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Resource polymorphism in a Patagonian fish *Percichthys trucha* (Percichthyidae): phenotypic evidence for interlake pattern variation

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Within-species differentiation in phenotypic characters related to resource use (resource polymorphism) is frequently thought to result from divergent natural selection in a heterogeneous environment with 'open niches'. In this study we found consistent resource-based polymorphism within three different populations of *Percichthys trucha*, a lake-dwelling fish native to the southern Andes. In each of three lakes we found two morphotypes that could be clearly distinguished by differences in gill raker length. However, the magnitude of the polymorphism, and the suite of phenotypic characteristics associated with the polymorphism, differed between lakes. Patterns of divergence were more similar between the two northern lakes which ultimately drain into a common river, than between these two lakes and a more southern, unconnected lake. The southern population, which had the largest divergence in gill raker length (32% vs. 16% and 19%), also showed substantial differences in diet. Evidence from the southern population suggests that polymorphism in *P. trucha* is present early during ontogeny. We conclude that while there are some strong parallels among lakes in the development of a trophic polymorphism, differences in environmental conditions and/or colonization history have led to substantial differences in the evolutionary history, resulting in different ecological roles of common morphotypes within different lakes. © 2003 The Linnean Society of London. *Biological Journal of the Linnean Society*, 2003, **78**, 497–515.

ADDITIONAL KEYWORDS: diet – divergence – fish – gill raker – morphology – Patagonia – variation in polymorphism.

INTRODUCTION

A long-standing goal in evolutionary biology has been to understand the processes that lead to speciation and to current patterns of species diversity. Natural selection and historical factors have both been identified as playing leading roles in phenotypic differentiation, adaptive radiation and speciation (Schluter, 1996, 2000; Losos *et al.*, 1998). While natural selection leads to deterministic patterns of differentiation, historical factors can produce different evolutionary outcomes even if environmental starting conditions and prevailing environmental conditions during a defined period are similar (Travisano *et al.*, 1995; Losos *et al.*, 1998). Although the two factors are often viewed as alternative explanations, both can be operating in the same system. For example, various morphologically similar but phylogenetically independent ecomorphs of *Anolis* lizards occur on various Greater Antilles islands suggesting that repeated adaptive radiation episodes in similar environments overcame historical contingencies to produce similar evolutionary outcomes (Losos *et al.*, 1998). Further, a pattern that appears deterministic can actually be the result of an

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interaction between historical contingency and natural selection (Taylor & McPhail, 2000). The occurrence of sympatric stickleback (*Gasterosteus aculeatus*) species pairs in postglacial lakes (McPhail, 1994) has been shown to be the result of 'double invasions' (historical events) by ancestral marine stickleback individuals, which then evolved independently but in a similar manner (determinism), into a limnetic and benthic pair in each lake (Taylor & McPhail, 2000).

Fish populations in post-glacial lakes provide opportunities to study cases of very recent, or incomplete, speciation and may thus be used as model systems to examine the processes underlying adaptive radiation (Schluter, 2000). Post-glacial lakes are relatively young environments where available or 'vacant' ecological niches are likely to be present. Such lakes tend to have few species, partly because there has been little time or opportunity for colonization and perhaps also because there has been less time for *in situ* speciation to have occurred. As a consequence, organisms that do colonize these environments are likely to encounter under- or unexploited resources and few competitors. Some populations of fish in many of these lakes are composed of two to several different morphological types (McPhail, 1994; Robinson & Wilson, 1994; Schluter, 1996, 2000; Smith & Skúlason, 1996; Bell & Andrews, 1997; Skúlason, Snorrason & Jónsson, 1999; Taylor, 1999). Such polymorphisms appear to be under varying degrees of genetic control (reviewed by Robinson & Schluter, 2000), and are generally related, at least in part, to feeding (Bentzen & McPhail, 1984; Ehlinger, 1990; McPhail, 1992, 1993; Skúlason *et al*., 1993; Schluter, 1995).

Most of the work referred to above has emphasized the striking parallels among species in patterns of polymorphism, and compilations examining variation in such patterns have generally focused on comparisons across species. Resource polymorphisms in freshwater fish usually (but not always) involve the exploitation of two very different habitat types (limnetic and benthic) with similar overall morphology of the forms in different species. Less attention has been paid to variation among populations in the magnitude and pattern of such polymorphisms, yet such variation does exist. Gross & Anderson (1984) described geographical variation in the gill rakers and diet of European threespine sticklebacks (*G. maculatus*). Griffiths (1994) reported a latitudinal increase in the magnitude of trophic polymorphism in Arctic charr (*Salvelinus alpinus*) and argued this was due to an increase in the intensity of competition resulting from increased seasonal changes in food abundance. Lu & Bernatchez (1999) and Gíslason *et al*. (1999) described a positive correlation between the magnitude of a polymorphism and levels of genetic divergence between sympatric morphs in whitefish (*Coregonus clupeaformis*) and Arctic char (*Salvelinus alpinus*), respectively. A recent study on trophically polymorphic populations of the pumpkinseed sunfish, *Lepomis gibbosus,* has shown that abiotic as well as biotic factors can be correlated with variation in the magnitude of the polymorphism (Robinson, Wilson & Margosian, 2000).

In this study, we examine variation among populations in the magnitude and pattern of a recently reported (Ruzzante *et al*., 1998) resource polymorphism within one southern temperate freshwater fish native to Patagonia and the southern Andes, *Percichthys trucha*. This species is endemic to, and widely distributed throughout southern Argentina and Chile (Ringuelet, Aramburu & Alonso de Aramburu, 1967; Arratia, Peñafort & Menu Marque, 1983). We found that populations from mountain lakes within one major watershed were composed of two morphs that differed most notably in gill raker length, but also in head and jaw dimensions. Although both morphs were predominantly benthic feeders, they showed differences in resource use. The morphs differed in the relative frequencies of prey types found in stomach contents (Ruzzante *et al*., 1998), and analysis of the fatty acid content of adipose and muscle tissues also suggested longer term differences in feeding patterns (Logan *et al*., 2000).

Here we extend our work on the evolutionary dynamics and polymorphism within *P. trucha* by asking (1) if the patterns of divergence are similar across lakes, and (2) if patterns of divergence can be detected early in the life history of the fish. We find evidence for parallel divergence in one key trait, gill raker length, but considerable variation in the patterns of differentiation in other traits. Large fish from the three populations we examine show divergence in gill raker length, and differentiation in this trait is already present in very small juvenile fish. However we also find variation among populations, in the magnitude of the divergence, in the pattern of differentiation of associated morphological traits, and in the way the morphotypes differed in diet.

METHODS

COLLECTION OF FISH

We examined the variation in morphology and diet within populations of *P. trucha* from three lakes located at altitudes between 500 and 1000 m above sea level within the temperate rainforest of the Patagonian Andes of south-western Argentina (Fig. 1). All lakes are well within the geographical range of the Pleistocene glaciation ice cap, and were thus formed after the retreat of the glaciers that took place between 15 000 and 14 500 years bp (Mercer, 1976; Clapperton, 1993). Lake Quillén, the northernmost lake, drains into the Aluminé River, which is part of

