

Invasive salmonids and lake order interact in the decline of puye grande *Galaxias platei* in western Patagonia lakes

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Abstract. Salmonid fishes, native to the northern hemisphere, have become naturalized in many austral countries and appear linked to the decline of native fishes, particularly galaxiids. However, a lack of baseline information and the potential for confounding anthropogenic stressors have led to uncertainty regarding the association between salmonid invasions and galaxiid declines, especially in lakes, as these have been much less studied than streams. We surveyed 25 lakes in the Aysén region of Chilean Patagonia, including both uninvaded and salmonid-invaded lakes. Abundance indices (AI) of *Galaxias platei* and salmonids (*Salmo trutta* and *Oncorhynchus mykiss*) were calculated using capture-per-unit-effort data from gillnets, minnow traps, and electrofishing. We also measured additional environmental variables, including deforestation, lake morphometrics, altitude, and hydrological position (i.e., lake order). An information-theoretic approach to explaining the AI of *G. platei* revealed that by far the strongest effect was a negative association with the AI of salmonids. Lake order was also important, and using structural equation modeling, we show that this is an indirect effect naturally constraining the salmonid invasion success in Patagonia. Supporting this conclusion, an analysis of an independent data set from 106 mountain lakes in western Canada showed that introduced salmonids are indeed less successful in low-order lakes. Reproductive failure due to insufficient spawning habitat and harsh environmental conditions could be the cause of these limits to salmonid success. The existence of this effect in Chilean Patagonia suggests that low-order lakes are likely to provide natural ecological refugia for *G. platei*. Finally, pristine, high-order lakes should be actively protected as these have become rare and irreplaceable unspoiled references of the most diverse, natural lake ecosystems in Patagonia.

Key words: AIC_c; Aplochiton; Chile; constraint to salmonid invasiveness; deforestation; Galaxiidae; information-theoretic approach; invasive trout; path analysis; Yulton Lake.

INTRODUCTION

Threats to native biodiversity are acute in fresh waters, and invasive species are considered a primary cause (Clavero and Garcia-Berthou 2005). However, direct confirmation of these effects has proven problematic owing to the absence of baseline data and the potential effects of other disturbances, such as habitat degradation, that took place concurrently to species invasions. This common inferential problem in invasion biology makes it difficult to firmly establish whether or not invaders are the root cause of problems for native species, and thus what might be the most effective management tools to achieve conservation goals (Didham et al. 2005, Light and Marchetti 2007, Hermoso et al. 2011). The effects of salmonid fishes (family Salmonidae) on native galaxiid fishes (family Galaxiidae) in temperate, Austral lakes pose precisely these challenges.

The salmonids rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*), native to the northern

hemisphere and regarded among the most common and disruptive invasive fishes worldwide, are now widespread in southern cold-temperate freshwaters (Cambray 2003, Casal 2006). One taxonomic group thought to be strongly impacted by these invaders is galaxiids, the most speciose taxon in the otherwise sparse freshwater fish fauna of the austral region (McDowall 2006). Although galaxiids have weathered a series of major environmental changes throughout their evolutionary history (geological activity, glaciations, and climate change), they are now seriously threatened (Cussac et al. 2004, McDowall 2006, Ruzzante et al. 2008). In an exhaustive review, McDowall (2006) found evidence of salmonids having adverse effects on 29 (58%) galaxioid species; but quantitative analyses of population-level impacts are rare, particularly for lake ecosystems and especially in Patagonia. This difficulty of robust inference stems largely from a general lack of pre-invasion baseline information and a rarity of known lakes that have not been invaded (Milano et al. 2002, Pascual et al. 2002, McDowall 2006, Lattuca et al. 2008a, Arismendi et al. 2009). In addition, the salmonid invasions were concurrent with other environmental changes, such as habitat degradation, and this increases uncertainty as to whether or not salmonids are the main

Manuscript received 29 June 2011; revised 18 November 2011; accepted 23 November 2011. Corresponding Editor (ad hoc): J. M. Moore.

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drivers of the galaxiid declines (e.g., Light and Marchetti 2007). This uncertainty has fundamental implications for conservation and management decisions in Patagonia, given that salmonid fishes are a lucrative part of the local economy (Arismendi and Nahuelhual 2007, Pascual et al. 2009). By dissipating some of this uncertainty, we seek to change the status quo in management and cultural attitudes that largely favor invading salmonids (Basulto 2003, Soto et al. 2006, Pascual et al. 2009).

A closer look at previous work suggests some likely effects and specific drivers and mediators thereof. For stream habitats, a substantial amount of observational and experimental evidence suggests pervasive negative effects of salmonids on galaxiids stemming from predation, habitat segregation, and competition, whereas key landscape features, such as insurmountable obstacles for dispersion or unstable river beds, have been shown to suppress salmonid dominance (McIntosh et al. 1992, Lintermans 2000, McIntosh 2000, McDowall 2006, Soto et al. 2006, Penaluna et al. 2009, Woodford and McIntosh 2010, Young et al. 2010). Although much less work has been done on lake communities, it too strongly suggests negative impacts of salmonids (McDowall 2006). For example, a recent longitudinal study from lakes in northern Chilean Patagonia showed a decline in the density of native fish (species aggregated) following an increase in salmonid density ostensibly due to escapes from industrial aquaculture (Arismendi et al. 2009). Our primary goal was to supplement and extend this previous body of work by formally evaluating empirical support for alternative hypotheses regarding patterns of abundance of a galaxiid species in lakes. In doing so, we also considered landscape features that might modulate the invasion success of salmonids (and hence their potential impacts on native fish) and thereby we also identified naturally occurring refugia for the natives. These observations then lead logically to a set of recommendations to aid native fish conservation while retaining some economic and social benefits that salmonids currently provide.

Our study was based on 25 western Patagonian lakes in a region of Chile (Aysén) that is sparsely populated by humans. This region has several properties relevant to our inferences. First, the native fish community is very simple with galaxiids being the only fishes in lakes. We focused specifically on the puye grande *G. platei* for statistical inference because it was the only galaxiid widely encountered and, indeed, was the only native fish in most lakes. Second, the salmonid invasion is relatively recent and uninvaded systems still exist for comparison to the more natural state. Third, other environmental changes are relatively minor and reasonably quantified, allowing us to more clearly separate potential influences. For instance, aquaculture, urbanization, and industry are rare in our study basins although deforestation and cattle grazing have been extensive (Woelfl et al. 2003, Yarrow and Torres 2009; see *Methods*). In particular, we

tested whether the abundance of *G. platei* was negatively correlated with the abundance of salmonids, and we challenged this basic model with a suite of alternatives drawn from theoretical considerations related to geography, hydrology, lake morphometrics, and anthropogenic land-cover change (Table 1). We hope improve our understanding of galaxiid population declines by weighing the evidence for alternative causal hypotheses.

METHODS

Study area

We studied 22 lakes in the Aysén watershed and three in the adjacent Cuervo and Baker watersheds in Chilean Patagonia (Fig. 1). (For a description of the region including historical perspectives, see Yarrow and Torres [2009].) These 25 lakes were selected in an effort to match apparently uninvaded lakes with physically comparable salmonid-invaded lakes (see next paragraph). In Chile and Argentina, most salmonid species were established during the first half of the twentieth century, with some more recent derivations from later stockings or from aquaculture escapes (Soto et al. 2001, Pascual et al. 2002, Basulto 2003, Correa and Gross 2008). In our study area, however, salmonid invasions began much later. The earliest known record of salmonid stocking in the Aysén watershed is from 1968, when 78 000 rainbow trout were released into Lago Pollux (Sakai 1989). Two years later, brown trout were introduced into Lago Elizalde and elsewhere in the region (Shiraishi 1985, cited in Blumberg-Muñoz 1996). These sparse written records agree with the opinion of local farmers, who assert that no salmonids existed in the area prior to the 1960s. (They were quick to take advantage of salmonids when they did appear in the local streams.) Trout have since proliferated, but detailed information on additional stocking events, rates of spread, or current distributions is lacking.

In each lake, we measured seven environmental variables and the units of measurement were sometimes transformed to stabilize variances and approach normality of residuals in linear models: lake area (measured in hectares, natural-log-transformed), shoreline development index (Bain and Stevenson 1999), maximum depth (measured in meters, natural-log-transformed), specific conductivity (μS), altitude (m), lake order (Strahler stream order at lake outlet; Donald 1987), and proportion ($\arcsin p^{1/2}$, where p is the proportion) of deforested area within a lake basin (see lake variables in Appendix A and deforestation analysis in Appendix B). That we were effective in sampling comparable uninvaded and salmonid-invaded lakes is confirmed by the fact that six out of the seven measured physical variables showed no significant difference between invaded and uninvaded lakes (Student's t tests, $df = 23$, $P > 0.05$). For the seventh variable, uninvaded lakes had a lower lake order than invaded lakes (mean [SD] of 1.9 [1.0] vs. 0.9 [1.1]; $P = 0.02$), although considerable overlap was still present between invaded and uninvaded lakes.

TABLE 1. Model-based hypothesis set.

