



Long term diet differences between morphs in trophically polymorphic *Percichthys trucha* (Pisces: Percichthyidae) populations from the southern Andes

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Divergent natural selection is often believed to be the driving force behind phenotypic differentiation in characters related to resource acquisition, leading to trophic polymorphism in fishes. Here we use variation in the fatty acid composition of adipose and muscle tissues to look at differences in resource use by two recently described sympatric morphs of *Percichthys trucha*, a common freshwater fish of the Andean and Patagonian regions of South America. Because dietary fatty acids are often stored in carnivorous animals with little modification after consumption, they can be used to infer information about dietary habits of individuals. We found that the two morphs differed in the overall composition of fatty acids in both adipose and muscle tissue, but that there were some differences in how the morphs differed in lakes from the northern vs southern part of the range. Furthermore, we found that certain fatty acids were correlated with diet as determined by gut content analysis. Consumption of anisopteran larvae was highly correlated with 14:0 in adipose and muscle tissue; and higher levels of longer chain unsaturated fatty acids (i.e. 20 and 22 carbons) were correlated with the presence of fish and also amphipods in the diets. Taken together, the results suggest that there are marked differences in the foraging ecology of the two morphs of *P. trucha* inhabiting southern Andean lakes.

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INTRODUCTION

Identifying the mechanisms responsible for the evolution of specialization, and the processes that ultimately lead to adaptive radiation, is fundamental to an understanding of the origins of diversity. Divergent natural selection in heterogeneous environments is probably the principal driving force causing phenotypic differentiation in characters related to resource acquisition. Such selection is often believed to lead to trophic polymorphism, the presence of two or more co-existing conspecific types that are morphologically distinct in trophic related characters (for a recent review see Smith & Skúlason, 1996). Trophic polymorphism has been described for a number of north temperate freshwater fish (Robinson & Wilson, 1994; Bell & Andrews, 1997; Skúlason, Snorrason & Jónsson, 1998). The variation in morphology is usually linked to structures that facilitate feeding and often improve feeding efficiency (Bentzen & McPhail, 1984; Meyer, 1989; Ehlinger, 1990; Schluter, 1995; Robinson, Wilson & O'Shea, 1996).

Recently, *Percichthys trucha*, a south temperate fish endemic to Patagonia and widely distributed within the region (Ringuelet, Aramburu & Alonso de Aramburu, 1967; Arratia, Peñafort & Menú Marque, 1983) has also been found to exhibit trophic polymorphism. Populations of *P. trucha* inhabiting a number of Andean lakes were composed of two morphs that differed most notably in gill raker length, but also in head and jaw dimensions (Ruzzante *et al.*, 1998). Both morphs were benthic feeders but one morph had longer gill rakers and was found more frequently in the littoral zone (long gill raker morph, or Morph 1), while the other had shorter gill rakers and was found more frequently in deeper benthic areas (short gill raker morph, or Morph 2).

Polymorphisms are thought to evolve most frequently in environments with 'open niches', where some resources are under-utilized, perhaps because the habitat has been colonized recently, is isolated, or for some other reason has few competitors (Bernatchez & Dodson, 1991; Robinson *et al.*, 1993; Schluter, 1996; Bell & Andrews, 1997). Under these conditions, dietary differences may emerge, as some individuals exploit the under-utilized resources, and assuming assortative mating and quantitative inheritance of relevant traits, evolution towards a more efficient exploitation of the resources drives an initially unimodal but variable population into a bimodal state (Doebeli, 1996a,b). The link between morphological variation and pattern of resource use (dietary differences) is thus critical to explaining why specific structures and behaviours have evolved.

The conventional method for determining differences in diet of freshwater fish is

the examination of stomach contents (e.g. Schluter & McPhail, 1992; Getachew, 1993; Ruben & Konopyla, 1994; Winemiller & Kelso-Winemiller, 1994). This method can provide information on the prey that have been consumed recently (typically the previous four to seven days for fish). However, estimates of consumption rate and prey species composition can be severely biased due to differences in digestion time or detectability (e.g. Jobling & Brieby, 1986). An alternative method, fatty acid analysis, can be used to provide additional information on diet (Iverson, 1993). When triglycerides and other forms of lipids are ingested, they are broken down into free fatty acids and monoglycerides before the body can incorporate them. Ingested fatty acids that are not utilized for immediate energy requirements are generally stored without substantial modification in adipose tissue (Iverson *et al.*, 1995). Chain elongation and insertion of double bonds may occur to some extent in specific organisms, or more generally, when fatty acids are incorporated into the cell membrane structure (Hagen, Kattner & Graeve, 1995; Pond *et al.*, 1997); and some biosynthesis can occur from other dietary components such as amino acids. However, despite these potential changes, differences in fatty acid composition are detectable in the tissues of animals that have been fed different diets; and these differences reflect a longer time integration of diet differences (e.g. Lee, Nevenzel & Paffenhofer, 1971; Sargent *et al.*, 1988; Fraser *et al.*, 1989; Iverson, 1993; Kirsch *et al.*, 1998).

The two recently described morphs of *P. trucha* showed some differences in prey consumption using stomach content analysis; the long gill raker morph consumed more Odonata than the short gill raker morph in five out of six lakes sampled while in the remaining lake it consumed more *Galaxias maculatus*, a small native fish common in littoral, vegetated areas of these lakes (Ruzzante *et al.*, 1998). However, there was little other evidence of inter-lake differences in diet. Our objective in the present study is to examine whether the dietary differences between the two morphs as determined from stomach content analysis are reflective of longer term feeding patterns. We examine the fatty acid composition of *P. trucha* and compare these results with those obtained by conventional examination of stomach contents. We conclude that by providing a 'time-integrated' perspective of diet, the fatty acid composition of adipose and muscle tissues demonstrates that there are persistent differences in resource use between morphs in trophically polymorphic populations of *P. trucha* in the Andean lakes of southern Argentina.