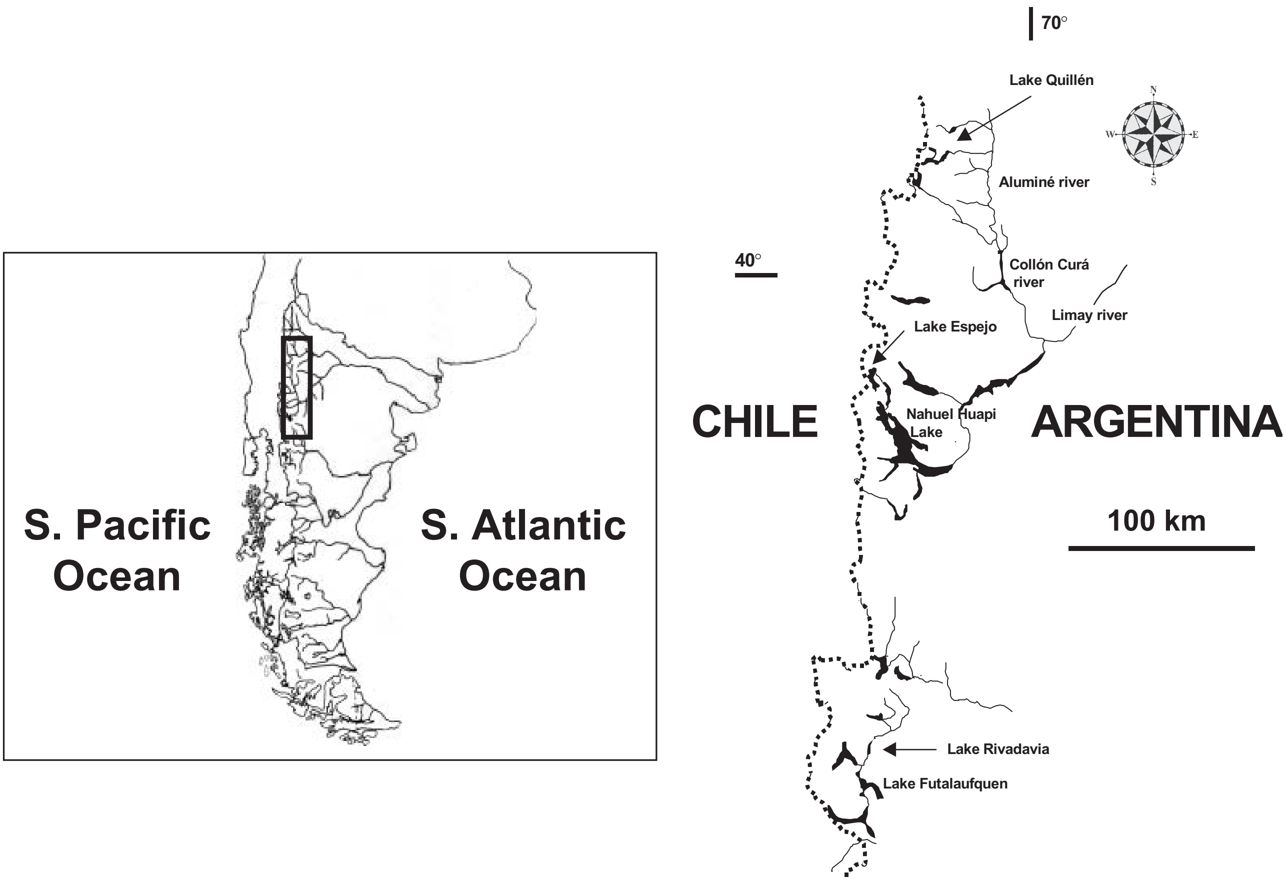

Figure 1. Geographic location of lakes. Two lakes, (1) Quillén (39°25′ S, 71°15′ W, 33.8 km^2, ~1000 m a.s.l.) and (2) Espejo (40°38′ S, 71°45′ W, 38.2 km^2, ~800 m a.s.l.), are situated within the Limay River basin within the Lanín and Nahuel Huapi National Parks, Provinces of Neuquén and Rio Negro, Argentina. The third lake, (3) Rivadavia (42°36′ S, 71°39′ W, 21.7 km^2, ~500 m a.s.l.), is situated within the Futalaufquen river basin within the 'Los Alerces' National Park in the province of Chubut. Other major lakes in the area are shown for completeness, but are not labelled for clarity.

the Limay River basin. Lake Espejo drains into the larger Lake Nahuel Huapi from which the Limay River originates. The Limay River system empties into the Atlantic Ocean. The southernmost samples were collected in Lake Rivadavia which is located in the Futaleufú river basin that drains across the Andes into the Pacific Ocean (Fig. 1). Lake Rivadavia is therefore not connected by any waterway to the other two lakes (Fig. 1). The lakes differ in size; lakes Quillén and Espejo are larger than Lake Rivadavia (Fig. 1). The lakes also differ somewhat in the composition of the fish assemblage and given that the diet of *P. trucha* could be influenced by other fish species present and what they feed on, we also report on the fish species composition for each lake.

Fish (*Percichthys trucha* and other native and introduced species) were collected with gillnets (stretched mesh sizes ranging from 30 to 140 mm) placed in three to five sites in each lake. Each lake was visited for up to a week. We placed gillnets in representative locations within the lakes, sites with standing or submerged vegetation (*Schoenoplectus californicus* Meyer Steud, and *Potamogeton linguatus* Hangstrom, respectively), or sites with rocky, exposed coastline, a steep bottom gradient and mud, sand, or stone substrate with little to no vegetation. At each site gillnets were set on the bottom at four depths: in the littoral zone near the surface of the water, and at 10, 30, and 50 m depth. In each lake we also placed a set of gillnets in the limnetic zone at midwater depth. Fishing effort was kept uniform among lakes and habitat type. The nets were set before dusk and hauled in after dawn the following morning. All fish were immediately removed and placed in 4% formalin. Where feasible we also collected fish from the littoral zone using a seine net. In Lake Rivadavia this method enabled us to collect larvae and juveniles of *P. trucha* in numbers sufficient for morphological analysis.

MORPHOLOGICAL ANALYSIS

All measurements were taken from formalin-preserved material. Each variable was measured twice to estimate repeatability or measurement error and consecutive measurements were taken at least one day apart. The means of the two measurements were used in the analysis. For all individuals ($N = 202$ adults and 79 small juveniles) we measured standard length (SL), head length (HL), mouth width (MW), length of the upper jaw (UJ), eye diameter (ED, mean of both eyes) and distance between eye sockets (interorbital distance, ID), depth of the caudal peduncle (CP), and length of the longest (usually the second) spine of the first dorsal fin (DF) (Fig. 2). We also counted the gill rakers, and measured the length of the four longest rakers, on the first left branchial arch (the longest rakers were usually those located side by side closest to the V-angle between the two branches of the arch). Gill rakers were first drawn under a stereomicroscope and later measured using image analyser software. Characters were selected based on their likely relationship to feeding and/or swimming ability. In addition, head length, mouth width, upper jaw length, and depth of caudal peduncle are diagnostic variables for species identification within the Percichthyidae (Ringuelet *et al*., 1967).

To control for differences in body size among the fish, we standardized all measured traits with respect to standard length using the relationship:

$$Y_i = \log(X_i) - b\,[\log(SL_i) - \text{Mean}(\log(SL))] \qquad (1)$$

where Y_i and X_i are the adjusted and original values for the character in individuals i ($i = 1, \ldots, N$), SL_i is the individual standard length, and b is the regression coefficient of the logarithm of X on the logarithm of SL. Allometric relationships did not differ between sexes for any lake, but they differed slightly among lakes for some of the variables. Variables were therefore standardized with sexes pooled, but separately for each lake. The same procedure of standardization to a common length was applied independently to the sample of larvae and juveniles ($N = 79$, median standard length = 16.8 mm) collected from Lake Rivadavia.

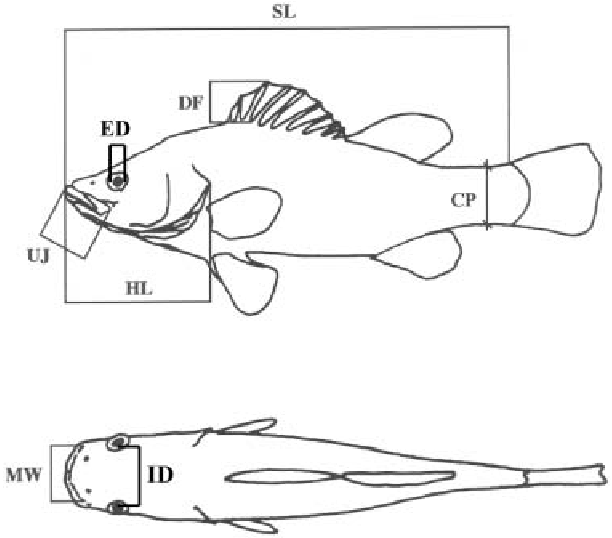

Figure 2. External morphological measurements of *Percichthys trucha*. SL: Standard length. HL: head length. UJ: length of upper jaw. MW: mouth width. ED: Eye diameter. ID: Distance between eye sockets. DF: height of dorsal spine. CP: Caudal peduncle depth.

We used several statistical approaches to examine the patterns of morphological variation. First, we used cluster analysis on various combinations of measured characters (standardized to a common standard length) to detect distinct morphological types within lakes (Fig. 2). In this analysis we used the method 'average' where the distance between clusters is the average of the distances between the points in one cluster and the points in the other cluster. We then used discriminant analysis to examine the extent to which different combinations of characters or single characters contribute to discriminating between defined clusters. Secondly, we examined the magnitude of the differences in the morphological characteristics between morphs, and the extent to which these differences were consistent across lakes. We used MANOVA to determine if there were significant differences between morphs in traits other than gill raker length. Subsequently we used classification and regression tree (CART) analysis (a non-parametric analysis, Venables & Ripley, 1999) to determine (1) which of the variables other than gill raker length most reliably distinguished the morphs, and (2) if these variables differed across lakes. Tree-based models such as CART characterize groups by first screening the entire set of variables (morphometric traits in this study) and algorithmically choosing a set that can be used to classify individuals into relatively homogeneous groups based on similarities in patterns. Nodes are defined with the variable 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 sample size of the node is too small ($N \le 4$). We recently used CART to examine dietary fatty acid signatures of the two morphotypes within *P. trucha* (Logan *et al*., 2000). All analyses were conducted with standard Splus statistical software (Mathsoft, 1998).