Model	Formula	Type	Underlying biological hypotheses
g01	trout	SB	The AI of trout is negatively correlated with the AI of <i>G. platei</i> (McDowall 2006). This effect could arise through a number of mechanisms (Introduction). This hypothesis in its simplest expression is represented by a linear model with three parameters (intercept, slope due to the trout effect, and residual variance).†
g02	area	Ph-Ch	Area can influence the variety and quantity of fish resources and ecological interactions (Milano and Vigliano 1997, Aigo et al. 2008, Dodson et al. 2009).
g03	depth	Ph-Ch	Deep lakes can provide ecological refugia to <i>Galaxias platei</i> as this species possesses distinct adaptations to life in the hypolimnion (Milano and Vigliano 1997, Milano et al. 2002, 2006, Cussac et al. 2004). Arguments outlined for model g02 also apply here.
g04	altitude	Ph-Ch	Lake altitude correlates with a variety of biotic and abiotic variables, such as temperature, precipitation, and freezing risk, which can affect species distribution (e.g., Hubert and Chamberlain 1996).
g05	conductivity	Ph-Ch	Total ion concentration, measured as specific conductance, can correlate positively with lake productivity and, hence, fish abundance (but see g04; Downing and Plante 1993, Soto and De Los Rios 2006, Diaz et al. 2007).
g06	order	Ph-Ch	Lake order describes lake position in a river network, and, therefore, several physical, hydrological, and ecological properties of lakes and streams, as well as connectivity that can influence salmonid invasion potential and success (Donald 1987, Kratz et al. 1997, McIntosh 2000). Therefore, lake order can directly or indirectly affect the abundance of <i>G. platei</i> .
g07	shoreline	Ph-Ch	The development of the littoral zone relative to open waters, measured by the shoreline development index (Bain and Stevenson 1999), could increase littoral productivity and refuge from piscivory (Aigo et al. 2008).
g08	trout + order	M	The abundance of <i>G. platei</i> is affected both by lake order and trout abundance.
g09	trout + order + trout × order	M-I	The effect of trout depends on lake order; trout may be especially detrimental in small headwater lakes as a consequence of a shortage of refugia or extreme oligotrophic conditions; or, to the contrary, milder due to shortage of suitable stream habitat, which is vital for salmonid colonization and persistence.
g10	trout + depth	M	The abundance of <i>G. platei</i> is affected both by lake maximum depth and trout abundance.
g11	trout + depth + trout × depth	M-I	The effect of trout depends on lake depth: deeper lakes minimize the negative effect of salmonids due to the availability of hypolimnetic refugia, during at least part of the life cycle of <i>G. platei</i> (see references from g03).
g12	trout + shoreline	M-I	The abundance of <i>G. platei</i> is affected both by lake shoreline development and trout abundance.
g13	trout + shoreline + trout × shoreline	M-I	The effect of trout depends on shoreline development; trout may be especially detrimental in lakes with little shoreline development as a consequence of a shortage of littoral refugia (Aigo et al. 2008).
g14	conductivity + is.shallow + conductivity × is.shallow	M-I	Hypothesis g05 is conditional on lake depth. It has been shown that the typically deep mixing of the epilimnion of Patagonian oligotrophic lakes due wind stress can substantially reduce productivity and ultimately may affect fish biomass (Soto 2002). Therefore, the productivity of shallower lakes (i.e., <25 m as proposed by Soto [2002]) should be more sensitive to variations in nutrients concentrations.
g15	trout-threshold	S-S	The effects of additional trout vary along the gradient of invasion intensity. Perhaps <i>G. platei</i> is not influenced by trout unless the latter are particularly abundant, or, conversely, the influence of trout increases until reaching a plateau at intermediate trout abundance. This model includes two different slopes pivoting at a threshold (Toms and Lesperance 2003).
g16	<i>S. trutta</i>	S-S	<i>S. trutta</i> is generally a more aggressive and piscivorous than is <i>O. mykiss</i> and might therefore have a greater effect (Crowl et al. 1992, Moyle and Light 1996, Young et al. 2010).
g17	<i>O. mykiss</i>	S-S	Complement to g16.

TABLE 1. Continued.

Model	Formula	Type	Underlying biological hypotheses
g18	deforestation	Co	Land-cover in the region of Aysén has been partially transformed through anthropogenic activity during the 20th century; the most dramatic changes took place during the 1930s and 1940s, when 50% of the old-growth forest within the Aysén watershed was burned for pasture and other uses (Appendix B; Yarrow and Torres 2009). These alterations likely changed fluvial geomorphology, increased land erosion, nutrient content, and carbon pathways, all of which can influence lake ecosystems (Rowe et al. 1999, Woelfl et al. 2003, Yarrow and Torres 2009).
g19	altitude + area + conductivity + deforestation + depth + order + trout	G1	Structure-rich model approaching a global model (Anderson 2008). Includes the effects of trout (species pooled) and all other environmental variables (lake altitude, area, conductivity, depth, deforestation, and order). Several terms (e.g., interactions) were omitted because of reduced sample size.
g20	naïve	Na	Naïve model. Uncertainty and stochasticity prevails. Fits a mean and residual variance to the data (Anderson 2008).

Notes: Types are: SB, basic hypothesis involving salmonids; Ph-Ch, hypothesis involving physical or chemical processes; M, hypothesis involving multiple effects; M-I, hypothesis involving multiple effects and a first-order interactions; S-S, hypothesis focusing on specific aspects of salmonid effects; Co, hypothesis involving confounding anthropogenic effects; G1, approximation to global model; Na, naïve hypothesis. Other abbreviations are AI, abundance index; is.shallow: variable specifying if a lake is shallow (<25 m) or not; trout-threshold, model with trout (trAI) as the only explanatory variable, and two slopes transitioning at a threshold point.

† The intercept and residual variance are implicit in subsequent models.

Fish community sampling

Our fish sampling took place in the austral summer and early fall (January–May) of 2007 and 2009. Although we sampled a few lakes in both years, and found no inter-annual differences in fish abundance estimates, we cannot rule out such effects for the lakes sampled in one year. We therefore made sure to sample a similar number of invaded vs. uninvaded lakes in each year (eight vs. four lakes in 2007, and nine vs. four in 2009). We also made sure to spread work on the two lake types across the entire study area; aside from these considerations, the sampling design was largely dictated by logistic constraints (Appendix A).

No single fishing gear is sufficient to accurately sample the fish community of a lake, and so we systematically employed a combination of three fishing techniques (Appendix A). (1) Gillnets were used to mainly target large fish (>50 g), particularly salmonids and large galaxiids in the littoral and demersal zone. (2) Minnow traps were used to sample smaller galaxiids from a broader range of microhabitats in the littoral and demersal zone. (3) Electrofishing was used to sample widely and indiscriminately in the littoral area. Our overall goal was to use a variety of complementary fishing methods that could be combined together into a single integrated estimate of relative abundance.

The gillnets (for gear specifications, see Appendix A) were sinking monofilament multipanel survey nets deployed overnight in both the littoral zone (perpendicular to the shoreline) and demersal zone (30–40 m deep, or deepest possible basin). In most lakes, we set one net per zone. In lakes Juncos and León, which had only littoral habitat, we set one and three littoral nets, respectively. In lakes Alta and Castor, we set both littoral and demersal gill nets in both years, and the data

were combined for analysis. Exclusion from the analyses of these four lakes with somewhat uneven fishing effort did not change our main conclusions (not shown).

The minnow traps were deployed overnight across all obvious habitat/substrate types over a wide area (generally >10 ha) ranging from 0.5 m to 35 m in depth (depending on lake depth). Generally we used 20 traps per lake (median = 20, range = 16–40; Appendix A). We mainly used unbaited traps, but we tested the effect of baiting. We split the traps of five lakes into baited and unbaited traps (we used fish flesh as bait) and we found that bait did not influence the capture rate of *G. platei* (Cochran-Mantel-Haenszel test, $\chi^2 = 2.407$, $df = 1$, $P = 0.121$; Appendix A). We therefore included both baited and unbaited traps in the analyses.

Electrofishing took place during the day in the shallow (<0.6 m) littoral zone of every lake. The method consisted in surveying, by the same person, any microhabitat where fish could hide in a number of transects per lake (median 4, range 2–8), spanning diverse habitats and shoreline conditions (including sedges, boulders, and woody debris). The electrofisher was activated only in the vicinity of potential hiding places such that the total discharge time was proportional to the number of microhabitats surveyed. Six lakes were surveyed in both 2007 and 2009, and no (paired) statistical differences were detected between years in the catch rate of *G. platei* (paired *t* test, $t = 0.895$, $df = 5$, $P = 0.412$) or salmonids ($t = -1.908$, $df = 4$, $P = 0.129$). The data from both years were therefore pooled for analysis.

Although fishing effort was standardized as much as possible across lakes, some slight variation (as above) was necessary owing to differences in lake size, available habitat, weather conditions, and logistics. However, this

