Ecological impacts of invasive trout in Patagonian lakes

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TABLE OF CONTENTS

TABLE OF CONTENTS	ii
DEDICATION	vi
ABSTRACT	. vii
RÉSUMÉ	ix
ACKNOWLEDGMENTS	xi
CONTRIBUTION OF AUTHORS	xiii
LIST OF TABLES	xiv
LIST OF FIGURES	XV
1. GENERAL INTRODUCTION	1
References	4
Exposing the harm	12
2. Invasive salmonids and lake order interact in the decline of puye grande <i>Galaxias platei</i> in western Patagonia lakes	15
2.1 Abstract	15
2.2 Introduction	16
2.3 Methods	18
Study area	18
Fish community sampling	19
Variables and model-based hypotheses	21
Model selection	23
Structural equation modeling	24
Probability of salmonid naturalization	25
2.4 Results	26
Fish assemblages	26
Model selection	26
Structural equation modeling	27

Probability of salmonid naturalization	28
2.5 Discussion	29
Hydrology and refuges from salmonids	31
The fatal naïveté	32
Outlook	34
2.6 References	35
2.7 Tables	43
2.8 Figures	49
2.9 Appendix A	55
2.10 Appendix B	59
Food web consequences	65
3. Reciprocal trophic niche shifts in native and invasive fish: salmonids and galaxiids in Patagonian lakes	66
3.1 Abstract	66
3.2 Introduction	67
3.3 Methods	69
Study area	69
Fish collections and density indicators	70
Elemental analysis and correction for preservation bias	71
Data analyses	72
3.4 Results	74
3.5 Discussion	76
Trophic height	76
Proportion of littoral carbon	78
Management implications	79
3.6 References	80
3.7 Tables	88
3.8 Figures	90

Understanding ecosystem-wide consequences	97
4. Disruption of top-down control of zooplankton by invasive salmonids	98
4.1 Abstract	98
4.2 Introduction	99
4.3 Methods	102
Study area & fish variables	102
Neolimnology – plankton	103
Analysis of community composition	104
Analysis of population size structure	105
Analysis of diel vertical migration	106
Paleolimnology – history, chronostratigraphy & plankton	107
Coring and chronostratigraphy	108
Recovery of subfossil remains	109
Proportion <i>Ceriodaphnia</i>	110
Ephippia morphology	110
Data analyses	111
4.4 Results	112
Community composition	112
Population size structure	114
Diel vertical migration	114
Historical change reconstruction	115
4.5 Discussion	117
Shifts in community structure	117
Shifts in morphology	120
Diel vertical migration	121

Conclusion	
4.6 References	
4.7 Tables	
4.8 Figures	
4.9 Appendix A	
5. GENERAL CONCLUSIONS	
References	
Appenxix 1: Diversity of the endangered <i>Aplochiton</i> (G insights from mtDNA	alaxiidea) in Patagonia: 155
A1.1 Abstract	
A1.2 References	
A1.3 Figures	
A1.4 Appendix	
Appenxix 2: Tissue preservation biases in stable isotope from Patagonian lakes	es of fishes and molluscs 172
A2.1 Abstract	
A2.2 References	
A2.3 Tables	
A2.4 Figures	
A2.5 Supporting Information	

DEDICATION

I dedicate this work to Robert ("Bob") M. McDowall (September 1939-February 2011), prolific and inspiring pioneer in the study of galaxioids worldwide.

ABSTRACT

Earth is undergoing an acute anthropogenic biodiversity crisis, and invasive species are a primary cause. Understanding the ecological impacts of two of the most disruptive cosmopolitan freshwater fish invaders, brown trout (Salmo trutta) and rainbow trout (Oncorhynchus mykiss), is a priority to develop conservation guidelines and learn about the functioning of natural ecosystems. The impacts of these salmonids native to the northern hemisphere are especially worrisome in cold-temperate freshwaters of the Southern Hemisphere, where they are now popular as sport fish. This region is the realm of the Galaxiidae, a family of freshwater and diadromous fishes considered amongst the most seriously threatened fishes known. The decline of galaxiids has been linked to trout invasions but data is often qualitative and ambiguous. Furthermore, little is known about the extent to which native ecosystems have been transformed, especially in lakes. My goal is to remedy some of these deficiencies by investigating Patagonian lakes. In order to isolate the impacts of trout on a single galaxiid species I chose an especially fish-depauperated study system -25 lakes with only galaxiids and/or trout. Results of fishing catch-per-unit-effort data showed a strong negative association between the density of trout and the density of the most widespread Patagonia-endemic galaxiid, Galaxias platei. Furthermore, I ruled out alternative natural and anthropogenic confounding effects, and identified a critical natural constraint to salmonid invasion success which is widely applicable. Given the strong population-level impacts on the native fish, I examined the possibility of food web disruptions by focusing on isotopic ($\delta^{15}C$ and $\delta^{13}N$) ontogenetic niche shifts. I looked at the reciprocal effects of the native and the invasive fish density on each other, a perspective rarely studied. I found strong effects whereby the galaxiid lost, at high trout density, its ability to enhance its trophic position during ontogeny, while trout achieved a high trophic position only at high G. platei density. Finally, by studying zooplankton along the galaxiid-trout density countergradient, I could demonstrate both a negative, strong

top-down control on zooplankton elicited solely by *G. platei*, and a positive, strong top-down control elicited by the cascading effects of trout. My dissertation delivers overdue evidence of the detrimental effects of invasive trout on Patagonian galaxiids, contributes to the understanding of salmonid invasion ecology, and provides several mechanistic hypotheses and new perspectives on the pervasive nature of the ecological impacts of invasive fish predators. Based on my results, I advocate the management strategy of culling trout from overpopulated lakes to simultaneously protect native fish and enhance a lucrative sport fishery for large trout, and encourage the active protection of rare relicts of unspoiled native biodiversity.

RÉSUMÉ

biodiversité est confrontée à une crise sans précédent de La transformations d'origine anthropique causée principalement par l'introduction d'espèces invasives. Au niveau des écosystèmes aquatiques d'eau douce, la compréhension des impacts écologiques engendrés par l'introduction de poissons prédateurs agressifs tels que les truites brunes (Salmo trutta) et les truites arc-enciel (Oncorhynchus mykiss) est une priorité pour le développement de lignes directrices en conservation. L'étude des changements causés par ces espèces fournit également l'opportunité de mieux comprendre les écosystèmes naturels. L'impact de ces salmonidaes natifs de l'hémisphère nord et maintenant populaires dans le domaine de la pêche sportive dans les écosystèmes tempérés de l'hémisphère sud est une préoccupation particulière. Cette région est également le domaine des galaxiidae, famille de poissons amphihalins natifs de cette région, qui sont sérieusement menacés. Le déclin des galaxiids est souvent lié à l'invasion des truites, mais les données à ce sujet sont souvent qualitatives et ambiguës. De plus, peu d'information est connue concernant le niveau de transformation des écosystèmes natifs, particulièrement dans les écosystèmes lacustres. Mon objectif est de remédier à ces lacunes en étudiant les lacs en Patagonie. Dans le but d'isoler l'impact de l'introduction des truites sur une espèce spécifique de galaxiids, j'ai choisi un système d'étude composé de 25 lacs, dont certains sont peuplés de galaxiids seulement, et d'autres de galaxiids et de truites. Les données capture-par-unité-d'effort ont démontré une forte relation négative entre la densité des truites et l'espèce la plus répandue de galaxiids endémiques en Patagonie, Galaxias platei. De plus, j'ai séparé les effets naturels des effets anthropogéniques, et j'ai identifié une contrainte naturelle critique très répandue pour le succès de l'invasion des espèces salmonidés. Étant donné le fort impact observé au niveau des populations sur les poissons natifs, j'ai également étudié les perturbations sur la composition de la chaîne alimentaire en examinant les changements isotopiques (δ^{15} C and δ^{13} N) de la niche ontogénétique. J'ai étudié les effets réciproques entre la densité des poissons natifs et la densité des poissons invasifs, une perspective rarement étudiée. J'ai observé qu'à de hautes densités de truites, les galaxiids on perdu leur habilité d'augmenter leur position trophique durant leur ontogénie, alors que les truites obtiennent une haute position trophique seulement en présence d'une haute densité de G. platei. Finalement, en étudiant la

communauté de zooplankton dans un gradient de densité de populations de truites et de galaxiids, j'ai démontré un fort effet négatif de contrôle par le haut sur le zooplankton causé seulement par *G. platei*, et un fort effet positif de contrôle par le haut causé par un effet de cascade induit par la présence de truites. Ma dissertation fournit une preuve longuement attendue des effets nuisibles des truites introduites en Patagonie sur les galaxiids, contribue à la compréhension de l'écologie de l'invasion des salmonidés, et propose plusieurs hypothèses méchanistiques ainsi que de nouvelles perspectives sur la nature envahissante des impacts écologiques de l'introduction des poissons prédateurs. En fonction de mes résultats, je supporte la stratégie de gestion de retirer les truites des lacs surpeuplés pour protéger à la fois les populations de poissons natifs, pour améliorer l'efficacité de la pêche sportive pour de grosses truites, et pour encourager une protection active des dernières reliques intactes de la biodiversité.

