomorphic LSR whitefish expanded their niche to utilize all habitat types, but contrary to expectations (Roughgarden 1972; Bolnick 2001) actually showed a more restricted diet, consuming more zooplankton prey. We suggest that these differences between monomorphic and trimorphic LSR whitefish reflect resource competition in trimorphic lakes, where the presence of the specialist whitefish morphs drives the generalist LSR morph to utilize

an alternative, less optimal niche (Werner & Hall 1979). This mirrors the situation in other examples of competitive release in adaptive radiations, including Darwin's finches and three-spined sticklebacks *Gasterosteus aculeatus* L. (Schluter & McPhail 1992; Grant 1999). In the Galapagos Islands, specialized finch species segregate available resources in sympatry, but when found in allopatry individual species are able to expand their niche (Grant 1999). In three-spined sticklebacks, allopatric populations are typically intermediate with regards to niche utilization, but when found in sympatry, show an exaggerated divergence into limnetic and benthic morphs (Schluter & McPhail 1992; Araújo *et al.* 2008). Our work extends these patterns, where monomorphic LSR whitefish had intermediate niche utilization, whereas adaptive divergence to littoral, pelagic and profundal was evident in trimorphic lakes. LSR whitefish are present in almost all lakes with fish in the study region, and this ancestral morph apparently only diverged into pelagic and profundal morphs in a subset of lakes (Østbye *et al.* 2005, 2006).

A crucial question in studies of adaptive radiation is the status of the common ancestor (Schluter 2000). The ecomorphology of monomorphic LSR whitefish might reflect the fundamental niche of whitefish, whilst that of trimorphic LSR whitefish represents adaptation to a realized niche (Hutchinson 1957), where these fish are subject to competition from planktivorous DR whitefish morph. For instance, monomorphic LSR whitefish consumed and assimilated more zooplankton than trimorphic LSR whitefish and were morphologically intermediate between polymorphic LSR and DR whitefish. In trimorphic populations, and under likely resource competition from the specialized DR and SSR whitefish, the realized niche of LSR whitefish is restricted to sub-littoral/littoral habitats inducing a morphology more associated with a benthivorous trophic niche. If polymorphic LSR whitefish are restricted to littoral habitats due to resource competition, they may have to widen their niche and non-selectively consume available (and possibly sub-optimal) prey. It is likely that ontogenetic diet shifts in trimorphic LSR whitefish will be restricted to benthic prey available in the littoral habitats (Kahilainen, Lehtonen & Könönen 2003), whilst monomorphic LSR whitefish are not as restricted in terms of habitat or diet choice. This permits selective consumption of prey, as indicated by a reduced niche breadth, increased consumption of pelagic zooplankton, ontogenetic diet shifts exploiting optimal prey resources in both pelagic and benthic habitats and an intermediate morphology. Such ecomorphology may also be typical in lakes that due to their limnological characteristics do not support habitat-based specialization.

All of the lakes studied here have almost identical fish fauna, and are exclusively dominated by whitefish, suggesting that resource competition is most evident within and among whitefish morphs (Kahilainen, Lehtonen & Könönen 2003; Kahilainen, Alajärvi & Lehtonen 2005). Whitefish represent the principal prey for many piscivores in all of the study lakes (Kahilainen & Lehtonen 2003; Jensen *et al.* 2008) and morph-selective predation likely affects mortality rates, life-

history traits and habitat selection of the different morphs. However, predation alone is unlikely to drive morph divergence, whilst resource availability represents a more likely explanation of divergence. Three of the study lakes were colonized by whitefish following glacial retreat along a similar timeframe (*c.* 10 000 years), but divergence to three morphs only apparently occurred in Muddus and Paadar which have a proportionally equal availability of the three major habitats. Vuontis is located close to the trimorphic lakes within the same watercourse, but has very restricted availability of pelagic and profundal habitats (9% of surface area), that likely explains the occurrence of a single LSR whitefish morph. In contrast, Kilpis has abundant availability of both profundal and pelagic (71%) habitats. However, it is located in a different watercourse, at higher altitude, is less productive and has a shorter ice-free period than the other lakes. In the case of Kilpis, prey resource limitation combined with a different colonization history probably represent the most likely reasons for a lack of specialized pelagic and profundal whitefish morphs.

Gill raker number is a highly heritable trait, and has been long used to characterize whitefish morphs (Svärdson 1979; Rogers & Bernatchez 2007) and gill raker traits are also closely associated to feeding ecology (Sanderson *et al.* 2001; Amundsen *et al.* 2004; Kahilainen & Østbye 2006). We also showed a strong association between gillraker number and shape, and whitefish could also be classified to morph with almost 100% accuracy using shape variation alone. The clustering of morphs across trimorphic lakes in the DFA either indicates similar morphological divergence in the different lakes (e.g. Losos, Warheit & Schoener 1997; Rundle & Nosil 2005; Østbye *et al.* 2006) or a common origin. In contrast, monomorphic LSR whitefish did not cluster with their counterparts from trimorphic lakes. The results of the shape analysis indicated an intermediate shape between LSR and DR whitefish, most likely reflecting adaptive morphology to generalized niche utilization in a particular lake in the absence of resource competition from specialized morphs.