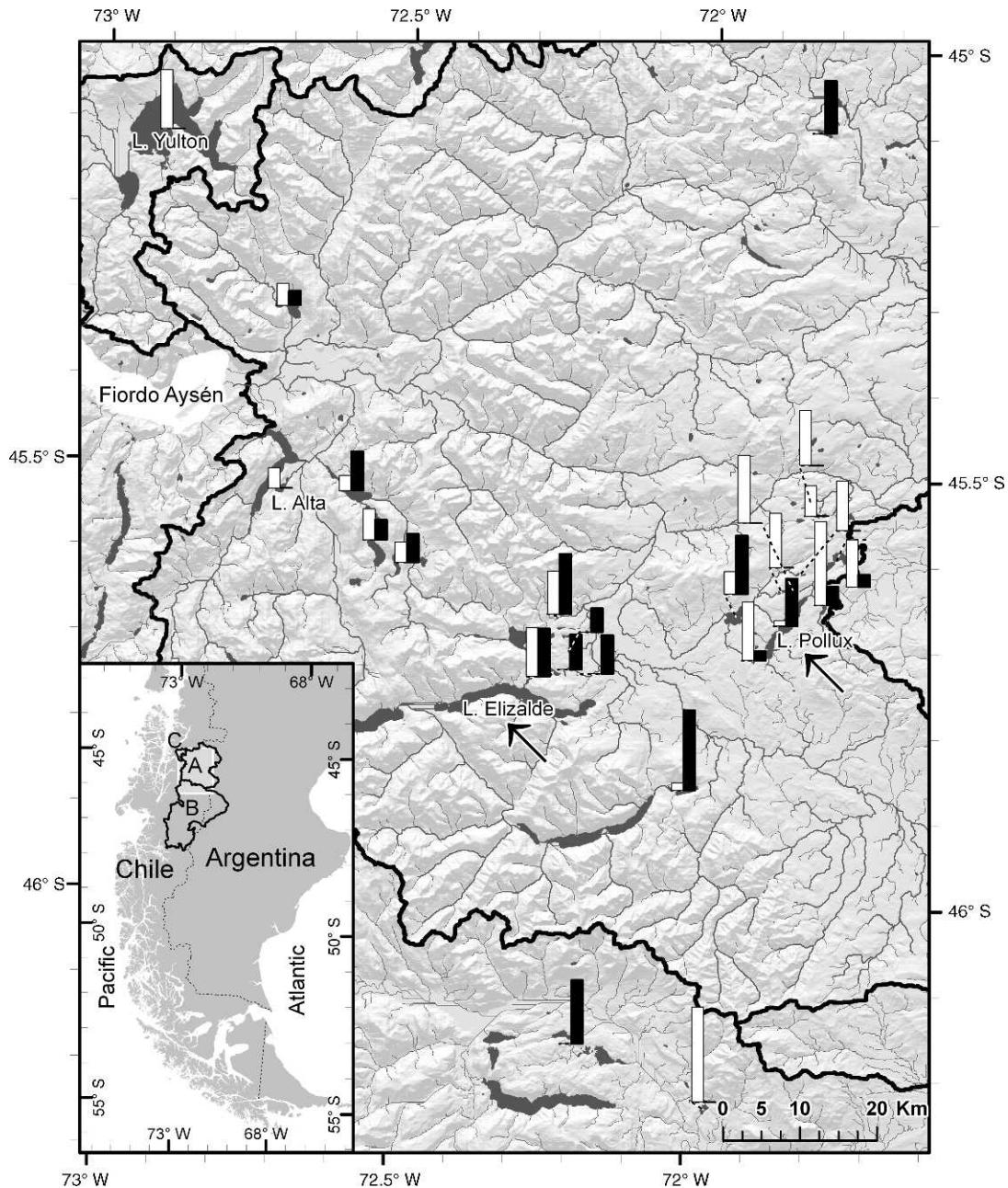


FIG. 1. Locations of the 25 lakes studied and corresponding abundance index (AI) estimates of native *Galaxias platei* (white bars) and invasive salmonids (black bars). The labeled lakes are those mentioned in the text (all lakes are identified in Appendix A). Most of the lakes reside within the Aysén watershed (insert; A), and three additional lakes are located in the Baker (B) and Cuervo (C) watersheds. Arrows indicate points of documented stockings of *Oncorhynchus mykiss* in Lago Pollux in 1968 and *Salmo trutta* in Lago Elizalde in 1970.

variation in effort was not correlated with any of the potential explanatory factors of interest: Pearson's product-moment correlations between effort (i.e., number of gill nets, traps, and electrofishing transects per lake) and the seven lake variables listed in Appendix A were $P > 0.05$ for 22 of 24 possible tests (increasing to all tests when a single outlier was removed). We therefore suggest that our results are not biased by

variation in sampling effort. We also took care not to miss salmonids simply because they had left the lake to spawn in adjacent streams; we timed our sampling so as not to coincide with the spawning season of rainbow or brown trout. We also systematically electrofished adjacent streams and did not find any spawning salmonid (data not shown). Finally, it is critical to note that we were interested in relative differences in the

overall abundance of fish species across lakes, not absolute numbers of fish in a given lake or unit area, and our methods are appropriate for inferring the former.

All fish were identified immediately after capture and most were released alive; indeed, mortality of *G. platei* was relatively low even in gillnets. However, samples were retained for future analyses, and voucher specimens were deposited in the Museo Nacional de Historia Natural de Chile.

Variables and model-based hypotheses

The fish capture data were used to calculate canonical variables representing abundance indices (AIs) for each taxon widely sampled: *G. platei* (GpAI), *S. trutta* (StAI), *O. mykiss* (OmAI), and “trout” (*S. trutta* and *O. mykiss* pooled; trAI). We deemed these integrated multi-gear AIs more appropriate than gear-specific values for several reasons. First, the different methods are complementary in their ability to catch fish of different species and sizes in different habitats. Combining data from the different gear types into a single variable therefore decreases the chance of false negatives and minimizes the potential biases or limitations of any one method (each method “borrows” strength from the others). Second, the use of a single metric avoids the shortcomings of multiple testing, particularly given that all methods intended to inform the same basic parameter: the relative number of fish in a lake. Lastly, estimates from the different gear types were correlated with each other (see principal components analysis [PCA] below), and post hoc statistical analyses using catch per unit effort (CPUE) yielded qualitatively similar yet some times quantitatively weaker conclusions. Although we include these analyses in the Supplement for the interested reader, here we illustrate our findings using the integrated AIs.

To develop an AI for each of the fish types, we first computed CPUE for each lake-by-gear combination. For gillnets, the average overnight catch per net was calculated and transformed for analysis ($x' = \ln(x + 1)$). For traps, a Poisson distribution was fit to the fish-per-trap frequency distribution data, and the parameter λ , which corresponds to the mean number of fish per trap, was then transformed for analysis ($y' = \ln(y + 0.1)$). For electrofishing, the catch per transect was divided by the total discharge time (minutes), and the resulting values were averaged across transects and then transformed ($z' = \ln(z + 1)$). Next, we combined the above lake-by-gear CPUE data into the first canonical axis (PC1) from principal components analysis (PCA) on correlations by taxon. (Traps were not included in the salmonid estimates because no trout were captured with this gear.) PC1 explained 70% of the variance in densities across lakes for *G. platei*, 86% for *S. trutta*, 91% for *O. mykiss*, and 85% for trout. Finally, we obtained the PC1 canonical scores for each lake and taxon, and these hereafter represent the (multivariate) AIs.

In all statistical models (see below), the GpAI was set as the response variable, whereas the OmAI, StAI, and trAI were used as explanatory variables. The environmental variables described above were also used as explanatory variables. We then identified 20 a priori statistical models representing alternative hypotheses about factors that might influence galaxiid abundance (Table 1).

Model selection

We used an information-theoretic approach (Anderson 2008) to evaluate the ability of the different models to explain GpAI. This approach is considered the best for situations where model uncertainty prevails, and it is widely used in ecology (Johnson and Omland 2004). Importantly, this approach does not accept or reject arbitrary models; it instead ranks a number of model-based a priori hypotheses according to the empirical evidence, and provides evidence ratios and posterior model probabilities that are easily interpretable for comparing the performance of different models. Alternative approaches to model selection, such as step-wise multiple regression, are now known to perform poorly and were therefore not used here (Mundry and Nunn 2009). In addition, we did not use logistic regression in models seeking to explain *G. platei* presence/absence (as opposed to our quantitative AI) because (1) this species was absent from only five lakes, and (2) we had good (and repeatable) quantitative information on relative GpAI that this simplification would ignore.

The 20 models were fit using the R functions `lm` and `nls` (R Development Core Team 2010). For each model, the sample-size corrected Akaike information criterion (AIC_c) was computed as $AIC_c = AIC + 2K(K + 1)/(n - K - 1)$, where $AIC = -2\log\text{-likelihood} + 2K$ (R function `AIC`), K = number of parameters in the model, and n is the number of observations (Anderson 2008). We ranked the model set on the basis of increasing AIC_c , and based further interpretation on model probabilities (w) and evidence ratios ($E_{i,j}$; Anderson 2008). In addition, top-ranked models were examined using standard frequentist variance partitioning methods and null hypothesis testing in order to assess model-conditional statistical performance (i.e., SE of slope coefficients, coefficients of determination, and ANOVAs with $\alpha = 0.05$). The R code and data are available in the Supplement.

Structural equation modeling

Results from the previous section indicated that lake order had a negative effect on GpAI, yet lake order was also positively correlated trAI suggesting that lake order might influence GpAI only indirectly. Hence, we addressed the corollary question: is the direct effect of lake order on GpAI statistically important after its indirect effect (through enhancing trout abundance) has been accounted for?

Structural equation modeling (SEM) provided an adequate quantitative approach. It permits the inclusion in a model of more than one response variable, unobserved (latent) variables, and a set of a priori hypothetical variable dependencies (or path structure) that can be tested statistically. We were specifically interested in weighing the statistical support of the direct vs. indirect paths of lake order on the density of *G. platei*, for which we adapted the PCA and SEM methods described in Grace (2003). In both cases, we defined the same core path structure (model A): lake order could have both an effect on trout density and on *G. platei* density, while trout density could only have an effect on *G. platei* density. We also defined a reduced model that had the direct effect of order on *G. platei* density set to zero (model B). Stronger support for the later model would indicate that the direct influence of lake order on *G. platei* density was statistically negligible after controlling for the indirect-effect pathway.

The PCA and SEM methods of Grace (2003) differ in which density variables were allocated to the core path. The PCA method used the GpAIs and trAI, as “observed” variables, in place of fish densities, because the AIs were derived from PCA). The SEM method used unobserved, latent variables in place of densities, and the standardized, lake-by-gear CPUE as indicators of these latent variables. In every case, response variables (including indicator variables) had associated random variables with regression coefficients fixed to one to accommodate residual variance. To attain identification in the SEM method, the residual variance of the latent variables was set to one. All models were fit by maximum likelihood in the software Amos 18.0 (Arbuckle 1995).