ANALYSIS OF DIET

To determine if the two morphs differed in diet, we examined the stomach contents of all individuals collected and estimated stomach fullness (proportion of stomach volume occupied by prey) visually. For each prey category 'i' (i.e. family, genus, or species) we counted the number of prey items consumed. For the juveniles we also calculated the proportion (by volume) that each prey type contributed to total volume.

Overall differences in diet between morphs within lakes were first determined by MANOVA, with prey categories as the dependent variables, and morph and length and their interaction as the independent variables. For adults this analysis was conducted on the absolute numbers of prey items (transformation log [x + 1]) consumed. For juveniles we conducted the MANOVA analysis using absolute numbers of prey in stomach content (transformation log [x +1]), as well as volume as a proportion of the total volume of prey items in individual stomachs (transformation: $2 \times \arcsin(\sqrt{x})$, where x is the proportion]. We also report the results of the univariate F-tests by taxon.

RESULTS

COMPOSITION OF THE FISH ASSEMBLAGE

The species composition of the fish assemblages differed from lake to lake (Table 1). Catch per unit effort (CPUE) for *P. trucha* was similar for Lakes Espejo and Rivadavia, but slightly lower in Lake Quillén (Table 1). Of the other native species, *Odontesthes hatcheri* (pejerrey) was far more common in Rivadavia than in the other two lakes, and *Galaxias platei* (puyen grande) was more abundant in Espejo and Rivadavia than in Quillén (Table 1). In addition, littoral seine netting yielded relatively high numbers of *Galaxias maculatus* (puyen chico) in Quillén and Espejo. This species was absent from Lake Rivadavia where, instead, we found a different species of small fish, *Aplochiton* sp. (peladilla) present in the littoral seine net collections. Total abundance of the three introduced salmonids differed strongly among lakes, with CPUE more than 5- and 3-fold higher in Rivadavia than in Quillén and Espejo, respectively. In Rivadavia, CPUE of the combined salmonids exceeded the catch of *P. trucha.* In Espejo, salmonid and *P. trucha* catches were roughly equivalent, and in Quillén, the catch rate of salmonids was less than half that of *P. trucha*.

MORPHOLOGICAL TYPES OF ADULT *P. TRUCHA:* VARIATION WITHIN AND AMONG LAKES

Identification of morphs

Exploratory cluster analyses, using up to seven (standardized) morphometric variables (head length (HL), upper jaw length (UJ), caudal peduncle depth (CP), dorsal spine height (DF), eye diameter (ED), interorbital distance (ID) and gill raker length) (Fig. 2) revealed that whenever gill raker length was used as one of the variables, two clusters emerged regardless of how many and which other variables were also included in the cluster analysis. We then used discriminant analysis to estimate the magnitude of the correlation between the various linear combinations of these variables and the clusters (morphs). We found that when morphs were defined (clustered) using gill

Table 1. Abundance (catch per unit effort (CPUE) and number of fish captured) and size (standard length in mm) of native and non-native fish caught in gill-net samples in three north-western Patagonian lakes

Lake		*Percichthys trucha*	*Oncorhynchus mykiss*	*Salvelinus fontinalis*	*Salmo trutta*	*Odontesthes hatcheri*	*Galaxias platei*	*Diplomystes viedmensis*
Quillén	CPUE (# fish)	3.45 (86)	0.95 (24)	0.24 (6)	0.08 (2)	0.04 (1)	–	–
	Length:							
	mean (SD)	265 (81)	323 (79)	330 (24)	472 (172)	345	–	–
	median	285	320	338	472	–	–	–
	range	84–375	120–455	300–355	350–593	–	–	–
Espejo	CPUE (# fish)	3.87 (57)	0.87 (29)	1.05 (34)	0.18 (6)	–	1.14 (38)	0.03 (1)
	Length:							
	mean (SD)	324 (63)	386 (116)	308 (84)	431 (131)	–	186 (31)	161
	median	345	435	331	474	–	194	–
	range	194–405	115–525	157–473	265–565	–	130–270	–
Rivadavia	CPUE (# fish)	3.87 (59)	5.66 (105)	1.18 (21)	0.06 (1)	1.90 (35)	1.01 (16)	–
	Length:							
	mean (SD)	241 (105)	328 (87)	274 (60)	706	258 (65)	199 (41)	–
	median	208	332	277	–	246	188	–
	range	89–450	133–510	197–433	–	160–428	150–290	–

raker length only, the correlations between the linear combination of the seven morphometric variables and the morphs were high for all three lakes ($r \geq 0.831$), and were not different from the correlations obtained when morphs were defined using combinations of variables ($t = -0.29$, d.f. = 4, P-value = 0.79). We therefore decided to define morphs on the basis of gill raker length only: a long gill raker morph and a short gill raker morph (Tables 2,3). Because a single variable was used there is no overlap in gill raker length between morphs.

There was no consistent difference between lakes in the average size of the two morphs (Table 2). Morphs did not vary significantly in standard length in Quillén ($P = 0.130$), in Espejo ($P = 0.873$), or in Rivadavia ($P = 0.080$) (Table 2). We caught more small fish (80–200 mm) in Rivadavia and Quillén than in Espejo. Sex ratios were usually female-biased (Table 2).

Morphological variation among morphs

Principal Component Analysis based on all seven morphometric characters indicate that the overall morphology of the two morphs differed significantly: the 95% confidence intervals for the two means in plots of components 1 vs. 2 and 1 vs. 3 did not overlap in any of the three lakes (Fig. 3). Together these three principal components accounted for 71%, 69.5%, and 73.4% of the total variance for lakes Quillén, Espejo, and Rivadavia, respectively (Fig. 3).

Next, we examined the magnitude of the differences in the morphological characteristics between morphs, and the extent to which these differences were consistent across lakes. For each trait and lake we calculated the percentage difference between morphs by subtracting the value of the trait in the short gill raker morph from that in the long gill raker morph and then dividing by the trait value in the short gill raker morph (×100).

The difference in gill raker length between the long and short gill raker morphs was significantly greater in Lake Rivadavia (32% difference in mean size) than in Lakes Quillén or Espejo (16–19% difference) ($P < 0.001$) (Tables 3,4). In addition, gill raker length, averaged across morphs, differed significantly between lakes, with individuals from Espejo having, on average, the longest gill rakers, and individuals from Quillén having the shortest ($P < 0.001$).

MANOVA run for each lake, with morph type as the independent variable, and all morphological characters (a log transformation was used to equalize variances) other than gill raker length as dependent variables (Table 3) revealed differences in several traits. In Quillén, the main difference between morphs was that the long gill raker morph had a longer upper jaw ($P = 0.004$). It also tended to have its eyes closer together ($P = 0.058$). In Espejo, the long-gill raker morph had a longer head ($P = 0.013$) and also tended to have a longer upper jaw ($P = 0.057$). However, its eyes were farther apart ($P = 0.004$), and it had a deeper caudal peduncle ($P = 0.021$). In Rivadavia, the long gill raker morph tended to have a shorter head ($P = 0.055$).

Next we determined whether the way the morphs differed in these characters was consistent among lakes, again using MANOVA, where lake and morph type were the independent variables (Table 4). We found that there was significant overall variation between lakes in the way the morphs differed from each other (morph type by lake interaction, $P < 0.02$). In particular, there was significant variation between lakes in the way the morphs differed in mean gill raker length ($P = 0.003$). Two characters differed consistently between morphs in all lakes; mean gill raker length ($P < 0.001$) and upper jaw length ($P = 0.012$). Upper jaw length always tended to be longer for the long gill raker morph. In addition, fish from the three lakes differed in average size of most characters except head and upper jaw length and mouth width (Table 4). Fish from Espejo tended to have longer gill rakers, larger eyes and deeper caudal peduncles than

Table 2. Number collected, size (standard length) and percent females for each morphotype of *Percichthys trucha* collected in three north-western Patagonian lakes

			Standard length (mm)			
Lake	Gill raker length	Number of fish	Mean	Median	Range	Percent females
Quillén	Long	49	253.7	255	84–375	59
	Short	37	281.2	310	129–410	62
Espejo	Long	18	342.7	360	194–380	56
	Short	39	323.8	345	194–405	69
Rivadavia	Long	36	259.6	220	92–450	69
	Short	23	210.7	190	89–415	45

Table 3. Mean size of measured morphological characters for adult *Percichthys trucha*. Measurements were standardized to a common fish size (standard length of 275 mm). Statistical differences between morphotypes are reported as *P*-values based on multivariate (Wilks' λ) and univariate *F*-tests. Percentage difference of means (% Diff) was estimated as the mean of the character in the long gill raker morph minus that in the short gill raker morph, divided by the mean in the short gill raker morph (×100). Also included is the average measurement error for each character, as a percent of the measurement. Sample sizes for the long and short gill raker morphs, respectively, were $N = 49$ and $N = 37$ for Quillén, $N = 18$ and $N = 39$ for Espejo, and $N = 36$ and $N = 23$ for Rivadavia