MATERIAL AND METHODS

Study sites and collection of samples

We used gill nets to collect fish from six lakes, ranging in size from 0.08 km² to 33.8 km², all located in the Limay River basin of southwestern Argentina (Fig. 1): Quillén, Ruca Choroí, Falkner-Villarino, Correntoso, Espejo and Morenito. The lakes can be divided into two geographic areas, where lakes within each area share the same drainage. Two lakes (Quillén and Ruca Choroí) are located in the northern region (G1), and the remaining lakes to the south, in G2. All sampling was conducted between January and March 1996; we sampled Lakes Correntoso, Espejo, Falkner-Villarino twice during this period, and the other lakes once. We placed sets of gill

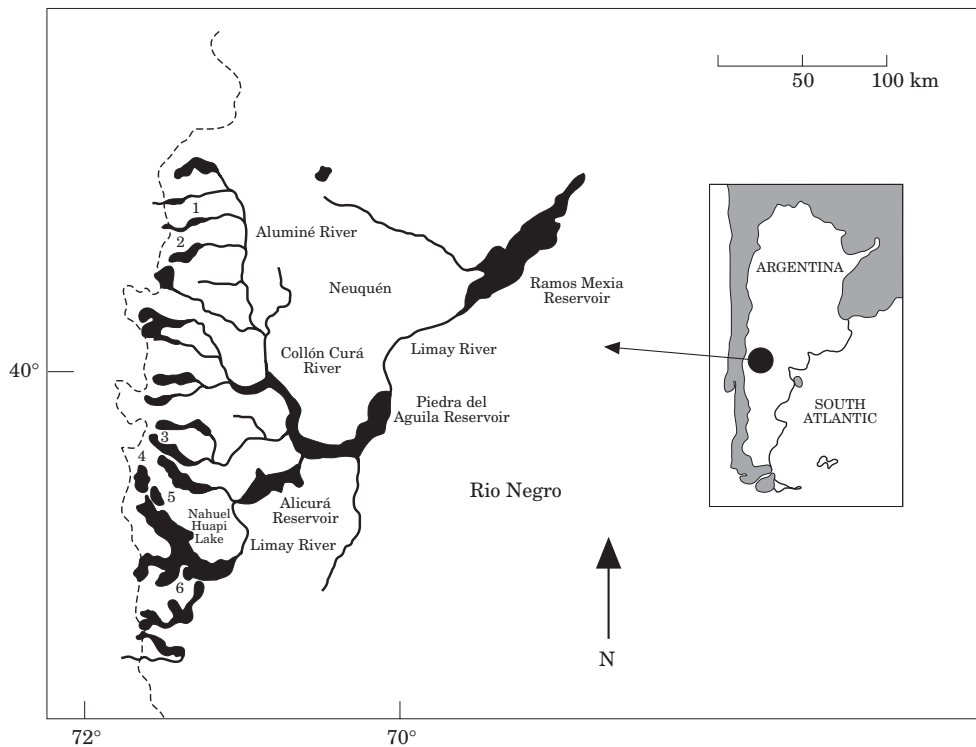


Figure 1. Map of the Limay River Basin. Lakes in G1 are (1) Ruca Choroí, and (2) Quillén. Lakes in G2 are (3) Villarino-Falkner, (4) Espejo, and (5) Correntoso and (6) Morenito.

nets with stretched mesh sizes ranging between 30 mm and 140 mm at two locations in each lake: one set in or near standing or submerged vegetation, and the second set in a location without vegetation cover, and usually with a steep bottom inclination. When possible, nets were placed at three depths at each site: surface, 10 m, and 20 m depth. The gill nets were set at dusk and the fish were collected after dawn the next morning. Immediately following collection we selected 8 to 10 individuals of *P. trucha* from each lake for fatty acid analysis. We chose individuals (in pairs) that under the naked eye differed most in external (mostly jaw and head length) morphometry. Over all lakes, we collected $n_1 = 55$ and $n_2 = 52$ samples of adipose and muscle tissue, respectively. Samples were placed immediately in glass vials containing a 2:1 (v/v) solution of chloroform/methanol and were then stored at -30°C . We used morphological measurements as described by Ruzzante *et al.* (1998) to determine the morph (M1 and M2) of each individual. Recent diet (prey type) was determined examining stomach contents. Contents of the intestine were excluded to minimize bias due to differential passage rates of prey. For each prey category (i.e. family, genus or species) we calculated an index of relative importance, RI, which takes into consideration the number of prey items of a particular type, their volume and the number of non-empty stomachs containing prey of that type (Pinkas, Oliphant & Iverson, 1971). Details of this analysis are found in Ruzzante *et al.* (1998).

Fatty acid analysis

Lipids were extracted using 2:1 chloroform/methanol according to Folch, Lees & Sloan-Stanley (1957) as modified by Iverson (1988), and fatty acids were transesterified according to Iverson, Frost & Lowry (1997b). We analysed the resulting fatty acid methyl esters using a Perkin Elmer Autosystem II Capillary FID temperature-programmed capillary gas-liquid chromatograph equipped with a 30 m × 0.25 mm id column coated with 50% cyanopropyl polysiloxane (0.25 µm film thickness; J&W DB-23; Folsom, CA, U.S.A.). Peaks were identified according to Iverson *et al.* (1997b) and integrated and quantified using Turbochrom 4.0 software (Perkin Elmer). Individual fatty acids are expressed as mass percentage of total fatty acids and designated by shorthand IUPAC nomenclature of carbon chain length:number of double bonds, and location ($n-x$) of the double bond nearest the terminal methyl group.

Data analysis

We used several statistical approaches to examine the pattern of variation in fatty acid content between morphs and between geographic regions.

First, we looked for overall differences in fatty acid concentration between morphs and between the northern and southern regions, using MANOVA and multidimensional scaling (MDS) analysis. For these analyses we used a subset of the total suite of identified fatty acids, consisting of the 16 most abundant and/or most important dietary fatty acids (Iverson, 1993). Patterns for adipose and muscle tissue were analysed separately. MANOVA provided a way of explicitly testing for an interaction between morph and region. We included the corresponding 2-way ANOVAs to indicate which of the fatty acids were probably mostly responsible for the multivariate patterns. For these multiple tests we adjusted significance levels according to the sequential Bonferroni method (Rice, 1989). MDS analysis (on untransformed data) provided a measure of the fraction of the total variance that is explained by each dimension. We used pairwise scatterplots of the MDS scores to determine the extent to which the various dimensions reflect differences between morphs since closer points reflect greater similarity.

