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Morphometric Response of *Galaxias maculatus* (Jenyns) to Lake Colonization in Chile

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Abstract: Body and head shape in fish responds to environmental factors such as water flow rate, food sources, and niche availability. However, the way in which fish respond to these environmental factors varies. In Central Chile, multiple river and lake systems along the coast provide an ideal study site to investigate these types of shape changes. We use geometric morphometrics to characterize shape differences in *Galaxias maculatus* (Jenyns) between river and lake populations. Lake fish converge on a shape with a more fusiform body, narrower head, and larger eyes, while river fish have a more robust body, rounder head, and smaller eyes. These shape changes are consistent with a shift to zooplanktivorous foraging in lakes, as evidenced in other systems. Unlike some fish species that develop polymorphisms in body shape after colonization (e.g., benthic and limnetic forms), *G. maculatus* in lakes exhibit a monomorphic limnetic form.

Keywords: geometric morphometrics; polymorphism; diversification; ecomorphological hypothesis

1. Introduction

The ecomorphological hypothesis suggests that the morphological traits of organisms should reflect the conditions of the environments they inhabit, and this idea has been acknowledged for over 100 years [1–5]. Organisms in lotic environments (rivers) tend to exhibit consistent and predictable morphometric differences compared to those in lentic environments (lakes). These general morphometric responses to lake colonization are primarily influenced by differences in flow rate [6–11]. In addition, many organisms exhibit location-dependent morphometric variation due to differential pressures from biotic factors (predator abundance, food availability, and resource competition) as well as abiotic factors (temperature, nutrient composition, and energy sources) [8,9,12–20].

Environmental conditions may alter the shapes of fish by the mechanism of natural selection leading to adaptive divergence [4,21,22] or via a phenotypic plasticity response [7,23–27]. Adaptation involves the multi-generational change of a population's phenotype by means of natural selection or the differential survival and reproduction of individuals. The phenotypes accumulated by adaptation are heritable and based on genetic differences [4,21,22]. Plasticity occurs when an individual expresses a different phenotype in response to different environmental conditions. The changes resulting from plasticity occur within an individual's lifetime and they are not heritable [23,27,28]. Both of these processes result in populations displaying traits that are suited to the conditions of their environments [3,4,10,21,23,26], and they are not mutually exclusive.

Morphometric differentiation in response to environmental variation can occur within species [7–9,11]. For example, river dwelling fish species often colonize lakes and establish isolated populations. This transition frequently leads to morphometric divergence between the source population and the colonizing population in response to the different flow rates present in rivers and lakes [7–11]. High flow environments tend to favor fish that are better suited for steady swimming [3,10,11]. This steady swimming is optimized by the development of a relatively fusiform body shape [3,11,29]. Meanwhile, lakes are relatively complex environments in which unsteady swimming is favored; unsteady swimming is associated with a more robust body shape [10,11,29]. These pressures result in lake fish generally developing relatively robust bodies [6–8,10]. However, this response is not universal. Several studies have found lake dwelling fish with more fusiform bodies than their river dwelling conspecifics [7,9]. Why some fish might respond differently to the colonization of lake environments remains poorly understood.

Galaxias maculatus is the most widely distributed diadromous fish in the southern hemisphere. It occurs in rivers and lakes in Australia, New Zealand, and Southern South America (Chile and Argentina) [30–32]. As adults, the individuals are small (about 100 mm) and elongate with generally slender bodies [33]. Although *G. maculatus* are typically river-dwelling [34], some populations in Chile have become isolated in lakes [31,35,36]. These landlocked populations have little to no gene flow with their original source river populations [32,34,37–39]. This genetic isolation is significant if the variation between populations is based on underlying, heritable differences rather than phenotypic plasticity. These river and lake systems provide an ideal study site for investigating the morphometric divergence between river and lake *G. maculatus*.

We characterized the morphometric response of multiple populations of *G. maculatus* to lake colonization in Chile. We used geometric morphometrics to quantify the general effects of lake colonization and body size on body and head shape among river and lake populations. We identified a convergent morphometric response to lake colonization in *G. maculatus* and a differential effect of body size on shape in river or lake environments.

