

## Combining resource use assessment techniques reveals trade-offs in trophic specialization of polymorphic perch

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**Abstract.** Trophic polymorphism has been found to be common in many taxa and is a suggested mechanism of ecological speciation. To characterize the trophic linkages of specific morphotypes of organisms as well as a time-integrated niche use, several methods are available. In this study, we present data of multiple techniques to investigate the trophic divergence of Eurasian perch (*Perca fluviatilis*) that displays well-studied trophic polymorphism associated with littoral and pelagic habitats in lakes. We combined bulk stable isotope and fatty acid analyses on the muscle tissue of perch from three different lakes in Sweden with analyses of stomach content. By comparing the three methods, we aimed at providing a broad and highly resolved picture on the trophic divergence in freshwater fish. The degree in morphological divergence varied between perch caught in the three different lakes. Generally, perch caught in the pelagic zone were more streamlined compared to the ones caught in the littoral zone that had a deeper body, as shown by geometric morphometrics. The three diet assessment methods revealed different levels of information. Data on stomach content showed some preferences for specific dietary items in littoral and pelagic perch, but general trophic specialization could not be concluded due to the small sample size. Analyses of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , however, confirmed these results as a long-term pattern connected to specific habitat use in two of the three lakes. Fatty acid signatures of perch reflected partly those of the prey items of the specific habitats. Although the proportions of the essential fatty acid 22:6n-3 were lower in littoral resources, the proportions in littoral fish were similar to the ones caught in the pelagic zone. We concluded that although a fundamental contribution from littoral resources exists in littoral phenotypes, a minor reliance on pelagic prey items is obviously needed to provide essential compounds. Thus, by combining the methods to characterize direct resource use (i.e., stomach analyses) with others that utilize trophic biomarkers (i.e., analyses of stable isotopes and fatty acids), we were able to illustrate the degree of variation in trophic divergence of perch but also shed some light on potential trade-offs that are related to resource specialization in freshwater fish.

**Key words:** carbon stable isotopes; ecological speciation; fatty acid analysis; geometric morphometrics; *Perca fluviatilis*; resource polymorphism; Special Feature: Biomarkers in Trophic Ecology; stomach content analysis.

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## INTRODUCTION

Resource polymorphism is a widespread phenomenon in the animal kingdom (Skúlason and Smith 1995, Smith and Skúlason 1996). Trophic specializations correlated with morphological adaptations are crucial factors in population divergence and early stages of speciation (Hendry 2009). In freshwater fish, trophic specialization has frequently been found along the littoral–pelagic axis of lakes (i.e., from the near-shore to open-water habitats) (Smith and Skúlason 1996, Puebla 2009). Examples include three-spined stickleback *Gasterosteus aculeatus* (e.g., Ravinet et al. 2013), Arctic charr *Salvelinus alpinus* (e.g., Malmquist et al. 1992), whitefish *Coregonus lavaretus* (e.g., Harrod et al. 2010), and Eurasian perch *Perca fluviatilis* (e.g., Svanbäck and Eklöv 2002). Commonly, morphotypes living and feeding in the littoral zones are characterized by deeper bodies that facilitate maneuverability in the structural complex habitat. In contrast, morphotypes living and feeding in the pelagic zones of lakes are defined by a more slender body form that supports a higher attack speed necessary for foraging on fast-moving pelagic prey and further reduces drag (Weihs 1989, Koehl 1996, Smith and Skúlason 1996).

Traditionally, stomach content analyses have been used to study the diet consumed by fish, providing a direct insight into foraging ecology (Hyslop 1980). This method has been widely used to study the divergence patterns in many freshwater fish species (e.g., Ravinet et al. 2013, Siwertsson et al. 2013, Theis et al. 2014). Stomach content analyses have the advantage of providing information on the genus or even species level of the prey consumed. However, this method presents only a “snapshot” of the recently consumed diet lacking longer-term dietary inferences and furthermore may underestimate soft-bodied prey items (Jackson et al. 1987).

Alternative techniques based on biochemical tracers such as stable isotope and fatty acid analyses provide a specific dietary “signature,” where consumers incorporate a trophic marker into their body tissue (Iverson et al. 2004, Layman et al. 2012).

Analyses of stable carbon isotopes are powerful tools to study the major carbon flows and food-web linkages (France 1995, Hecky and Hesslein

1995). They provide a time-integrated depiction of a consumer’s diet, from weeks to months in the muscle tissue of ectotherms, depending primarily on body size and temporal variation (Hesslein et al. 1993, Perga and Gerdeaux 2005, Vander Zanden et al. 2015). Nitrogen stable isotopes serve as an indicator of the trophic level of a consumer, whereas carbon stable isotopes reflect the different sources of primary production at the base of the food web (Peterson and Fry 1987). The latter can be used to differentiate the carbon source of different habitats. In lakes, carbon signatures (expressed as  $\delta^{13}\text{C}$ ) typically have more negative (i.e.,  $^{13}\text{C}$ -depleted) values in pelagic species and more positive (i.e.,  $^{13}\text{C}$ -enriched) values in littoral species (France 1995). Isotope mixing models can further be applied to quantify the reliance on resources of the specific habitats. In many polymorphic fish species, stable isotope analyses have often been used to demonstrate habitat-specific niche use (e.g., Harrod et al. 2010, Matthews et al. 2010, Knudsen et al. 2014).

Analyses of the fatty acids represent another valuable tool to study trophic ecology, recently gaining an increasing interest (Iverson et al. 2004, Kelly and Scheibling 2012). Fatty acids are the compounds of lipids that play major functional and structural roles in cell membranes and many other physiological processes, such as immune response, hormone release, and disease susceptibility (Parrish 2009); especially long-chain polyunsaturated fatty acids (PUFAs) are physiologically valuable and considered essential for the growth and reproduction of consumers (Brett and Müller-Navarra 1997). Fatty acid signatures of consumers are tightly linked to those of their prey, as the majority of them cannot be synthesized by animals “de novo” (Tocher 2003, Iverson 2009). Thus, the analyses of fatty acids augment a study on trophic specialization with aspects on the functional importance of specific dietary items, providing additional data on the quality of resources. Furthermore, fatty acids can also be used as trophic markers as a number of primary producers have characteristic fatty acid compositions, which will be reflected in the tissue of a consumer (Dalsgaard et al. 2003, Kelly and Scheibling 2012). Previous studies have shown that morphotypes in polymorphic fish can be characterized by a unique fatty acid signature (Kuusipalo and Käkälä 2000, Logan et al. 2000).

Therefore, a combination of available methods is a promising technique to resolve the patterns of trophic specialization, further unraveling the evolutionary drivers of population divergence and understanding the ultimate causes of ecological speciation.

In this study, we present data on the trophic specialization of Eurasian perch *P. fluviatilis*, a common predator in boreal lakes that show habitat-specific morphological adaptations that has been well studied (e.g., Svanbäck and Eklöv 2002, 2003, Quevedo et al. 2009, Bartels et al. 2012). In many lakes, individual perch specialize on foraging in either the littoral zone, feeding more on benthic macroinvertebrates favoring a deeper body form, or the pelagic zone where diet consists mostly of pelagic zooplankton and foraging favors a more streamlined body (Svanbäck and Eklöv 2002, 2003). Evolutionary drivers shaping this divergence pattern are growth rate and predation (Eklöv and Svanbäck 2006), but intraspecific resource competition has been identified as the strongest selective force (Olsson et al. 2006, Svanbäck et al. 2008). Furthermore, water transparency has been identified as one of the key drivers of divergence (Bartels et al. 2012). Genetic studies have demonstrated that morphological divergence in perch is mainly driven by phenotypic plasticity, but also include a genetic component (Svanbäck and Eklöv 2006, Faulks et al. 2015). This may explain the general high variability found in the degree of divergence across multiple lakes (Bartels et al. 2012, Faulks et al. 2015).

To investigate the trophic specialization of perch, we combined the morphological analyses with three analyses of resource use: stomach content, bulk carbon stable isotope, and fatty acid analyses. We predicted that the time resolution in resource use by perch would substantially increase by using a combination of three different assessment techniques. By combining the resource use assessment with morphological analyses, we were also able to test different time perspectives of morphological variation that allow us to infer a deeper and broader understanding of trophic linkages in freshwater fish.

## MATERIALS AND METHODS

### Study areas and sampling

We surveyed three clear-water lakes (Secchi depth > 2.9 m) located in central Sweden (Table 1), where perch divergence has previously been reported (Bartels et al. 2012). Necessary permits were obtained from the land owners and respective authorities. In August and September 2014, fish were caught using multimesh gill nets (littoral nets: 30 × 1.5 m; pelagic nets: 27.5 × 6 m). The nets were set in shallow, near-shore (littoral), and open-water (pelagic) zones for up to 12 h to catch littoral and pelagic perch, respectively. For the fatty acid analysis, only fresh fish with red gills were used. Fish were stored on ice until further analysis. In the laboratory, the fish were partially thawed and measured to the nearest

Table 1. Main characteristics of the surveyed lakes.