We then used a classification and regression tree (CART) analysis, to determine if the individual fish could be correctly classified into morph and region using knowledge of their fatty acid composition. CART also allowed us to identify the variables responsible for the groupings. We used the entire set of fatty acids (69) for this analysis, since classification trees or tree-based models have no limit to the number of variables that can be considered regardless of sample size (Venables & Ripley, 1994). At each step of the analysis the method screens the entire set of fatty acids and algorithmically chooses a set that can be used to classify individuals into relatively homogeneous groups based on similarities in patterns of fatty acid proportions. Such tree-based methods use a statistical criterion (change in deviance) to select the appropriate subset of fatty acids at each step (Smith, Iverson & Bowen, 1997). Nodes are defined with the variable (fatty acid) that provides the largest difference between groups and the split value (proportion) that best separates the groups. This analysis is then carried out for the two groups that are created by the split in a tree-like fashion until the variability within the node is minimized or the

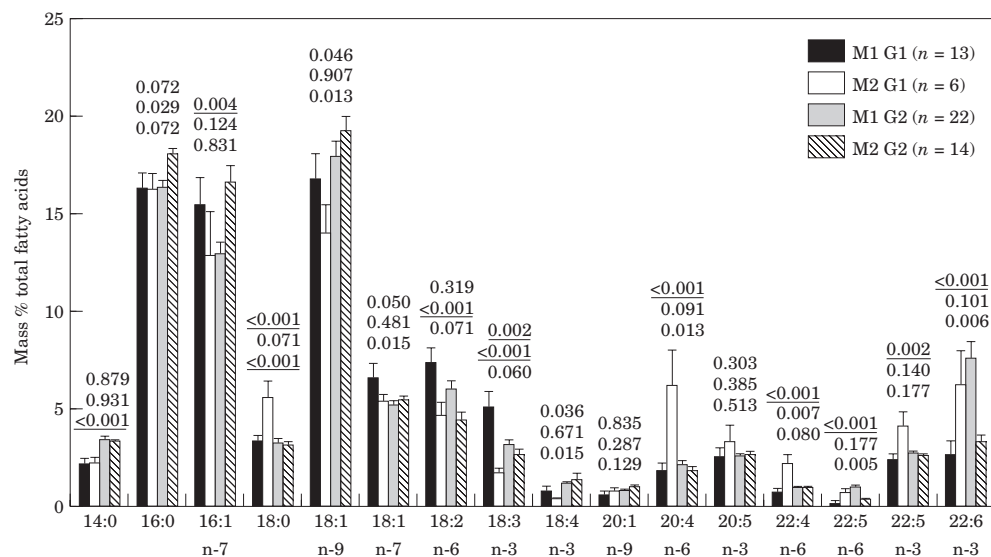


Figure 2. Selected fatty acids (mean \pm SEM) in adipose tissue collected from individuals ($n=55$) of *P. trucha*, separated into M1 (morph 1, long gill raker morph) and M2 (morph 2, short gill raker morph) within geographic regions G1 and G2. Differences between morphs and regions were tested by 2-way ANOVA on arcsin transformed data. The top, middle and bottom terms above each fatty acid are the P -values for the interaction term, morph and region, respectively. Underlined P -values are significant after sequential Bonferroni correction (initial $\alpha=0.05/16=0.003$).

sample size of the node is too small ($n \leq 4$). CART has recently been used to examine fatty acid signatures in studies of the foraging ecology of seals (Smith *et al.*, 1997; Iverson, Arnould & Boyd, 1997a; Iverson *et al.*, 1997b).

Finally, we examined the patterns of correlations between the proportions of the 16 most abundant/important fatty acids in individuals of *P. trucha* and their most recent diet as determined from the number of prey items in the stomach contents (from Ruzzante *et al.*, 1998). Fatty acid concentrations were arcsine transformed for the MANOVA and correlation analyses, and prey abundance data were log transformed [$\log(X+1)$] for the correlation analysis. After sequential Bonferroni adjustment for initial $K=112$ tests (16 fatty acids, 7 food groups), the initial significance level for these tests was set at $\alpha=0.05/112=0.0004$ (Rice, 1989). All analyses were conducted with standard Splus[®] statistical software.

RESULTS

We identified and quantified a total of 69 fatty acids in all samples. Adipose and muscle tissue generally contained the same abundant fatty acids, although composition varied between tissues, morphs and geographic areas. The 16 fatty acids selected for MANOVA, MDS and correlation analysis are shown in Figures 2 and 3.

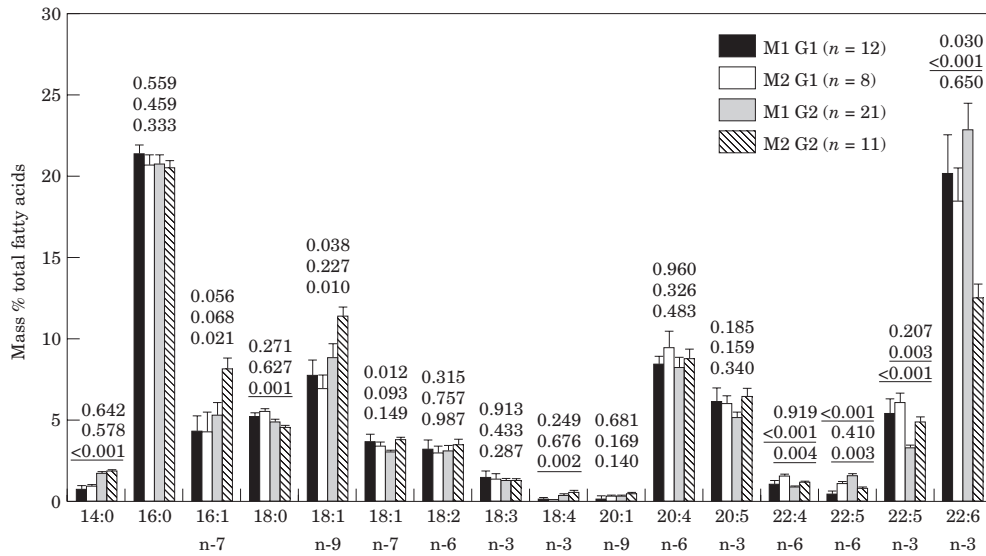


Figure 3. Selected fatty acids (mean \pm SEM) in muscle tissue collected from individuals ($n=52$) of *P. trucha*, separated into M1 (morph 1, long gill raker morph) and M2 (morph 2, short gill raker morph) within geographic regions G1 and G2. See Fig. 2 legend for further explanation. Underlined P -values are significant after sequential Bonferroni correction (initial $\alpha=0.05/16=0.003$).