Translation by Maryse Boisjoly

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CONTRIBUTION OF AUTHORS

The ideas and experimental designs contained in this thesis are the result of my own long-term interests and intellectual work, and were influenced by the guidance of my supervisor, Andrew Hendry, my co-supervisor Martin Thiel, and the members of my supervisory committee Irene Gregory-Eaves and Anthony Ricciardi. I am the principal author of all the manuscripts included in this thesis. I conducted the data analyses and the initial writing that formed the basis for further improvement thanks to the contributions of my coauthors, referees, and so forth (but see details about Appendix 1 below). In all three core chapters (§ 2-4) Andrew Hendry coauthored. He contributed providing support and guidance throughout the project execution, and helped during manuscripts writing. Andrea Bravo coauthored § 3. She helped during field and lab work with sample collection and processing. Irene Gregory-Eaves coauthored § 4. She was involved in the experimental design and writing, and provided key guidance and advice especially regarding paleolimnology. In the case of the manuscript in Appendix 1, I am the second author though the first and second author contributed equally to that manuscript. That work is coauthored by Dominique Aló and Leyla Cárdenas. The hypotheses and experimental design were conceived collectively; Aló collected samples, conducted the genetic analyses in Cárdenas's lab, and wrote the first draft. I collected most of the samples, conducted the morphological analysis, made the figures, and helped improving the manuscript. Cardenas helped at various stages. Finally, I am the sole author in the manuscript in Appendix 2.

LIST OF TABLES

Table 1: Model-based hypothesis set. 43 Table 2: Number of lakes in which fish species occurred alone (diagonal, in brackets); alone or in combination with other species (diagonal, no brackets); and together with another species (off diagonal). 46 Table 3: Model set evaluation. 47 Table A1: Environmental variables of the lakes studied and summary of fishing effort. 55 Table B1: Reclassification of land-cover data. 62 Table B2: Recent land-cover within the basins of the 25 lakes studied. 63 Chapter 3 7 Table 1: Lake descriptors and sample sizes of the molluses and fish analysed	Chapter 2
Table 2: Number of lakes in which fish species occurred alone (diagonal, in brackets); alone or in combination with other species (diagonal, no brackets); and together with another species (off diagonal)	Table 1: Model-based hypothesis set. 43
Table 3: Model set evaluation	Table 2: Number of lakes in which fish species occurred alone (diagonal, in brackets); alone or in combination with other species (diagonal, no brackets); and together with another species (off diagonal)
Table A1: Environmental variables of the lakes studied and summary of fishing effort	Table 3: Model set evaluation. 47
Table B1: Reclassification of land-cover data.62Table B2: Recent land-cover within the basins of the 25 lakes studied.63 Chapter 3 Table 1: Lake descriptors and sample sizes of the molluscs and fish analysed 88 Chapter 4 Table 1: Quartiles of lake variables.134 Appendix 2	Table A1: Environmental variables of the lakes studied and summary of fishing effort
Table B2: Recent land-cover within the basins of the 25 lakes studied	Table B1: Reclassification of land-cover data. 62
Chapter 3 Table 1: Lake descriptors and sample sizes of the molluscs and fish analysed 88 Chapter 4 Table 1: Quartiles of lake variables	Table B2: Recent land-cover within the basins of the 25 lakes studied. 63
Table 1: Lake descriptors and sample sizes of the molluscs and fish analysed 88 Chapter 4 Table 1: Quartiles of lake variables. 134 Appendix 2	Chapter 3
Chapter 4 Table 1: Quartiles of lake variables	Table 1: Lake descriptors and sample sizes of the molluscs and fish analysed 88
Table 1: Quartiles of lake variables. 134 Appendix 2	Chapter 4
Appendix 2	Table 1: Quartiles of lake variables. 134
	Appendix 2

Table I: Lake environmental descriptors and sample sizes for the experiment. 184

LIST OF FIGURES

Chapter 2

Figure 4: Effect of lake order on (A) presence of salmonids in 25 Patagonian lakes (this study), and (B) naturalization success 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 are present or naturalized with greater probability in lakes with relatively high Strahler stream order at lake outlet. Curves indicate probability of presence/naturalization (left y-axis), and bars the frequency of success (top) and failure (bottom) (see statistics in Results: Probability of salmonid naturalization). Furthermore, salmonids in colonized lakes tended to occur in higher densities (measured as capture per unit effort, CPUE') in highorder 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 catch*h⁻¹*(100-m littoral net)⁻¹ following Donald [1987], however CPUE' comparisons between studies might be inappropriate due uncontrolled

Chapter 3

Figure 1: Stable isotope data for each lake (sorted alphabetically). The snail *Chilina* sp. (\Box) is taken to represent the isotopic baseline for the littoral food web and the mussel *Diplodon chilensis* (\circ) is taken to represent the same for the

Chapter 4

Figure 1: Non-metric, multidimensional scaling (NMDS) plot of zooplankton composition (stress = 0.14). Lakes are labelled with a three-letter-code that corresponds to the initial letters of lake's names, and black circles whose size is proportional to the mean catch-per-trap of G. platei [ln(mean-catch * trap⁻¹ +1)]. Lakes where G. platei was not detected by any of the three fishing gears are marked with an X. The ordination of zooplankton taxa is shown by three-letter codes (in larger font) which correspond to the cladocerans Bosmina (Neobosmina) spp. (Bos), Ceriodaphnia dubia (Cer), and Daphnia curvirostris (Dap); calanoid copepods of the genus Boekella (Boe); and cyclopoid copepods (Cyc). The vectors (arrows) are linear representations of variables fitted a posteriori where arrows in black indicate variables significant at $\alpha = 0.1$ (or gray if $\alpha > 0.1$). Vectors are also labelled with two-letter codes that correspond to fish-catch-perunit-effort: trout captured by gillnetting (TG) or electrofishing (TE), and G. platei captured by gillnetting (PG), electrofishing (PE) or trapping (PT). Finally, PC1 corresponds to the vector derived from the PC1-scores defining the environmental gradient. The NMDS ordination explained ~35% of the variance of PT (permutation test, P = 0.0210), ~28% of TE (P = 0.0544), ~23% of TG (P =

Figure 3: *Bosmina* dominance as a function of overnight mean catch per trap of G. *platei*. Bars represent number of lakes in which *Bosmina* was (top) or not (bottom) dominant. The black line corresponds to the fitted logistic regression

Figure 4: Body length of two pelagic cladoceran species as a function of *G. platei* catch-per-unit effort. Individual measurements are shown in gray (jittered in the *x*-axis); lake-coefficient estimates and (multi-level) regression lines, in black..... 136

Appendix 1

Figure 1: Distribution range of *Aplochiton* (insert), collection sites, and COI haplotypes. For each site, numbers correspond to sample sizes, and letters to haplotypes. Abbreviations: AT, *Aplochiton taeniatus*; AM, *Aplochiton marinus;*,