Characteristics	Ljustjärn	Långsjön	Erken
Location	59°54' N, 15°23' E	60°01' N, 17°34' E	59°50' N, 18°33' E
Area (km <sup>2</sup> )†	0.12	2.5	23.7
Maximum depth (m)†	11.0	12.5	21.0
Dissolved organic carbon (mg/L)‡	3.0 ± <0.1	6.0 ± 0.2	10.9§
Total phosphorus (µg/L)‡	7.1 ± 1.6	21.1 ± 1.4	27.2 ± 11.8
Secchi depth (m)¶	6.2	2.9	3.4
Proportion of perch on CPUE (%)	4.5	16.0	49.9
Proportion of roach on CPUE (%)	67.3	25.8	29.4
Proportion of predatory fish on CPUE (%)	28.2	0.8†	10.8†

† Values were adapted from Bartels et al. (2012).

‡ Values represent the average values (± SD) of three samples at 1 m depth, taken in August 2014.

§ Sample was taken in June 2014.

¶ Samples were taken in August 2014.

1 mm (total length) and weighed to the nearest 0.1 g. Perch typically undergo a ontogenetic niche shift from being pelagic zooplanktivores as juveniles to being omnivorous including benthic invertebrates in the diet at intermediate size classes and becoming piscivorous at large size classes (Persson 1986). We targeted individuals within the intermediate size spectrum ( $115 \pm 17$  mm) that are known to feed on both invertebrates and zooplankton. A photograph was taken of the left side of the fish with fins stretched out. Stomachs were removed for diet analysis and stored at  $-20^{\circ}\text{C}$ . One piece of the dorsal muscle tissue was removed and kept at  $-20^{\circ}\text{C}$  and later used for the fatty acid analysis, whereas another one was dried in an oven at  $60^{\circ}\text{C}$  that was used for stable isotope analysis. Stable isotope and fatty acid analyses were restricted to seven and six individuals per habitat/lake combination, respectively.

Zooplankton samples were collected with several vertical hauls using zooplankton nets (mesh width 60 and 100  $\mu\text{m}$ ), and littoral macroinvertebrates were collected from stones or by using a sweep net and sorted into major taxonomic groups. All the samples were kept frozen ( $-20^{\circ}\text{C}$ ) until the analyses. In the laboratory, the

samples were carefully thawed and zooplankton was separated into cladocerans and copepods under a stereomicroscope. The samples of resources for the stable isotope analyses were oven-dried at  $60^{\circ}\text{C}$ , whereas the samples for the fatty acid analyses were freeze-dried at  $-51^{\circ}\text{C}$  for 12 h.

#### Geometric morphometrics

We estimated the perch morphology using landmark-based geometric morphometrics (Bookstein 1991). Digital photographs were transferred to TPSdig2 (<http://life.bio.sunysb.edu/morph/>), and 16 landmarks were digitized sensu Bartels et al. (2012) (Fig. 1). Variation in the morphology between littoral and pelagic perch of the three lakes was assessed using MorphoJ (Klingenberg 2011). We checked for outliers using the "Find outliers" function. To correct the shape data for body size, we used a regression of the shape scores (Procrustes coordinates) on size (centroid size) for each lake separately and the residuals of this regression were used for all further analyses. We used discriminant function analyses (DFA) to assess the significance of shape differences (Mahalanobis distance, MD) between littoral and pelagic perch for each lake.

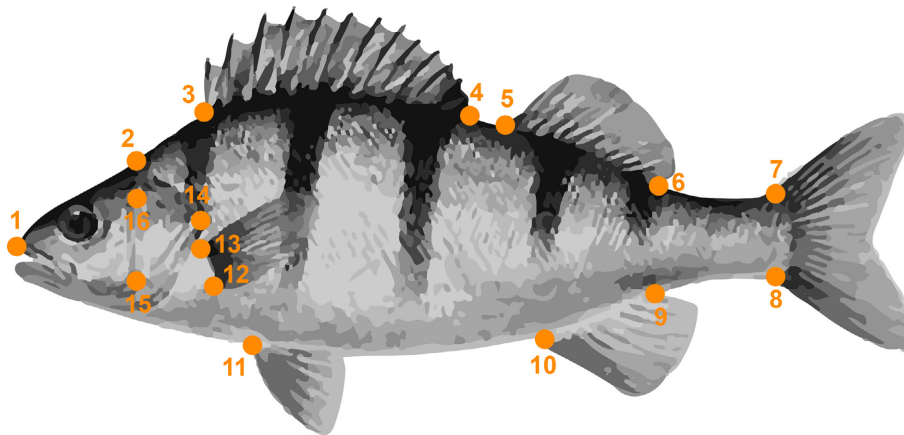


Fig. 1. Positions of the 16 digitized landmarks used in geometric morphometrics: 1: anterior tip of snout; 2: transition from head to body; 3: anterior insertion of anterior lobe of dorsal fin; 4: posterior insertion of anterior lobe of dorsal fin; 5: anterior insertion of posterior lobe of dorsal fin; 6: posterior insertion of posterior lobe of dorsal fin; 7: superior insertion of caudal fin; 8: inferior insertion of caudal fin; 9: posterior insertion of anal fin; 10: anterior insertion of anal fin; 11: insertion of pelvic fin; 12: ventral insertion of pectoral fin; 13: dorsal insertion of pectoral fin; 14: posterior margin of principal opercular bone; 15: ventral margin of principal opercular bone; 16: dorsal margin of principal opercular bone.

### Stomach content analysis

The stomach content of perch was analyzed using an established method (e.g., Svanbäck and Eklöv 2002, 2003, Bartels et al. 2012) that has been adapted from the numeric method from Hyslop (1980). In our method, prey items were identified to the smallest taxonomic unit under a dissecting microscope. Prey categories used in the stomach content analysis included littoral Diptera (Chironomidae, Ceratopogonidae), pelagic Diptera (*Chaoborus* spp.), Ephemeroptera (*Cloeon* spp., *Baetis* spp., *Leptophlebia* spp.), Isopoda (*Asellus aquaticus*), Gammaridae (*Gammarus* spp.), copepods (cyclopoida, calanoida), cladocerans (Daphniidae, Bosminidae, *Holopedium gibberum*, *Chydorus* spp., *Alona* spp.), and predatory cladocerans (*Polyphemus* spp., *Leptodora* spp., *Bytotrephes* spp.). The body length of 10 prey was measured in each group and in groups consisting of <10 individuals, all prey were measured to the nearest 0.1 mm. The average length was converted to biomass (mg dry weight) using our own length/weight regressions and the proportions of biomasses of each category consumed were calculated. Nonparametric Mann–Whitney *U* tests (using IBM SPSS Statistics 21 [IBM Corporation, Armonk, New York, USA]) were used to assess the variation in pelagic prey consumed between perch phenotypes. Schoener's similarity index (*S*) was used to estimate the overlap between littoral and pelagic perch (Schoener 1970):

$$S = 1 - 0.5 \left( \sum_{i=1}^n |p_{li} - p_{pi}| \right)$$

where  $p_{li}$  is the mean proportion of prey category in the diet of littoral perch,  $p_{pi}$  is the mean proportion of prey category  $i$  in the diet of pelagic perch, and  $n$  is the number of prey categories. A value of  $S > 0.6$  (60% of diet overlap) is generally assumed to reflect the high overlap in resources used (Wallace 1981).

### Stable isotope analysis and mixing model

Samples for bulk stable isotope analysis of fish muscle tissue and macroinvertebrates were ground to a fine powder using a mortar and pestle. Of all the samples, approximately 1.00 mg

was weighed into tin capsules. Elemental and stable isotope analyses of carbon and nitrogen were conducted at the University of California, Davis Stable Isotope Facility, California, USA, using a PDZ Europa ANCA-GSL elemental analyzer coupled to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Cheshire, UK). The results are expressed in the  $\delta$  notation, using the ratios of samples to an international standard. Thirty-one percent of the samples were analyzed in duplicate, and the analytical error was 0.1‰. We did not apply the corrections for lipid normalization, as mean ( $\pm$ SD) C/N ratios were low ( $3.32 \pm 0.07$ ) (Kiljunen et al. 2006).

To estimate the dietary proportions of perch caught in the littoral zone and the pelagic zone, we used the Bayesian mixing model MixSIAR version 3.0.2 (Stock and Semmens 2013) in R (R Core Team 2013). We generally followed the advice of Phillips et al. (2014) on conducting the stable isotope mixing models. We pooled the isotope data of pelagic (zooplankton) and littoral (benthic invertebrates) to estimate the proportion of pelagic and littoral resources in the diet of perch (see isotope ratios depicted in isotope biplots, Fig. 4). Pooling of resources allowed us to receive more constrained and less diffuse solutions in contrast to the alternative underdetermined model of substantially more resources than isotopes (Phillips et al. 2005). We used a fractionation factor of  $0.4 \pm 1.3$  for  $\delta^{13}\text{C}$  and  $3.4 \pm 1.0$  for  $\delta^{15}\text{N}$  to correct for trophic fractionation (Post 2002). Prior to model runs, we carefully inspected the isotope biplots to check for valid mixing geometry and distinct values of resources. For the model evaluation, we used the Gelman–Rubin and Geweke diagnostics included in the model to check for chain convergence. Furthermore, we inspected the probability distribution of the posteriors in the model output, as a broader distribution of the possible solutions would indicate a low accuracy of the model, which is further reflected by high SDs of the mean output. As suggested by Ward et al. (2010), we included informative prior distributions. These were obtained from the previous study of Bartels et al. (2012), who have been estimating the littoral and pelagic contribution to littoral and pelagic perch by using stable isotopes in the same lakes before (for the values of informative priors, see Appendix S1). For all results

of the Bayesian mixing model, we refer to trends in mean values and give SDs in the figures, but do not refer to significant differences between the means in the sense of frequentist statistics.