In order to ensure that the pattern of variances and covariances in the data were consistent with those implied by the models (i.e., to diagnose adequate models’ fit), we relied on various indices that are part of the software’s output, including the incremental fit index (IFI) and the goodness-of-fit index (GFI). These indices vary between 0 and 1 and values close to one are indicative of good fit (Arbuckle 1995). We also conducted goodness-of-fit chi-square tests ($\alpha = 0.05$) for all but model B, PCA method, because this model was saturated with no degrees of freedom left for the test.

The difference between the fit of models A and B for each method were then statistically compared using the chi-square likelihood ratio test (LRT) and a significance level of 0.05. The models were also ranked according to increasing values of AIC_c . Finally, we based model interpretation on the effect sizes as measured by standardized regression weights (also called structural or path coefficients); total and partial effects; and coefficients of determination of response variables. Because of the small sample size ($n = 25$ lakes) and possible departure from multivariate normality, SE and 95% bias-corrected CI for regression weights were

obtained from 1000 nonparametric bootstrap samples; regression weights were considered significant if the CI did not encompass zero (Arbuckle 1995). For further details on these methods, please refer to Pugsek et al. (2003).

Probability of salmonid naturalization

We sought additional evidence to confirm the finding that a low lake order can suppress trout populations. To this end, we analyzed an independent and larger data set containing lake order and salmonid naturalization success (i.e., self-sustainable population establishment; binary response) after salmonid stockings in 106 mountain lakes in western Canada (Donald 1987). This set of lakes was suitable to test our hypothesis because they, like our Patagonian lakes, opposed little biotic resistance to incoming salmonids and the physical environment was ostensibly the main determinant of salmonid naturalization (Donald 1987). We used binomial logistic regression to model the probability of salmonid naturalization with respect to lake order. The same analysis was conducted on our own data for comparison: presence of salmonids in our samples was used as a surrogate of naturalization. We compared the results of the analysis of the two data sets based on the 95% confidence intervals of expected probabilities. The statistical effect of lake order was tested by comparing models with and without the lake-order effect (i.e., LRT implemented in the R function ANOVA; R Development Core Team 2010).

RESULTS

Fish assemblages

We captured a total of 1828 individual fish of five species, with a median of 43 fish (range 10–509) per lake. Native fish were represented only by galaxiids: puye grande (*G. platei*; 20 lakes), puye chico or whitebait (*G. maculatus*; 2 lakes), and peladilla or zebra trout (*Aplocheilichthys zebra*; 1 lake). Exotic fish were represented only by salmonids: brown trout (*S. trutta*; 13 lakes) and rainbow trout (*O. mykiss*; 7 lakes). Eight lakes had no salmonids, with *G. platei* occurring alone in all but one of these uninvaded lakes. By contrast, five lakes had no galaxiids, with *S. trutta* occurring alone in three of these lakes. Most multispecies assemblages had trout (14 out of 15 lakes), and 12 of these multispecies lakes had only two species (Table 2). Because *G. platei* was present in all 20 lakes with galaxiids and was the sole galaxiid species in 18 of these lakes, subsequent quantitative analyses used GpAI as the response variable.

Model selection

The GpAI was best explained by hypotheses involving trAI, because all top-ranked models included such effects (Table 3). Furthermore, our main hypothesis in its simplest form (model g01, GpAI is negatively correlated with trAI) had the highest empirical support. This model reveals a strong negative effect of trAI on

TABLE 2. Number of lakes in which fish species occurred alone (diagonal, in brackets); alone or in combination with other species (diagonal, outside brackets); and together with another species (off diagonal).

Species	<i>Galaxias platei</i>	<i>Galaxias maculatus</i>	<i>Aplochiton zebra</i>	<i>Oncorhynchus mykiss</i>	<i>Salmo trutta</i>
<i>G. platei</i>	20 (7)	2	1	5	8
<i>G. maculatus</i>	2	2 (0)	1	1	1
<i>A. zebra</i>	1	1	1 (0)	0	1
<i>O. mykiss</i>	5	1	0	7 (0)	3
<i>S. trutta</i>	8	1	1	3	13 (3)

Note: Only two lakes had more than two species: Lago Monreal-2 had *G. platei*, *S. trutta*, and *O. mykiss*; and Lago Los Palos had *A. zebra*, *G. platei*, *G. maculatus*, and *S. trutta*.

GpAI (mean slope of -0.67 [SE 0.19]; ANOVA, $F_{1,23} = 18.16$, $P = 0.001$, Fig. 2). Indeed, the addition of other explanatory variables contributed only marginally to model fit and did not alter the importance of salmonids. For instance, R^2 was 0.36 (adjusted $R^2 = 0.33$) for the best model with trAI alone (model g01) vs. 0.54 (adjusted $R^2 = 0.31$) for the model with seven additional explanatory variables (model g19; Table 3).

The linear form of the trAI model was more likely than the piecewise threshold form (evidence ratio: $E_{g01,g15} = 4.0$), although the fit was slightly better for the latter. In particular, the negative association between trAI and GpAI seemed stronger at low to medium trAI, than at higher trAI. Although the model with AI of both trout species combined had the greatest support, the

model with StAI alone had much more support than the model with OmAI alone ($E_{g16,g17} = 16.0$). At least some of the relative weakness of the latter model (g16) might be due to the small number of lakes with *O. mykiss* compared to the number of lakes with *S. trutta* (Table 2).

Although non-salmonid explanatory variables were substantially less important than those involving salmonids, three of those other effects deserve mention. In particular, the second- and third-best models (g10, g08) show additional, although small, negative associations between GpAI and lake depth or lake order, whereas the forth-best model a small positive association with shoreline development (g12). Considered without trout in the model, the effect of lake order (g06) becomes

TABLE 3. Model set evaluation.

Model	Formula (parameter estimates)†	<i>k</i>	RSS	Adjusted R^2	AIC _c	Model likelihood‡	<i>w</i>
g01	trout (0.00; -0.67)	3	32.41	0.33	84.58	1.00	0.33
g10	trout + depth (1.14; -0.63 ; -0.35)	4	30.02	0.35	85.52	0.63	0.21
g08	trout + order (0.28; -0.60 ; -0.18)	4	31.71	0.32	86.89	0.31	0.11
g15	trout-threshold ($b_0 = -1.04$; $b_1 = -1.20$; $b_2 = 0.07$; brk = 0.48)	5	28.47	0.36	87.36	0.25	0.08
g12	trout + shoreline (-0.19 ; -0.67 ; 0.10)	4	32.37	0.30	87.40	0.24	0.08
g16	<i>S. trutta</i> (0.00; -0.56)	3	37.61	0.22	88.30	0.16	0.05
g11	trout + depth + trout \times depth (1.17; -0.56 ; -0.36 ; -0.02)	5	30.01	0.32	88.67	0.13	0.04
g09	trout + order + trout \times order (0.05; -0.94 ; -0.11 ; 0.19)	5	30.44	0.31	89.03	0.11	0.04
g13	trout + shoreline + trout \times shoreline (-0.07 ; -0.39 ; 0.04; -0.16)	5	32.26	0.27	90.48	0.05	0.02
g06	order (0.82; -0.51)	3	42.68	0.12	91.46	0.03	0.01
g20	naïve (0.00)	2	50.58	0.00	93.11	0.01	0.00
g03	depth (1.60; -0.49)	3	45.67	0.06	93.15	0.01	0.00
g04	altitude (-0.79 ; 0.00)	3	45.95	0.05	93.30	0.01	0.00
g17	<i>O. mykiss</i> (0.00; -0.32)	3	46.07	0.05	93.37	0.01	0.00
g18	deforestation (1.08; -1.43)	3	47.55	0.02	94.16	0.01	0.00
g05	conductivity (-0.39 ; 0.01)	3	49.76	-0.03	95.30	0.00	0.00
g07	shoreline (-0.07 ; 0.04)	3	50.57	-0.04	95.70	0.00	0.00
g02	area (-0.00 ; 0.00)	3	50.58	-0.04	95.71	0.00	0.00
g14	conductivity + is.shallow + conductivity \times is.shallow (0.09; -0.01 ; 0.20; 0.02)	5	44.13	0.00	98.31	0.00	0.00
g19	altitude + area + conductivity + deforestation + depth + order + shoreline + trout (1.77; 0.00; 0.29; 0.00; -1.40 ; -0.82 ; -0.21 ; 0.37; -0.43)	10	23.13	0.31	104.72	0.00	0.00

Notes: Models are sorted by increasing values of the Akaike information criterion adjusted for small sample sizes (AIC_c). Fish taxa in the formulas denote abundance index (AI) for the corresponding taxon. Other variables are: *k*, number of parameter estimates; RSS, residual sum of squares; and *w*, conditional model probability (model likelihood of model *i* divided by the sum of model likelihoods). Regression coefficients in g15 are: *b*₀, intercept; *b*₁, first slope; *b*₂: second slope, brk, breakpoint (in the *x*-axis) connecting the two segments of the regression line.

† Least-squares parameter estimates: intercept followed by slope(s).

‡ Relative to the best model.