TABLE 1. MANOVA analysis for the effect of morph and geographic region and their interaction on the concentration of the 16 most abundant and/or influential fatty acids. (A) Adipose tissue; (B) muscle tissue

| (A) | | | | | | |
|-----------------|----|-----------------|------------|--------|----------|------------|
| Variable | Df | Wilks λ | Approx F | Num df | Denom df | P -value |
| Morph | 1 | 0.421 | 3.101 | 16 | 36 | 0.002 |
| Geographic area | 1 | 0.317 | 4.848 | 16 | 36 | <0.001 |
| Interaction | 1 | 0.396 | 3.437 | 16 | 36 | 0.001 |
| Residuals | 51 | | | | | |
| (B) | | | | | | |
| Variable | Df | Wilks λ | Approx F | Num df | Denom df | P -value |
| Morph | 1 | 0.504 | 2.030 | 16 | 33 | 0.042 |
| Geographic area | 1 | 0.250 | 6.186 | 16 | 33 | <0.001 |
| Interaction | 1 | 0.420 | 2.849 | 16 | 33 | 0.005 |
| Residuals | 48 | | | | | |

Patterns of variation in fatty acid composition between morphs and across regions

Taken as a whole, the fatty acid composition of both adipose and muscle tissues of individuals of *P. trucha* depended on both morph (M) and geographic region (G) (MANOVA results, Table 1). However, the significant interaction between morph and region indicates that while the two morphs of *P. trucha* differed in the proportions of many of the most abundant fatty acids, the direction and/or magnitude of

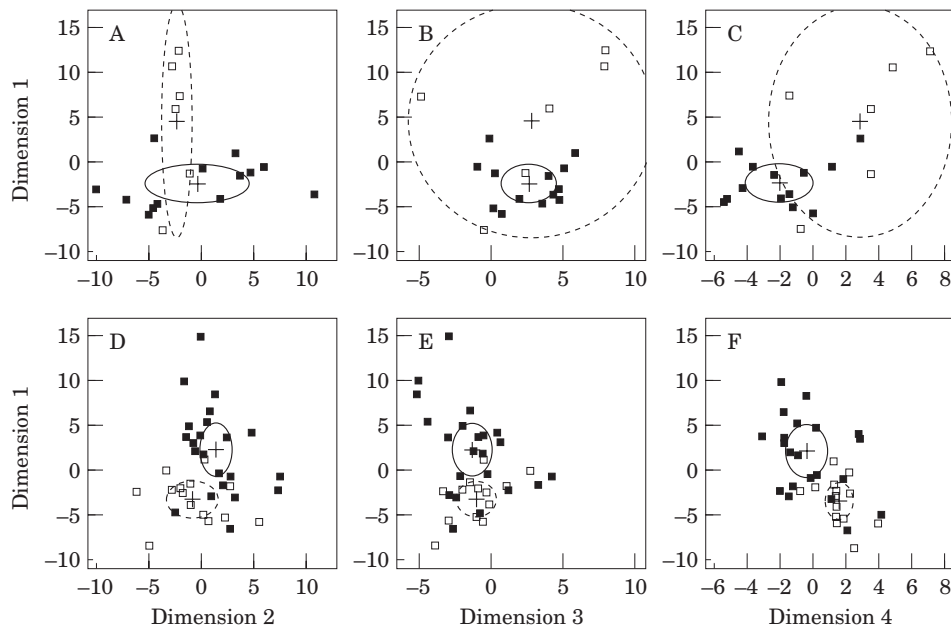


Figure 4. Scatterplots of Multidimensional Scaling analysis based concentration (mass % of total fatty acids) of the 16 most abundant and/or important fatty acids in adipose tissue of *P. trucha*. The top three panels reflect variation in the northern region (G1) and the bottom three panels reflect variation in the southern region (G2). Panels (A) and (D): dimensions 1 (41.3% of total variation) vs dimension 2 (22.4% of variation). Panels (B) and (E): dimensions 1 vs 3 (15% of variation). Panels (C) and (F): dimensions 1 vs 4 (10.6% of variation). Solid squares: morph 1 (long gill rakers). Open squares: morph 2 (short gill rakers). +: population means and ellipses are the 95% confidence intervals for the population means.

these differences were often not consistent between geographic regions. This was particularly evident in adipose tissue, where there was a significant interaction between morph and region for 8 out of the 16 most abundant fatty acids ($\alpha \leq 0.005$; Fig. 2). In the majority of these cases, there was a difference between morphs only in the northern region. For one fatty acid the difference between morphs was clearly consistent between regions: Levels of 18:2 n -6 were always higher in morph 1 (long gill raker) than in morph 2 (short gill raker). There was also one consistent difference between geographic regions, in that fish from the southern region (G2) had more 14:0 than fish from the northern region (G1; Fig. 2).

There was less evidence from muscle tissue that differences between morphs in particular fatty acids varied with geographic region; only one interaction (22:5 n -6) was significant (Fig. 3). Instead, several fatty acids in muscle tissue showed consistent differences across geographic regions and/or across morphs (Fig. 3). Among the fatty acids that showed an effect due to region: 14:0 and 18:4 n -3 were lower in the north (G1) than in the south (G2), whereas 18:0, 22:4 n -6, and 22:5 n -3, were higher in the north (Fig. 3). Two of the three fatty acids that showed a morph effect, 22:4 n -6 and 22:5 n -3, were higher in morph 2 than in morph 1. Fatty acid 22:6 n -3 was higher in morph 1 than in morph 2.

Scatterplots of MDS analysis further showed how the overall difference in fatty acid composition between morphs differed between regions (Figs 4 and 5). For

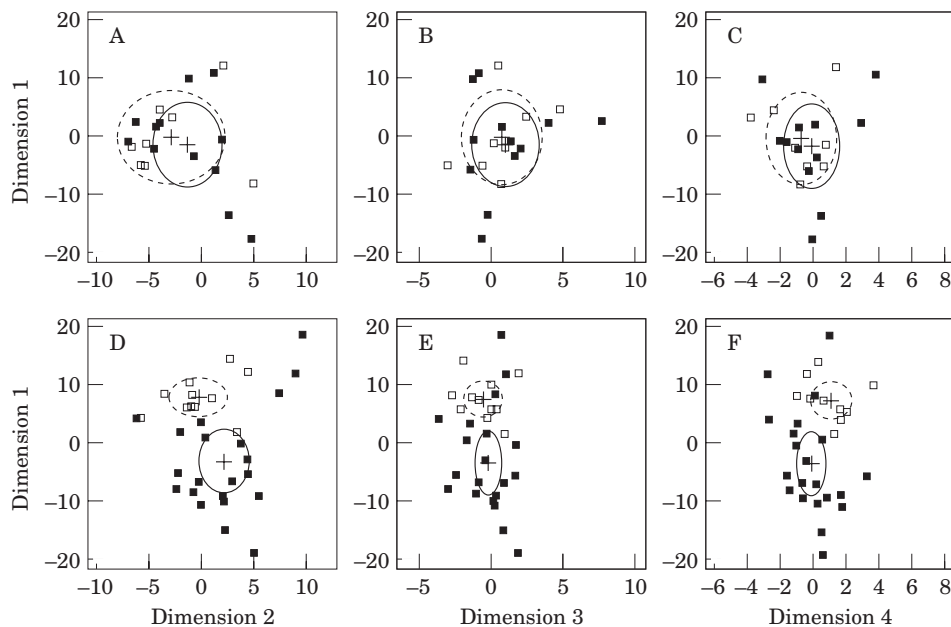


Figure 5. Scatterplots of Multidimensional Scaling analysis based concentration (mass % of total fatty acids) of the 16 most abundant and/or important fatty acids in muscle tissue of *P. trucha*. The top three panels reflect variation in the northern region (G1) and the bottom three panels reflect variation in the southern region (G2). Panels (A) and (D): dimensions 1 (72.8% of total variation) vs dimension 2 (16.7% of total variation). Panels (B) and (E): dimensions 1 vs 3 (3.9% of total variation). Panels (C) and (F): dimensions 1 vs 4 (2.7% of total variation). Solid squares: morph 1 (long gill rakers). Open squares: morph 2 (short gill rakers). +: population means and ellipses are the 95% confidence intervals for the population means.