#### *Fatty acid analysis*

To estimate the fatty acid compositions of perch, approximately 200 mg of the fresh muscle tissue was used. Samples of zooplankton and macroinvertebrates were freeze-dried, and an amount of 1.2–13 mg of dry mass was used. Lipids were extracted in chloroform/methanol mixture (2:1, by volume), and 0.88% potassium chloride in water was added to remove the nonlipids. The extraction procedure was repeated, and after vortexing and centrifuging, both organic phases were pooled. Sonification (10 min) was used to enhance the extraction, and the total lipid extracts were concentrated under a nitrogen stream. Lipids were dissolved in hexane and transmethylated at 90°C for 90 min, using acidic catalyst (1% H<sub>2</sub>SO<sub>4</sub> in methanol).

Analyses of the fatty acid methyl esters (FAME) were conducted at the University of Eastern Finland using an Agilent 6890 N Gas Chromatograph (Agilent Technologies, Santa Clara, California, USA) equipped with a DB-23 column (length 30 m, ID 0.25 mm, film thickness 0.25 µm; Agilent). For fish samples, split injection (20:1) was applied with an initial temperature of 180°C for 8 min, which was then increased by 2°C/min to 210°C, which was finally maintained for 2 min. For zooplankton and macroinvertebrate samples, a splitless injection was used with an initial temperature of 50°C. The inlet temperature was held for 1 min and first raised 15°C/min to 150°C and then 1°C/min to 190°C, which was then increased by 2°C/min to 210°C, which was finally maintained for 10 min. In both methods, helium was used as a carrier gas with an average velocity of 36 cm/s.

FAME peaks were identified using retention times and mass spectra, and a heneicosanoic acid (Nu-Chek Prep, Elysian, Minnesota, USA) was used as an internal standard. Fatty acid concentrations were calculated using the calibration curves based on the standard solutions of known lipid mixtures (Nu-Chek Prep).

#### *Multivariate analysis*

We used Primer 7.0.6 (Primer E, Plymouth, UK) with the PERMANOVA add-on for multivariate

analyses of arcsine-square-root-transformed fatty acids (% fatty acids) of perch and resources. Ordination was based on Euclidean distance matrices.

PERMANOVA, the nonparametric analog of MANOVA, was used to test the differences in the fatty acid composition of perch between lakes, habitats (habitats nested within lake) as fixed factors and the total length as a covariate. To identify the significant differences in the fatty acid composition of resources (zooplankton and macroinvertebrates), lake was used as another fixed factor. Significance of analyses was determined using permutation of residuals under a reduced model (9999 permutations) with type I sums of squares used for the model including a covariate and type III sums of squares used for the model without a covariate (Anderson et al. 2008). Proportion of variance explained (%) was calculated from the sums of squares. Nonmetric multidimensional scaling (nMDS) was used to depict the variation in the fatty acid composition of fish and resources. To identify the fatty acids, respectively, that contributed most to the observed differences between littoral and pelagic perch as well as between littoral and pelagic resources (i.e., zooplankton and macroinvertebrates), we used the similarity percentages routine (SIMPER) on the transformed data.

## RESULTS

Overall, there were differences in shape between perch caught in the littoral and pelagic zones in all three lakes, with littoral individuals having a deeper body compared to pelagic individuals that were more streamlined (Fig. 2). The differences were clear in Långsjön (Mahalanobis distance  $D = 3.38$ , depicting the distance between the group means in shape space) and Erken ( $D = 2.06$ ), but less pronounced in Ljustjärn ( $D = 1.59$ ). The differences were only significant for comparison in Erken, primarily attributable to the low sample size of fish caught in the other two lakes. However, DFA classified always more than 63% of the individuals caught in the littoral and pelagic zones correctly in the respective group (Table 2).

We analyzed the stomach contents of 131 perch individuals. Fourteen stomach sample contents were empty (11 in Erken, two in Ljustjärn, and

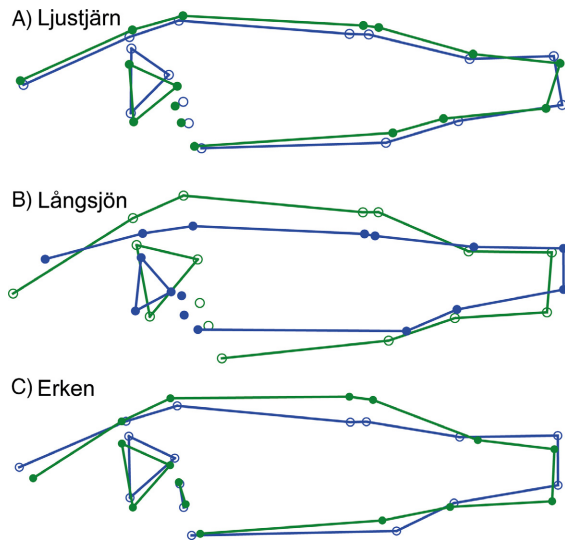


Fig. 2. Shape differences between the pelagic (blue line) and littoral (green line) perch of the three lakes studied: (A) Ljustjärn, (B) Långsjön, and (C) Erken. Shape-change outlines of discriminant function analysis are magnified fivefold.

one in Långsjön) and two samples were lost during the sample preparation. The composition of the stomach content varied between the different lakes and habitats (Table 3). Strong specialization on few categories was found in the pelagic fish of Ljustjärn (cladocera, especially *H. gibberum*) and Erken (cladocera and predatory cladocera) (Table 3). Diet overlap between the littoral and pelagic perch caught in the same lake varied between 42% and 75% (Table 3). Prey consumed was most similar between the littoral and pelagic perch caught in Långsjön and most distinct in Erken. Consumption of cladocerans and predatory cladocerans was the highest in the pelagic perch, and the proportion of littoral Diptera was higher in the stomachs of littoral perch

(Table 3). Generally, the pelagic perch consumed more pelagic prey than the perch caught in the littoral zone, albeit this effect was significant only in Erken perch (Mann–Whitney  $U$  tests; Fig. 3).

The  $\delta^{13}\text{C}$  of the muscle tissue was generally more depleted in the pelagic perch, compared to the littoral perch (Fig. 4). The Bayesian mixing model revealed a strong difference in the reliance on pelagic carbon in the perch caught in the different lakes, ranging from an average of  $9.5\% \pm 0.1\%$  SD in Långsjön up to an average of  $90.3\% \pm 0.1\%$  SD in Erken (Fig. 5). Although the mean reliance on pelagic carbon of the perch caught in the pelagic zone was consistently higher than in littoral perch (Fig. 5), a high overlap in SDs in Ljustjärn suggests a similar resource use in this lake.

We identified 37 fatty acids from the perch muscle tissues (Table 4). The highest proportion in the fatty acid composition came from 22:6n-3 (Table 4). There was a significant difference in the fatty acid composition of the perch caught from the different lakes (PERMANOVA: pseudo- $F_{2,35} = 14.13$ ;  $P = 0.0001$ , 37.4% of the variance explained). Furthermore, there were a significant habitat effect (littoral vs. pelagic zones) (PERMANOVA: pseudo- $F_{3,35} = 4.91$ ;  $P = 0.0001$ , 19.5% of variation) and a significant effect of the total length as covariate (PERMANOVA: pseudo- $F_{1,35} = 3.67$ ;  $P = 0.014$ , 4.8% of variance) on the fatty acid composition (Fig. 6). Due to the significant lake position effect, we repeated PERMANOVA to test for the consistency of significant differences in the fatty acid compositions between the littoral and pelagic perch in each of the lakes using the habitat as a fixed factor and the total length as a covariate. In Erken (PERMANOVA: pseudo- $F_{1,11} = 3.24$ ;  $P = 0.033$ , 20.9% of variance) and Långsjön (PERMANOVA: pseudo- $F_{1,11} = 5.79$ ;  $P = 0.006$ , 34.8% of variance), the fatty acid composition was significantly different between the littoral and

Table 2. Results of shape analyses of perch caught in the three different lakes, including the sample size ( $N$ ).

Lakes	$N$ pelagic	$N$ littoral	Mahalanobis distance $D$	Pelagic correctly classified (%)	Littoral correctly classified (%)
Ljustjärn	11	21	1.59	95.2	63.6
Långsjön	19	7	3.38	100	100
Erken	37	36	2.06*	83.8	83.8

Notes: Mahalanobis distance  $D$  of discriminant function analyses depicts the distance of group means (littoral or pelagic) in shape space, and percentages show the proportion of individuals caught in littoral or pelagic habitats that were classified in the respective group. An asterisk indicates significance at  $P = 0.05$ .

Table 3. Diet composition (the percentage of total stomach biomass of each individual) from the three study lakes, including the sample size (*N*).