		Quillén				Espejo				Rivadavia			
Trait	Gill raker length	Mean (SD) (mm)	*P*-value	% Diff	% Error (SD)	Mean (SD) (mm)	*P*-value	% Diff	% Error (SD)	Mean (SD) (mm)	*P*-value	% Diff	% Error (SD)
Head length	Long	86.09 (2.60)	0.257	0.8	0.5 (0.3)	86.53 (3.32)	0.013	2.8	0.4 (0.7)	84.37 (3.04)	0.055	–1.6	0.8 (0.5)
	Short	85.43 (2.73)				84.19 (3.18)				85.74 (1.99)			
Upper jaw length	Long	28.17 (1.91)	0.004	4.4	1.7 (0.3)	29.21 (1.14)	0.057	2.8	2.0 (0.5)	27.91 (1.43)	0.293	1.4	2.8 (0.5)
	Short	26.98 (1.70)				28.41 (1.59)				27.52 (1.37)			
Mouth width	Long	21.90 (1.80)	0.109	2.9	3.7 (0.6)	22.34 (1.87)	0.826	–1.3	2.9 (1.4)	21.50 (1.80)	0.573	–1.2	3.5 (0.6)
	Short	21.28 (1.67)				22.60 (1.97)				21.77 (1.81)			
Eye diameter	Long	13.59 (0.81)	0.763	0.4	2.8 (0.3)	13.07 (0.84)	0.431	1.3	3.6 (0.4)	13.33 (0.61)	0.625	0.8	2.4 (0.3)
	Short	13.53 (0.74)				12.90 (0.67)				13.22 (0.65)			
Interorbital distance	Long	17.45 (1.03)	0.058	–2.5	1.4 (0.3)	18.95 (1.00)	0.004	4.5	4.4 (0.6)	18.22 (1.04)	0.190	–1.6	1.1 (0.2)
	Short	17.90 (1.14)				18.13 (0.91)				18.51 (0.95)			
Peduncle depth	Long	28.70 (1.59)	0.731	–0.3	1.1 (0.3)	30.28 (1.52)	0.021	4.0	1.4 (0.5)	29.25 (1.47)	0.302	–1.1	1.0 (0.3)
	Short	28.80 (1.34)				29.12 (1.81)				29.59 (1.12)			
Dorsal spine length	Long	29.87 (2.46)	0.224	–2.4	1.0 (0.2)	28.87 (3.17)	0.778	1.0	1.2 (0.5)	26.96 (2.35)	0.104	–4.8	1.2 (0.3)
	Short	30.60 (2.82)				28.58 (2.96)				28.32 (3.49)			
Gill raker length	Long	5.91 (0.40)	<0.001	18.9	1.1 (0.1)	6.46 (0.28)	<0.001	16.2	1.1 (0.9)	6.49 (0.54)	<0.001	31.6	1.1 (0.1)
	Short	4.97 (0.26)				5.55 (0.31)				4.93 (0.43)			
# gill rakers	Long	19.8 (0.18)	0.559	1.0		18.9 (0.31)	0.741	–0.1		18.4 (0.21)	0.657	–0.2	
	Short	19.6 (0.17)				19.0 (0.21)				18.6 (0.27)			
Multivariate *P*-values from Wilks' λ	Excluding gill rakers		0.179				0.039				0.073		
	Including gill rakers		<0.001				<0.001				<0.001		

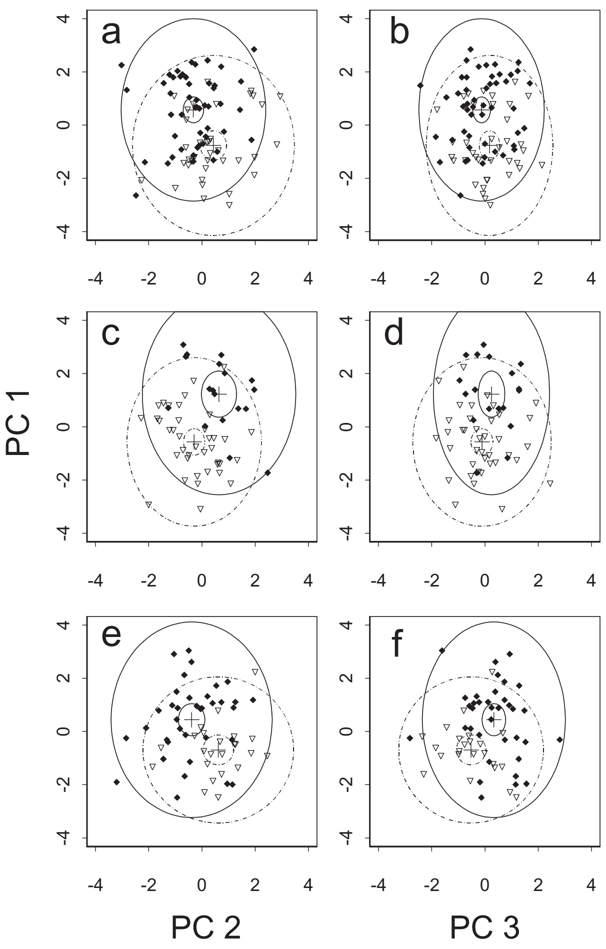

Figure 3. Scatter-plot of Principal component analysis (PCA) scores for adult *Percichthys trucha* with relatively long (◆) and short (▽) gill rakers. The symbols + indicate the means for each of the two morphotypes. The small ellipses are the 95% confidence intervals for the means of the two morphs. The large ellipses are the 95% prediction intervals for individuals. Left panels are plots of PC1 vs. PC2 and right panels are plots of PC1 vs. PC3. (a,b) Lake Quillén, (c,d) Lake Espejo (e,f) Lake Rivadavia. PC1, PC2 and PC3 explain, respectively: 35.3%, 22.0% and 13.8% of the total variance for Lake Quillén. For Lake Espejo they explain, respectively, 36.6%, 18.5%, and 14.3% of the total variance, and for Lake Rivadavia they explain 30.5%, 23.6% and 19.3% of the total variance, respectively.

fish from the other two lakes. Fish from Quillén had the shortest, but most numerous gill rakers, and had their eyes closest together. Fish from Rivadavia had gill rakers of intermediate length and had their eyes furthest apart.

Finally, we used CART analysis to determine which characters, other than gill raker length, best differentiated the two morphs in each lake. Within each lake, between 70% and 84% of the individuals could be correctly classified to morph type using only non-gill raker characters (Fig. 4). However, the characters that emerged as most important in distinguishing the morphs differed between lakes. In Quillén, the variables responsible for separating the morphs were, in order of importance, upper jaw length, eye diameter and interorbital distance (Fig. 4a). In Espejo, most individuals were classified using interorbital distance, the depth of the caudal peduncle, and the head length (Fig. 4b), while in Rivadavia head length and eye diameter were the variables responsible for the primary and secondary splits, respectively (Fig. 4c).

In summary, we found that morphs from the southernmost lake, Rivadavia, showed the greatest divergence in gill raker length. The three populations showed different patterns of divergence in other morphological traits; the only common feature was a tendency for the long gill raker morph to have longer upper jaws in all three lakes.

Identification of morphs in larvae and juveniles (Lake Rivadavia)

We examined a sample ($N = 79$) of small juvenile fish collected in the littoral zone of Lake Rivadavia to determine if similar patterns of morphological variation could be seen in young fish. We found that we could again quite clearly distinguish two morphs, with long ($N_1 = 23$) and short ($N_2 = 56$) gill rakers using a combination of cluster and discriminant analysis (correlation between linear combination of variables and of morphs $r = 0.73$).

All but one of the individuals belonging to the long gill raker morph were less than 20 mm in standard length (mean ± SD: 14.7 ±1.7, range 11.8–17.6), but those belonging to the short gill raker morph belonged to two size classes, a small size class (mean ± SD: 13.1 ±1.9, range 10.6–18.8) and a larger size class (mean ± SD: 56.3 ±13.4, range: 33.4–77.8). Of the fish belonging to the smaller group, individuals belonging to the long gill raker morph were significantly larger than those of the short gill raker group ($P = 0.002$).