AZ, *Aplochiton zebra*; SAN, Santo Domingo River, Valdivia; RIÑ, Riñihue Lake; PAL, Palos Lake; CAR, Caro Lake; BAK, Baker River; QUE, Quetru Lake.... 167

Appendix 2

1. GENERAL INTRODUCTION

We must make no mistake: we are seeing one of the great historical convulsions in the world's fauna and flora Charles S. Elton (1958)

By means of transportation technology humans are breaching an otherwise patchy world, removing biogeographical barriers that for millennia have separated diverse evolutionary trajectories of life. As a result, virtually every biome on earth have recently - in the last few centuries - received species from foreign biogeographical regions at an accelerate rate, some of which became established in the new range, and even invasive (i.e. widespread and dominant; stage V in Colautti & MacIsaac, 2004; stage E in Blackburn et al., 2011). Invasive species lead to complex suites of novel ecological interactions in the receiving communities, which can have profound impacts on biodiversity, ecosystems, and human society. These realizations were neatly elaborated for the first time by Charles S. Elton (1958) in his seminal book¹ that became the foundation of Invasion Ecology, one of the most prominent sub-disciplines of ecology. Half a century later, biological invasions are regarded as one of the leading causes of an acute anthropogenic biodiversity crisis (Mack et al., 2000; Mooney et al., 2004; Clavero & García-Berthou, 2005; Ricciardi, 2007). Paradoxically, biological invasions are also regarded as great unplanned experiments that allow a much better understanding of the processes that control biodiversity at spatial and temporal scales far beyond the scope of manipulative experiments (Mooney & Cleland, 2001; Freeman & Byers, 2006; Sax et al., 2007; Yoshida et al., 2007; Whitlow, 2010). The focus of my dissertation is on the ecological impacts of predatory fish invasions on native communities, with the double-purpose of addressing biodiversity threats, and understanding natural ecosystem functioning and invasion ecology in Patagonian lakes.

The anthropogenic biodiversity crisis is particularly dire in freshwater ecosystems (Ricciardi & Rasmussen, 1999; Abell, 2002; Clavero & García-Berthou, 2005), especially where top-predators have been introduced (Casal, 2006; Eby et al., 2006). Freshwater ecosystems, like islands, tend to host more

¹ Elton, C. S. 1958. The ecology of invasions by animals and plants. Methuen, London.

endemic and predator-naïve lineages than adjacent terrestrial habitats and therefore face higher risks of irreplaceable biodiversity losses (Cox & Lima, 2006). Invasive top-predator fishes are common in freshwaters worldwide, and most such invasions likely originated from the widespread practice of stocking large, predatory game or aquaculture fish in lakes and rivers (Casal, 2006; Eby et al., 2006). These additions can have individual-, population-, community- and ecosystem-level consequences, mainly by boosting direct and indirect top-down control mechanisms (Elton, 1958; Brooks & Dodson, 1965; Carpenter et al., 1985; Townsend, 2003; Eby et al., 2006). Salmonid fishes, native to the northern hemisphere, are very common among freshwater invasive top-predators. Among them, rainbow and brown trout (*Oncorhynchus mykiss* and *Salmo trutta*, respectively) are infamous disruptive cosmopolitan invaders (Welcomme, 1988; Alcaraz et al., 2005; Casal, 2006; Fausch et al., 2009).

Salmonid invaders are of particular concern in cold-temperate freshwater ecosystems of the southern hemisphere which host a distinct icthyofauna characterized by a paucity of piscivorous fish predators like rainbow and brown trout (Townsend & Crowl, 1991; Modenutti et al., 1998; Soto, 2002). The most specious and widespread fishes in the relatively sparse freshwater fish fauna of this Austral region are the galaxioid fishes, a group derived from amongst the lower euteleostean fishes (Johnson & Patterson, 1996). Galaxioids comprise three extant families with Galaxiidae dominating (McDowall, 2006). Despite surviving for millennia the rifting of the mega continent Gondwanaland, rise of mountain ranges, and the spread of ice sheets, galaxioids are currently amongst the most threatened fishes known (Cussac et al., 2004; McDowall, 2006; Zemlak et al., 2008; Zemlak et al., 2010; Zemlak et al., 2011). Seventy percent of all 56 galaxoid species are listed as threatened, 23% are critically endangered, and one has gone extinct (McDowall, 2006). One of the greatest threats to galaxoid fishes appears to be invasive trout, which have been broadly introduced into virtually every corner of the realm of galaxioids (Jackson, 1981; Crowl et al., 1992; Townsend, 1996; McDowall, 2003; McDowall, 2006). In an exhaustive literature review, McDowall (2006) found evidence of (rainbow and brown) trout posing a serious threat to 32 galaxoid species (56% of all galaxoid species; and 63% of the threatened species). Closer examination of the evidence alluded, however, reveals a striking lack of scientific data to support these assertions, particularly in lakes. The main problems appear to be a lack of comparison data from before invasions, or from still-pristine sites, as well as the potential for confounding effects.

Likewise, recent critical evaluations of the alleged detrimental effects of other invasive species in other regions of the world generally expose a lack of rigorous evidence. It has been proposed that the correlation between invasive species and loss of native biodiversity might be caused by responses to a common driver, particularly habitat degradation (Gurevitch & Padilla, 2004; Didham et al., 2005; Bauer, in press). This general problem has motivated the careful assessment of the impacts of invasive species in light of alternative effects. The results have been mixed, suggesting that the impact of invasive species relative to other covariates is context dependent (Didham et al., 2005; Light & Marchetti, 2007; Hermoso et al., 2011; Bauer, in press).

The situation in Chilean and Argentinean Patagonia does not escape from the generalities introduced above. Most salmonid species were likely established during the first half of the twentieth century, yet there are more recent derivations from stockings and aquaculture escapes (Sakai, 1989; Soto et al., 2001; Basulto, 2003; Gajardo & Laikre, 2003; Pascual et al., 2007; Correa & Gross, 2008; Consuegra et al., 2011). Traditionally, salmonids have been the focus of research and protection due to their economic benefits (i.e., world-class sport-fishery and aquaculture), and only recently there is growing interest among scholars about their ecological impacts (Campos, 1970; Modenutti et al., 1998; Milano et al., 2002; Pascual et al., 2002; Milano et al., 2006; Reissig et al., 2006; Soto et al., 2006; Balseiro et al., 2007; Macchi et al., 2007; Pascual & Ciancio, 2007; Vigliano et al., 2007; Young et al., 2008; Pascual et al., 2009; Penaluna et al., 2009; Vigliano et al., 2009; Habit et al., 2010; Young et al., 2010; Habit et al., 2012). Five general conclusions emerge from this body of work: (i) the impacts are seemingly pervasive with regard to geography and ecological hierarchy; (ii) the impacts are better understood in streams than in rives and lakes; (iii) inferences seldom identify specific ecological interactions; (iv) population-level impacts are seldom demonstrated, and the focus is often on species richness; and (v) the ecology of rare uninvaded aquatic systems is poorly understood. Overall, inferential difficulties commonly emerge from the fact that salmonids are widespread, often have been dominant for a long time, and usually there is insufficient baseline information about native fish distribution, abundance and ecology.

The goal of my dissertation is to advance and disambiguate our understanding of the ecological impacts of invasive salmonids in Patagonian lakes. To succeed in this endeavour, I chose an especially depauperate study

system - 25 lakes with only galaxiids and/or trout - and adopted a multidisciplinary approach to identify impacts at different levels in the ecological hierarchy. In Chapter 2 (\S 2), I investigate the effects of trout densities as well as other natural and anthropogenic variables on population densities of a widespread Patagonia-endemic galaxiid, Galaxias platei. I also describe a general natural constraint to trout invasion success. Having exposed population-level consequences, it becomes apparent the potential for trout-imposed trophic niche demands on the native fish, and reciprocally, the potential of a feedback-loop affecting the trophic niche of the invasive fish. Hence, in § 3, I study, by means of stable isotope analyses, the ontogenetic trophic-position changes of native and invasive fish. The combined results of § 2 and § 3 suggest that trout could relax top-down control on zooplankton through cascading effects. Therefore, in § 4, I examine how the native-invasive fish-density coutergradient affects zooplankton behaviour, morphology, density and community composition. In sum, my dissertation delivers overdue quantitative evidence of the detrimental effects of invasive trout on Patagonian galaxiids in lakes, contributes to the understanding of salmonid invasion potential anywhere in the world, and provides mechanistic hypotheses and new perspectives on the transversal nature of the ecological impacts of predatory fish invasions in lakes.