Order	Ljustjärn pelagic ( <i>N</i> = 21)		Ljustjärn littoral ( <i>N</i> = 13)		Långsjön pelagic ( <i>N</i> = 8)		Långsjön littoral ( <i>N</i> = 15)		Erken pelagic ( <i>N</i> = 31)		Erken littoral ( <i>N</i> = 29)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Littoral Diptera	3.2	13.6	8.9	27.5	25.2	34.0	26.5	35.8	0.3	1.1	38.9	40.6
Ephemeroptera	0	0	0	0	0	0	0.6	2.3	0	0	16.1	26.5
Trichoptera	0	0	0	0	0	0	0.0	0.0	0	0	1.2	3.5
Isopoda	4.5	14.6	0	0	0	0	12.3	26.2	0	0	0.7	3.8
Gammaridae	0	0	0	0	0	0	4.8	14.5	0	0	0	0
Pelagic Diptera	0	0	0	0	1.4	3.0	0.2	0.6	0	0	0	0
Copepoda	12.9	6.6	42.6	22.2	16.7	24.2	1.9	5.8	1.2	4.9	2.3	7.5
Cladocera	77.7	20.8	48.5	24.0	47.3	32.4	53.7	42.9	44.1	25.5	24.4	34.8
Predatory cladocera	1.7	5.5	0	0	9.5	26.8	0	0	54.4	27.0	16.5	28.3
∑ littoral prey	7.7	19.2	8.9	27.5	25.2	32.9	44.1	42.4	0.3	1.1	56.9	48.6
∑ pelagic prey	92.3	19.2	91.1	27.5	74.8	40.4	55.9	42.4	99.7	1.1	43.1	48.6

Notes: SD indicates the standard deviation within each population. Schoener's similarity index (*S*) on the dietary overlap of pelagic and littoral perch by lake is as follows: Ljustjärn, 64.6%; Långsjön, 74.6%; and Erken, 42.4%.

pelagic fish, but this was not the case in the perch caught in Ljustjärn (PERMANOVA: pseudo- $F_{1,11} = 0.78$ ;  $P = 0.446$ , 4.6% of variance).

In nMDS, Primer states a stress level that indicates the deviation of the ordination from the initial distance matrix. Albeit stress level of 0.14 of the two-dimensional nMDS represents a "good" fit of ordination into the multidimensional space

(Anderson et al. 2008), we further refer to an animation in the three-dimensional space (stress level of 0.05) for a better inspection of the data (see Video S1). Factors included in the model were able to explain only 61.7% of the variation in the fatty acid composition of perch. The remaining 38.3% of the variation could be explained by high variability in seasonal or spatial differences in the fatty acid composition, as previously shown in phytoplankton and zooplankton (Hiltunen et al. 2015, Strandberg et al. 2015a, b).

Eight fatty acids contributed together to 74.0% of the separation between the littoral and pelagic groups using SIMPER analysis: 22:6n-3 (18.2% contribution), 16:1n-7 (12.4% contribution), 18:3n-3 (9.6% contribution), 20:5n-3 (8.5% contribution), 20:4n-6 (7.8% contribution), 22:5n-6 (7.2% contribution), 18:4n-3 (5.2% contribution), and 18:2n-6 (5.1% contribution). Highest proportions of 22:6n-3 were found in fish from Ljustjärn, with higher proportions found in the littoral compared to the pelagic fish (Table 4). In general, higher proportions of 18:3n-3, 20:5n-3, 20:4n-6, and 18:4n-3 were found in the pelagic fish, whereas 16:1n-7, 22:5n-6, and 18:2n-6 were higher in the fish caught in the littoral zone (Table 4, Fig. 6).

For zooplankton and macroinvertebrates, 49 fatty acids were identified and the fatty acid composition differed significantly between these two groups ( $N = 19$ ; PERMANOVA: pseudo- $F_{2,18} = 8.43$ ;  $P = 0.0001$ ; Tables 5, 6), explaining 30.9% of the fatty acid variation (Fig. 6 for

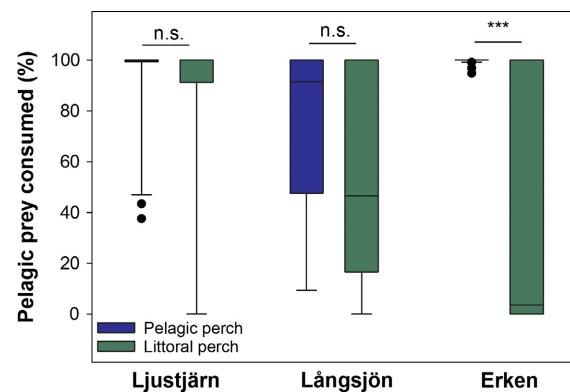


Fig. 3. Proportion of pelagic prey consumed depicted by stomach content analyses, of pelagic (blue bars) and littoral (green bars) perch caught in the three different lakes (Ljustjärn, Långsjön, and Erken). Results of pairwise comparison (Mann-Whitney *U* tests) between lake habitats are shown. \*\*\* $P \leq 0.001$ . Boxplots with mean values and 25th and 75th percentiles are shown, while whiskers represent 10th and 90th percentiles. Outliers are represented by dots.

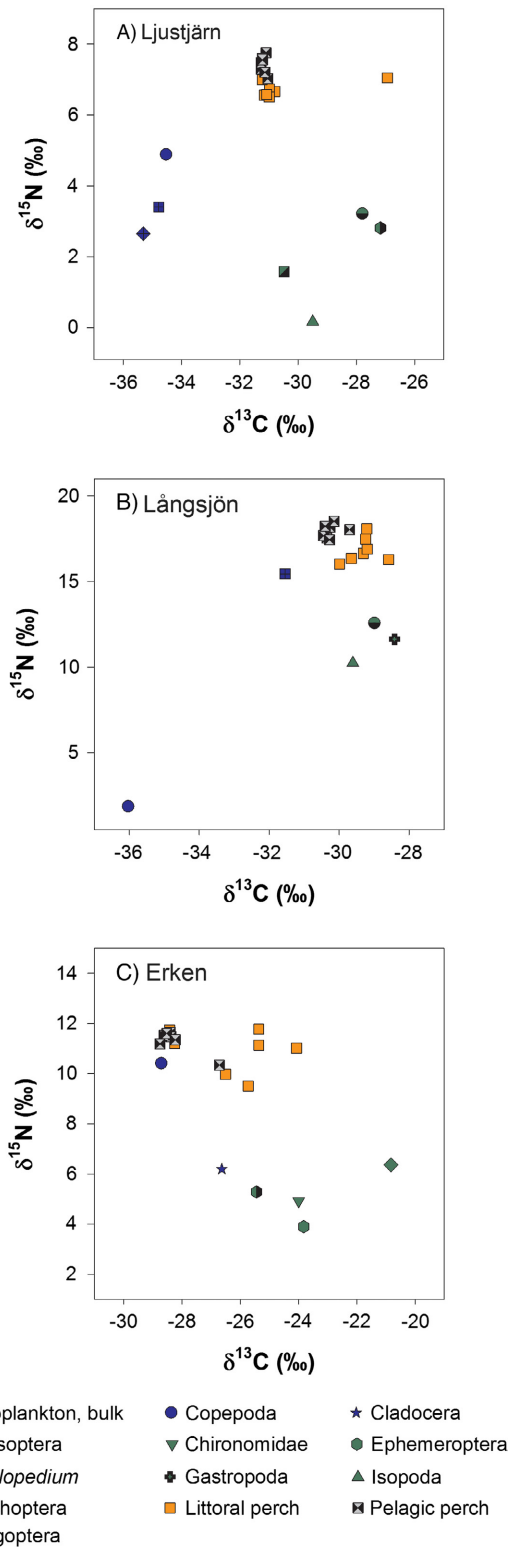


Fig. 4. Biplots of stable carbon and nitrogen isotope ratios of perch and pelagic resources of the three different lakes studied: (A) Ljustjärn, (B) Långsjön, and (C) Erken. All pelagic (blue) and littoral (green) resources of each lake were averaged and entered into the Bayesian mixing model MixSIAR. Note the different axes scaling.

the 2D presentation [stress level of 0.11] and Video S2 for the 3D presentation [stress level of 0.06]). Lake showed no significant effect on the variation in the fatty acid composition (PERMANOVA: pseudo- $F_{1,18} = 1.82$ ;  $P = 0.053$ , 13.3% of the variance explained). Nine fatty acids contributed together to 72.0% of the explained variance between zooplankton and macroinvertebrates using SIMPER analysis: 22:6n-3 (21.8% contribution), 16:1n-7 (12.8% contribution), 18:4n-3 (9.7% contribution), 14:0 (7.5% contribution), 18:1n-7 (4.9% contribution), 18:2n-6 (4.4% contribution), 18:3n-3 (3.7% contribution), 20:4n-6 (3.6% contribution), and 18:1n-9 (3.6% contribution). High proportions of 22:6n-3, 18:4n-3, 14:0, and 18:3n-3 were found in the zooplankton, whereas higher proportions of 16:1n-7, 18:1n-7, 18:2n-6, and 18:1n-9 were found in the benthic macroinvertebrates (Table 6, Fig. 7). High proportions of 20:4n-6 were found in the pelagic cladocerans, but also in the littoral Isopoda and Chironomidae (Table 6, Fig. 7).

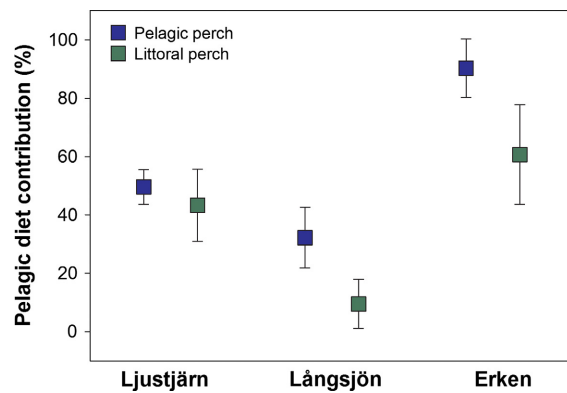


Fig. 5. Pelagic reliance of perch, as calculated by the Bayesian mixing model MixSIAR, of the pelagic (blue) and the littoral (green) perch caught in the three different lakes (Ljustjärn, Långsjön, and Erken). Error bars with mean values and SD of each population are depicted.