2. Materials and Methods

2.1. Study Site and Collection

To quantify the general effect of lake colonization, we took advantage of the natural variation in the environments where *G. maculatus* is found in Chile. *Galaxias maculatus* is widespread in many river systems and has become isolated in a large number of lakes. Selecting populations from rivers and lakes across a latitudinal gradient and in different drainage basins allowed us to test for a general morphometric response to lake colonization. These lake populations represent multiple independent transitions from river to lake environments and, along with the extant river populations, provide the critical comparison needed to determine the general response to lake colonization.

We collected *G. maculatus* from a total of 16 populations in Chile (7 rivers, 9 lakes; Figure 1). All collections were done under the auspices of Dirección de Investigación, Universidad de Concepción, following Institutional Animal Care and Use Committee (IACUC) protocols (Resolución Exenta N° 3738, 8 November 2005 of the Undersecretariat of Fisheries). We collected 30 specimens from each population in March 2006 with seines and backpack electrofishing equipment. All individuals were considered pre-reproductive based on their size, the time of year they were collected, and the undeveloped nature of their gonads. All fish were considered to be about the same age and represented individuals that had hatched the previous November and would have spawned the following November. *Galaxias maculatus* is considered sexually monomorphic in terms of its body shape and coloration [33,35,40], so we did not differentiate between males and females in our sample. Specimens collected from the seven river populations ($n = 210$) had a mean standard length of 55.5 mm (± 10.8 , SD). Specimens collected from the nine lake populations ($n = 270$) had a mean standard length of 49.3 mm (± 5.4 mm, SD). We euthanized specimens with an overdose of BZ-20 (20% ethyl p-aminobenzoate), measured the standard length of

each fish, and assigned them ID numbers. We photographed the body and head of each freshly-killed fish from the left lateral view for morphometric analysis. Voucher specimens were deposited in the EULA-Chile Center (University de Concepción, Concepción, Chile); however, all specimens were destroyed as a consequence of the earthquake in 2010.