Most of the content of this thesis is contained in five stand-alone manuscripts [three core chapters (§ 2-4) and two Apendices)] which are published or intended for publication in peer-review scientific journals. To emphasize the continuity of my research program, each core chapter is preceded by a short connecting statement. I close with a summary of the main conclusions.

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Exposing the harm

Does trout abundance compromise native fish abundance? This is the cornerstone question of my dissertation, and the first logical step in a program to elucidate the ecological impacts of predatory invasive fish. As stated in the *General Introduction*, there are a number of reasons why a negative association between trout and galaxiids is expected, yet the evidence is often evasive or anecdotal, especially for lakes. Answering this question is not only a prerequisite to address more interesting scientific questions but is also essential to inform management decisions, and to stimulate changes in the *status quo* of cultural attitudes that for over a century have unconditionally favoured the charismatic exotic salmonids (Basulto, 2003; Pascual et al., 2007). The objective of the following chapter (§ 2) is, therefore, to expose the harm invasive trout inflict on a relatively common native galaxiid, *Galaxias platei*, while gauging the effects of alternative natural and anthropogenic variables that might affect fish distribution and abundances.

Before concluding this prelude, I elaborate on the issue of why focusing on a relatively common galaxiid species instead of on endangered species such as *Aplochiton taeniatus* and *A. zebra* (Campos et al., 1998; Habit et al., 2006; Comisión Nacional del Medio Ambiente-Comité Clasificación de Especies Silvestres, 2009). I originally intended to also study the latter genus in greater depth. However, *Aplochiton* was less common than anticipated and *G. platei* was the only galaxiid reasonably common thus allowing overdue and essential quantitative assessments (Pascual et al., 2007; Young et al., 2010). An additional problem was that the taxonomy of the *Aplochiton* is still poorly understood and morphological classification is sometimes equivocal.

To address the latter problem, in collaboration with D. Aló we set out to revise the taxonomy of *Aplochiton* using morphological and molecular methods. Our results and those of others who simultaneously embarked in a similar endeavour show higher phylogenetic affinities among *A. taeniatus* and *A. zebra* than previously accepted, and set limits to the usefulness of morphological species

identification (Appendix 1; Vanhaecke et al., 2012). Furthermore, our results clearly reject the synonymy proposed for *A. taeniatus* and *A. marinus* (McDowall, 1971), by showing that the latter species is the most distinctive both from the morphological and genetic viewpoints (Appendix 1). Although recent studies are beginning to demonstrate the role of invasive salmonids in the dramatic *Aplochiton* decline in streams (Young et al., 2010), the ability to delineate species is central to achieving conservation objectives.

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

Correa, C., & A.P. Hendry (2012). Invasive salmonids and lake order interact in the decline of puye grande *Galaxias platei* in western Patagonia lakes. *Ecological Applications* 22(3): 828-842.

2.1 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 dataset 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.

2.2 Introduction

Threats to native biodiversity are acute in fresh waters, and invasive species are considered a primary cause (Clavero & García-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 & Marchetti, 2007; Hermoso et al., 2011). The effects of salmonid fishes (family Salmonidea) 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 amongst 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 through their evolutionary history galaxiids have weathered a series of major environmental changes through their evolutionary history (geological activity, glaciations, and climate change), they are nowadays 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 drivers of the galaxiid declines (e.g., Light & 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 & 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 & 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 & 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.

2.3 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 & 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 & Gross, 2008). In our study area, however, salmonid invasions begun 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 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 [ln(ha)], shoreline development index (Bain & Stevenson, 1999), maximum depth [ln(m)], specific conductivity (μ S), altitude (m), lake order (Strahler stream order at lake outlet; Donald, 1987), and proportion (arcsin p^{1/2}) 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) 1.9(1.0) vs. 0.9(1.1); *P* = 0.02], although considerable overlap was still present between invaded and uninvaded lakes.

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 can not 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 (8 vs. 4 lakes in 2007, and 9 vs. 4 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 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 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 forth we illustrate our findings using the integrated AIs.

To develop an AI for each of the above 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 $[x' = \ln(x + 1))$, where ln is natural logarithm]. 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 (min), 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 & 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 which 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 & 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 twenty 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 (AICc) was computed as AICc = AIC + 2K(K + 1) / (n - K - 1), where AIC = $-2 * \log$ -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 AICc, 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 electronic Supplement (Ecological Archives).

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) which 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 of *G. platei* density. We also defined a reduced model which 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 densityvariables 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 which are part of the software's output, including the incremental fit index (IFI) and the goodness-of-fit index (GFI). These indices vary between 0-1 and values close to one are indicative of good fit (Arbuckle, 1995). We also conducted goodness-of-fit chi-squared 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-squared likelihood ratio test and a significance level of 0.05 (LRT). The models were also ranked according to increasing values of AICc. 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 1,000 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 (Pugesek 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 dataset containing lake order and salmonid naturalization success (i.e., selfsustainable 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 datasets 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).

2.4 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 (*Aplochiton 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 twelve 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 GpAI [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 piece-wise 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 more negative and significantly different from zero [slope of -0.51 (SE 0.25); ANOVA F(1, 23) = 4.26, P = 0.050, R^2 adj. = 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 (Results: Structural equation modeling). The predictive value of log-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 variances-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-squared 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 AICc value (delta AICc > 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 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 stem 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 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)]. Figure 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; Figure 4). The fitted logistic models for the two datasets were not statistically distinguishable between regions of the world, and the model fitted to the pooled data was highly significant [Pr(trout naturalization) = logit⁻¹(-0.73 + 1.08 * 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 loworder lakes (order 0 – 1) compared to high-order lakes (order 2 – 4; inserts in Figure 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.

2.5 Discussion

We found a strong negative association between indices of abundance (AI) for *G. platei* versus 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 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 & 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; Lattuca et al., 2008b; Young et al., 2008; Penaluna et al., 2009; Vigliano et al., 2009; Young et al., 2010). 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 galaxioid 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 pers. obs.). 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 (Figure 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 & 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 versus 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 highorder lakes. High-order lakes typically feature a high diversity of native fishes in Patagonia and yet uninvaded, 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; Figure 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. (this study) and Aplochiton 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 hydro-electric impoundment in Río Cuervo would flood lakes Yulton and Meullin and degrade the littoral habitat that is vital to the fish survival (Energía Austral Ltda., 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; this study), 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 & Light, 1996) to invasive salmonids, and instead might even facilitate salmonid invasion. Differences in reproductive strategy might beneficiate salmonids to 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, non-migratory *G. platei* larva and small juveniles occupy limnetic and littoral habitats in lakes, where they are vulnerable to salmonid predation (Correa unpublished data; also see Barriga et al., 2002). Galaxiids are soft bodied with no armor or spines, and do not appear to have co-evolved 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 hypolimnon, which were probably vital during past glacial cycles, could now serve for salmonid avoidance (Milano & 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 & 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 & 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 *Aplochiton*. Without these sites, we lose an important and irreplaceable window into the natural state of affairs in Patagonian lakes.

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2.7 Tables

Table 1: Model-based hypothesis set.