Table 4. Fatty acid composition (%) of perch.

Fatty acids	Ljustjärn		Långsjön		Erken	
	Pelagic	Littoral	Pelagic	Littoral	Pelagic	Littoral
14:0	1.3 ± 0.1	1.4 ± 0.4	0.9 ± 0.1	0.6 ± 0.3	1.3 ± 0.2	0.9 ± 0.2
i-15:0	0.1 ± <0.1	0.2 ± <0.1	0.1 ± <0.1	0.1 ± <0.1	0.3 ± 0.1	0.2 ± 0.1
15:0	0.4 ± <0.1	0.4 ± <0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± <0.1	0.4 ± 0.1
i-16:0	0.4 ± 0.1	0.3 ± 0.2	0.6 ± <0.1	1.0 ± 0.4	0.4 ± 0.2	0.7 ± 0.2
16:0	20.1 ± 0.6	19.3 ± 1.0	23.0 ± 1.2	22.8 ± 1.5	21.6 ± 1.0	21.7 ± 0.8
16:1	0.3 ± <0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± <0.1	0.2 ± <0.1
16:1n-9	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.4 ± 0.2
<b>16:1n-7</b>	<b>0.7 ± 0.1</b>	<b>1.2 ± 0.8</b>	<b>1.2 ± 0.3</b>	<b>2.0 ± 0.7</b>	<b>2.6 ± 0.5</b>	<b>3.8 ± 1.7</b>
16:1n-5	0.5 ± <0.1	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± <0.1	0.4 ± <0.1
i-17:0	0.3 ± <0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± <0.1	0.2 ± 0.1
17:0	0.7 ± 0.1	0.7 ± 0.3	0.6 ± 0.1	0.5 ± 0.1	0.7 ± <0.1	0.7 ± 0.1
18:0	7.2 ± 0.1	7.1 ± 0.3	7.2 ± 0.4	8.2 ± 0.8	6.6 ± 0.4	7.4 ± 0.6
18:1n-9	4.7 ± 0.6	5.0 ± 0.6	6.1 ± 0.5	5.6 ± 0.9	6.1 ± 0.5	5.2 ± 1.0
18:1n-7	2.7 ± 0.3	2.7 ± 0.8	2.5 ± 0.2	2.8 ± 0.6	3.2 ± 0.2	4.3 ± 0.7
<b>18:2n-6</b>	<b>1.2 ± 0.2</b>	<b>2.0 ± 1.3</b>	<b>2.6 ± 0.5</b>	<b>2.3 ± 1.1</b>	<b>2.3 ± 0.2</b>	<b>2.7 ± 0.6</b>
<b>18:3n-3</b>	<b>1.2 ± 0.2</b>	<b>1.6 ± 0.4</b>	<b>1.7 ± 0.5</b>	<b>0.4 ± 0.2</b>	<b>3.0 ± 0.5</b>	<b>1.6 ± 0.5</b>
<b>18:4n-3</b>	<b>0.6 ± 0.1</b>	<b>0.9 ± 0.4</b>	<b>0.4 ± 0.2</b>	<b>0.1 ± 0.1</b>	<b>1.2 ± 0.2</b>	<b>0.5 ± 0.2</b>
<b>20:4n-6</b>	<b>12.1 ± 1.6</b>	<b>9.4 ± 2.2</b>	<b>10.8 ± 0.9</b>	<b>9.5 ± 0.6</b>	<b>8.1 ± 0.5</b>	<b>7.4 ± 1.2</b>
20:4n-3	0.2 ± <0.1	0.2 ± <0.1	0.1 ± <0.1	0.1 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
<b>20:5n-3</b>	<b>14.0 ± 1.4</b>	<b>11.2 ± 1.8</b>	<b>14.8 ± 1.9</b>	<b>11.2 ± 2.8</b>	<b>14.2 ± 0.7</b>	<b>14.4 ± 1.3</b>
22:4n-6	0.6 ± 0.2	0.5 ± 0.2	0.4 ± <0.1	0.5 ± 0.2	0.5 ± 0.1	0.4 ± 0.1
<b>22:5n-6</b>	<b>2.6 ± 0.5</b>	<b>4.2 ± 1.4</b>	<b>1.7 ± 0.3</b>	<b>1.9 ± 0.5</b>	<b>1.6 ± 0.1</b>	<b>1.5 ± 0.2</b>
22:5n-3	1.9 ± 0.5	1.4 ± 0.6	2.0 ± 0.5	2.6 ± 0.7	2.1 ± 0.3	2.3 ± 0.3
<b>22:6n-3</b>	<b>24.2 ± 4.0</b>	<b>26.8 ± 5.9</b>	<b>20.1 ± 1.6</b>	<b>24.6 ± 5.3</b>	<b>19.6 ± 2.3</b>	<b>20.6 ± 2.2</b>
24:1n-9	0.8 ± 0.1	1.0 ± 0.2	0.7 ± 0.1	1.0 ± 0.2	1.0 ± 0.1	0.9 ± 0.1
∑ SFA	30.8 ± 0.3	30.2 ± 0.7	33.2 ± 0.8	34.0 ± 1.3	32.2 ± 1.0	32.6 ± 0.8
∑ MUFA	10.3 ± 1.1	11.3 ± 2.0	11.9 ± 1.1	12.5 ± 1.7	14.6 ± 1.3	15.6 ± 2.9
∑ PUFA	58.8 ± 1.0	58.4 ± 2.4	54.8 ± 0.9	53.4 ± 2.0	53.2 ± 1.1	51.9 ± 2.2
∑ n-6	16.6 ± 1.3	16.3 ± 1.7	15.6 ± 0.6	14.4 ± 1.0	12.6 ± 0.5	12.3 ± 0.9
∑ n-3	42.2 ± 2.0	42.1 ± 4.1	39.3 ± 1.2	39.1 ± 2.4	40.6 ± 1.3	39.6 ± 2.4
n-3/n-6	2.6 ± 0.3	2.6 ± 0.5	2.5 ± 0.1	2.7 ± 0.3	3.2 ± 0.2	3.3 ± 0.4

Notes: Averages of six individual perch analyzed from each habitat and lake ± SDs are listed (total N = 36). Sums (∑) of saturated fatty acids (SFA) list the fatty acids without a double bond, excluding branched and odd-chain saturated fatty acids. Sums of monounsaturated fatty acids (MUFA) list the fatty acids with a single double bond, and sums of polyunsaturated fatty acids (PUFAs) list the fatty acids with more than one double bond. Only those FA are presented that account for > 0.3% at least in one group. Fatty acids highlighted in boldface are those that contributed most to the differences between the littoral and the pelagic perch.

## DISCUSSION

In this study, we used the stomach content, stable isotope, and fatty acid analyses combined with the morphological variation to investigate the trophic specialization in Eurasian perch *P. fluviatilis*. Fish caught in the littoral habitats of lakes were generally deeper bodied, whereas pelagic individuals had a more streamlined body shape. Thus, these results are in line with previous studies of divergence of perch along the pelagic–littoral axes in Swedish lakes (Svanbäck and Eklöv 2002, Quevedo et al. 2009, Bartels et al. 2012). However, the variation in degree

of morphological divergence of the fish studied herein was high, similar to the results of Faulks et al. (2015) that suggested a high degree of phenotypic plasticity in divergence pattern of perch. Previous studies (e.g., Svanbäck and Eklöv 2002, Quevedo et al. 2009, Bartels et al. 2012) that related this morphological divergence to specialization on specific dietary items have been based on the results of stomach content or stable isotope analyses, but so far, studies on the fatty acids have not been conducted. By combining these three different techniques, we compiled a broader picture on the pattern of trophic divergence than gained from each method alone.

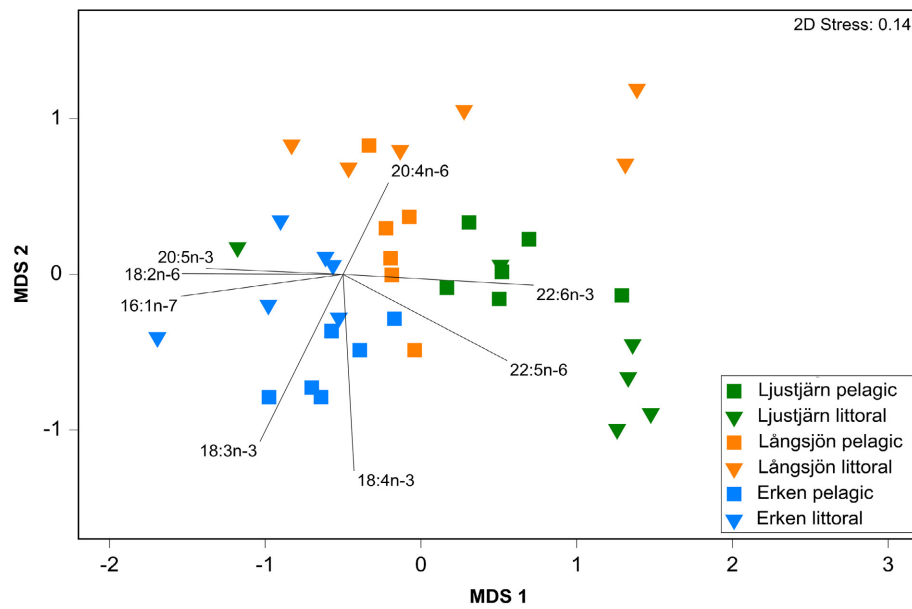


Fig. 6. Two-dimensional representation of nonmetric multidimensional scaling (nMDS) of fatty acid composition (%) from the perch caught in the littoral and pelagic zones of the three lakes surveyed. Relative length of vectors from fatty acids identified to contribute most to the observed difference in the composition between littoral and pelagic perch depicts the strength in positioning in the respective dimension.