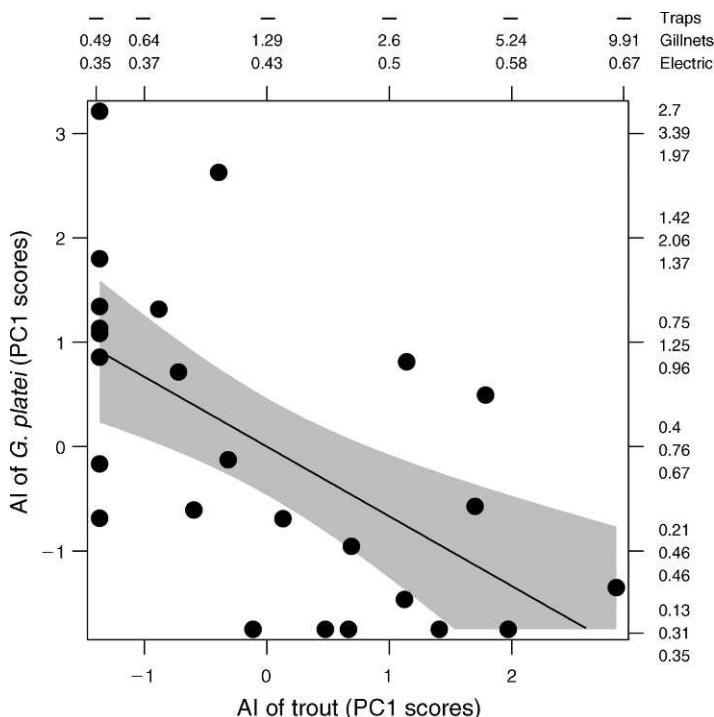


FIG. 2. Negative association between the abundance indices of the invasive salmonids (*S. trutta* and *O. mykiss* pooled; trAI) and the native puye grande *G. platei* (GpAI). Data points represent individual lakes ($n = 25$ lakes). The shaded area is the 95% CI of the regression line. Top (trout) and right-hand (*G. platei*) axes illustrate how PC1 scores translate into expectations of back-transformed units of catch per unit effort (CPUE) (a dash indicates no data). Out of the 17 models evaluated, this model (g01) had the highest empirical support.

more negative and significantly different from zero (mean slope of -0.51 [SE 0.25]; ANOVA $F_{1,23} = 4.26$, $P = 0.050$, adjusted $R^2 = 0.12$) yet the model loses substantial support ($E_{g08,g06} = 10.3$). This is likely because there is a positive correlation between lake order and trAI ($r = 0.49$, $t = 2.68$, $df = 23$, $P = 0.013$) and, hence, the slopes of these effects on GpAI are related (also see *Results: Structural equation modeling*). The predictive value of depth and shoreline development, on the other hand, remained always low. The addition of interaction terms (g09, g11, and g13) did not enhance the fit of these models (Table 3). The naïve and subsequent models had little empirical support given the data and the model set and are not discussed further ($E_{g01,g20} = 100.00$).

Structural equation modeling

The variance-covariance structures of the data were consistent with those implied by the four models (i.e., models A and B for methods PCA and SEM). The IFI and the GFI were close to one (both > 0.95 for the PCA method; for the SEM method IFI > 0.90 and GFI > 0.87) and the goodness-of-fit chi-square tests showed no statistically significant difference (PCA method, model B $\chi^2 = 0.524$, $df = 1$, $P = 0.469$; SEM method model A $\chi^2 = 11.031$, $df = 7$, $P = 0.137$; model B $\chi^2 = 11.449$, $df = 8$, $P = 0.178$).

The results indicated that the effect of lake order on *G. platei* density is primarily indirect: lake order had a positive effect on trout density, which led to an indirect, negative effect on *G. platei* density. Both the PCA and SEM methods supported this conclusion. Although models A and B were not statistically different for a given method (likelihood-ratio test, PCA method $\chi^2 = 0.524$, $df = 1$, $P = 0.469$; SEM method $\chi^2 = 0.418$, $df = 1$, $P = 0.518$), model B should be preferred for two reasons: (1) It is more parsimonious and had a lower AIC_c value ($\Delta AIC_c > 3$ for both methods). (2) The direct effect of lake order on *G. platei* density in model A was unimportant; its low regression weight was not statistically different from zero (95% CI encompassed zero). That is why constraining this parameter to take a value of zero (i.e., model B) did not compromise the overall model fit. In contrast, the indirect effect of order on *G. platei* density was supported both by model A and B; the regression weights of the two paths between lake-order-trout and trout-*G. platei* had relatively high absolute values and were significantly different from zero using either method. The lack of statistical support for the direct effect of lake order on the density of *G. platei*, while accounting for the indirect effect, could have stemmed from the small sample size (and low statistical power). However, when both paths were possible in models A, the effect size of the indirect paths were

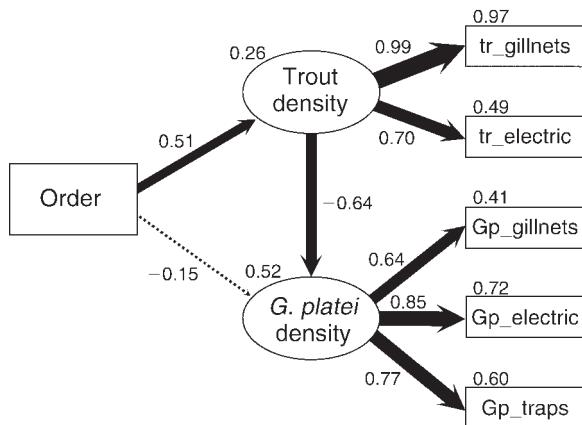


FIG. 3. Path diagram of model A, structural equation modeling (SEM) method, illustrating the effects of lake order and trout density on *G. platei* density. Ellipses represent latent (unobserved) variables modeling fish density as a function of indicator (observed) variables in boxes, and the structural model outlined by arrows. Arrow widths are proportional to effect sizes, which are shown nearby. Significant effect sizes are depicted with solid arrows; arrows are dashed otherwise. Note how the negative effect of lake order on *G. platei* density is mainly indirect, mediated by a positive effect on trout density. The coefficients of determination are reported on the top left side of each response variable; for simplicity, residual variance variables are not shown.

substantially stronger (the mean [SE] was -0.26 [0.11] for the PCA method, and -0.32 [0.16] for the SEM method) than the effect size of the direct path (-0.13 [0.15] and -0.15 [0.18]). Fig. 3 illustrates this finding using the path diagram of the SEM method; the other path diagrams show similar patterns and were omitted for the sake of space.

Probability of salmonid naturalization

Not only did lake order have a positive significant effect on the probability of finding trout in Patagonian lakes (LRT $\chi^2 = 5.731$, $df = 1$, $P = 0.017$), it also had a similar effect in stocked Canadian mountain lakes (LRT $\chi^2 = 22.442$, $df = 1$, $P < 0.001$; Fig. 4). The fitted logistic models for the two data sets were not statistically distinguishable between regions of the world, and the model fitted to the pooled data was highly significant ($\text{Pr}(\text{trout naturalization}) = \text{logit}^{-1}(-0.73 + 1.08 \times \text{lake order})$; LRT $\chi^2 = 27.279$, $df = 1$, $P < 0.001$). Hence, on average, 33% of lakes with no obvious associated streams (lake-order zero) and 97% of lakes with outlets and substantial tributaries (lake-order four) can support salmonid populations. Furthermore, in accordance with the results of the previous section, the salmonid catch per unit effort in salmonid-colonized lakes was lower in low-order lakes (order 0–1) compared to high-order lakes (order 2–4; inserts in Fig. 4, catch-per-unit-effort units as in Donald [1987]). These results suggest that lake order can effectively be used to identify lakes with

high potential to naturally resist salmonid invasions in different regions of the world.

DISCUSSION

We found a strong negative association between the abundance index (AI) for *G. platei* vs. salmonids and these effects were much stronger than those of other potential environmental or land use variables that might influence *G. platei* abundance. These findings point to

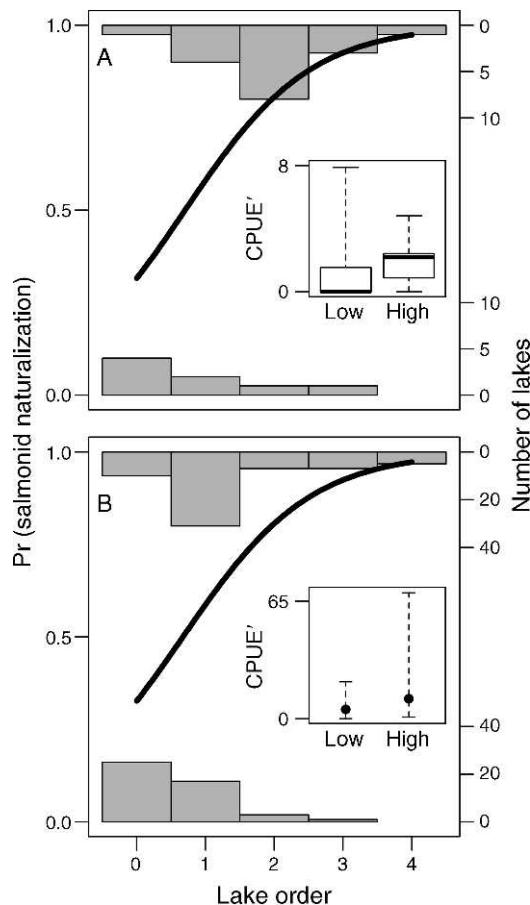


FIG. 4. Effect of lake order on (A) occurrence of salmonids in 25 Patagonian lakes (this study) and (B) fate of salmonid introductions in 106 mountain lakes with little biological resistance in western Canada (data from Tables 2 and 3 in Donald [1987]). Salmonids in the panel A comprise rainbow trout (*O. mykiss*) and brown trout (*S. trutta*); in panel B, rainbow trout, cutthroat trout (*O. clarkii*), and brook char (*Salvelinus fontinalis*). In the southern and northern hemisphere, salmonids naturalized with greater probability in lakes with relatively high Strahler stream order at lake outlet (black curves; see statistics in *Results: Probability of salmonid naturalization*). Salmonids in colonized lakes tended to occur in higher densities (measured as capture per unit effort, CPUE') in high-order lakes (order 2–4) compared to low-order lakes (order 0–1; panel A insert, quartiles, one-tailed Mann-Whitney *U* test, $U = 45$, $P = 0.039$; panel B insert, only means and ranges for a subset of 45 lakes were available). (Note: CPUE' was here defined as $\text{catch} \cdot \text{h}^{-1} \cdot (100\text{-m littoral net})^{-1}$ following Donald [1987]; however, CPUE' comparisons between studies might be inappropriate due uncontrolled methodological differences.)

salmonids as the primary driver of population declines of this Patagonia-endemic fish. Although a correlative study such as ours cannot provide definitive proof, the weight of evidence from ours and other studies strongly supports this inference. In addition, we found evidence that *S. trutta* had stronger effects on *G. platei* than did *O. mykiss*, although we interpret this finding cautiously because *S. trutta* was present in about twice as many lakes as *O. mykiss*. Nonetheless, bearing in mind that *S. trutta* is generally more piscivorous than *O. mykiss*, this finding is consistent with the idea that predation is a stronger contributor to impacts on native species than competition (Crowl et al. 1992, Moyle and Light 1996, Young et al. 2010). Finally, we identified a landscape feature that can constrain the salmonid invasion success and hence provide natural ecological refugia for the native fish.