Model	Word formula	Туре	Underlying biological hypotheses
g01	trout	SB	The AI of trout is negatively correlated with the AI of <i>G. platei (McDowall, 2006)</i> . 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 & Vigliano, 1997; Aigo et al., 2008; Dodson et al., 2009).
g03	depth	Ph-Ch	Deep lakes can provide ecological refugia to <i>G. platei</i> as this species possesses distinct adaptations to life in the hypolimnon (Milano & Vigliano, 1997; Milano et al., 2002; Cussac et al., 2004; Milano et al., 2006). 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 & 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 g14; Downing & Plante, 1993; Soto & De los Ríos, 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 & Stevenson, 1999) - could increase littoral productivity and refuge from piscivory
			(Aigo et al., 2008).
g08	trout + order	М	The abundance of G. platei is affected both by lake order and trout abundance.
g09	$trout + order + trout \times order$	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	М	The abundance of G. platei is affected both by lake maximum depth and trout abundance.
g11	trout + depth + trout \times 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 G. platei (see refs.
			from g03).
g12	trout + shoreline	M-I	The abundance of G. platei is affected both by lake shoreline development and trout abundance.
g13	trout + shorline + trout ×	M-I	The effect of trout depends on shoreline development; trout may be especially detrimental in lakes with
	shoreline		little shoreline development as a consequence of a shortage of littoral refugia (Aigo et al., 2008).
g14	conductivity + is.shallow +	M-I	Hypothesis g05 is conditional on lake depth. It has been shown that the typically deep mixing of the
	conductivity × is.shallow		epilimnon 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., \leq
			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 G. platei 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 & Lesperance, 2003).

g16	S. trutta	S-S	S. trutta is generally a more aggressive and piscivorous than is O. mykiss and might therefore have a			
			greater effect (Crowl et al., 1992; Moyle & Light, 1996; Young et al., 2010).			
g17	O. mykiss	S-S	Complement to g16.			
g18	deforestation	Co	Land-cover in the region of Aysén has been partially transformed through anthropogenic activity during			
			the 20 th century; the most dramatic changes took place during the 1930s and 1940s, when 50% of the			
			old-growth forest within the Aysén watershed was burnt for pasture and other uses (Appendix B;			
			Yarrow & 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 & Torres, 2009).			
g19	altitude + area + conductivity	Gl	Structure-rich model approaching a global model (Anderson, 2008). Includes the effects of trout (species			
	+ deforestation + depth +		pooled) and all other environmental variables (lake altitude, area, conductivity, depth, deforestation,			
	order + trout		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; Gl, 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.

Table 2: Number of lakes in which fish species occurred alone (diagonal, in brackets); alone or in combination with other species (diagonal, no brackets); and together with another species (off diagonal).

	G. platei	G. maculatus	A. zebra	O. mykiss	S. trutta	
G. platei	20 (7)	2	1	5	8	
G. maculatus	2	2 (0)	1	1	1	
A. zebra	1	1	1 (0)	0	1	
O. mykiss	5	1	0	7 (0)	3	
S. trutta	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*).

Table 3: Model set evaluation.

				Adjuste		Model	
Model	Word formula (parameter estimates)*	k	RSS	d R ²	AICc	likelihood**	W
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 (b0 = -1.04; b1 = -1.20; b2 = 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 + inter. (1.17; -0.56; -0.36; -0.02)	5	30.01	0.32	88.67	0.13	0.04
g09	trout + order + inter. (0.05; -0.94; -0.11; 0.19)	5	30.44	0.31	89.03	0.11	0.04
g13	trout + shoreline + trout × 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	O. mykiss (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;	5	44.13	0.00	98.31	0.00	0.0

	-0.01; 0.20; 0.02)						0
g19	altitude + area + conductivity + deforestation + depth + order +	10	23.13	0.31	104.72	0.00	0.00
	shoreline + trout (1.77; 0.00; 0.29; 0.00; -1.40; -0.82; -0.21; 0.37; -						
	0.43)						

Note: Models are sorted by increasing values of the Akaike information criterion adjusted for small sample sizes (AICc). 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).

* Least-square parameter estimates: intercept followed by slope(s). In g15 parameters are b0, intercept; b1, first slope; b2: second slope, brk, breakpoint (in the *x*-axis) connecting the two segments of the regression line.

** Relative to the best model.

2.8 Figures

Figure 1: Locations of the 25 lakes studied and corresponding abundance index (AI) estimates of native *G. platei* (white bars) and invasive salmonids (black bars). The labelled 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 *O. mykiss* in Lago Pollux, 1968, and *S. trutta* in Lago Elizalde, 1970.

Figure 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). The shaded area comprises 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.

Figure 3: Path diagram of model A, 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. Indicator variables correspond to the standardized lake-by-gear CPUE. Arrow widths are proportional to effect sizes, which are shown nearby. Significant effect sizes are depicted with solid arrows; 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 printed on the top-left side of each response variable; for simplicity, residual variance variables are not shown.

Figure 4: Effect of lake order on (A) presence of salmonids in 25 Patagonian lakes (this study), and (B) naturalization success 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 are present or naturalized with greater probability in lakes with relatively high Strahler stream order at lake outlet. Curves indicate probability of presence/naturalization (left y-axis), and bars the frequency of success (top) and failure (bottom) (see statistics in Results: Probability of salmonid naturalization). Furthermore, salmonids in colonized lakes tended to occur in higher densities (measured as capture per unit effort, CPUE') in highorder 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 catch*h⁻¹*(100-m littoral net)⁻¹ following Donald [1987], however CPUE' comparisons between studies might be inappropriate due uncontrolled methodological differences.)

Figure 1



Figure 2


Figure 3:



Figure 4



2.9 Appendix A

Site name	Easting	Northing	Altitude	Order	Area	Shoreline	Max.	Specific	Deforest	Year	#	#	#
			(m)		(ha)	index	Depth	Cond.	ation		Gillnets	Electric	Traps
							(m)	(µS *cm⁻¹)	(%)			fishing	
1 Alta	679152	4955342	80	2	89	1.38	55	27	9	2007	2	1	
										2009	2	3	19
2 Barroso	714559	4931448	334	2	50	1.87	43	33 55		2007	2	3	20
										2009		1	
3 Brava	749873	5001332	840	1	73	1.59	41	51	68	2009	2	3	20
4 Burgos	716734	4934348	375	2	24	2.04	30	59	93	2009	2	3	20
5 Castor	751243	4945575	696	2	449	2.67	52	79	49	2007	2	3	24
										2009	2	4	
6 Cea	715377	4938670	289	2	25	1.50	11	32	40	2007	2	2	22
										2009		1	
7 Chacano	716888	4883167	510	2	13	1.87	14	112	64	2009	2	4	20
8 Escondida	748081	4953254	695	1	79	2.04	17	99	29	2007	2	2	19
9 Espejo	717753	4931206	336	2	10	1.18	21	76	84	2009	2	4	20
10 Frío	738304	4938597	513	4	424	1.58	15	74	47	2007	2	2	33
										2009		2	
11 Gemela	748775	4951683	759	0	25	1.54	14	93	57	2009	2	6	20
12 Juncos	745691	4942090	748	0	4	1.15	3	57	44	2007	1	1	18
										2009		1	

Table A1: Environmental variables of the lakes studied and summary of fishing effort.

13 Leon	746576	4943994	756	0	27	1.85	8	62	57	2009	3	4	21	
14 Mercedes	716737	4931753	349	1	20	1.53	22	75	76	2009	2	4	20	
15 Monreal1	744219	4941773	753	0	36	1.54	14	49	50	2007	2	3	21	
16 Monreal2	731450	4916049	603	2	232	1.90	51	53	37	2009	2	4	40	
17 Palos	680287	4979082	0	3	513	1.62	60	24	10	2007	2	2	29	
18 Pollux	744585	4937380	679	3	879	2.52	55	72	42	2007	2	4	19	
										2009		4		
19 Portales	688378	4955007	53	3	577	2.13	150	24	30	2007	2	3	20	
										2009		5		
20 Quijada	740643	4932928	727	0	32	1.24	10	65	63	2009	2	3	16	
21 Rengifo	694268	4945678	110	1	198	1.69	20	25	40	2009	2	4	19	
22 Sepulveda	734074	4874583	421	1	68	1.54	21	111	61	2009	2	3	19	
23 Thompson	750051	4940097	748	1	120	2.12	15	72	44	2007	2	2	18	
24 Yulton	665236	5002049	488	3	6104	2.60	150	25	4	2009	2	2	20	
25 Zenteno	691476	4948603	95	2	678	2.47	50	25	41	2009	2	5	20	_
Total effort											54	98	537	