adipose tissue, M1 individuals (solid squares) had lower scores than M2 individuals along Dimension 1 in the northern region, but had higher scores in the southern region (Fig. 4). Dimension 1 explained 41.3% of the total variation in fatty acid composition. Little difference was seen between morphs in either region along dimensions 2 (22.4% of variance) or 3 (15% of variance), except perhaps a relatively wide range along dimension 2 for morph 1 in the north but not in the south (Fig. 4A,D). M1 individuals tended to have lower scores than M2 individuals along Dimension 4 (10.6% of the variance) in both regions (Fig. 4C,F).

Muscle tissue scatterplots of MDS scores (Fig. 5) indicated a larger influence of morph in the south than in the north, particularly along dimension 1 (explaining 72.3% of the variance). Little difference between morphs was seen in either region along dimensions 2 (16.7% of variance), 3 (3.9% of variance), or 4 (2.7% of variance) (Fig. 5).

Classification of individuals into morphs and geographic region

Fatty acid composition could be used to correctly classify most of the individuals as to morphological type. CART analysis correctly classified 93% (51/55) of the individuals in M1 vs M2 morphs using adipose tissue and 92% (48/52) using muscle.

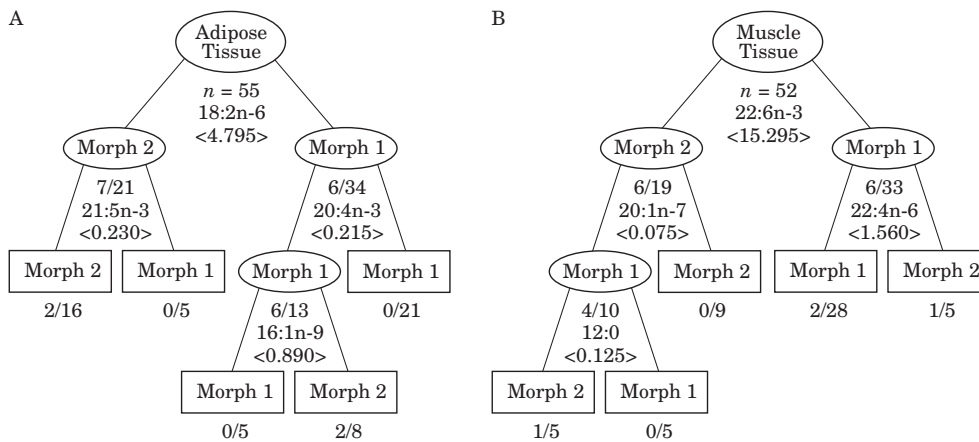


Figure 6. Classification tree of (A) adipose and (B) muscle tissue based on phenotype (i.e. morph) of *P. trucha*. Ellipses represent intermediate nodes, and boxes represent terminal nodes; labels within an ellipse or rectangle indicate the classification at that node as represented by the largest number of observations in that node. Root (first) and intermediate nodes include the variable and value (mass %) used to create the split. Samples containing < splitting value are classified on the left, and samples containing > this value are classified on the right. Fractions under each intermediate and terminal node indicate the number of misclassifications over the total number of observations in that node. Total misclassification rate was (A) 4/55 and (B) 4/52. Proportions of morphs correctly classified were (A) adipose tissue, 93%, i.e. 31 of 35 in the long gill raker morph (morph 1), and 20 of 20 in the short gill raker morph (morph 2); (B) muscle tissue, 92%, i.e. 31 of 33 in the long gill raker morph (morph 1), and 17 of 19 in the short gill raker morph (morph 2).

For both tissues, the majority of individuals of each morph were separated at the primary split (Fig. 6A,B). CART used 18:2n-6 for the primary split in adipose tissue, where M1 individuals had concentrations greater than 4.795%, and M2 individuals less (Fig. 6A). For muscle, the primary split used 22:6n-3, where most individuals of M1 had a concentration greater than 15.295% (Fig. 6B). After the initial split, the remaining misclassified individuals of each tissue type were successfully separated in a second or third split using fatty acids found at much lower concentrations (ones not included among the 16 most abundant fatty acids shown in Figs 2 and 3). M1 and M2 were classified with 89% and 100% accuracy, respectively, using adipose tissue, and with 94% and 90% accuracy, using muscle tissue (Fig. 6). The percentages of correctly classified individuals decreased but were still relatively high if only the fatty acid at the primary split was considered. M1 and M2 morphs were correctly classified with 80% and 70% accuracy, using only 18:2n-6 in adipose, and with 82% and 70% accuracy using only 22:6n-3 in muscle tissue.

Fatty acid composition could also be used to classify individuals of *P. trucha* into geographic regions, but more splits were required for the same level of accuracy. CART analysis indicated that 95% and 92% of individuals could be correctly classified as to geographic region using adipose tissue and muscle tissues, respectively (Fig. 7). With adipose tissue, individuals were successfully separated into regions within the first two splits (Fig. 7A). In contrast, muscle tissue required more splits to correctly separate regions using fatty acid composition (Fig. 7B).