Stomach content analyses provided the most detailed information on the diet consumed by perch. A high amount of variation among individual stomachs was depicted. Significant differences between the stomach contents of fish caught in the littoral and pelagic zones were observed only in Erken, which was the largest and deepest study lake. In this lake, pelagic fish predominantly fed on cladocerans and predatory cladocerans, whereas littoral perch consumed higher proportions of benthic macroinvertebrates, especially on littoral chironomids. In the other lakes, Schoener's similarity index indicated a high overlap of the resources used. These findings point out the downside of stomach content analyses when used to study trophic divergence, as they only provide a short-term insight into the recently consumed diet, which might eventually deviate from the more general and long-term patterns. To receive broader and more general inferences on dietary preferences, sample size should be large and multiple samplings should be conducted to provide a sufficient coverage across the seasons. When compared to the results of Bartels et al. (2012) that conducted stomach content analyses of perch in the same lakes, the potential yearly

variation becomes apparent. For example, the littoral perch in Erken consumed on average 39% of littoral prey in the study of Bartels et al. (2012), whereas we found that almost 57% of the stomach content consisted of littoral prey.

Although foraging on littoral resources was higher in fish caught in the littoral zone of Erken, overall proportions of pelagic prey remained high (43.1–91.1%). Pelagic dietary contributions were exceptionally high in the perch from Ljustjärn and the diet consisted mostly of cladocerans. In this lake, zooplankton species composition was dominated by the cladoceran *Holopedium gibberum* (K. Scharnweber and M. Marklund, *personal observation*) that can reach high abundance during short times in summer in boreal lakes (Stenson 1973, Lehtovaara et al. 2014). *H. gibberum* also dominated the stomach content of the pelagic and littoral perch in this lake. *H. gibberum* has been shown to be a stronger competitor to *Daphnia* due to its low calcium requirements and has a strong predation defense against predatory cladocera and *Chaoborus* larvae by a mucopolysaccharide jelly capsule (Jeziorski et al. 2015). Fatty acid composition of *H. gibberum* was comparable to those of other cladocerans, therefore

Table 5. Fatty acid composition (%) of Cladocera, Copepoda, and Chironomidae of Ljustjärn (Lj), Långsjön (La), and Erken (E).

Fatty acids	Cladocera			Copepoda			Chironomidae			
	Lj		La	E	Lj	La	E	Lj	La	E
	a	b								
<b>14:0</b>	<b>10.3</b>	<b>12.5</b>	<b>3.4</b>	<b>4.6</b>	<b>10.2</b>	<b>4.0</b>	<b>4.4</b>	<b>0.9</b>	<b>1.1</b>	<b>3.4</b>
4,8,12 TMTD	0.2	0.1	<0.1	0.1	0.5	0.1	1.5	<0.1	0.1	0.3
i-15:0	1.2	1.4	0.6	0.8	1.1	0.5	0.7	0.4	8.9	0.7
ai-15:0	0.5	0.6	0.1	0.2	0.5	0.1	0.1	0.1	12.7	0.2
15:0	0.8	0.6	0.8	0.7	0.8	0.7	0.5	0.6	0.5	1.0
16:0	17.3	14.2	27.3	22.8	16.6	27.5	26.7	15.3	12.2	21.1
16:1n-9	0.9	0.5	0.7	1.0	0.2	0.2	0.3	0.2	<0.1	0.2
<b>16:1n-7</b>	<b>2.7</b>	<b>2.7</b>	<b>3.2</b>	<b>9.2</b>	<b>2.3</b>	<b>2.0</b>	<b>3.1</b>	<b>3.1</b>	<b>6.9</b>	<b>15.9</b>
16:1n-5	0.7	0.7	0.3	0.5	0.6	0.1	0.2	0.3	<0.1	0.4
i-17:0	0.5	0.4	0.5	0.7	0.5	0.5	0.5	0.4	0.4	0.2
16:2n-6	0.1	0.1	0	0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1
ai-17:0	0.6	0.6	0.2	0.2	0.5	0.2	0.2	0.5	0.5	0.1
16:2n-4	0.1	0.1	0	0.5	<0.1	<0.1	0.1	<0.1	<0.1	0.4
17:0	0.7	0.4	1.8	1.4	0.8	3.0	1.2	2.4	1.5	1.6
Phytanate	<0.1	<0.1	0	<0.1	0.1	0.4	0.7	<0.1	<0.1	<0.1
17:01	0.1	<0.1	0	0	<0.1	<0.1	0	<0.1	<0.1	1.1
i18:0	0.3	0.1	0	0.1	0.3	0.1	0	<0.1	<0.1	0
18:0	5.1	4.3	6.9	5.2	5.1	7.8	4.9	9.3	8.0	8.4
<b>18:1n-9</b>	<b>8.2</b>	<b>6.5</b>	<b>14.1</b>	<b>9.6</b>	<b>3.3</b>	<b>8.9</b>	<b>6.8</b>	<b>12.3</b>	<b>7.8</b>	<b>8.2</b>
<b>18:1n-7</b>	<b>3.8</b>	<b>2.8</b>	<b>4.0</b>	<b>6.3</b>	<b>1.7</b>	<b>2.0</b>	<b>2.0</b>	<b>9.1</b>	<b>9.7</b>	<b>6.1</b>
<b>18:2n-6</b>	<b>3.0</b>	<b>3.3</b>	<b>7.1</b>	<b>4.8</b>	<b>2.4</b>	<b>5.4</b>	<b>5.8</b>	<b>13.1</b>	<b>14.5</b>	<b>10.7</b>
18:3n-6	0.2	0.3	<0.1	0.3	0.3	0.1	0.1	0.2	<0.1	0.4
<b>18:3n-3</b>	<b>6.3</b>	<b>6.3</b>	<b>9.8</b>	<b>8.9</b>	<b>4.8</b>	<b>8.1</b>	<b>13.9</b>	<b>6.1</b>	<b>2.0</b>	<b>3.0</b>
<b>18:4n-3</b>	<b>6.5</b>	<b>10.0</b>	<b>2.6</b>	<b>2.8</b>	<b>6.0</b>	<b>3.0</b>	<b>6.0</b>	<b>0.1</b>	<b>&lt;0.1</b>	<b>0.3</b>
20:1n-9	<0.1	<0.1	0	0	0.2	0.3	0.4	<0.1	<0.1	<0.1
<b>20:4n-6</b>	<b>8.0</b>	<b>8.1</b>	<b>2.4</b>	<b>4.9</b>	<b>2.6</b>	<b>1.4</b>	<b>1.3</b>	<b>9.4</b>	<b>4.8</b>	<b>3.5</b>
20:5n-3	19	20.1	7.4	11.7	11	6.2	7	14.7	7.2	11.5
22:0	0.1	0.1	0.3	0.2	0.2	0.2	0.1	0.3	<0.1	0.1
<b>22:6n-3</b>	<b>1.6</b>	<b>1.9</b>	<b>5.8</b>	<b>1.4</b>	<b>20.1</b>	<b>14.3</b>	<b>8.3</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>
24:1n-9	<0.1	<0.1	0	<0.1	1.5	0.3	0.9	0.4	<0.1	<0.1
∑ SFA	20.5	21.6	15	14.3	20.6	17.8	13	15.1	34.4	16.4
∑ MUFA	16.5	13.3	22.2	26.5	10.1	13.8	13.6	25.8	24.6	31.9
∑ PUFA	45.6	50.8	35.4	36.3	52.0	40.3	44.5	43.7	28.6	30.1
∑ n-6	11.7	12.0	9.8	10.3	9.4	7.8	8.7	22.7	19.3	14.6
∑ n-3	33.8	38.7	25.7	25.2	42.5	32.3	35.6	20.9	9.3	15.0
n-3/n-6	2.9	3.2	2.6	2.4	4.5	4.1	4.1	0.9	0.5	1.0

Notes: Sums ( $\Sigma$ ) of saturated fatty acids (SFA) list the fatty acids without a double bond, excluding branched and odd-chain saturated fatty acids. Sums of monounsaturated fatty acids (MUFA) list the fatty acids with a single double bond, and sums of polyunsaturated fatty acids (PUFAs) list the fatty acids with more than one double bond. Only those FA are presented that account for >0.5% at least in one group. Values for cladocerans of Ljustjärn list separately (a) Daphniidae and Bosminidae and (b) *Holopedium gibberum* (only frequently abundant in Ljustjärn). Fatty acids highlighted in boldface are those that contributed most to the differences between zooplankton and macroinvertebrates.

representing a valuable food source. However, other studies highlighted a lower content of essential nutrients of *H. gibberum* that is channeled up the food chain, such as phosphorus and calcium (Andersen and Hessen 1991, Jeziorski et al. 2015).