Our study focused on *G. platei* because this species was still reasonably common in the study area. Although we found negative effects of salmonids on this species, it is important to recognize that effects on other galaxiid species were probably more severe. For instance, *Aplochiton* spp. and *G. maculatus* were seldom encountered in our study area. These species have been regarded as (formerly) common and abundant in Patagonia and Falkland Islands (McDowall 2006), and can establish landlocked populations, although diadromy is common (Cussac et al. 2004). No obvious contemporary physical impediment should be restricting their distributions in our study area. It therefore seems likely that their distributions have contracted over the last four decades, presumably due to the impacts of invasive salmonids (Habit et al. 2010). Alternatively, these taxa might be common only in sites neighboring the ocean, which were underrepresented in our study (Cussac et al. 2004). Although direct evidence of range contraction is thus lacking, negative interactions with salmonids would seem likely: *G. maculatus* are small and presumably susceptible to predation and *Aplochiton* show considerable niche overlap with salmonids including similar habitat needs (Lattuca et al. 2008a, b, Young et al. 2008, 2010, Penaluna et al. 2009, Vigliano et al. 2009). In short, the galaxiid species (*G. platei*) for which we documented impacts of salmonids may, actually, be the most resilient in the face of salmonid invasion. Low-order lakes can reduce the exposure of *G. platei* to salmonids yet other galaxiids absent from or not adapted to low-order lakes might have less ability to avoid exposure and the detrimental effects of salmonid invasions.

Overall then, just how detrimental are the effects of salmonids on galaxiid fishes? Salmonids certainly seem to have driven the extirpation of many galaxiid populations in Australasia and the Falkland Islands, and the extinction of the New Zealand “grayling,” *Prototroctes oxyrhynchus* (McDowall 2006). Salmonids probably had similar effects on galaxiid extirpation in the five lakes we studied where salmonids were present

but galaxiids were absent. Although no pre-invasion baseline data exists for these lakes, locals have asserted the past presence of abundant native fish (C. Correa, *personal observation*). Furthermore, these sites have good connectivity with river networks and no outlying lake variable, rendering unlikely a scenario of historical native fish absence, particularly in light of the high tolerance of *G. platei* to various environmental conditions (Cussac et al. 2004). We suggest that our spatial analysis, therefore, provides a reasonable approximation of the historical changes that occurred during salmonid invasion—and these were severe.

Hydrology and refuges from salmonids

We found that salmonids were abundant and *G. platei* rare in high-order lakes, with the reverse being true in low-order lakes. This result suggests that the success of salmonids, and hence their negative effects on *G. platei*, is constrained by lake hydrological position, herein measured as lake order. This interpretation is supported by the model-selection approach, structural equation models (SEMs), and the analysis of salmonid stocking experiments in Canadian mountain lakes (Fig. 4). Lakes closer to headwaters (i.e., low-order lakes) might be less likely to support salmonids for several reasons: (1) they are less likely to have been deliberately stocked, (2) they are less accessible for self-induced range expansion, (3) they have little connection to stream habitats necessary for salmonid reproduction, and (4) they are more prone to winter hypoxia under ice and snow cover (Greenbank 1945, Devito and Dillon 1993). (The latter two mechanisms might not hold true in other regions, where lower elevation lakes can be subject to higher temperature, eutrophication, and oxygen depletion.) Mechanisms 1 and 2 might occasionally apply in Patagonia, but salmonids too frequently “get rides” by humans to the most remote locations (tales by locals; Basulto 2003, McDowall 2006, Vigliano et al. 2007). Certainly mechanisms 3 and 4 seem the most plausible for the Canadian lakes where salmonids were directly introduced (Donald 1987), and also seem likely in Patagonia. Further work will be required to confirm the specific mechanisms that are most important in Patagonian lakes. In contrast to the constrained distribution of salmonids to higher order lakes, *G. platei* is distributed widely regardless of hydrological position, presumably because it has had more geological time to spread (Zemlak et al. 2008); it can complete its life cycle entirely within lakes (Barriga et al. 2002); and it shows high endurance to hypoxia (Cussac et al. 2004). These different requirements for the invaders vs. the natives mean that low-order lakes might often provide at least some galaxiid species with a refuge from salmonids.

The association of hydrological position, salmonid invasion, and galaxiid declines also makes clear the value of preserving any remaining un-invaded high-order lakes. High-order lakes typically feature a high diversity of native fishes in Patagonia and yet unin-

vaded, high-order lakes have become very rare (Pascual et al. 2002, Cussac et al. 2004). In this study, only two uninvaded lakes had moderate to substantial connected stream habitat (order two and three; Fig. 4). In particular, the Río Cuervo watershed, containing Lago Yulton, might be by far the largest Patagonian system that remains uninvaded by salmonids. On a much smaller scale, Laguna Alta, in the Río Aysén watershed, also features streams that could sustain trout yet remains uninvaded. These rare pristine conditions still exist because remoteness hampers anthropogenic introductions and waterfalls limit self-colonization by salmonids. These systems, thus, represent a truly unique opportunity to study the ecology of *Galaxias* spp. (as we show) and *Aplocheilichthys zebra* (Soto et al. 2006), as well as natural ecosystem functioning of western Patagonian catchments. Sadly, these systems are at imminent risk of invasion by salmonids: a public road was recently built to Laguna Alta, and another road is underway to Lago Yulton. Such easy access will greatly increase the risk of trout introductions (McDowall 2006). Furthermore, a proposed project to build a hydroelectric impoundment in Río Cuervo would flood lakes Yulton and Meullin and degrade the littoral habitat that is vital to the native fishes' survival (Energía Austral 2009). The potential ecological impacts of this project are under consideration by the Chilean environmental protection authority, and impoundments have caused major galaxiid declines elsewhere (McDowall 2006).

The fatal naïveté

Accepting the mounting evidence that salmonids negatively impact galaxiids in western Patagonian lakes (Arismendi et al. 2009), we now consider more carefully why this is so. Our goal in this discussion is to help generate hypotheses that might be considered explicitly in further work. Although we focus on *G. platei*, similar arguments likely apply to other galaxiid species. First, *G. platei* clearly does not impose strong biological resistance (Moyle and Light 1996) to invasive salmonids, and instead might even facilitate salmonid invasion. Differences in reproductive strategy might benefit salmonids at the expense of *G. platei*. *G. platei* is one of the larger galaxiid species (reaching >30 cm in length) and could potentially prey on young salmonids. However, the main nursing habitat of juvenile salmonids is in swift streams, where *G. platei* is uncommon. In contrast, nonmigratory *G. platei* larva and small juveniles occupy limnetic and littoral habitats in lakes, where they are vulnerable to salmonid predation (C. Correa, unpublished data; also see Barriga et al. 2002). Galaxiids are soft bodied with no armor or spines, and do not appear to have coevolved with strong fish predators. In short, the evolutionary naïveté of galaxiids to salmonids is likely the root cause of the negative impacts, as has also been suggested for many other situations where invasive predators have decimated native prey (see also Sih et al. 2010).

Perhaps some galaxiids can escape from this trap, and *G. platei* could yield some clues as to how it could happen. One possibility is the existence of refuges within lakes. For instance, adaptations of *G. platei* to life in the hypolimnion, which were probably vital during past glacial cycles, could now serve for salmonid avoidance (Milano and Vigliano 1997, Cussac et al. 2004, Ruzzante et al. 2008). Alternatively littoral habitat might confer ecological refugia to native Patagonian fish under certain conditions (Aigo et al. 2008, Lattuca et al. 2008a). Although both hypotheses seem reasonable, we find no evidence for them in our survey; salmonids had a strong negative effect on *G. platei* regardless of lake depth (a surrogate for the amount of hypolimnion) and shoreline development index (a surrogate for the relative amount of littoral habitat). More detailed studies could address within-lake habitat ecology to test these hypotheses more rigorously. In addition to the use of within-lake habitat refugia, prey might implement a series of innovations that reduce susceptibility to, or the consequences of, predation, such as evasive behaviors, trophic niche changes, morphological changes, and life history trait changes (Reznick et al. 2001, Milano et al. 2002, Gardmark et al. 2003, Cussac et al. 2004, Stuart-Smith et al. 2008). And these changes might be implemented through adaptive evolution or phenotypic plasticity, both of which have been observed in other fish systems (Brönmark and Miner 1992, Langerhans et al. 2004, Kinnison et al. 2008). Ongoing work is examining some of these possibilities for *G. platei*.