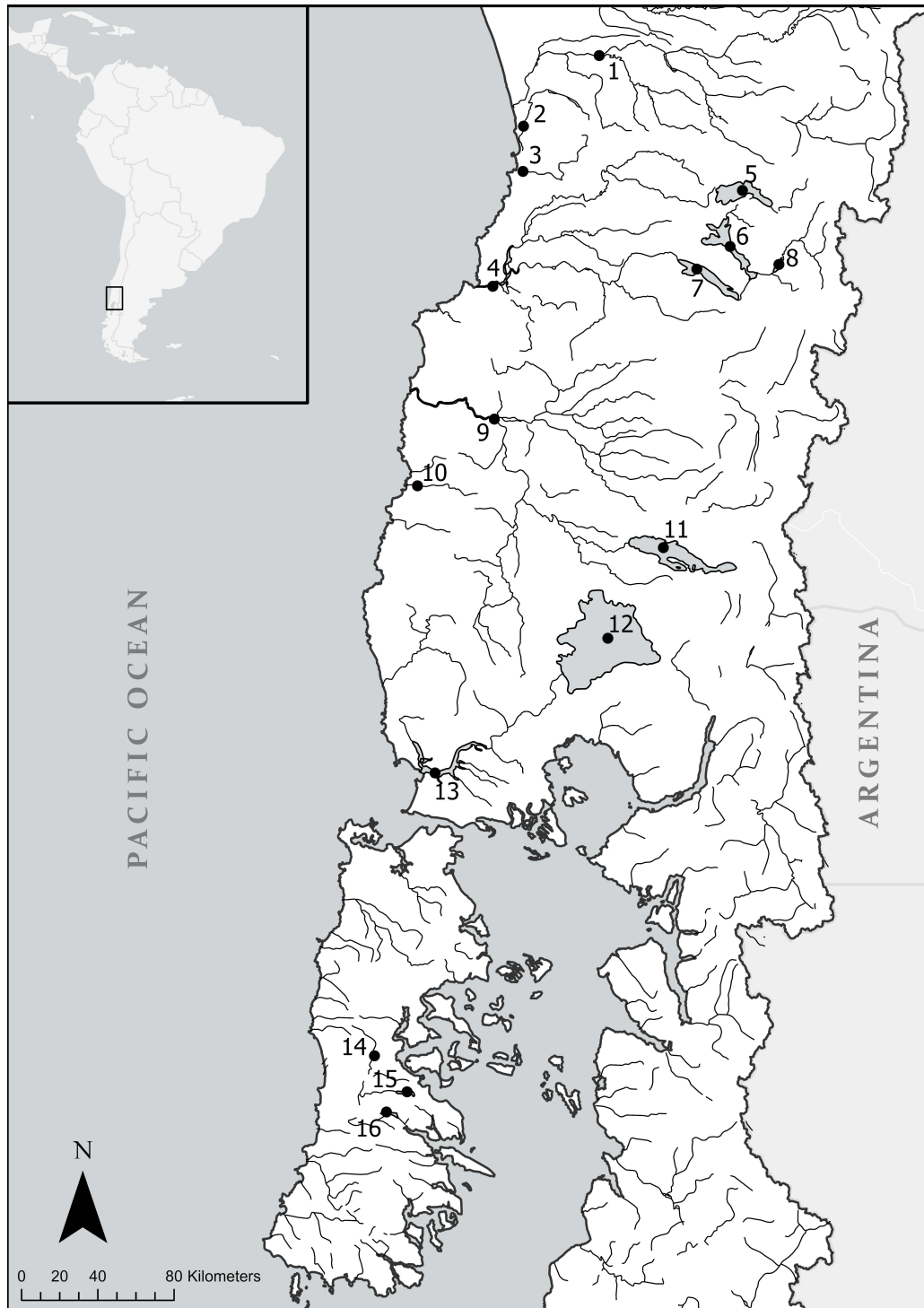


Figure 1. Collection sites for the *G. maculatus* populations considered in this study. 1: Toltén River, 2: Queule River, 3: Lingue River, 4: Valdivia River, 5: Calafquen Lake, 6: Panguipulli Lake, 7: Riñihue Lake, 8: Neltume Lake, 9: Bueno River, 10: Contaco River, 11: Rupanco Lake, 12: Llanquihue Lake, 13: Maullín River, 14: Huillínco Lake, 15: Natri Lake, 16: Tarahuin Lake.

2.2. Geometric Morphometrics

We used landmark-based geometric morphometrics to characterize body shape. Using the program tpsDig, we digitized landmarks and semi-landmarks [41,42]. To characterize body shape, we used 10 landmarks on 477 specimens (Figure 2). We included all 30 specimens per location except for 3 specimens that were not positioned correctly (Bueno River, $n = 1$; Natri Lake, $n = 2$). Our landmarks included (1) anterior maximum of nose, (2) juncture of the ventral margin of the operculum with the ventral outline of the body, (3) projection of the x-value of landmark 2 on the dorsal outline, (4) anterior origin of the dorsal fin, (5) anterior origin of the pelvic fin, (6) midpoint between landmarks 1 and 4 on the dorsal outline, (7) midpoint between landmarks 1 and 5 on the ventral outline, (8) posterior extent of the body at the vertical midpoint, (9) midpoint between landmarks 4 and 8 on the dorsal outline, and (10) midpoint between landmarks 5 and 8 on the ventral outline. Landmarks 6, 7, 9, and 10 were sliding semi-landmarks.

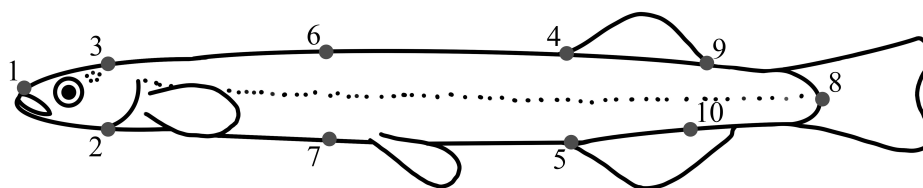


Figure 2. Landmarks used in the analysis of the shape of the lateral body view of *G. maculatus*.

To characterize the shape of the head, we used 11 landmarks on 186 specimens (Figure 3). We randomly subsampled 12 specimens from each population, with the exception of Natri Lake. Only 6 specimens from Natri Lake were of suitable image quality, and subsampling was not possible. Our landmarks included (1) anterior maximum of nose, (2) dorsal maximum of eye, (3) ventral maximum of eye, (4) posterior maximum of eye projected onto the dorsal outline, (5) posterior maximum of eye projected onto the ventral outline, (6) anterior maximum of eye projected onto the ventral outline, (7) anterior maximum of eye projected onto the dorsal outline, (8) projection of the x-value of landmark 9 on the dorsal outline, (9) juncture of the ventral margin of the operculum with the ventral outline of the body, (10) midpoint between landmarks 1 and 7 on the dorsal outline, and (11) midpoint between landmarks 1 and 6 on the ventral outline. Landmarks 10 and 11 were sliding semi-landmarks.