We treated the two groups separately in our analyses, although mean values for morphological characters were very similar for the two size groups (Table 5). The difference in gill raker length between the long and the short gill raker morphs was 16–17% (Table 5). Other traits also varied or showed a tendency to vary between morphs, e.g. the long gill raker morph had longer heads ($P = 0.044$) and larger eyes ($P = 0.004$, Table 5). A scatter-plot of principal component analysis scores based on the same seven morphological traits as those used in the PC scores scatter-plot of adults (HL, UJ, CP, DF, ED, ID (Fig. 2) and gill raker length) revealed no overlap between the means of the two morphs (Fig. 5). This plot also shows that a number of individuals within the long

Table 4. Variation among lakes in morphological characters. Results from MANOVA, where morphotype and lake were independent variables and all morphological traits were included as dependent variables. Reported *P*-values are from univariate *F*-tests and from the multivariate test statistic, Wilks' λ. Differences among lakes were determined by pairwise comparisons (Tukey's test), and are presented as different for $P < 0.05$

Trait	Difference among lakes (Tukey's test)	*P*-values Morph × Lake	Lake	Morph
Head length	Quill = Esp = Riv	0.105	0.081	0.149
Upper jaw length	Esp = Quill = Riv	0.112	0.390	0.012
Mouth width	Quill = Riv = Esp	0.089	0.854	0.715
Eye diameter	Esp > Quill = Riv	0.644	0.009	0.064
Interorbital distance	Riv > Esp > Quill	0.481	<0.001	0.294
Caudal peduncle depth	Esp > Quill = Riv	0.847	0.007	0.806
Dorsal spine length	Quill > Esp = Riv	0.690	<0.001	0.226
Mean gill raker length	Esp > Riv > Quill	0.003	<0.001	<0.001
Number of gill rakers	Quill > Esp = Riv	0.728	<0.001	0.825
Multivariate *P*-value based on Wilks' Lambda		0.020	<0.001	<0.001

gill raker morph fall outside the prediction interval for individuals of the opposite morph (Fig. 5). These results suggest that in Lake Rivadavia *P. trucha* already exhibits evidence of polymorphism early in development.

DIFFERENCES IN DIET AMONG MORPHS OF *P. TRUCHA*

Adults

Although most of the principal prey items included in the diet of *P. trucha* were the same across lakes (Anisoptera, Amphipoda, Trichoptera), stomach content, i.e. number of food items in the stomach, and the relative contribution of these and other prey to the diet differed greatly between lakes (Table 6). The most important interlake differences in number of prey found in stomach contents were that far more Anisoptera were consumed in Espejo than in Quillén or Rivadavia ($P < 0.001$), and that Cladocera were eaten more frequently in Rivadavia ($P < 0.001$). In addition gut fullness differed significantly among lakes ($P < 0.001$), with individuals from Rivadavia having the lowest gut fullness (Table 6) and those from Espejo having marginally fuller guts than those from Quillén ($P = 0.056$).

We tested for diet differences among morphs within each lake, taking into account variation among sites and depth. For each lake we included only those sites where we had collected a minimum of five fish of each morph type (three sites for Quillén, two sites for Espejo, and one site for Rivadavia). In the MANOVA for each lake, we excluded very rare taxa and included only those taxa where a minimum of ten items had been found over all stomach contents from that lake (a minimum of five for the fish, *Galaxias maculatus* or puyen).

In Quillén (Table 6a), there was no significant variation in consumption rate of any prey type as a function of site or depth. The two morphs did differ somewhat in their diets, with the short gill raker morph consuming slightly more Anisoptera ($P = 0.038$), and the long gill raker morph tending to consume more chironomid larvae ($P = 0.076$).

The long and short gill raker types found in Espejo did not differ significantly in diet (Table 6b). There was spatial variation in diet within the lake, where both morphs consumed more Anisoptera ($P = 0.032$) and probably amphipods ($P = 0.088$) in the deep than in the littoral zone.

The greatest differences in diet between the morphs were found in Rivadavia. In the littoral zone, the long gill raker morph had fuller guts ($P = 0.041$), while both morphs were similarly full in the deep zone ($P = 0.457$). In contrast to the other two lakes, neither morph consumed large numbers of Anisoptera. The long gill raker morph consumed more amphipods than did the short gill raker morph ($P = 0.036$) and, overall, amphipods were consumed more often in the littoral zone. Terrestrial insects ($P = 0.003$) and Cladocera ($P = 0.046$) were consumed more often by fish caught in the deep zone, and the short gill raker morph tended to consume higher numbers of both (terrestrial insects, $P = 0.002$ and Cladocera, $P = 0.073$).

Juveniles

We examined the diet of the small juveniles (<20 mm) collected from one site in Lake Rivadavia. Only one of

Table 5. Mean size of measured morphological characters for the juvenile (<20 mm standard length) *P. trucha* collected in the littoral zone of Lake Rivadavia (long gill raker morph: $N = 22$; short gill raker morph: $N = 40$). Measurements were standardized to a common fish size (16.8 mm standard length). Statistical differences between morphotypes are reported as *P*-values based on multivariate (Wilks' λ) and univariate *F*-tests. Percentage difference of means (% Diff) between morphs was estimated as the mean of the character in the long gill raker morph minus that in the short gill raker morph divided by the mean in the short-gill-raker morph (×100)

Trait	Gill raker length	Mean (SD) (mm)	*P*-value*	% Diff*
Head length	Long (<20 mm)	6.34 (0.19)	0.044	1.9
	Short (<20 mm)	6.22 (0.23)		
	(>30 mm)	6.18 (0.14)		
Upper jaw length	Long (<20 mm)	2.28 (0.11)	0.384	1.3
	Short (<20 mm)	2.25 (0.16)		
	(>30 mm)	2.26 (0.09)		
Mouth width	Long (<20 mm)	2.15 (0.19)	0.149	3.9
	Short (<20 mm)	2.07 (0.20)		
	(>30 mm)	2.06 (0.17)		
Eye diameter	Long (<20 mm)	1.79 (0.07)	0.004	2.9
	Short (<20 mm)	1.74 (0.06)		
	(>30 mm)	1.75 (0.09)		
Interorbital distance	Long (<20 mm)	2.04 (0.10)	0.096	2.0
	Short (<20 mm)	2.00 (0.10)		
	(>30 mm)	2.00 (0.14)		
Caudal peduncle depth	Long (<20 mm)	2.03 (0.12)	0.065	3.0
	Short (<20 mm)	1.97 (0.10)		
	(>30 mm)	1.95 (0.08)		
Dorsal spine length	Long (<20 mm)	2.68 (0.46)	0.196	6.3
	Short (<20 mm)	2.52 (0.38)		
	(>30 mm)	2.43 (0.20)		
Mean gill raker length	Long (<20 mm)	0.486 (0.028)	<0.001	16.5
	Short (<20 mm)	0.417 (0.031)		
	(>30 mm)	0.412 (0.028)		
Multivariate *P*-values based on Wilks' λ				
Excluding gill raker length	0.019			
Including gill raker length	<0.001			

**P*-values and % Diff are for the small juveniles (<20 mm) only.

22 fish from the long gill raker morph (5%) had no prey in its gut, and six of 38, or 16% of the short gill raker morph had not been feeding.

Numerically, the gut contents of the small juveniles that had been feeding were dominated by chironomid larvae, *Daphnia* sp., and cyclopoid copepods (Table 7). The number of prey consumed differed between morphs ($P = 0.001$) and differed with the size of the fish ($P < 0.001$; MANOVA, Table 7). Of the fish that had been eating, the long gill raker morph consumed more prey and, in particular, consumed significantly more cyclopoid copepods and more cladocerans (*Daphnia* and Chydoridae) than did the short gill raker morph (Table 7).

When prey were considered as a proportion of total prey volume, consumption by juvenile fish was dominated by chironomid larvae and pupae (over 50%) and *Daphnia* (20–30%). There was an overall difference between morphs in the contribution of the different prey to total gut contents ($P = 0.009$), but there were no statistically significant differences in individual prey taxa (Table 7). Inspection of the data suggests that the overall difference was driven by a greater proportion of the short gill raker morph's diet being composed of chironomids, while *Daphnia* and cyclopoid copepods contributed relatively more to the long gill raker morph's diet.

If we consider the proportion of fish feeding on particular prey, we also see a greater emphasis on *Daphnia* and cyclopoid copepods by the long gill raker morph. *Daphnia* and/or cyclopoids were found in 95% of the guts of the long gill raker morph (all that had been feeding), while only 61% of the short gill raker morph had eaten these prey. In contrast, roughly the