Outlook

Our study helps to dissipate some of the previous uncertainty regarding the effects of invasive salmonids on native fishes of Patagonian lakes, and generates the novel hypothesis that invasion success, and therefore impact on native species, is mediated by a lake's hydrological position indexed as lake order. With regard to the first point, the strong negative spatial association between salmonid and galaxiid abundance suggests that galaxiids have declined drastically in most lakes as a result of four decades of widespread salmonid propagation. With regard to the second point, isolated lakes and lakes with few or no associated streams have now become refugia for some galaxiids. These results suggest that other refugia could be discovered or created by holding salmonid colonization and density in check through careful management.

Patagonian freshwater fish management has traditionally focused on the promotion of salmonid game fishes, whereas little effort has been devoted to the protection of native fish. It is therefore important to search for incentives to promote conservation without abandoning the economic and social mandates that protect salmonid populations (Pascual et al. 2009). One such incentive could be the promotion of galaxiid-salmonid coexistence in certain lakes. For example, catch quotas or fishing weirs aimed at reducing salmonid

abundance might allow partial galaxiid recovery, while simultaneously reducing salmonid intra-specific competition and increase their foraging base, which would help enhance the quality of the fishery (through faster growth and larger fish; Langeland and L'Abée-Lund 1996, Amundsen et al. 2007) and its attendant social benefits (Vigliano et al. 2007, Pascual et al. 2009, Arismendi et al. 2011). Furthermore, manipulations of this sort would allow the assessment of native fish resilience while facilitating adaptive responses to salmonid invasion. Finally, it is important to keep salmonids out of the few remaining high-order uninvaded lakes, and out of sites of populations with increasingly rare species, such as *Aplocheilichthys*. Without these sites, we lose an important and irreplaceable window into the natural state of affairs in Patagonian lakes.

ACKNOWLEDGMENTS

We dedicate this work to Robert ("Bob") M. McDowall (September 1939–February 2011), prolific and inspiring pioneer in the study of galaxioids worldwide. We are especially thankful to A. Bravo for her continuous support. We also thank our outstanding field assistants: A. Bravo, C. Cortéz, G. Orellana, and S. Vasquez. We are also grateful to D. Cayun, F. Durot, M. Hendry, J. Hudson, P. Ortiz, J. J. Ortiz, V. Peralta, B. Reid, and C. Serrano for their assistance in the field. Kyle Young and three anonymous reviewers provided constructive criticism to earlier drafts. We appreciate the support of the scientists B. Dyer, G. Gajardo, C. García de Leaniz, C. Meier, and staff members of the Centro de Investigación en Ecosistemas de la Patagonia, especially B. Reid. We thank the people scattered across the landscape in Aysén for their unconditional cooperation in the field. Thanks to M. Kinnison, and Freshwater Illustrated for lending their gear. This research was funded by the National Geographic Society (CRE grant), the Canadian Association of Universities (LACREG grant), and the McGill School of Environment. C. Correa held a scholarship from Comisión Nacional de Investigación Científica y Tecnológica, Gobierno de Chile (CONYCIIT).

LITERATURE CITED

- Aigo, J., V. Cussac, S. Peris, S. Ortubay, S. Gómez, H. López, M. Gross, J. Barriga, and M. Battini. 2008. Distribution of introduced and native fish in Patagonia (Argentina): patterns and changes in fish assemblages. *Reviews in Fish Biology and Fisheries* 18:387–408.
- Amundsen, P.-A., R. Knudsen, and A. Klemetsen. 2007. Intraspecific competition and density dependence of food consumption and growth in Arctic charr. *Journal of Animal Ecology* 76:149–158.
- Anderson, D. 2008. *Model based inference in the life sciences: a primer on evidence*. Springer Verlag, New York, New York, USA.
- Arbuckle, J. L. 1995. *Amos 18 user's guide*. Amos Development Corporation, Crawfordville, Florida, USA.
- Arismendi, I., and L. Nahuelhual. 2007. Non-native salmon and trout recreational fishing in lake Llanquihue, southern Chile: economic benefits and management implications. *Reviews in Fisheries Science* 15:311–325.
- Arismendi, I., B. Penaluna, and D. Soto. 2011. Body condition indices as a rapid assessment of the abundance of introduced salmonids in oligotrophic lakes of southern Chile. *Lake and Reservoir Management* 27:61–69.
- Arismendi, I., D. Soto, B. Penaluna, C. Jara, C. Leal, and J. León-Muñoz. 2009. Aquaculture, non-native salmonid invasions and associated declines of native fishes in Northern Patagonian lakes. *Freshwater Biology* 54:1135–1147.
- Bain, M. B., and N. J. Stevenson. 1999. *Aquatic habitat assessment: common methods*. American Fisheries Society, Bethesda, Maryland, USA.
- Barriga, J. P., M. A. Battini, P. J. Macchi, D. Milano, and V. E. Cussac. 2002. Spatial and temporal distribution of landlocked *Galaxias maculatus* and *Galaxias platei* (Pisces: Galaxiidae) in a lake in the South American Andes. *New Zealand Journal of Marine and Freshwater Research* 36:345–359.
- Basulto, S. 2003. El largo viaje de los salmones. una crónica olvidada. Propagación y cultivo de especies acuáticas en Chile. Maval, Santiago, Chile.
- Blumberg-Muñoz, C. 1996. Impactos de la introducción de especies exóticas en Aysén. Comité Nacional Pro Defensa de la Flora y la Fauna (CODEFF), Coyhaique, Chile.
- Brönmark, C., and J. G. Miner. 1992. Predator-induced phenotypical change in body morphology in crucian carp. *Science* 258:1348–1350.
- Cambray, J. A. 2003. The global impact of alien trout species—a review with reference to their impact in South Africa. *African Journal of Aquatic Science* 28:28:61–67.
- Casal, C. M. V. 2006. Global documentation of fish introductions: the growing crisis and recommendations for action. *Biological Invasions* 18:3–11.
- Claverro, M., and E. Garcia-Berthou. 2005. Invasive species are a leading cause of animal extinctions. *Trends in Ecology and Evolution* 20:110.
- Correa, C., and M. Gross. 2008. Chinook salmon invade southern South America. *Biological Invasions* 10:615–639.
- Crowl, T. A., C. R. Townsend, and A. R. McIntosh. 1992. The impact of introduced brown and rainbow trout on native fish: the case of Australasia. *Reviews in Fish Biology and Fisheries* 2:217–241.
- Cussac, V., S. Ortubay, G. Iglesias, D. Milano, M. E. Lattuca, J. P. Barriga, M. Battini, and M. Gross. 2004. The distribution of South American galaxiid fishes: the role of biological traits and post-glacial history. *Journal of Biogeography* 31:103–121.
- Devito, K. J., and P. J. Dillon. 1993. Importance of runoff and winter anoxia to the P and N dynamics of a beaver pond. *Canadian Journal of Fisheries and Aquatic Sciences* 50:2222–2234.
- Diaz, M., F. Pedrozo, C. Reynolds, and P. Temporetti. 2007. Chemical composition and the nitrogen-regulated trophic state of Patagonian lakes. *Limnologia—Ecology and Management of Inland Waters* 37:17–27.
- Didham, R. K., J. M. Tylianakis, M. A. Hutchison, R. M. Ewers, and N. J. Gemmill. 2005. Are invasive species the drivers of ecological change? *Trends in Ecology and Evolution* 20:470–474.
- Dodson, S. I., A. L. Newman, S. Will-Wolf, M. L. Alexander, M. P. Woodford, and S. Van Egeren. 2009. The relationship between zooplankton community structure and lake characteristics in temperate lakes (Northern Wisconsin, USA). *Journal of Plankton Research* 31:93–100.
- Donald, D. B. 1987. Assessment of the outcome of eight decades of trout stocking in the mountain national parks, Canada. *North American Journal of Fisheries Management* 7:545–553.
- Downing, J., and C. Plante. 1993. Production of fish populations in lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 50:110–120.
- Energía Austral. 2009. Estudio de impacto ambiental proyecto Central Hidroeléctrica Cuervo. Comisión Nacional del Medio Ambiente, Región de Aysén, Coyhaique, Chile.
- Gardmark, A., U. Dieckmann, and P. Lundberg. 2003. Life-history evolution in harvested populations: the role of natural predation. *Evolutionary Ecology Research* 5:239–257.
- Grace, J. B. 2003. Examining the relationship between environmental variables and ordination axes using latent