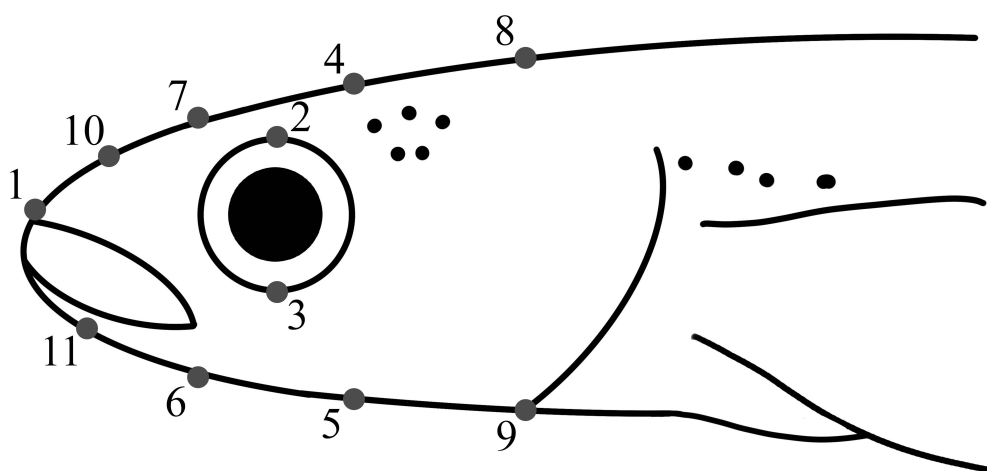


Figure 3. Landmarks used in the analysis of the shape of the lateral head view of *G. maculatus*.

We used our landmark coordinates in the program tpsRelW to generate shape variables [41]. We aligned the specimens and used a generalized Procrustes analysis to remove non-shape variation [43].

We then generated shape variables in the form of partial warps and uniform components (**W** or weight matrix). We used a principal component analysis of the **W** (weight) matrix to produce relative warps as our measures of shape for analysis. Relative warps are therefore linear combinations of uniform and non-uniform shape components that are orthogonal to each other [42,44,45]. Since relative warps are the principal components of the **W** matrix, relative warps concentrate shape variation in the first few relative warps compared to later relative warps. Relative warps analysis allows a reduction in the number of shape variables to be used in further analysis [18,46]. We conducted a statistical analysis of shape variation, using the first nine of sixteen relative warps for body shape and the first nine of eighteen relative warps for head shape as our response variables. The first 9 relative warps accounted for 98% of the shape variation in the body view and 97% of the shape variation in the head view.

2.3. Statistical Analysis

We used a multivariate linear mixed model to determine the effects of river or lake environments and centroid size (i.e., a multivariate measure of size derived from the shape analysis) on shape variation in *G. maculatus*. We ran a separate analysis for body and head shape. The response variable in both analyses was shape, as characterized by the first 9 relative warps. A mixed model framework assumes a univariate response variable, so we vectorized the shape variables such that each row represented one response variable, but each individual was represented by multiple rows of data [47]. Thus, the first row represented relative warp 1 for the first specimen, the second row represented relative warp 2 for the first specimen, and so forth until all relative warps were represented in successive rows for the first individual. The same pattern was repeated for all individuals, each with nine rows. The predictor variables were river/lake environment; centroid size; an index variable to account for the order of relative warps [11,48,49]; interactions between the main effects and the index variable; and the three-way interaction between river/lake environment, centroid size, and the index variable. The index variable preserved the order of the relative warps such that comparisons between groups (e.g., river/lake environment) were made by matching each relative warp to the same relative warp in each group (i.e., relative warp 1 in the river environment was compared to relative warp 1 in the lake environment). Thus, it was the three-way interaction between the main effects and the index variable that tested the hypothesis of interest (i.e., does shape vary on at least some of the relative warps between river and lake environments with centroid size?). Main effects by themselves were tested for an average effect across all relative warps. Since relative warps are principal components, they have a mean of 0; more importantly, they have an arbitrary ordination. Thus, a single individual may have a positive score on some relative warps and a negative score on other relative warps so that their mean score across all relative warps may be near 0. It was only by matching relative warps in the same order (by using the index variable as a predictor) that we could accurately test the hypothesis of interest [50].