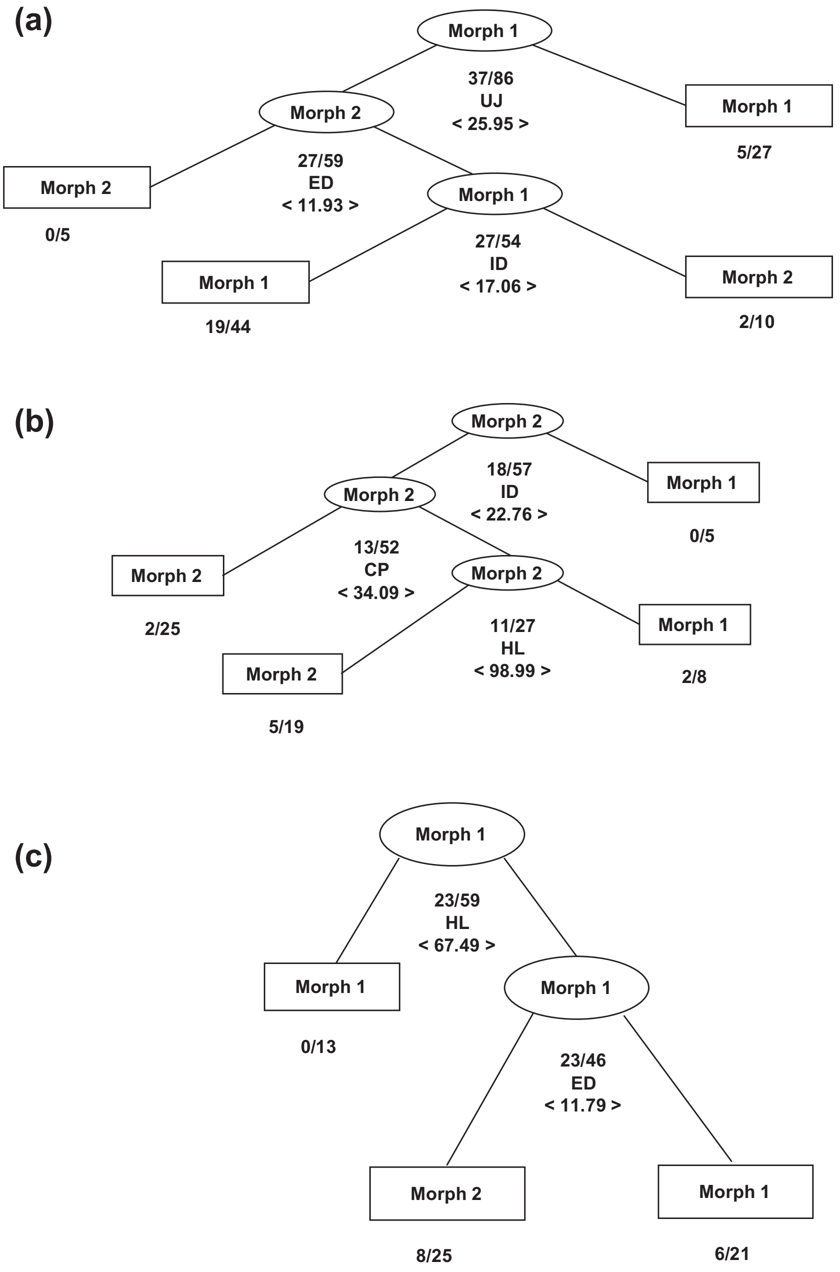

Figure 4. Classification trees obtained for morphs of *Percichthys trucha* (defined by gill raker length) using morphometric traits other than gill raker length. The trees were based on the five morphometric variables with relatively low measurement error, i.e. upper jaw (UJ), head length (HL), depth of caudal peduncle (CP), eye diameter (ED), and distance between eyes (interorbital distance, ID). 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 (in mm) 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. Overall misclassification rates using these variables were (a) Quillén: 26/86 (30%), (b) Espejo: 9/57 (16%), and (c) Rivadavia: 14/59 (24%). Proportions correctly classified, by morph (long gill raker, LG, and short gill raker, SG) were: (a) Quillén, LG: 96%, SG: 41%, (b) Espejo, LG: 61%, SG: 95%, and (c) Rivadavia, LG: 78%, SG: 74%.

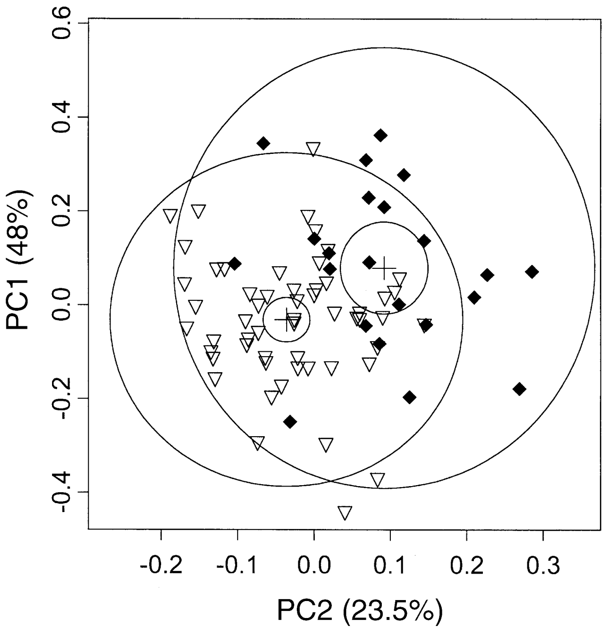

Figure 5. Scatter-plot of principal component analysis (PCA) scores for larvae and juvenile *Percichthys trucha* with relatively long (◆) and short (▽) gill rakers collected from Lake Rivadavia. The symbol + indicates the means for each of the two morphotypes. The small ellipses are the 95% confidence intervals for the means of the two morphs, the large ellipses are the 95% prediction intervals for individuals. PC1 and PC2 explain, respectively, 48% and 23.5% of the total morphological variance.

same proportion of both morphs included chironomid larvae as part of their diet: 62% of the long gill raker morph and 58% of the short gill raker morph.

We thus have evidence suggesting that early differences in morphology may also be associated with differences in diet. The long gill raker morph appears to consume more prey and, in particular, more pelagic prey: *Daphnia*, cyclopoid copepods and chydorids.

DISCUSSION

In this study we found resource-based polymorphism within different populations of *Percichthys trucha*, including within small juvenile fish. However, the magnitude of the polymorphism, and the suite of phenotypic characteristics associated with the polymorphism, differ between lakes. As we had shown previously (Ruzzante *et al.*, 1998), the trait that best distinguished the two morphs in all lakes was gill raker length. However, the percentage difference of means between morphs in this trait was 31.6% in the southernmost lake, which drains to the west, but only 16–19% in the two northern lakes, which drain to the east, ultimately into a common river. The consistency among lakes in certain key morphological differences (gill raker and upper jaw lengths) suggests a common cause, thus supporting proposals that divergent natural selection for the use of heterogeneous resources generates the same patterns of polymorphism across lakes (Endler, 1986; Futuyma, 1998). For the traits other than gill raker length, however, we found a number of interlake differences in the magnitude and, occasionally, the direction of the differences between morphs, suggesting an important role for historical factors or independent evolution within lakes. The evidence of polymorphism, and of correlated differences in diet among larvae and juveniles, indicates that the polymorphism seen in adult *P. trucha* begins early during ontogeny.

POLYMORPHISM AND DIET

The key distinguishing trait, gill raker length, proved also to be the most important differentiating character in small juvenile fish in Rivadavia (the only location we were able to obtain significant numbers of small fish). The functional significance of the gill raker apparatus in feeding has been studied mostly in connection with the difference among filter, ram, and suction feeding (e.g. Lauder, 1985). Generally, fish that predominantly live on small, planktonic prey (the limnetic form in many north temperate fish polymorphisms or species pairs) have more and longer gill rakers (Svärdson, 1961; Magnuson & Heitz, 1971; Gross & Anderson, 1984; Schluter & McPhail, 1992, 1993; Robinson & Wilson, 1994; Wood & Foote, 1996; Bell & Andrews, 1997; Foote *et al.*, 1999). The increase in number of gill rakers is assumed to reduce pore size during filtering, which presumably allows the fish to retain small particle sizes. The morphs of *P. trucha* did not differ in number of gill rakers, only in length. Unlike the majority of examples from northern temperate lakes (see Jónsson & Skúlason, 2000 for one exception), both morphs of *P. trucha* are predominantly bottom feeders as adults, probably because the lakes are too oligotrophic (Modenutti *et al.*, 1998) to support planktivorous species or morphs of fish. Thus the primary feeding modes of adult *P. trucha* are likely to include the sorting of sediments to extract small prey (amphipods and chironomids), and the individual capture of larger prey (Odonata and small fish). Appropriate biomechanical methods to optimize sediment sorting are probably quite different from that of classic filtering.