The profundal specialist benthivore SSR morph had the most distinct shape, with a series of characteristics of a benthic-feeding mode: a deep, robust body, large eye, subterminal mouth and low gill raker counts (Webb 1984). These traits are likely to aid the location and retention of patchily-distributed scarce benthic prey located in soft sediments in the dark profundal zone (Kahilainen, Lehtonen & Könönen 2003). SCA and SIA both indicated that SSR whitefish ingested and assimilated their energy from profundal sources. LSR whitefish from trimorphic lakes, had intermediate gill-raker-counts with subterminal mouths, and SCA data showed that they largely exploited benthic resources. However the use of isotope mixing models revealed that these were largely of a littoral origin (c.f. with profundal benthic resources utilized by SSR). Planktivorous DR whitefish had a fusiform body, a superior mouth, extreme gillraker-counts and an eye positioned lower in the head relative to other morphs. Such morphology is characteristic of pelagic specialist

zooplanktivores, such as vendace, *Coregonus albula* (L.) (Svärdson 1976), or herring *Clupea harengus* (L.) (Gibson 1988). SIA and SCA of DR whitefish confirmed a marked reliance on pelagic prey resources. Taken collectively, SIA and GM data both supported results from the more traditional means of describing PE-correlations.

Similarly strong PE-correlations have been described in studies of classic adaptive radiations across several vertebrate lineages. Probably the most studied example is morphological variation in Galapagos ground-finches (*Geospiza* spp.), where variation in beak shape is strongly correlated with the size and hardness of seeds selected both by individual birds and different species (Grant 1999). In *Anolis* lizards, hindlimb length is correlated with niche-specific structural characteristics of the trees utilized by different lizards (Losos 1990). In three-spined sticklebacks, the most important traits correlated with environment are overall body size and shape, and the number and length of gillrakers (Schluter & McPhail 1992). All of these classic vertebrate model studies were able to directly or indirectly show the fitness consequences of morphological divergence. Our results are indicative of specialization extending beyond the pelagic-littoral axis to the profundal niche. However, our results are correlative, and the contribution of morphological variation on the fitness of morphs in the different major niches needs to be tested experimentally.

Our multidisciplinary approach provided a powerful tool to evaluate variation and relationships between shape, habitat use and both short- (SCA = recently ingested prey) and long-term (SIA = assimilated prey) niche selection in polymorphic species. Although ecologists typically rely on SCA or similar methods to assess diet, our approach has underlined the utility of SIA as a tool for evolutionary ecologists interested in rapid divergence in closely-related taxa as it provides a long-term signal of individual diet and habitat use, not available through SCA. In this study, we have described a spectacular level of ecomorphological divergence within a single species comparable to that typically associated with different biological species. In lake whitefish *Coregonus clupeaformis* (Mitchill), populations with the greatest morphological differences were also genetically most divergent (Lu & Bernatchez 1999). This suggests significant genetic differences may exist between the sympatric morphs described here. Genetic studies are underway to examine the ancestral origin (mtDNA) and level of reproductive isolation (nDNA) in these populations.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Some limnological (A) and biotic (B) characters of the lakes where allopatric (Kilpis and Vuontis) and sympatric (Muddus and Paadar) whitefish morphs (DR = densely rakered, LSR = large sparsely rakered and SSR = small sparsely rakered whitefish) were sampled. Fish community structure is based on sampling with multimesh (12–60 mm knot-to-knot, Kahilainen *et al.* 2004) gillnets in 2002 for Kilpis, 2000–2001 for Muddus, and 2004 for Paadar and Vuontis. Interannual variation in fish community structure is minor in these lakes (Kahilainen *et al.* 2004, 2003).

Table S2. Mean ± SD δ¹³C and δ¹⁵N values of putative prey from different habitats for the four study lakes.

Table S3. Discriminant function analysis (DFA) outputs for morphological shape variation. Variation was explained with seven DF axes, with the first two explaining *c*. 80% of shape variation of whitefish morphs.

Table S4. Jack-knifed classification matrix of whitefish morphs (SSR = small sparsely rakered, LSR = large sparsely rakered and DR = densely rakered) – comparison of *a priori* classification by gill raker characteristics and classification after multivariate analysis of shape through DFA. Values in bold indicate the number of individuals correctly classified to individual morph/lake combinations.

Fig. S1. Lateral view of a whitefish, showing the locations of 20 anatomical landmarks used to capture shape for geometric morphometric analyses.

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