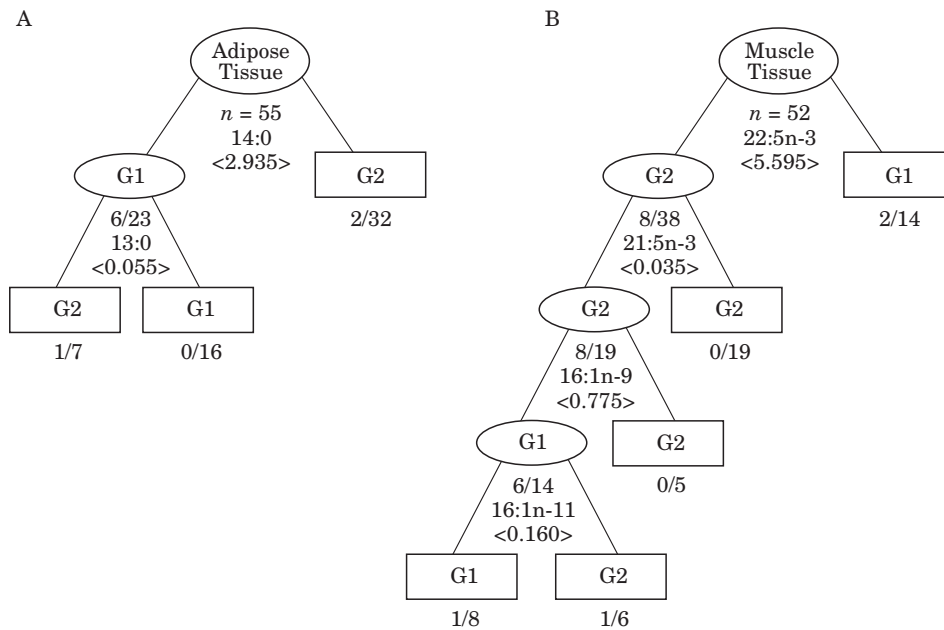


Figure 7. Classification tree of (A) adipose and (B) muscle tissue of *P. trucha* based on geographic region of *P. trucha* (see Fig. 1). See legend to Fig. 6 for explanation. Total misclassification rate was (A) 3/55 and (B) 4/52. Proportions of morphs correctly classified were (A) adipose tissue, 95%: 16 of 19 in G1, and 36 of 36 in G2; (B) muscle tissue, 92%: 19 of 20 in G1, and 29 of 32 in G2.

Correlation between recent diet (stomach contents) and fatty acid composition

Adipose tissue

There were three significant ($\alpha < 0.0004$) correlations between prey abundance in the stomach contents of individual *P. trucha* and fatty acid content in adipose tissue. Anisoptera in the diet were positively correlated with fatty acid 14:0 (Table 2A), and fish (two *Galaxias* species) in the diet were positively correlated with fatty acids 22:5n-6 and 22:6n-3 (Table 2A). In adipose tissue there were an additional 11 correlations between fatty acids and stomach content which did not reach statistical significance after Bonferroni correction for 112 simultaneous tests but which might indicate some tentative relationships (Table 2A). For example, the crustaceans *Aegla* sp and *Samastacus* sp were positively correlated with 18:1n-9, and amphipods were correlated with both 22:4n-6 and 22:5n-3. In general, *P. trucha* with gut contents containing fish and amphipods had relatively high percentages of relatively long-chain (22 carbon) polyunsaturated fatty acids in their adipose tissue, whereas those that had recently consumed Anisoptera and two large crustacean species tended to have higher percentages of short (14:0) and medium (18:1n-9) length fatty acids, respectively.

Muscle tissue

There was only one significant ($\alpha < 0.0004$) correlation between stomach contents and fatty acid concentration in muscle; the percentage of 14:0 was again highly correlated with the quantity of Anisoptera in the diet (Table 2B). There were also

TABLE 2. Correlations between stomach contents and fatty acid composition of (A) adipose ($n=53$) and (B) muscle ($n=50$) tissues of *P. trutta*. Values in bold are significant after sequential Bonferroni adjustment for 112 simultaneous tests (initial $\alpha<0.0004$). The P -values for correlations that were not significant after Bonferroni adjustment but which may nevertheless indicate tentative relationships are also indicated