Alternatively, or in addition, the divergent morphologies seen among adults might be primarily a response to divergent selection early in ontogeny. Differences in morphology and diet are present among the very small juveniles of Lake Rivadavia. Most notably, the long gill raker morph had eaten more

Table 6. Average stomach fullness and diet of the long and short gill raker morphs of *Percichthys trucha* adults, as assessed from stomach contents. Average number of prey consumed per fish are given for the whole lake as well as by depth (Littoral: ≤2 m, Deep: 5–10 m), and by sampling location (Site no.) for (a) Quillén, (b) Espejo, and (c) Rivadavia. Only locations with at least five individuals of each morph were included in the analysis. *P*-values indicate whether stomach fullness and number of prey items consumed (individually by taxon and simultaneously) differed between morphs, depth of capture (littoral vs. deep), between sites or due to interactions between morph and depth (M × D), and between morphs and sites (M × S)

(a) Quillén

				Prey taxon							
	Morph	No. of fish	Stomach fullness	Anisoptera	Amphipoda	Trichoptera	Chironomid larvae	Chironomid pupae	Ephemeroptera	Terrestrial insects	*Galaxias* (puyen)
Lake	Long	34	0.52	2.1	19.4	2.5	6.8	1.0	1.2	0.7	0.03
	Short	30	0.58	6.0	22.4	1.4	4.3	0	0.0	0.03	0.03
Littoral	Long	26	0.47	1.8	21.0	3.2	8.9	1.2	1.4	1.0	0.04
	Short	16	0.55	3.4	31.2	2.6	7.5	0	0.0	0.06	0
Deep	Long	8	0.66	2.9	14.3	0	0	0.3	0.5	0	0
	Short	14	0.60	8.9	12.4	0.1	0.6	0	0	0	0.7
Site 2	Long	9	0.57	3.9	5.3	3.8	0.0	0	0	0	0
	Short	15	0.56	7.5	11.6	0.1	0.6	0	0	0	0.07
Site 3	Long	6	0.33	0.5	29.2	0	31.7	5.0	0	0	0
	Short	5	0.60	6.2	0.2	7.4	0	0	0	0.2	0
Site 5	Long	19	0.55	1.7	23.0	2.6	2.2	0.2	2.2	1.3	0.05
	Short	10	0.59	3.6	49.8	0.4	12.0	0	0	0	0
Morph (*P*-value)			ns	0.038	ns	ns	0.076	ns	ns	ns	ns
Depth (*P*-value)			ns	ns	ns	ns	ns	ns	ns	ns	ns
Site (*P*-value)			ns	ns	ns	ns	ns	ns	ns	ns	ns
M × D			ns	ns	ns	ns	ns	ns	ns	ns	ns
M × S			ns	ns	ns	0.085	ns	ns	ns	ns	ns
Wilk's λ											
Morph				0.369							
Depth				0.813							
Site				0.804							
M × D				0.956							
M × S				0.521							

(b) Espejo

				Prey taxon					
	Morph	No. of fish	Stomach fullness	Anisoptera	Amphipoda	Trichoptera	Chironomid pupae	*Galaxias* (puyen)	*Samastacus*
Lake	Long	13	0.74	9.9	51.2	5.1	3.7	0.2	0.2
	Short	22	0.79	18.1	23.5	6.6	0.3	0.05	0.05
Littoral	Long	4	0.69	5.5	0	13.5	0	0	0.3
	Short	7	0.66	7.1	0	16.1	0	0	0.1
Deep	Long	9	0.76	11.9	73.9	1.3	5.3	0.3	0.2
	Short	15	0.85	23.3	34.5	2.1	0.5	0.07	0
Site 2	Long	8	0.61	9.1	82.5	1.4	0	0.4	0.3
	Short	11	0.71	21.0	14.5	0.1	0.6	0.1	0
Site 5	Long	5	0.95	11.2	1.0	11.0	9.6	0	0.2
	Short	11	0.86	15.3	32.6	13.1	0	0	0.1
Morph (*P*-value)			ns	ns	ns	ns	ns	ns	ns

Table 6. *Continued*

	Morph	No. of fish	Stomach fullness	Prey taxon					
				Anisoptera	Amphipoda	Trichoptera	Chironomid pupae	*Galaxias* (puyen)	*Samastacus*
Depth (*P*-value)			ns	0.032	0.088	ns	ns	ns	ns
Site (*P*-value)			ns	ns	ns	0.058	ns	ns	ns
M × D			ns	ns	ns	ns	ns	ns	ns
M × S			ns	ns	ns	ns	ns	ns	ns
Wilk's λ									
Morph				0.888					
Depth				0.104					
Site				0.125					
M × D				0.975					
M × S				0.783					

(c) Rivadavia

	Morph	No. of fish	Stomach fullness	Prey taxon					
				Anisoptera	Amphipoda	Trichoptera	Chironomid larvae	Terrestrial insects	Cladocera
Lake (Site 2)	Long	29	0.46	0.7	51.7	3.1	6.4	0.2	1.1
	Short	10	0.25	0.2	0	0.5	0.2	10.1	4.1
Littoral	Long	23	0.53	0.3	65.2	3.6	8.0	0.3	0
	Short	8	0.19	0.3	0	0.6	0.3	0.1	5.1
Deep	Long	6	0.20	2.3	0	1.2	0	0	5.3
	Short	2	0.50	0	0	0	0	50.0	0
Morph (*P*-value)			0.041	ns	0.036	ns	ns	0.002	0.073
Depth (*P*-value)			ns	ns	0.044	ns	ns	0.003	0.046
M × D			0.089	ns	ns	ns	ns	0.001	0.099
Wilk's λ									
Morph				0.024					
Depth				0.018					
M × D				0.015					

pelagic prey, *Daphnia* and cyclopoid copepods, than had the short gill raker morph. As these prey were presumably captured in the water column among the vegetation in the littoral zone, there is some association between longer gill rakers and pelagic feeding, at least among the very small fish in Rivadavia. In addition, the long gill raker morph appeared to be more successful at this stage of development and in this location; overall they had more prey in their stomachs, and individuals were, on average, slightly larger. This pattern also carried over to the adults, where the long gill raker morph in Rivadavia had fuller guts than did the short gill raker morph. However, the per cent difference in gill raker length was smaller in the small juveniles, 17% as compared to 31.6% in the adults caught in the same lake, suggesting that pressures leading to the divergence may continue to operate during later ontogenetic stages.

We also saw differences in diet between morphs for adult fish in one lake and a tendency for differences in a second lake. In Quillén there was a tendency for the short gill raker morph to eat more anisopteran larvae, which tend to be found on the substrate surface or on the submerged vegetation, while the long gill raker

Table 7. Diet (stomach contents) of *Percichtys trucha* juveniles collected from a single littoral site in Lake Rivadavia (long gill raker morph $N = 21$, short gill raker morph $N = 34$). Diet is analysed as absolute number of prey items (N), and as proportion of the total volume of prey in the stomach (Vol %). For each prey category, the minimum consumed was always zero. *P*-values are reported for *F* (ANOVA) and for Wilk's λ (MANOVA). Prey numbers were log-tranformed [log(n + 1)], and volumes were arcsin transformed [2 × arcsin (√ Y)] prior to analysis

	Gill raker morph	Chironomid larvae		Chironomid pupae		Harpacticoid copepods		Calanoid copepods		Cyclopoid copepods		Cladocera *Daphnia* sp.		Cladocera Chydoridae		Cladocera *Bosmina* sp.	
		N	Vol %	N	Vol %	N	Vol %	N	Vol %	N	Vol%	N	Vol%	N	Vol%	N	Vol%
Mean	Long	4.29	38.3	0.67	12.0	0.67	0.5	0.19	3.0	10.9	14.3	3.67	31.6	1.86	0.3	0.19	<0.1
	Short	2.72	51.7	0.34	12.6	0.34	3.3	0.03	0.4	3.8	7.9	1.56	21.8	0.53	0.4	0.13	2.0
Median	Long	2	47.4	0	0	0	0	0	0	6	4.32	3	21.8	0	0	0	0
	Short	1	65.2	0	0	0	0	0	0	1	0.8	1.3	0	0	0	0	0
Max	Long	16	89	6	67	4	6	2	32	41	100	14	99	12	2	2	0.2
	Short	15	100	6	99	3	89	1	13	28	99	13	100	3	6	1	53
Morph (*P*-value)		ns	ns	ns	ns	ns	ns	ns	ns	0.002	ns	0.001	ns	0.037	ns	ns	ns
Length (*P*-value)		0.086	ns	ns	ns	ns	ns	ns	ns	<0.001	ns	<0.001	ns	ns	ns	ns	0.079
Morph * Length (*P*-value)		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.076	ns	ns	ns	ns	
Wilk's λ																	
Morph		0.001	0.009														
Length		<0.001	0.032														
Morph * Length		0.887	0.477														

morph tended to eat relatively more of the taxa that tend to be within organic or inorganic sediments (chironomid larvae). There were no statistically significant differences in diet for the Espejo morphs, but the patterns were in the same direction, the short gill raker morph tended to eat more Anisoptera, particularly in the deep zone, and the long gill raker morph tended to eat more chironomids. In Rivadavia, long gill raker morph adults were feeding primarily on amphipods, chironomids and trichopterans. The short gill raker morph was not feeding much, and was eating mostly organisms that were rarely consumed in the other lakes, namely terrestrial insects and Cladocera, prey likely to be captured in the water column. It is clear, however, that in all lakes, both morphs are capable of feeding on all prey types, and so the differences in diet must lie in preference or efficiency, or differences in habitat use.