Compared to the stomach content analyses, stable isotopes provide long-term dietary inferences

that acknowledged the trophic specialization in perch as an established pattern in all three lakes. Resources from pelagic habitats were more depleted in  $^{13}\text{C}$  and this was further transferred to the pelagic perch, having more  $^{13}\text{C}$ -depleted carbon isotope values compared to the littoral perch. This pattern was further detected in other freshwater fishes that show the divergence along

Table 6. Fatty acid composition (%) of Trichoptera, Isopoda, and Ephemeroptera of Ljustjärn (Lj), Långsjön (La), and Erken (E).

Fatty acids	Trichoptera			Isopoda			Ephemeroptera		
	Lj	Lå	E	Lj	Lå	E	Lj	Lå	E
<b>14:0</b>	<b>1.0</b>	<b>2.5</b>	<b>2.8</b>	<b>0.4</b>	<b>1.3</b>	<b>1.5</b>	<b>0.7</b>	<b>2.8</b>	<b>2.6</b>
4,8,12 TMTD	0.1	0.1	1.4	0.1	0.1	0.1	<0.1	<0.1	<0.1
i-15:0	0.1	0.6	<0.1	0.4	0.4	0.5	0.2	0.5	0.4
ai-15:0	0.1	0.3	<0.1	0.2	0.2	0.1	<0.1	0.1	<0.1
15:0	0.2	1.0	0.2	0.4	0.6	0.4	0.2	0.6	0.9
16:0	15.3	18.1	27.7	17.1	19.5	23.3	18.7	26.5	21.2
16:1n-9	0.1	0.4	0.1	0.2	0.5	0.5	0.1	0.4	0.4
<b>16:1n-7</b>	<b>2.1</b>	<b>8.6</b>	<b>24.7</b>	<b>8.2</b>	<b>21.0</b>	<b>11.7</b>	<b>5.4</b>	<b>19.2</b>	<b>18.3</b>
16:1n-5	<0.1	0.2	0.2	0.5	0.5	0.9	0.2	0.6	0.8
i-17:0	0.2	0.5	0.1	0.3	0.2	0.5	0.1	0.1	0.2
16:2n-6	<0.1	0.1	0.5	0.1	0.2	0.1	<0.1	<0.1	0.1
ai-17:0	0.2	0.5	0.2	0.2	0.2	0.1	<0.1	<0.1	<0.1
16:2n-4	<0.1	<0.1	3.9	<0.1	0.4	0.2	<0.1	0.6	0.7
17:0	1.9	2.5	0.1	0.7	0.6	0.8	0.7	0.5	1.1
Phytanate	<0.1	<0.1	<0.1	<0.1	1.1	0.5	<0.1	<0.1	0.2
17:01	<0.1	0.6	0.1	<0.1	0.6	0.4	<0.1	0.4	1
i18:0	0.5	<0.1	1.7	0.9	0.1	<0.1	<0.1	<0.1	0.1
18:0	10.5	9.9	2.0	4.3	4.1	5.0	9.8	3.8	4.4
<b>18:1n-9</b>	<b>18.6</b>	<b>13.2</b>	<b>12.4</b>	<b>19.8</b>	<b>9.4</b>	<b>13.6</b>	<b>14.6</b>	<b>7.9</b>	<b>9.1</b>
<b>18:1n-7</b>	<b>4.5</b>	<b>6.2</b>	<b>1.0</b>	<b>10.5</b>	<b>9.1</b>	<b>7.3</b>	<b>6.3</b>	<b>12.1</b>	<b>10.4</b>
<b>18:2n-6</b>	<b>10.9</b>	<b>0.1</b>	<b>2.8</b>	<b>12.2</b>	<b>3.6</b>	<b>6.3</b>	<b>12.5</b>	<b>3.9</b>	<b>5.1</b>
18:3n-6	<0.1	0.1	0.3	0.2	0.5	0.3	0.1	0.6	0.5
<b>18:3n-3</b>	<b>8.6</b>	<b>4.2</b>	<b>4.8</b>	<b>5.1</b>	<b>1.4</b>	<b>7.3</b>	<b>16.8</b>	<b>5.1</b>	<b>4</b>
<b>18:4n-3</b>	<b>0.1</b>	<b>0.2</b>	<b>1.9</b>	<b>0.1</b>	<b>0.5</b>	<b>0.7</b>	<b>0.3</b>	<b>1.6</b>	<b>1.1</b>
20:1n-9	<0.1	<0.1	<0.1	0.6	0.4	0.4	<0.1	<0.1	<0.1
<b>20:4n-6</b>	<b>6.9</b>	<b>4.2</b>	<b>0.1</b>	<b>9.5</b>	<b>9.5</b>	<b>5.7</b>	<b>2.9</b>	<b>2.1</b>	<b>3.6</b>
20:5n-3	17.9	14.5	5.9	5.5	10.8	8.6	9.3	9.2	12.5
22:0	<0.1	0.3	<0.1	0.4	0.5	0.6	0.6	0.2	0.4
<b>22:6n-3</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>0.7</b>	<b>1.2</b>	<b>1.4</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>
24:1n-9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
∑ SFA	14.8	18.9	7.2	8.9	8.8	9.8	12.6	8.8	10.5
∑ MUFA	25.3	29.4	38.6	39.9	41.5	35.1	26.6	40.8	40.1
∑ PUFA	44.5	33.5	25.1	33.9	28.9	31.2	42.0	23.5	28.1
∑ n-6	17.8	14.4	3.7	22.1	13.9	12.5	15.6	6.6	9.3
∑ n-3	26.7	19.0	16.2	11.8	14.1	18.3	26.5	16.2	18.0
n-3/n-6	1.5	1.3	4.4	0.5	1.0	1.5	1.7	2.5	1.9

Notes: Sums ( $\Sigma$ ) of saturated fatty acids (SFA) list fatty acids without a double bond, excluding branched and odd chained saturated fatty acids. Sums of mono-unsaturated fatty acids (MUFA) list fatty acids with a single double, and sums of poly-unsaturated fatty acids (PUFAs) list fatty acids with more than one double bond. Only those FA are presented that account for >0.5% at least in one group. Fatty acids highlighted in bold are those that contributed most to differences between zooplankton and macroinvertebrates.

the littoral–pelagic axis (Harrod et al. 2010, Matthews et al. 2010, Knudsen et al. 2014). Bayesian isotope mixing models provided a tool to quantify the contribution of pelagic prey and depicted a clear decrease in reliance on littoral phenotypes of Erken and Långsjön. The model provides a sufficient precision to allow a general comparison between pelagic carbon contributions of the littoral and pelagic fish. However, absolute

numbers have to be considered with caution, as a temporal variation in isotope ratios in resources, especially in zooplankton, can be high (Syväranta et al. 2006, Syväranta and Rautio 2010), and our data set did not allow a high temporal resolution. Furthermore, we assumed the values on trophic fractionation factor and its variability from the published literature (Post 2002) because true fractionation and isotopic turnover rates are difficult

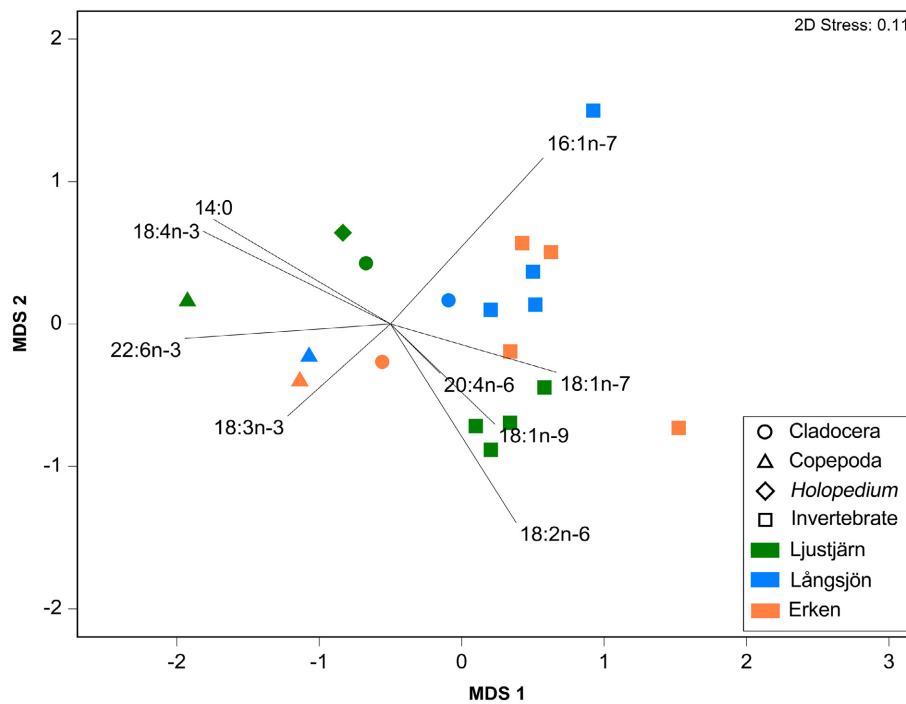


Fig. 7. Two-dimensional representation of nonmetric multidimensional scaling (nMDS) of fatty acid composition of cladocera (circles), copepoda (triangles), *Holopedium gibberum* (diamond, only present in Ljustjärn), and macroinvertebrates (squares) of the three lakes surveyed (depicted by different colors). Relative length of vectors from the fatty acids identified to contribute most to the observed difference in the composition between zooplankton and invertebrates depicts the strength in positioning in the respective dimension.

to assess and can be highly variable (e.g., del Rio et al. 2009).