Notes: **Coordinates** in UTM, zone 18S, datum PSAD69. Lake **altitude** (m) was determined using ArcGIS 9.1 by calculating the median altitude in lake polygons superimposed onto a digital elevation model (USGS/NASA SRTM-90m, Jarvis et al., 2008). Lake **order**, defined as Strahler stream order at lake outlet (Quinlan et al., 2003), was determined by examining Google Earth Imagery with superimposed GPS data and a stream network model (see Appendix S2), taking into account field observations. Lakes with no visible tributary where assigned an order of zero. We measured lake **area** (ha) from lake polygons in CONAF-CONAMA (1999) using software ArcGIS 9.1. **Shoreline development index** was calculated as: perimeter (km) * (2 * (area (km²) * π)^{1/2})⁻¹ (Bain & Stevenson, 1999) – a circular lake has an index of one; more convoluted shorelines have values > 1. Maximum **depth** (m) was determined by

surveying with sonar (Hummingbird PirañaMax20 or Fishfinder 565), except for lakes Pollux, Yulton, Frio, and Palos, where maximum depth was extracted from (Sakai, 1989; PRAMAR ambiental consultores, 2007). Specific conductivity (µS) was measured at <1 m deep in a single location, using a handheld probe (YSI Model 85). Percent of lakes's basins deforested (see Appendix B for details). The gillnets (Sterling Nets Inc.) were sinking monofilament multipanel survey nets 38 m long and 1.8 m deep. In each lake, at least one net (square mesh size of 13, 19, 25, 32 and 38 mm) was set overnight in the littoral zone perpendicular to the shoreline. Additionally, in all lakes except two that had only littoral habitat (Juncos and León), at least one net (square mesh size of 25, 38, 51, 64, 76, and 89 mm) was set on the benthic zone at 30–40 m deep, or in the deepest part of shallower lakes. In lakes Alta and Castor, littoral-deep gillnet-pairs were deployed in both years and the data were pooled for nalysis. A number of one-pass, open transects were surveyed using a backpack electrofisher (Smith Root Inc. model 12-B) by shocking periodically, particularly in the close proximity of potential refugia. At the end of each transect of variable length (10-100 m), according to the habitat types encountered and physical impediments, the catch and total discharge time (from shocker's timer) was recorded. In each of six lakes (all with G. platei; 5 with salmonids) one specific transect was sampled both in 2007 and 2009; there were no statistical differences in the catch rate between years for G. platei (paired t-test, t = 0.8948, df = 5, P = 0.4119) and salmonids (t = -1.9077, df = 4, P = 0.1291). Traps (dimensions 10" x 10" x 17"; Miller Net Company Inc.) were made of brown mesh (square mesh size 1/8") mounted on a collapsible metal frame, and had 2" diameter funnel entrances at either end. In five lakes (Brava, Escondida, Monreal-1, Quijada and Thompson), about half of the traps were baited (with fish or cheese) and the other half were un-baited. In the twenty other lakes, all traps were either baited (8 lakes) or unbaited (12 lakes). Traps captured only galaxiids; baiting did not significantly alter the frequency of traps with G. platei catches in the lakes trapped both ways (Cochran-Mantel-Haenszel Test, $\gamma^2_{CMH} = 2.4066$, df = 1, P = 0.1208), and baiting was not correlated to overall lake density estimates of G. platei [ANOVA F(1, 18) = 0.42, P = 0.5230] or trout [ANOVA F(1, 18) = 0.42, P = 0.5230] or trout [ANOVA F(1, 18) = 0.42, P = 0.5230] or trout [ANOVA F(1, 18) = 0.42, P = 0.5230] or trout [ANOVA F(1, 18) = 0.42, P = 0.5230] or trout [ANOVA F(1, 18) = 0.42] 0.86, P = 0.3657] across the other lakes.

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2.10 Appendix B

Deforestation for pasture in Aysén, Chilean Patagonia

The land-cover in Aysén underwent an extensive transformation during the first half of the 20th century, due to the sudden arrival of settlers and livestock companies sponsored by the government. Broad extensions of burnt trunks scattered on pastures are still an eloquent proof of the dramatic changes to the landscape (Figure B1). In order to study the long-tem effects of deforestation on galaxiid fishes, we calculated the % area deforested within the basins of the lakes sampled, and used it as an explanatory variable of galaxiid density.



Figure B1: Pasture in deforested area by Lago Castor. Note the old, burnt trunks still scattered, and patches of secondary forest in the background. Photo by C. Correa, January 2009.

We based our land-cover classification on a digital map from CONAF-CONAMA (1999). We used Gajardo's (1994) 'vegetation associations' as a reasonable hypothesis of the composition and distribution of forests in Aysén before colonization (Yarrow & Torres, 2009). [The classification of the Aysén region sensu Gajardo was provided by CONAF, along with the attribute table of their main dataset (CONAF-CONAMA 1999).] Within the basins of the 25 lakes sampled in this study, eight of Gajardo's vegetation associations were represented; these were re-classified to distinguish originally forested areas from areas naturally devoid of forests (a few additional naturally-devoid-of-forest areas were identified using CONAF-CONAMA 1999). Potentially forested areas comprised evergreen and deciduous forests, while areas naturally devoid of forest high Andean peaks, rivers, lakes, Patagonian steppe, high-mountain shrubs, and outcrops.

We then used data from the a relatively recent assessment of land-cover in the region (CONAF-CONAMA 1999) to quantify deforestation. First, we reclassified the land use data into six categories: old-growth native forest, secondary native forest (regrowth), pasture, shrub, exotic forest, and naturally devoid of forest (Table B1). Then, we calculated the % of lakes' basins² covered by each of these categories³, excluding from the analysis the areas naturally devoid of forest (see previous paragraph). We treated pastures, shrubs (but Gajardo's high-mountain shrubs), and exotic forest (CONAF-CONAMA 1999) as deforested areas, while regrowth forest as native forest, assuming that substantial forest ecosystem functions have, for decades, been restored.

² We obtained lake basins and stream networks from the hydrology model Soil and Water Assessment Tool (SWAT), implemented in the ArcGIS extension ArcSWAT 1.0.7 (Arnold et al., 1998; Gassman et al., 2007). Input data were the digital elevation model (DEM; Jarvis et al., 2008) and a parameter that defined the minimum drainage area necessary to originate a stream, which we set to 200 ha. We enhanced the fit by editing small cell-clusters of the DEM, at four locations. Lake polygons (CONAF-CONAMA 1999) were then superimposed onto the new stream network to guide the manual placement of lakes' outlets points, which were subsequently selected in ArcSWAT to obtain lake basins's polygons.

³ Using the reclassification in Table B1, we created a thematic raster, with cell size equal to that of the DEM. Then, we based the calculation of land-cover proportion within basins on this raster and the basins' polygons, using Hawth's tools (Beyer, 2004).

The results indicate little to complete deforestation within the basins of the lakes studied, with a median deforestation of 49 % (Table B2; Figure B2). The proportions of deforested area per basin were transformed before being used as explanatory variable (see main article).



Figure B2: Deforestation in the study area. Besides lakes (blue), all coloured areas were formerly forested; grey indicates areas naturally devoid of forest such as high peaks and Patagonian steppe, or no data (in Argentina). Black contours show boundaries of the lake basins studied.