| | 14:0 | 16:0 | 16:1 <i>n</i> -7 | 18:0 | 18:1 <i>n</i> -9 | 18:1 <i>n</i> -7 | 18:2 <i>n</i> -6 | 18:3 <i>n</i> -3 |
|----------------------|-----------------------------|-------------------|----------------------|-----------------------|-----------------------|-----------------------------|-----------------------|-----------------------------|
| (A) Stomach content | | | | | | | | |
| Anisoptera | 0.496 ($P=0.0002$) | 0.163 | 0.241 | -0.325 | 0.334 ($P=0.0140$) | -0.209 | -0.076 | -0.128 |
| Aegla/Samastacus | -0.188 | 0.040 | -0.198 | 0.061 | 0.421 ($P<0.0017$) | 0.053 | 0.254 | 0.166 |
| Galaxias spp. (fish) | 0.373 ($P=0.0060$) | -0.262 | -0.316 | -0.269 | -0.256 | -0.309 | -0.113 | -0.051 |
| Trichoptera larvae | 0.096 | -0.004 | 0.219 | -0.252 | 0.295 | -0.067 | -0.138 | 0.009 |
| Ephemeroptera | -0.142 | 0.215 | 0.370 ($P=0.0065$) | -0.073 | -0.131 | 0.223 | 0.077 | 0.091 |
| Amphipoda | -0.058 | -0.012 | 0.040 | 0.167 | -0.266 | -0.020 | -0.373 ($P=0.0057$) | -0.361 ($P=0.0079$) |
| Diptera | -0.207 | -0.154 | -0.014 | 0.313 | -0.382 ($P=0.0048$) | -0.017 | -0.051 | -0.027 |
| Stomach content | 18:4 <i>n</i> -3 | 20:1 <i>n</i> -9 | 20:4 <i>n</i> -6 | 20:5 <i>n</i> -3 | 22:4 <i>n</i> -6 | 22:5 <i>n</i> -6 | 22:5 <i>n</i> -3 | 22:6 <i>n</i> -3 |
| Anisoptera | 0.145 | 0.096 | -0.315 | -0.208 | -0.263 | 0.083 | -0.232 | 0.093 |
| Aegla/Samastacus | -0.199 | 0.196 | -0.033 | -0.099 | -0.021 | -0.169 | -0.252 | -0.170 |
| Galaxias spp. (fish) | 0.334 ($P=0.015$) | -0.033 | 0.010 | -0.008 | -0.006 | 0.619 ($P<0.0001$) | 0.116 | 0.521 ($P=0.0001$) |
| Trichoptera larvae | -0.099 | 0.166 | -0.209 | -0.229 | -0.161 | -0.132 | -0.213 | -0.103 |
| Ephemeroptera | -0.037 | -0.131 | -0.137 | 0.133 | -0.209 | -0.263 | -0.146 | -0.198 |
| Amphipoda | -0.207 | 0.287 | 0.346 ($P=0.0110$) | 0.181 | 0.449 ($P=0.0008$) | 0.209 | 0.356 ($P=0.0009$) | 0.078 |
| Diptera | -0.181 | -0.099 | 0.323 | 0.169 | 0.311 | -0.116 | 0.333 | -0.119 |
| (B) Stomach content | c14:0 | c16:0 | c16:1 <i>n</i> -7 | c18:0 | c18:1 <i>n</i> -9 | c18:1 <i>n</i> -7 | c18:2 <i>n</i> -6 | c18:3 <i>n</i> -3 |
| Anisoptera | 0.497 ($P=0.0002$) | -0.221 | 0.408 ($P=0.0033$) | -0.354 ($P=0.0117$) | 0.391 ($P=0.0049$) | 0.092 | 0.114 | -0.126 |
| Aegla/Samastacus | 0.039 | -0.145 | -0.033 | -0.055 | -0.053 | -0.065 | 0.283 | 0.199 |
| Galaxias spp. (fish) | -0.076 | 0.098 | 0.006 | -0.118 | -0.153 | 0.068 | -0.220 | -0.066 |
| Trichoptera larvae | 0.273 | -0.212 | 0.386 ($P=0.0096$) | -0.402 ($P=0.0038$) | 0.338 ($P=0.0163$) | 0.263 | 0.013 | -0.080 |
| Ephemeroptera | 0.008 | -0.014 | 0.330 | -0.063 | 0.083 | 0.434 ($P=0.0016$) | 0.122 | 0.092 |
| Amphipoda | -0.118 | 0.095 | -0.098 | 0.007 | -0.189 | -0.098 | -0.192 | -0.047 |
| Diptera | -0.233 | 0.026 | -0.168 | 0.259 | -0.251 | -0.050 | -0.063 | 0.079 |
| Stomach content | c18:4 <i>n</i> -3 | c20:1 <i>n</i> -9 | c20:4 <i>n</i> -6 | c20:5 <i>n</i> -3 | c22:4 <i>n</i> -6 | c22:5 <i>n</i> -6 | c22:5 <i>n</i> -3 | C22:6 <i>n</i> -3 |
| Anisoptera | 0.364 ($P=0.0094$) | 0.100 | -0.331 | -0.161 | -0.293 | 0.081 | -0.299 | -0.147 |
| Aegla/Samastacus | -0.179 | 0.164 | 0.342 ($P=0.0150$) | 0.134 | 0.167 | 0.005 | 0.007 | -0.209 |
| Galaxias spp. (fish) | -0.061 | -0.036 | 0.046 | 0.097 | 0.191 | 0.162 | 0.097 | -0.020 |
| Trichoptera larvae | 0.071 | 0.275 | -0.238 | -0.081 | -0.060 | -0.126 | 0.003 | -0.296 |
| Ephemeroptera | 0.023 | 0.088 | -0.142 | 0.144 | -0.013 | -0.159 | 0.020 | -0.211 |
| Amphipoda | -0.093 | -0.056 | 0.194 | 0.094 | -0.159 | 0.201 | 0.168 | 0.024 |
| Diptera | -0.122 | -0.082 | 0.012 | 0.113 | 0.323 | -0.130 | 0.447 ($P=0.0011$) | -0.029 |

a number of weaker correlations. Four fatty acids (16:1*n*-7 and three 18 carbon acids) tended to increase with Anisoptera. Individuals with Trichoptera larvae had high levels of 16:1*n*-7 and 18:1*n*-9, and low levels of 18:0. Diets of Ephemeroptera and Diptera larvae were linked to high levels of 18:1*n*-7 and 22:5*n*-3, respectively.

DISCUSSION

We recently described two sympatric morphs of *P. trucha* from a number of south temperate Andean lakes in Patagonia, one with longer gill rakers that tended to be found in shallower waters, and the second with shorter gill rakers, and with a deeper distribution (Ruzzante *et al.*, 1998). Here we show that the two morphs also differ in the fatty acid composition of their adipose and muscle tissues, and that some of the differences are sufficiently marked and consistent that one can correctly classify most individuals as to morph using an array of fatty acids. In addition, we find geographic variation in the fatty acid composition of both muscle and adipose tissues. At least some of the differences in fatty acid composition appear to broadly reflect differences in diet as shown by correlations with abundance of prey found in stomach contents. The results therefore provide evidence of persistent or long-term differences in dietary patterns between morphs in these trophically polymorphic populations of *P. trucha*, and thus suggest a link between morphological variation and pattern of 'time-integrated' resource use.

The two morphs differed in the concentration of a number of fatty acids, and taken together, these differences allowed us to correctly classify more than 90% of the individuals into the two morphs. This is all the more striking because, while we had previously shown some differences in diet based on stomach contents and some separation in habitat use (depth at capture), there was considerable overlap between morphs in both characteristics (Ruzzante *et al.*, 1998). The clear separation into the two morphs, based on fatty acid composition, strongly suggests that the small differences we detected earlier were of ecological significance, and that the morphs likely differ more in long-term dietary patterns than was indicated by the analysis of stomach contents. CART analysis suggested that the most consistent difference between morphs (across geographic regions) was the concentration of 18:2*n*-6 in adipose tissue and 22:6*n*-3 in muscle tissue. These fatty acids were also among those that differed significantly in the univariate ANOVA analyses, but did not differ in direction with geographic region. The emergence of 22:6*n*-3 as a critical fatty acid for morph differentiation, with the littoral morph (M1) having higher concentrations, is particularly interesting, as this fatty acid tends to be higher in fish than in crustaceans, and lowest of all in insects (see below). We know that in at least one of the lakes, M1 fed extensively on a small fish, *Galaxias* (Ruzzante *et al.*, 1998).

We found a considerable amount of variation in fatty acid composition between fish from the northern versus southern lakes, both in adipose and muscle tissue. In addition, the magnitude, and sometimes even the direction of differences between the two morphs varied between the two sets of lakes. This geographical variation could have originated through any of a number of mechanisms. Numerous studies have demonstrated the often conservative transfer of fatty acids up marine or aquatic food chains. For example, fatty acids 18:2*n*-6 and 18:3*n*-3 are heavily linked to primary production. Plants are capable of *de novo* synthesis of *n*-3 and *n*-6 fatty acids

in general and are some of the only organisms that have the enzymes capable of placement of these double bonds (Gurr & James, 1980). Indeed, each family of plant in both marine (Ackman *et al.*, 1964; Lee *et al.*, 1971; Gurr & James, 1980; Sargent *et al.*, 1988; Fraser *et al.*, 1989) and lacustrine environments (Gurr & James, 1980; Renaud *et al.*, 1994; Leveille, Amblard & Bourdier, 1997) tends to exhibit characteristic fatty acid compositions. Hence, differences could arise in the fatty acid composition of herbivores and planktivores in different habitats due to differences in their consumption of specific primary producers (e.g. phytoplankton vs vascular plants), which could in turn contribute to differences in the fatty acid composition of the morphs occupying different habitats. Alternatively, or in addition, morphs could simply differ in their prey selection within and/or between habitats. Whether the observed differences are driven by differences in prey type consumed or differences in where these prey are found, the differences in fatty acid composition between morphs within each area supports the hypothesis that resource acquisition varies between morphs and is thus the likely driving force for the morphological differences in this species as has been suggested or shown for other freshwater fish (Ehlinger, 1989, 1990; Meyer, 1989; Schluter & McPhail, 1993; Robinson *et al.*, 1996).