Finally, we included two random effects in our model, individual ID and location. We included individual ID as a random effect in our model because we had multiple shape variables per individual. We included location as a random effect in our model to adjust for the variation that is inherent to a natural experiment, including the variation along the latitudinal gradient and between drainage basins. This allowed us to test for a general morphometric response to lake colonization [50]. Degrees of freedom were estimated using the Kenward–Roger method [51]. We used Proc MIXED in SAS to run all analyses (SAS version 9.4, SAS Institute Inc., Cary, NC, USA).

To visualize the effects of river and lake environments on shape, we plotted least squares means of shape on relative warps 1 and 2 (error bars represent 95% confidence intervals of the mean). To visualize the effect of variation in centroid size on body and head shape, we plotted lines with endpoints represented by ± 1 SD of mean centroid size (i.e., mean centroid size plus one standard deviation represents shape of larger individuals and mean centroid size minus one standard deviation represents shape of smaller individuals). This allows us to visualize the difference in direction and magnitude of shape variation associated with variation of centroid size. Relative warps 1 and 2

accounted for about 55% of the body shape variation and about 60% of the head shape variation. We used thin-plate spline representations of shape at positive and negative extremes of relative warps 1 and 2 to characterize the range of shape variation represented on each relative warp [52].

3. Results

The body and head shape of *G. maculatus* differed significantly depending on whether an individual was from a river or lake environment and on its size (three-way interaction with index variable; Table 1). River fish had robust bodies, whereas lake fish had fusiform bodies. The change in body shape associated with size was four times greater in river environments than lake environments over the same size range (Figure 4). River fish had smaller eyes and rounder heads, whereas lake fish had larger eyes and narrower heads which came to a sharper point. The change in head shape associated with size was about the same in river and lake environments over the same size range (Figure 5).

Table 1. Multivariate analysis of covariance effects for body shape and head shape.

Source	Degrees of Freedom	F-Value	p-Value
Body shape			
River/Lake (RL)	1, 1375	3.00	0.0834
Index	8, 1726	14.96	<0.0001
Centroid size (CS)	1, 1665	43.38	<0.0001
CS*Index	8, 1726	15.19	<0.0001
River/Lake*Index	8, 1726	7.01	<0.0001
CS*RL*Index	9, 1710	5.78	<0.0001
Head shape			
River/Lake (RL)	1, 1055	10.35	0.0013
Index	8, 684	49.47	<0.0001
Centroid Size (CS)	1, 1055	186.08	<0.0001
CS*Index	8, 684	49.28	<0.0001
RL*Index	8, 684	7.42	<0.0001
CS*RL*Index	9, 675	6.44	<0.0001

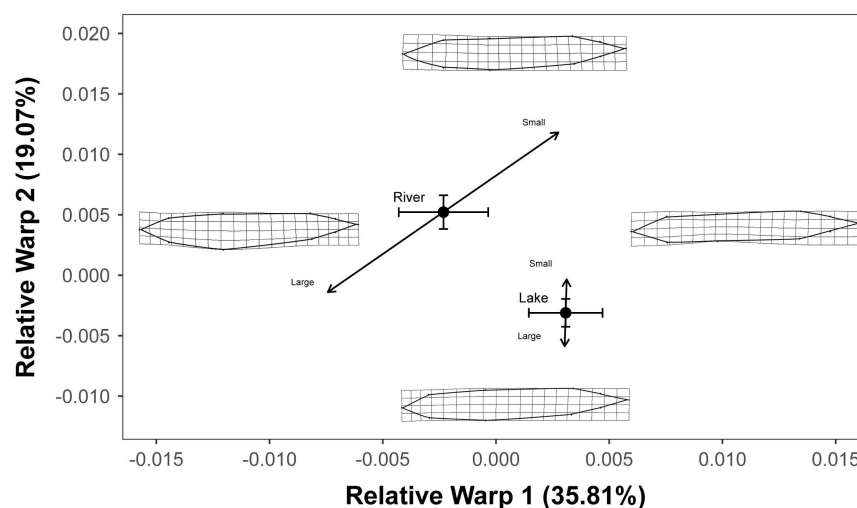


Figure 4. Least squares means of body shape (error bars represent 95% confidence intervals of the mean) of *G. maculatus* for river and lake populations on relative warps 1 and 2. Arrows represent ± 1 SD for centroid size. Thin-plate spline figures represent visual shape variation at the extremes of relative warps 1 and 2. The head is to the left in the thin-plate spline representations.