CONAF-CONAMA 1999	Correa and Hendry, this article
Field: DESC_USO	Field: RECENT_COV
ROTACION CULTIVO-PRADERA	Pasture
ESTEPA PATAGONICA	Naturally devoid of forest
MATORRAL ABIERTO	Shrub
MATORRAL ARBORESCENTE ABIERTO	Shrub
MATORRAL ARBORESCENTE SEMIDENSO	Shrub
MATORRAL PRADERA ABIERTO	Shrub
MATORRAL PRADERA SEMIDENSO	Shrub
MATORRAL SEMIDENSO	Shrub
PRADERAS PERENNES	Pasture
BOSQUE NAT.ACHAPARRADO ABIERTO	Old-growth native forest
BOSQUE NAT.ACHAPARRADO DENSO	Old-growth native forest
BOSQUE NAT.ACHAPARRADO SEMIDENSO	Old-growth native forest
BOSQUE NAT.ADULTO-RENOVAL ABIERTO	Old-growth native forest
BOSQUE NAT.ADULTO-RENOVAL SEMIDENSO	Old-growth native forest
BOSQUE NATIVO ABIERTO	Old-growth native forest
BOSQUE NATIVO ADULTO DENSO	Old-growth native forest
BOSQUE NATIVO ADULTO SEMIDENSO	Old-growth native forest
BOSQUE NATIVO ADULTO-RENOVAL DENSO	Old-growth native forest
PLANTACION	Exotic forest
RENOVAL ABIERTO	Regrowth native forest
RENOVAL DENSO	Regrowth native forest
RENOVAL SEMIDENSO	Regrowth native forest
ÑADIS HERBACEOS Y ARBUSTIVOS	Naturally devoid of forest
OTROS TERRENOS HUMEDOS	Naturally devoid of forest
VEGAS	Naturally devoid of forest

Table B1: Reclassification of land-cover data.

AFLORAMIENTOS ROCOSOS	Naturally devoid of forest
CORRIDAS DE LAVA Y ESCORIALES	Naturally devoid of forest
TERRENOS SOBRE LIMITE VEGETACION	Naturally devoid of forest
NIEVES	Naturally devoid of forest
LAGOS-LAGUNAS-EMBALSES-TRANQUES	Naturally devoid of forest
RIOS	Naturally devoid of forest

Table B2: Recent land-cover within the basins of the 25 lakes stud

Land-cover	Land-cover modified from	mean, median &						
	CONAF-CONAMA (1999)	range % cover						
Deforested	Pasture	21, 2 (0 – 63)						
	Shrub	27, 9 (0 – 93)						
	Exotic forest	0, 0 (0 – 5)						
	Subtotal deforested	48, 47 (4 – 93)						
Native forest	Old-growth	41, 45 (0 – 91)						
	Regrowth	12, 8 (0 – 39)						
	Subtotal native forest	52, 53 (7 - 96)						

Notes: Only areas forested before colonization were included in this analysis.

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Food web consequences

The previous chapter (§ 2) revealed a stark countergradient in galaxiid and trout abundance suggestive of strong antagonistic ecological interactions. In particular, it was hypothesized that trout face little or no biological resistance from galaxiids in the invading range. On the contrary, galaxiids – which are evolutionarily naïve to the trout's insults – might facilitate trout invasions by providing food. From the perspective of the galaxiids, trout cause novel challenges in the struggle for survival. One possibility is the need to adjust foraging behaviour to evade predation by trout. Another possibility is that galaxiids and trout engage in exploitative competition. Therefore, both the invasive and the native fish are exposed to reciprocal interactions that might result in trophic niche shifts, and hence food web alterations. In the next chapter (§ 3) I study these possibilities using stable isotopes to track important possible sources of food, both for the native and the invasive fish, along the native-invasive fish countergradient.

Due to technical difficulties related to the use of stable isotopes in remote locations, I conducted a study about the effects of sample preservation on the isotopic signatures of C and N of the fishes and molluscs analyzed in the next chapter (§ 3). This study is included in Appendix 2.

3. Reciprocal trophic niche shifts in native and invasive fish: salmonids and galaxiids in Patagonian lakes

Correa, C. and A.P. Hendry (2012). Reciprocal trophic niche shifts in native and invasive fish: salmonids and galaxiids in Patagonian lakes. *Freshwater Biology* **57:**1769-1781.

3.1 Abstract

Rainbow (Oncorhynchus mykiss) and brown trout (Salmo trutta) are widespread and invasive salmonids with important lethal effects as predators, although indirect effects are also possible. We used stable isotope analyses (δ^{15} N. δ^{13} C) to explore how the density of invasive trout in 25 Patagonian lakes alters the trophic niche of a widespread native fish, Galaxias platei (Galaxiidae). We also explored how the density of the galaxiid influences the trophic niche of invasive trout. We quantified two aspects of the trophic niche: a) the proportion of littoral carbon (PL) and, b) trophic height (TH) (i.e. the 'height' at which the fish feeds in the food web). We related these measures of trophic niche in a given species to the density of other species (as estimated by catch-per-unit-effort). As G. platei body size increased, their PL increased (increasing littoral feeding) in several lakes. However, none of the fish species investigated showed changes in PL with increasing density of the other fish species. Trophic height increased with body size in all three species. In addition, the TH of large G. platei declined with increasing trout density and, reciprocally, the TH of large S. trutta decreased with decreasing G. platei density. The reciprocal effects of native and the invasive fish on trophic height were as large as a shift of one trophic level. This pattern is consistent with an exhaustion of galaxiid prey for both piscivorous G. platei and S. trutta in lakes with high trout density. These finding support the suggested management strategy of culling trout from overpopulated lakes, which should simultaneously protect native fish and enhance a lucrative sport fishery for large trout.

3.2 Introduction

A consistent effect of introduced predators is a decline in the density of native prey populations owing to the lethal effect of predation (Elton, 1958; Preisser et al., 2005; Salo et al., 2007), although indirect effects can also be dramatic (Werner & Peacor, 2003; Bolnick & Preisser, 2005; Preisser et al., 2005; Sih et al., 2010). One such effect is a change in the trophic niche of native species, which might be brought about via exploitative competition or behavioural interactions (Preisser et al., 2005; Bøhn et al., 2008; Sih et al., 2010). Because native species were presumably sufficiently well adapted to their original niches before invasion, induced by invaders niche shifts might reduce growth and survival and thereby contribute to population decline in the natives. Interestingly, reciprocal effects of natives on invaders are also likely. For instance, variation in the abundance, distribution and behaviour of native prey can cause shifts in the trophic niche of an invader (Sidorovich et al., 2010), which might then influence its growth, survival, and population dynamics. Our goal was to evaluate the potential reciprocal effects on the trophic niches of natives and invaders in a simple food web where such effects should be most easily detected (Elton, 1927; Strong, 1992; Moyle & Light, 1996; Duffy et al., 2007; Sih et al., 2010).

Our study focussed on interactions between a native galaxiid fish and introduced salmonids in post-glacial lakes of Chilean Patagonia. Galaxiids are confined to the Southern Hemisphere and are evolutionarily naive with respect to the salmonid predators that have been deliberately introduced from the Northern Hemisphere (Cox & Lima, 2006; McDowall, 2006). These salmonids, particularly brown trout (*Salmo trutta* L.) and rainbow trout (*Oncorhynchus mykiss* [Walbaum]), are extremely disruptive invasive top predators (Cambray, 2003; Casal, 2006) known to have negative effects on galaxiid populations (McDowall, 2006; Young et al., 2010; Correa & Hendry, 2012). Although direct predation is probably the leading cause of these negative effects, some indirect effects have also been documented, including habitat shifts (McIntosh et al., 1992; Edge et al., 1993; Penaluna et al., 2009) and reduced growth (Young et al., 2008). Here we test hypotheses about postulated reciprocal effects: that is, invasive salmonids should influence the TN of galaxiids and native galaxiids should influence the TN of trout. To evaluate these hypotheses, we consider two dimensions of TN (e.g., Matthews et al., 2010): (1) a 'vertical' dimension indicating the 'height' in the food web at which consumers assimilate nutrients (measured as trophic height; TH), and (2) a 'horizontal' dimension indicating the importance of littoral versus pelagic food web resources (measured as proportion of littoral carbon; PL).