The use of fatty acid analysis broadened our approach for the study of trophic specialization in perch by including the aspects of the quality of the resources assimilated. Fatty acid composition of resources varied greatly between the two habitats. Generally, the composition of benthic macroinvertebrates was characterized by higher proportions of fatty acids with one or two double bonds, such as 16:1n-7, 18:1n-7, 18:2n-6, and 18:1n-9. These fatty acids are commonly found to be prevalent in littoral macroinvertebrates (Lau et al. 2012, 2013), therefore serving as littoral biomarkers, indicating, for example, contributions from epilithic diatoms (16:1n-7) or terrestrial detritus (18:2n-6) (Napolitano 1999). In contrast to the littoral habitats, more n-3-PUFAs such as 22:6n-3, 18:4n-3, and 18:3n-3 were found in the pelagic sources (i.e., cladocera and copepoda), also generally reflected in higher n-3/n-6 ratios in these organisms. Furthermore, SIMPER analysis

identified 14:0 as being higher in the pelagic than in the littoral resources. This fatty acid is more prevalent in the pelagic sources, as it is abundant in many algae taxa (Dalsgaard et al. 2003). Thus, our results are in accordance with those of previous studies, showing a prevalence of essential PUFAs in pelagic habitats (Napolitano 1999, Brett et al. 2009, Lau et al. 2012). Unfortunately, we did not analyze the fatty acid composition of predatory cladocerans such as *Bythotrephes*, which were identified to be an important prey item (on average 54.5% contribution in the pelagic perch from Erken) from the data on stomach content analyses. However, previous studies showed higher proportions of important PUFAs, such as 20:4n-6, 22:6n-3, and 20:5n-3, in predatory cladocerans, for example, *Bythotrephes*, compared to herbivorous ones (Persson and Vrede 2006, Hiltunen et al. 2015).

Fatty acid signatures of resources in the different habitats were also partly reflected in the fatty acid composition of the perch muscle tissue.

Littoral perch were characterized by abundant littoral fatty acids, such as 16:1n-7 and 18:2n-6. Similarly, 16:1n-7 was found to be an important benthic indicator in yellow perch (*Perca flavescens*) of Lake Michigan, USA (Happel et al. 2015). Fatty acid composition of pelagic phenotypes was also high in proportions of 18:3n-3, 20:4n-6, and 18:4n-3, which were also common in the pelagic resources. However, n-3/n-6 ratios were not generally higher in fish caught in the pelagic zone. Long-chain (>C18) polyunsaturated fatty acids are essential compounds of the cell membranes in vertebrates, playing an important role in the growth and development of fish (Sargent et al. 1999, Bell and Sargent 2003, Tocher 2003). Generally, it is assumed that fish are not able to synthesize long-chain polyunsaturated FAs (>C18) de novo, and an uptake via the diet is crucial to maintain an optimal physiological status (Yang and Dick 1994). However, evidence exists that freshwater fish are, to a certain degree, capable of converting shorter-chain fatty acid precursor such as 18:2n-6 and 18:3n-3 to longer-chain ones such as 20:4n-6, 20:5n-3, and 22:6n-3 (Xu and Kestemont 2002, Tocher 2003, Murray et al. 2014). One of the most essential PUFAs, 22:6n-3, is especially important for the neural development with high concentrations in brain, eyes, and nerves and is generally found in high concentrations in freshwater fish (Parrish 2009); especially abundant in pelagic resources, 22:6n-3 reaches highest proportions in selective feeding copepods (see also Persson and Vrede 2006, Ravet et al. 2010). Interestingly, the proportions of 22:6n-3 were similar in the littoral and the pelagic fish, even though we observed a high degree of specialization on littoral resources that were generally of lower 22:6n-3 proportions in the littoral phenotypes. One possible explanation for this pattern would be that the littoral fish would compensate for low 22:6n-3 availability by converting shorter-chain analogs by elongation and desaturation (Xu and Kestemont 2002, Tocher 2003, Murray et al. 2014). This would come at a cost, as metabolic support is required to maintain the enzymatic machinery, especially specific desaturases. Another possibility could be that specific gut endosymbionts may help to take up essential fatty acids of food sources of lower quality. Experiments with mice have shown that specific microbes may help to produce PUFA-derived

metabolites (Druart et al. 2015). However, the research field on gut microbiota and the connection to their host metabolism has just started to develop and many pathways and specific adaptations are still unknown. Future studies need to resolve the possible degree of variation connected to enzymatic pathways as well as the role of gut endosymbionts involved in synthesizing important PUFAs in natural populations.

Using compound-specific carbon isotope analysis of fatty acids, Koussoroplis et al. (2010) showed an overall higher reliance of gray mullet *Liza saliens* on 22:6n-3 from the planktonic sources even after the ontogenetic niche shift from the zooplankton- to a benthic-based diet. Thus, it can be reasonably assumed that a minimum reliance on pelagic resources involving the selective feeding on specific prey items of high quality prevails even after a diet shift to more benthic resources. Feeding on high-quality prey items may provide littoral perch with essential fatty acids as they are lacking essential biogeochemical compounds (i.e., 22:6n-3). However, this would require a habitat shift potentially leading to a trade-off between acquiring resources of high quality in the pelagic and foraging on the littoral resource. In turn, this trade-off could potentially influence the intraspecific competition that has been identified as the major driver of habitat distribution, trophic specialization, and divergence in perch (Svanbäck et al. 2008). This would then be a potential example for how the spatial variation in food quality would contribute to population divergence. Unraveling such patterns would only be possible with the combined approach of gut content, stable isotope, and fatty acid analyses with morphological analyses as demonstrated in this study.

Ecological speciation occurs along a continuous gradient, from early stage of weak population differentiation all the way to a complete, irreversible genome-wide divergence (Hendry 2009, Langerhans and Riesch 2013). However, often the process of speciation has been found to be rather incomplete and early stages without reproductive isolation are common in many taxa (Nosil et al. 2009). Eurasian perch in Swedish lakes is one example of an organism that could be placed at the beginning of this continuum. Morphological divergence is occurring frequently (Svanbäck and Eklöv 2002, 2003, Bartels et al.

2012, Faulks et al. 2015), but no correlation with genetic divergence has been found and resource polymorphism varies and may be predominately caused by phenotypic plasticity (but see Faulks et al. 2015 for evidence on assortative mating suggesting an influence of mate choice). Bartels et al. (2012) have identified the potential factors that influence the degree of population divergence in perch. Water transparency was found to act as a key driver, but the occurrence of roach (*Rutilus rutilus*), a strong interspecific competitor (Persson and Greenberg 1990), and piscivores in the littoral zone was further identified to contribute to the development of resource polymorphism (Svanbäck et al. 2008). In Ljustjärn, high abundance of roach and piscivores together with high abundance of pelagic resources of *H. gibberum* may explain the rather low degree of morphological and trophic divergence in this lake of high water transparency. Therefore, our results further support the recent findings that the trophic specialization and the connected change in morphology in perch are a rather flexible, plastic trait that may allow the responses to variable predatory and resource regimes (Svanbäck and Eklöv 2006). This was also experimentally demonstrated in Olsson and Eklöv (2005), showing that morphotypes could be induced by habitat structure and feeding mode and that this pattern could be reversed within 4 weeks of time.

Furthermore, our results suggest that the requirements of essential fatty acids could potentially constrain the development of advanced stages of ecological speciation in perch. It is possible that the littoral perch need to obtain, directly or indirectly, a specific proportion of their diet from the pelagic, high-quality resources to meet the metabolic requirements for growth and reproduction. However, further studies are necessary to specify the role of food quality in trophic specialization and the possible inhibiting effects of essential biochemical compounds in ecological speciation.

### Conclusions

By combining different methods with resource use assessment techniques, we provide a deeper understanding of the trophic specialization of perch. Data on stomach content provided information on the food assimilated that would not have been detected if only stable isotope

or fatty acid analyses would have been applied, for example, the high specialization on single prey items such as *H. gibberum* in Ljustjärn. However, if the sample size is small and obtained from a restricted time period only, general specialization patterns could be overlooked as it would have been the case in Långsjön, if assumption were purely based on the calculation on the dietary overlap. In contrast, stable isotope analyses provided data on diet assimilated over a longer time period, indicating that the trophic specialization in perch of Långsjön and Erken is a long-term and established pattern. Fatty acids added a new layer of comprehension in the context of trophic specialization due to the information gained on the quality of resources assimilated and the potential physiological constraints involved in the processes of resource polymorphism. All three different resource use assessment techniques of this study showed a high degree of variation between the different lake ecosystems, potentially caused by specific biotic or abiotic factors, such as interspecific competition and predation pressure. Further field-based research is needed to unravel the underlying mechanisms of the strength of trophic specialization in teleost fish.

The information obtained by a suite of methods can be valuable to better understand the relative costs and benefits and the potential trade-offs involved in generating and maintaining resource polymorphism, which has been identified to be a common feature of many vertebrate populations (Skúlason and Smith 1995, Smith and Skúlason 1996).

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