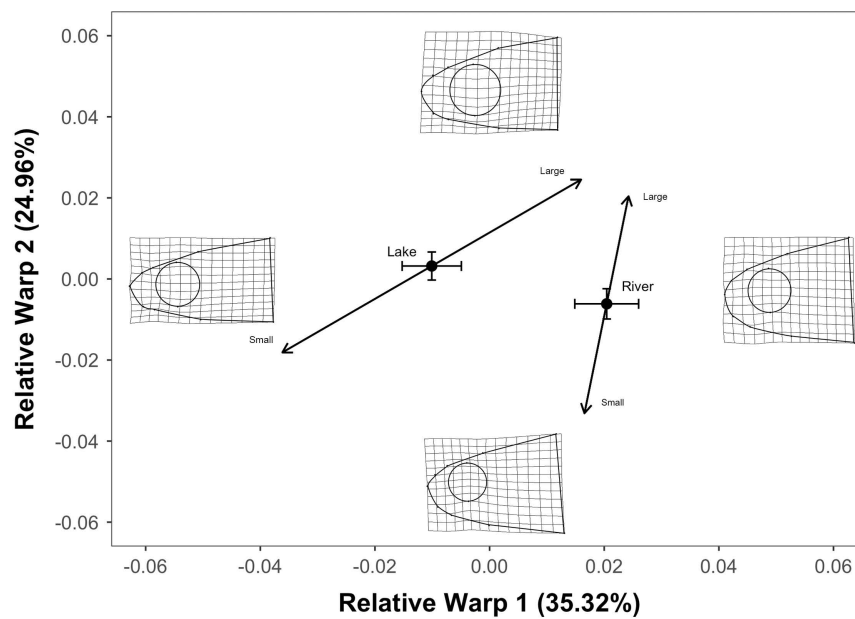


Figure 5. Least squares means of head shape (error bars represent 95% confidence intervals of the mean) of *G. maculatus* for river and lake populations on relative warps 1 and 2. Arrows represent ± 1 SD for centroid size. Thin-plate spline figures represent visual shape variation at the extremes of relative warps 1 and 2.

4. Discussion

Lake populations of *G. maculatus* exhibit a fusiform body relative to their riverine counterparts. In fish, robust bodies are typically favored in complex environments which require unsteady swimming, whereas fusiform body shapes are favored in high-flow environments which require steady swimming [3,6,10,32,39,53,54]. However, several studies, including our own, found the opposite pattern; river fish were robust whereas lake fish were fusiform [9,11,55,56]. These studies suggest that factors such as niche availability and diet, in addition to flow rate, could affect the difference in shape between river and lake fish [11,55]. The development of a fusiform body is consistent with zooplanktivorous feeding. Other studies have also determined that zooplanktivorous fish generally tend toward a fusiform body [57–60].

Lake *G. maculatus* have larger eyes and narrower mouths. Larger eyes are generally observed in organisms with better vision [39,61–63] and are associated with factors like active foraging (such as zooplanktivory) [64–67], high levels of competition [68], and low levels of predation [69–71]. Fish that specialize in zooplanktivorous feeding strategies tend to have a greater eye size than fish occupying other niches [72–75]. This is associated with their greater need for visual acuity. In addition, zooplanktivorous fish have smaller, upturned mouths, allowing them to feed efficiently by picking individual zooplankton out of the water column [74,75]. The narrow mouth and larger eyes of *G. maculatus* may facilitate zooplanktivorous, visual feeding in lakes [32,37,63,76–78].