While a number of factors can contribute to the fatty acid composition of animal tissues, one of the most important in carnivorous or piscivorous species is dietary intake of fatty acids (e.g. Sargent *et al.*, 1988; Iverson *et al.*, 1995; Kirsch *et al.*, 1998). Whole body fatty acid composition of fish has been shown to be far more directed by diet than by other environmental factors (Kirsch *et al.*, 1998). Although other factors such as ambient temperature and salinity can lead to modifications of body lipids in fish, this is primarily confined to the phospholipids of cell membranes (Greene, 1990), rather than the fat contained in adipocytes. Adipose tissue is primarily a storage tissue for lipids and thus should most directly reflect dietary intake. In contrast, a greater proportion of the lipids in muscle tissue are found in membranes, which are predominantly composed of structural fatty acids found in both phospholipids and lipoproteins. Although compositional changes of membranes are affected by diet, the fatty acids tend to be relatively more conserved and are also affected by the function of the membrane (Cowey & Sargent, 1972). In this study we found significant differences between morphs and geographic regions in both tissues.

We used the most recent meal as determined by stomach contents as an indicator of individual diet. Given the wide trophic spectrum characteristic of *P. trucha* (Macchi, 1991; Ferriz, 1989, 1993–1994; Grosman, 1993–1994), we cannot expect this to give a complete representation of the long-term diet of individuals, but it can be used as an index. We did not have samples of prey species of *P. trucha* for fatty acid analysis, but some general inferences can be made across broad taxonomic groups from literature values for freshwater insects, crustaceans, and fish larvae (e.g. Thompson, 1973; Hanson *et al.*, 1985; Blomquist, Borgeson & Vundla, 1991; Mjaavatten, 1997). Within the same area or ecosystem, fatty acids 22:5 n -3 and 22:6 n -3 tend to be higher in fish than in crustaceans and higher in crustaceans than in terrestrial or aquatic insects. Levels of 22:6 n -3 tend to be particularly high in some fish. Aquatic crustaceans, on the other hand, tend to differ from fish and insects in having higher levels of 20:4 n -6 (Mjaavatten, 1997).

The predominant prey type in the stomach contents of both morphs was Anisoptera larvae and, except for one lake, the primary difference between morphs was a

generally greater presence of these larvae in the long gill raker morph (M1) than in the short gill raker morph (M2) (Ruzzante *et al.*, 1998). The quantity of Anisoptera larvae in the stomach (as a proportion of total volume) was strongly and positively correlated with fatty acid 14:0 in adipose and muscle tissue, and also weakly associated with at least three other fatty acids (Table 2). Significant amounts of fatty acid 14:0 in animal tissue is predominantly the result of dietary intake (Nelson, 1992). Thus, the lack of any difference in proportions of 14:0 between morphs (Figs 2 and 3) was somewhat surprising, but could have resulted from the consumption of other prey types which were equally high in 14:0. Nevertheless, diets of *P. trucha* as determined from stomach contents differed among morphs in other respects which showed stronger relationships with specific fatty acids. For example, in the northern lakes (G1), amphipod crustaceans were particularly high in M2 and absent from M1 stomach contents; M1 diets contained only insect larvae (Ruzzante *et al.*, 1998). This could account for the higher levels of 20:4 n -6, in particular, but also 22:5 n -3 and 22:6 n -3 in the adipose tissue of M2 compared to M1 within this region (Fig. 2). In contrast, in some of the southern lakes (G2), amphipods were present in both M1 and M2 diets, but fish larvae, when present, were found only in M1 diets. This would be consistent with the very high levels of 22:6 n -3 in M1 adipose tissue compared to M2 in the southern region (Fig. 2). Furthermore, the level of 22:6 n -3 in the adipose tissue of *P. trucha* was strongly correlated with recent consumption of *Galaxias*, and it was also the fatty acid selected by CART to separate the two morphs using muscle tissue. Thus, some of the differences in fatty acids among morphs can be, very tentatively, linked to dietary differences. The correlations of specific tissue fatty acids with recent diet as determined from stomach contents, also suggests that the fish are probably somewhat conservative in their dietary habits.

To our knowledge, the only other study to date to have used lipids to detect functional trade-offs between or among coexisting phenotypes within a single species is that conducted by Robinson *et al.* (1996), who compared the amount of extractable lipids in skeletal muscle with relative growth rate in sunfish. Their methods and objectives were quite different, however. They measured total lipids rather than examining fatty acid composition, and their objective was to measure the 'condition' of the fish, i.e. to determine whether they had been on an adequate plane of nutrition. They concluded that fish with extreme benthic and limnetic phenotypes were on average superior to fish with intermediate morphologies, especially in the pelagic habitat.

In conclusion, we find some consistent differences among morphs in particular fatty acids, and each morph can be characterized by a suite of fatty acids. However, just as important, there appears to be considerable variation between regions as each morph perhaps specializes on prey according to their availability and abundance in that lake or region. However, within each lake or region, morphs do continue to differ from one another, not only in morphology but also in resource use. Thus the pattern we find is one of an overall difference in resource use (as indicated by their fatty acid signatures), but considerable regional variation in the details of that resource use, as indicated by fatty acid and stomach content analysis. This suggests that there is a general selection driving the formation of the two morphs, but that the details and nature of that selection differ between lakes. At present we do not know whether the morphological differences result from environmental influences during ontogeny, from genetic differences between morphs or from a combination of both factors (Ruzzante *et al.*, 1998). However, in addition to the differences in

pattern of fatty acid composition between the southern and northern lakes that we report here, we are now finding that the degree of morphological differentiation between morphs varies considerably among drainages (Ruzzante, Walde, Cussac, unpublished data). Our results are thus consistent with a hypothesis that the degree of reproductive isolation and, consequently, the degree to which this trophic polymorphism constitutes a stepping stone towards speciation in these fishes, may also vary among lakes or drainages.

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