AMONG-LAKE VARIATION IN THE POLYMORPHISM

The patterns of morph differentiation were more similar between Quillén and Espejo than between these lakes and Rivadavia. In all lakes, the long gill raker morph tended to have longer upper jaws, but the difference between morphs in this character was most pronounced in Quillén and Espejo. In addition to a longer jaw, the long gill raker morph in Quillén tended to have a wider mouth, and eyes closer together; all three are characteristics associated with another species of *Percichthys*, the more piscivorous *P. colhuapiensis*. In fact, the characters used to distinguish *P. trucha* from *P. colhuapiensis* include traits that differed between morphs in these lakes (i.e. head length, upper jaw length, mouth width, eye diameter and interorbital distance; Ringuelet *et al*., 1967). *Percichthys colhuapiensis* has never been found in the lakes of the Andean mountains, but is found alone and coexisting with other *Percichthys* species, including *P. trucha*, in some of the lakes, rivers and reservoirs to the east of the mountains (Arratia, 1982; Arratia *et al*., 1983; Cussac *et al*., 1998), a region largely beyond the limit of the Pleistocene ice caps (Mercer, 1976; Clapperton, 1993). In Espejo there was also some tendency for the long gill raker morph to qualitatively resemble *P. colhuapiensis*, but the differences were not as strong, and reversed in the case of interorbital distance. In contrast, the morphs from Rivadavia appear to have differentiated along quite different axes; they showed the smallest difference in jaw length, and the long gill raker morph actually had a shorter head (Table 3). (Note that this must develop during ontogeny, as the long gill raker morph for the small juveniles had a longer head.) There are several, non-mutually exclusive explanations for the greater similarity of the morphological differentiation in Quillén and Espejo than in Rivadavia. First, one possibility is that the original invaders to Quillén and Espejo, which presumably came from populations to the east, may have been more similar to each other than to the original invaders of Rivadavia, which would probably have come from the west. As a result, the genetic material available for subsequent evolution may have been more similar for the two northern lakes than for Rivadavia. Significant here is the fact that in some of the lakes to the west of the Andes, in Chile, *P. trucha* coexists, not with *P. colhuapiensis*, but with another species, *Percichthys melanops* (Ringuelet *et al*., 1967). It is intriguing that adults of *P. melanops* appear to have a shorter head than do adults of *P. trucha* (Campos & Gavilán, 1996). This explanation suggests that historical factors could be at least partially responsible for the different patterns of polymorphism across lakes. Second, the patterns of resource diversity in Lakes Quillén and Espejo might be more similar to each other than to those of Lake Rivadavia, resulting in more similar evolutionary or developmental pathways. Third, if the divergence between morphs were truly caused by divergent selection, then differences in the amount of separation (but most likely not in the pattern of polymorphism) could simply be due to the amount of time since divergence. Fourth, fish feeding mechanisms are often plastic in response to both the type and the nutritive value of the food consumed. Differences in plasticity in either morph could be responsible for differences between lakes in the degree and pattern of divergence. Finally, genetic drift could also be responsible for differences among lakes. At present we do not have sufficient information to clearly distinguish between these possibilities, and efforts to expand the geographical coverage and to examine the genetics of this complex are ongoing.

The biotic environments of the three lakes do differ in ways that could influence the evolution of these fish. Notwithstanding the fact that food availability will vary seasonally and may vary independently among lakes, prey availability in the present study appeared to be highest in Espejo, second in Quillén and lowest in Rivadavia, as indicated by differences in gut fullness. This could have been due to lower absolute prey densities in this lake (not assessed), or to higher levels of intra- and/or interspecific competition due to higher fish densities (cf. Robinson *et al*., 2000). Based on our catches, densities of *P. trucha*, and therefore the potential for intraspecific competition, should have been highest in Rivadavia and Espejo. *Oncorhynchus mykiss* and *S. fontinalis* have diets that parallel that of *P. trucha* through their ontogeny (Macchi *et al*., 1999), and thus are also potentially important competitors of *P. trucha*. Catches of these salmonids were much higher in Rivadavia than in the other lakes. Thus the high combined densities of potential intraand interspecific competitors in Rivadavia, coupled with evidence of emptier guts there, suggest a higher level of competition in this lake. That intensity of competition can influence the extent to which divergence in a trophic polymorphism is realized has been suggested before (Robinson *et al*., 2000; Schluter, 2000, 2001). For example, a latitudinal gradient in the degree of polymorphism in Arctic char has been correlated with an increase in the seasonal variation in food supply (Griffiths, 1994).

In addition, the patterns of diet differentiation between the long and short gill raker morphs were not consistent between lakes. In Quillén diet differentiation between the two morphs was weak and differences can only be interpreted as tendencies. The long gill raker morph tended to concentrate its feeding in the littoral zone, eating chironomid larvae and pupae, and Ephemeroptera, with chironomid pupae and Ephemeroptera virtually absent from the diet of the short gill raker morph. The short gill raker morph tended to concentrate more on Anisoptera, and more of this feeding was in the deep than the littoral zone. Espejo appeared to have the highest availability of prey, as judged by gut fullness of the captured *P. trucha* and no significant difference in diet between the morphs was found in this lake. It is, of course, possible that food is only seasonally abundant, and that food shortage and diet differentiation occur at other times of the year. The greatest magnitude of both morphological and diet differentiation was found in Rivadavia. The diet of *P. trucha* in this lake was quite different from that in the other lakes. Anisoptera were relatively unimportant, whereas this taxon has been one of the most important prey types in every other lake we have looked at thus far. In addition, *P. trucha* collected from Rivadavia had higher rates of consumption of Cladocera and terrestrial insects than did *P. trucha* from the other lakes. The long gill raker morph was clearly consuming more food overall than was the short gill raker morph, and this was particularly so in the littoral zone. Although we did not catch disproportionately more of either morph in the littoral vs. the deep zones, as we had previously (Ruzzante *et al*., 1998), there was some evidence in Rivadavia that feeding did vary with depth. The long gill raker morph seemed to be concentrating its feeding in the littoral zone, consuming primarily amphipods, trichopteran larvae and chironomid larvae. In contrast, the short gill raker morph used both zones, feeding mostly on Cladocera in the littoral zone and on terrestrial insects (presumably caught at the surface) in the deep zone.

Thus, there were identifiable differences in adult diet between morphs in one of the three lakes, but in all lakes there was also a considerable overlap in diet between the morphs. We were able to obtain small juveniles from one lake only, and therefore we are unable to assess between-lake variation in morphology and diet of these early life history stages. However, the presence of morphological and diet differences in these very small fish suggests that one explanation for the weak correspondence between morphology and adult diet is that morphological divergence has occurred primarily in response to selection pressures earlier in development. A lack of close and consistent correspondence between morphology and trophic ecology has also been noted recently among ciscoes (*Coregonus*) in the North American Great Lakes. Here, diet breadth seemed to characterize diet differences better than selection or preference for particular prey types (Turgeon *et al*., 1999), a pattern that might also be present in some of our data.

CONCLUSIONS

In summary, we have found evidence for parallel divergence in the morphology of one species, *P. trucha*, in three Patagonian lakes; in all lakes two morphological types are present, one with relatively long gill rakers and upper jaws, the other with shorter gill rakers and jaws. These lakes are relatively young, the populations of fish must have colonized following the last glacial maximum, *c*. 15 000 years ago. One of the lakes is not connected to the other two, and has most likely not been connected over this period of time. Thus the presence of similar morphological types in all three lakes suggests an important role for divergent natural selection in generating the polymorphism. The variation across lakes in the magnitude of the polymorphism in gill raker length and in the pattern of polymorphism in associated morphological characters (e.g. head length and interorbital distance) suggests, however, that selection, to some extent, operates differently in the different lakes or that historical factors (historical contingency, Travisano *et al*., 1995) have played an important role in generating the polymorphism. We will clearly need information on the genetics of the *Percichthys* complex to improve our understanding of the mechanisms responsible for the polymorphism in *P. trucha*, since at this point we do not know if the degree of morphological differentiation correlates with the degree of reproductive isolation. However, we do know that the polymorphism is already present in very small fish, and additional study of the early life history stages may help determine if most of the divergence occurs early in development, as well as to identify the relevant selection pressures.

The morphological divergence within the *Percichthys* complex developed over a time frame (12 000–15 000 years bp) similar to that of many of the examples of resource polymorphisms and adaptive radiation in fishes from post-glacial lakes in the northern hemisphere (McPhail, 1984, 1993; Taylor, McPhail & Schluter, 1997; Bernatchez, Chouinard & Lu, 1999; Taylor & McPhail, 1999; Taylor, 1999). The time frames available for colonization and adaptation, as well as the broad environmental conditions (depauperate, oligotrophic, cold-water lakes) were similar in both hemispheres, and thus it is perhaps not surprising to find similar patterns of differentiation in both southern and northern temperate lakes. The patterns of trophic polymorphism within the *Percichthys* complex in recently deglaciated lakes of the southern Andes indicate, however, that differentiation of fishes within these lakes can take place not only along a pelagic–benthic feeding axis, but can also involve two predominantly benthic feeders. Whether the differentiation in *P. trucha* is the consequence of two different modes of benthic feeding or is primarily the result of a pelagic–benthic divergence among very small fish during the larval and early juvenile stages remains to be determined.

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