The shape variation we observed in *G. maculatus* could be due to either adaptive divergence in response to selection or adaptive divergence via phenotypic plasticity (i.e., environmentally induced change). Documented genetic isolation between river and lake populations [32,34,37–39] demonstrates that these systems provide the conditions necessary for selection-based adaptive divergence to occur. However, phenotypic plasticity can also result in adaptive shape variation among environments [23–26], even when gene flow is high among populations. For example, cranial shape differences observed in contrasting environments in a Central American cichlid appear to be mostly due to phenotypic plasticity [23]. Plasticity is also an important mechanism for differentiation among lake and river populations of three-spined sticklebacks (*Gasterosteus aculeatus*), where it accounts for the majority of shape variation [24–26]. Thus, phenotypic plasticity could be partially or completely responsible

for the morphological variation observed in *G. maculatus*. Divergence in form between contrasting environments as an expression of phenotypic plasticity is often the first step toward heritable adaptive divergence due to selection and eventual speciation [28]. The variation observed in our study likely resulted from adaptive divergence via natural selection or phenotypic plasticity as a consequence of the consistent environmental differences between rivers and lakes.

Although water flow is a common environmental factor that impacts the morphometric response to lake colonization, location specific environmental factors such as niche availability, geological age of the system, and genetic history can have comparable effects [2,4,7,15–20]. Niche availability is typically associated with the development of polymorphism, in which fish diverge into multiple forms adapted to specialized feeding strategies. The more niches available, the more ecological “space” there is for multiple forms in an ecosystem [25,79]. Polymorphism is a relatively common response to lake colonization. Polymorphism after lake colonization has been documented in many species, including arctic char (*Salvelinus alpinus*), whitefish (*Coregonus* spp.), and three-spined stickleback (*Gasterosteus aculeatus*) [80–84]. Multiple populations of three-spined sticklebacks have diverged into two distinct forms upon lake colonization [60]. Benthic (bottom dwelling, invertivorous) individuals have deeper bodies and smaller eyes, while limnetic (open water dwelling, zooplanktivorous) individuals have narrower bodies and larger eyes [59,85]. These polymorphic sticklebacks colonized lakes that lacked competing planktivores and thus were able to fill empty niche space, reducing intraspecific resource competition [60,80,81,86].

We found no evidence for polymorphism in *G. maculatus*. Competition for a limited number of niches may result in specialization, which reduces adaptive potential and results in convergence into one form [87]. *Galaxias maculatus* shares much of its Chilean range and habitat with its relative, *Galaxias platei* [31,32,88]; *G. platei* is naturally occurring in all of the lakes we sampled for this study (E. Habit, personal observation). It is possible that these fish colonized the same lakes around the same time [31,88]. In these lakes, *G. platei* currently occupies the benthic zone, while *G. maculatus* occupies the limnetic zone [31,32,88]. These fish may have become specialized to their respective zones upon lake colonization in order to reduce interspecific competition for similar prey items. In addition, the invasion of other fish species prior to *G. maculatus*’ colonization could have further reduced available niches, limiting *G. maculatus*’ potential for polymorphism.

Environmental variation among sampling locations may have contributed to the variation we observed in the effect of size on shape (three-way interaction with index variable; Table 1). In river environments, body shape changes more with an increase in size than it does in lake environments. As fish in rivers grow, they become more robust with increased size, whereas lake fish change less in shape. *Galaxias maculatus* in rivers feed mainly on benthic macroinvertebrates and allochthonous organisms [32,37,77,89–92]. In addition, river fish have access to many different habitats, and some spend part of their lives in the ocean [32,34,36]. Since river fish engage in a generalist feeding strategy and occupy a variety of habitats, it may be that shape varies more with size in rivers than in lake environments.

In any comparative study of a natural system, there are multiple influences that are likely to impact the trait of interest. A slight variation in shape existed between *G. maculatus* in each population we sampled. This implies that location-specific factors (such as lake depth and habitat structure) may have influenced the shape of *G. maculatus* at each study site [84,93,94]. Location specific factors may also help explain other studies that found the opposite pattern in *G. maculatus*; these studies had a smaller breadth than ours and were conducted in different areas [32,39]. To account for these location-specific factors—which are inherent in any natural experiment—we included location as a random effect in our model. Despite the presence of these location-specific factors, we identified consistent shape differences between river and lake populations of *G. maculatus*, which is evidence of a generalized response to lake colonization.

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Methodology, R.C., E.H., and M.C.B.; Project administration, E.H. and M.C.B.; Resources, E.H.; Supervision, M.C.B.; Visualization, M.M., P.C.S., R.C., and M.C.B.; Writing—original draft, M.M., P.C.S., and R.C.; Writing—review & editing, M.M., P.C.S., E.H., and M.C.B. All authors have read and agreed to the published version of the manuscript.

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