- variables and structural equation modeling. Pages 171–193 in B. H. Pugesek, A. Tomer, and A. von Eye, editors. Structural equation modeling: applications in ecological and evolutionary biology. Cambridge University Press, Cambridge, UK.
- Greenbank, J. 1945. Limnological conditions in ice-covered lakes, especially as related to winter-kill of fish. *Ecological Monographs* 15:343–392.
- Habit, E., P. Piedra, D. Ruzzante, S. Walde, M. Belk, V. Cussac, J. Gonzalez, and N. Colin. 2010. Changes in the distribution of native fishes in response to introduced species and other anthropogenic effects. *Global Ecology and Biogeography* 19:697–710.
- Hermoso, V., M. Clavero, F. Blanco-Garrido, and J. Prenda. 2011. Invasive species and habitat degradation in Iberian streams: an analysis of their role in freshwater fish diversity loss. *Ecological Applications* 21:175–188.
- Hubert, W., and C. Chamberlain. 1996. Environmental gradients affect rainbow trout populations among lakes and reservoirs in Wyoming. *Transactions of the American Fisheries Society* 125:925–932.
- Johnson, J. B., and K. S. Omland. 2004. Model selection in ecology and evolution. *Trends in Ecology and Evolution* 19:101–108.
- Kinnison, M., M. Unwin, and T. Quinn. 2008. Eco-evolutionary vs. habitat contributions to invasion in salmon: experimental evaluation in the wild. *Molecular Ecology* 17:405–414.
- Kratz, T. K., K. E. Webster, C. J. Bowser, J. J. Maguson, and B. J. Benson. 1997. The influence of landscape position on lakes in northern Wisconsin. *Freshwater Biology* 37:209–217.
- Langeland, A., and J. H. L'Abée-Lund. 1996. Habitat use, size and age structure in sympatric brown trout (*Salmo trutta*) and Arctic charr (*Salvelinus alpinus*) stocks: resistance of populations to change following harvest. *Ecology of Freshwater Fish* 5:49–58.
- Langerhans, R. B., C. A. Layman, A. M. Shokrollahi, and T. J. DeWitt. 2004. Predator-driven phenotypic diversification in *Gambusia affinis*. *Evolution* 58:2305–2318.
- Lattuca, M., M. Battini, and P. Macchi. 2008a. Trophic interactions among native and introduced fishes in a northern Patagonian oligotrophic lake. *Journal of Fish Biology* 72:1306–1320.
- Lattuca, M., D. Brown, L. Castiñeira, M. Renzi, C. Luizon, J. Urbanski, and V. Cussac. 2008b. Reproduction of landlocked *Aplocheilichthys zebra* Jenyns (Pisces, Galaxiidae). *Ecology of Freshwater Fishes* 17:394–405.
- Light, T., and M. Marchetti. 2007. Distinguishing between invasions and habitat changes as drivers of diversity loss among California's freshwater fishes. *Conservation Biology* 21:434–446.
- Lintermans, M. 2000. Recolonization by the mountain galaxias *Galaxias olidus* of a montane stream after the eradication of rainbow trout *Oncorhynchus mykiss*. *Marine and Freshwater Research* 51:799–804.
- McDowall, R. M. 2006. Crying wolf, crying foul, or crying shame: alien salmonids and a biodiversity crisis in the southern cool-temperate galaxioid fishes? *Reviews in Fish Biology and Fisheries* 16:233–422.
- McIntosh, A. R. 2000. Habitat- and size-related variations in exotic trout impacts on native galaxiid fishes in New Zealand streams. *Canadian Journal of Fisheries and Aquatic Sciences* 57:2140–2151.
- McIntosh, A. R., C. R. Townsend, and T. A. Crowl. 1992. Competition for space between introduced brown trout (*Salmo trutta* L.) and native galaxiid (*Galaxias vulgaris* Stokell) in a New Zealand stream. *Journal of Fish Biology* 41:63–81.
- Milano, D., V. E. Cussac, P. J. Macchi, D. E. Ruzzante, M. F. Alonso, P. H. Vigliano, and M. A. Denegri. 2002. Predator associated morphology in *Galaxias platei* in Patagonian lakes. *Journal of Fish Biology* 61:138–156.
- Milano, D., D. E. Ruzzante, V. E. Cussac, P. J. Macchi, R. A. Ferriz, J. P. Barriga, J. C. Aigo, M. E. Lattuca, and S. J. Walde. 2006. Latitudinal and ecological correlates of morphological variation in *Galaxias platei* (Pisces, Galaxiidae) in Patagonia. *Biological Journal of the Linnean Society* 87:69–82.
- Milano, D., and P. Vigliano. 1997. Nuevos registros de *Galaxias platei* Steindachner, 1898 en lagos andino-patagónicos (Teleostei: Osmeriformes: Galaxiidae). *Neotropica* 43:109–111.
- Moyle, P. B., and T. Light. 1996. Biological invasions of freshwater: empirical rules and assembly theory. *Biological Conservation* 78:149–161.
- Mundry, R., and C. Nunn. 2009. Stepwise model fitting and statistical inference: turning noise into signal pollution. *American Naturalist* 173:119–123.
- Pascual, M. A., J. L. Lancelotti, B. Ernst, J. E. Ciancio, E. Aedo, and M. García-Asorey. 2009. Scale, connectivity, and incentives in the introduction and management of non-native species: the case of exotic salmonids in Patagonia. *Frontiers in Ecology and the Environment* 7:533–540.
- Pascual, M., P. Macchi, J. Urbansky, F. Marcos, C. R. Rossi, M. Novara, and P. Dell'Arciprete. 2002. Evaluating potential effects of exotic freshwater fish from incomplete species presence-absence data. *Biological Invasions* 4:101–113.
- Penaluna, B., I. Arismendi, and D. Soto. 2009. Evidence of interactive segregation between introduced trout and native fishes in northern Patagonian rivers, Chile. *Transactions of the American Fisheries Society* 138:839–845.
- Pugesek, B. H., A. Tomer, and A. von Eye, editors. 2003. Structural equation modeling: applications in ecological and evolutionary biology. Cambridge University Press, Cambridge, UK.
- R Development Core Team 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org
- Reznick, D., M. J. Butler IV, and H. Rodd. 2001. Life history evolution in guppies. VII. The comparative ecology of high and low predation environments. *American Naturalist* 157:126–140.
- Rowe, D., B. Chisnall, T. Dean, and J. Richardson. 1999. Effects of land use on native fish communities in east coast streams of the North Island of New Zealand. *New Zealand Journal of Marine and Freshwater Research* 33:141–151.
- Ruzzante, D. E., S. J. Walde, J. C. Gosse, V. E. Cussac, E. Habit, T. S. Zemplak, and E. D. M. Adams. 2008. Climate control on ancestral population dynamics: insight from Patagonian fish phylogeography. *Molecular Ecology* 17:2234–2244.
- Sakai, M. 1989. Final report of aquaculture project in Chile. Japan International Cooperation Agency (JICA), Junin de los Andes, Argentina.
- Sih, A., D. I. Bolnick, B. Luttbeg, J. L. Orrock, S. D. Peacor, L. M. Pintor, E. Preisser, J. S. Rehg, and J. R. Vonesh. 2010. Predator-prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos* 119:610–621.
- Soto, D. 2002. Oligotrophic patterns in southern Chilean lakes: the relevance of nutrients and mixing depth. *Revista Chilena de Historia Natural* 75:377–393.
- Soto, D., I. Arismendi, J. González, J. Sanzana, F. Jara, C. Jara, E. Guzman, and A. Lara. 2006. Southern Chile, trout and salmon country: invasion patterns and threats for native species. *Revista Chilena de Historia Natural* 79:97–117.
- Soto, D., and P. De Los Rios. 2006. Influence of trophic status and conductivity on zooplankton composition in lakes and ponds of Torres del Paine National Park (Chile). *Biologia* 61:541–546.

- Soto, D., F. Jara, and C. Moreno. 2001. Escaped salmon in the inner seas, southern Chile: facing ecological and social conflicts. *Ecological Applications* 11:1750–1762.
- Stuart-Smith, R., R. White, and L. Barmuta. 2008. A shift in the habitat use pattern of a lentic galaxiid fish: an acute behavioural response to an introduced predator. *Environmental Biology of Fishes* 82:93–100.
- Toms, J., and M. Lesperance. 2003. Piecewise regression: a tool for identifying ecological thresholds. *Ecology* 84:2034–2041.
- Vigliano, P., M. Alonso, and M. Aquaculture. 2007. Salmonid introductions in Patagonia: a mixed blessing. Pages 315–331 in T. M. Bert, editor. *Ecological and genetic implications of aquaculture activities*. Springer, Dordrecht, The Netherlands.
- Vigliano, P. H., D. A. Beauchamp, D. Milano, P. J. Macchi, M. F. Alonso, M. I. G. Asorey, M. A. Denegri, J. E. Ciancio, G. Lippolt, M. Rechencq, and J. P. Barriga. 2009. Quantifying predation on galaxiids and other native organisms by introduced rainbow trout in an ultraoligotrophic lake in northern Patagonia, Argentina: a bioenergetics modeling approach. *Transactions of the American Fisheries Society* 138:1405–1419.
- Woelfl, S., L. Villalobos, and O. Parra. 2003. Trophic parameters and method validation in Lake Riñihue (North Patagonia: Chile) from 1978 through 1997. *Revista Chilena de Historia Natural* 76:459–474.
- Woodford, D. J., and A. R. McIntosh. 2010. Evidence of source–sink metapopulations in a vulnerable native galaxiid fish driven by introduced trout. *Ecological Applications* 20:967–977.
- Yarrow, M., and M. Torres. 2009. The ecological and cultural landscape of the Aysén river basin. Pages 341–356 in R. Neves, J. Baretta, and M. Mateus, editors. *Perspectives on integrated coastal zone management in South America*. IST Press, Lisbon, Portugal.
- Young, K. A., J. B. Dunham, J. F. Stephenson, A. Terreau, A. F. Thailly, G. Gajardo, and C. Garcia de Leaniz. 2010. A trial of two trouts: comparing the impacts of rainbow and brown trout on a native galaxiid. *Animal Conservation* 13:399–410.
- Young, K., J. Stephenson, A. Terreau, A.-F. Thailly, G. Gajardo, and C. Garcia de Leaniz. 2008. The diversity of juvenile salmonids does not affect their competitive impact on a native galaxiid. *Biological Invasions* 11:1955–1961.
- Zemlak, T. S., E. M. Habit, S. J. Walde, M. A. Battini, E. D. M. Adams, and D. E. Ruzzante. 2008. Across the southern Andes on fin: glacial refugia, drainage reversals and a secondary contact zone revealed by the phylogeographical signal of *Galaxias platei* in Patagonia. *Molecular Ecology* 17:5049–5061.

SUPPLEMENTAL MATERIAL

Appendix A

Environmental variables of the lakes studied and summary of fishing effort (*Ecological Archives* A022-045-A1).

Appendix B

Deforestation for pasture in Aysén, Chilean Patagonia (*Ecological Archives* A022-045-A2).

Supplement

The data and R source describing statistical analyses (particularly the information-theoretic approach) (*Ecological Archives* A022-045-S1).