Potential shifts in trophic niches resulting from interactions between native and invasive species need to be considered in the context of natural ontogenetic niche shifts. For example, TH increases with body size in many fish species, because larger individuals can consume prey over a wider size range (e.g., Werner & Gilliam, 1984; Hjelm et al., 2001; Post, 2003). In addition, PL should increase with body size in G. platei that change from planktonic to littoral habitats, or decrease for G. platei that change from planktonic or littoral habitats to a profundal benthic mode of life (Barriga et al., 2002; Habit et al., 2010; Correa & Hendry, 2012). The question remains as to how such natural ontogenetic niche shifts might be modified by interactions between native and invasive species? First, we predicted that small G. platei, which are particularly susceptible to predation by trout, might show an earlier shift in PL (a switch to littoral resources) in invaded lakes as they avoid the risk of trout predation in open water lacking refugia (Barriga et al., 2002; Macchi et al., 2007; Stuart-Smith et al., 2007; Aigo et al., 2008). Second, we predicted that the TH of small G. platei would decrease (they would feed at a lower trophic height) if foraging for small fish larva and predatory invertebrates is suppressed at high trout abundance (Vander Zanden et al., 1999), or if the exploitation of littoral and benthic resources becomes more important (Matthews et al., 2010). Third, we predicted that large G. platei would show a decrease in TH in invaded lakes because a reduced density of conspecifics limits cannibalism, a behaviour that can otherwise be common (Milano et al., 2006). On the other hand, we predicted that trout would show a decrease in their TH as populations of native fish decline. We tested these predictions by analysing stable isotope (SI) ratios of carbon $({}^{13}C/{}^{12}C;$ denoted by δ^{13} C) and nitrogen (15 N/ 14 N; δ^{15} N).

3.3 Methods

In principle, the δ^{15} N and δ^{13} C signatures of consumers in lakes can be used to calculate time-integrated quantitative measures of TH and PL, respectively. In particular, snails that scrape periphyton from littoral substrates are typically enriched in δ^{13} C relative to mussels that filter pelagic phytoplankton, and predatory fish that exploit both the pelagic and littoral food webs show intermediate δ^{13} C (PL) and higher δ^{15} N (TH) compared to the molluscs (Vander Zanden & Rasmussen, 1999; Post, 2002; Matthews et al., 2010). Trophic height and PL should be calculated relative to lake-specific isotopic baselines, particularly if inter-lake comparisons are required. Here we follow the recommendations of Post (2002) in using long-lived primary consumers (snails and mussels) as calibration baselines, coupled to a two-end member mixing model to estimate PL and TH of fish.

We used the snail *Chilina* sp. Gray (Basommatophora: Chilinidae) and the mussel *Diplodon chilensis* (Gray) (Unionacea: Hyriidae) as calibration baselines because they are abundant and widely distributed in Patagonia (Parada & Peredo, 2006; Valdovinos, 2006). These taxa (1) frequently coexist in our study lakes (13 out of 25 lakes in the present analysis), (2) capture pelagic or littoral primary productivity and detritus due to their feeding habits [scraper- (snail) and filter-feeder (mussel)] (Estebenet et al., 2002; Lara et al., 2002; Post, 2002), and (3) are long-lived compared to most other primary consumers (>30 years for *D. chilensis*; Parada et al., 1989; 1 year for *Chillina sp*. Bosnia et al., 1990). We collected these species by hand from the littoral zone of all lakes that contained them.

Study area

Our study took place in 25 lakes of the Aysén region in western Patagonia, Chile (~46°S) [Table 1; also see Correa & Hendry (2012) for a map and further description of study area]. Trout were first introduced into the region in 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). Both species quickly became established and spread (naturally or artificially) to colonize most lakes. Some lakes, however, remained uncolonized, either because obstacles to trout dispersal made them inaccessible or because they were lacking streams that could support trout reproduction (Correa & Hendry, 2012). Across lakes, the density of galaxiids is currently negatively correlated with the density of trout, and in several lakes galaxiids have apparently gone extinct (Correa & Hendry, 2012). This variation among lakes in the density of trout and galaxiids provides us with an opportunity to investigate trophic niches along an invasion gradient.

As our goal was to study the effect of trout and galaxiids on each other, we sought to minimize variation among lakes in other environmental variables. Therefore we chose invaded lakes (i.e. trout were detected) and uninvaded lakes (i.e. trout were not detected) that were otherwise similar. To confirm this similarity, we compared invaded (n = 17) and uninvaded lakes (n = 8) with respect to lake area [ln(ha)], shoreline development index, maximum depth $[\ln(m)]$, specific conductivity (μ S cm⁻¹), altitude (m), stream order at lake outlet, and proportion (arcsine \sqrt{p}) of deforested area within a lake basin (Table 1). Six of the seven variables showed no differences between invaded and uninvaded lakes (Student's t-tests, df = 23, P > 0.05). Lake order was significantly higher in invaded than uninvaded lakes [mean(SD) 1.9(1.0) vs. 0.7(1.2); Student's t-test, df = 23, P = 0.03], although considerable overlap was evident. Uninvaded lakes were often of lower order because they were less likely to be connected to streams that were appropriate for trout reproduction. Lake order, however, had no significant effect on the density of G. platei after controlling for the negative effect of trout density (Correa & Hendry, 2012). Thus, any differences in trophic niches between invaded and univaded lakes are expected to be the result of trout invasion rather than confounding environmental variables.

Fish collections and density indicators

Our sampling methods are detailed elsewhere (Correa & Hendry, 2012), and so we here describe them only briefly. During the austral summer and early autumn (January–May) of 2007 and 2009, we conducted quantitative fish sampling with three types of fishing gear. (1) Multi-panel survey gillnets targeted large fish (>50 g), particularly trout and large *G. platei*. Two gillnets were usually set per lake, one in the littoral zone and one in a deep location at the lake bottom (demersal). (2) Minnow traps, usually 20 per lake, targeted smaller *G. platei* in a broad range of microhabitats from the littoral to the demersal zone. (3) Electrofishing targeted small trout and galaxiids in the shallow littoral zone of all lakes and, owing to logistical constraints, this was the only fishing method used in Toro Lake. This combination of approaches secured samples including a broad range of body sizes of each species, allowing an analysis of ontogenetic, interspecific, and inter-lake variation in trophic niche.

Correa & Hendry (2012) calculated and analysed multivariate indices (PC1 across the three gear types) of capture-per-unit-effort (CPUE) as a proxy for the densities of *G. platei* and trout. Here, however, we use univariate CPUE data, because this allowed us to target more precisely the demographic classes most relevant to our specific hypotheses, such as large predatory trout or small (prey) *G. platei*. As the explanatory variable for testing the effects of trout on the TN of *G. platei*, we used trout CPUE from gillnets (pooling data from *O. mykiss* and *S. trutta*): ln(number of trout gill netted / number of overnight gillnets + 1). As the explanatory variable for testing the effects of *G. platei* trapped / number of overnight traps + 1). *Post hoc* analyses using other univariate and multivariate CPUE-based proxies for fish density led to the same conclusions as reached here. In short, our results are robust to variation in the specific fish density indices, which were in any case were correlated with each other (Correa & Hendry, 2012).

Elemental analysis and correction for preservation bias

Shortly after collection in the field, fish were killed with an overdose of MS222 or clove oil. A small portion of muscle tissue (about 3-5 mm in cross-section and 1 cm in length) was then removed from the anterodorsal region of the fish, avoiding dorsal spines. A similar quantity of muscle was obtained from the foot of mussels and snails. These tissue samples were preserved and stored in 95% denatured ethanol (see below): the denaturing agent was 0.02% diethyl

phthalate (Cruz Verde). Individual samples were kept in 1.5 mL Eppendorf tubes, whereas samples from several invertebrates of different species were kept together in 25 mL scintillation vials. In the laboratory, the samples were dried at 50°C for 24 h, 1.0 – 1.3 mg (analytical balance Mettler Toledo, Canada, model AB104) sub-samples were removed and placed in tin-capsules. The dried samples were then sent to the Stable Isotope Facility of the University of California, Davis, where they were measured for δ^{15} N and δ^{13} C using a PDZ Europa ANCA-GSL elemental analyser attached to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Stable isotope values were specified in δ notation: δ^{15} N or δ^{13} C = [($R_{sample}/R_{standard}$) – 1]1000, where *R* is 13 C/ 12 C or 15 N/ 14 N relative to international standards (Vienna PeeDee Belemnite for carbon and atmospheric air for nitrogen).

Ethanol preservation is not ideal for stable isotope analysis because it can produce systematic bias in isotopic values. In our study, however, ethanol preservation was necessary because sampling took place in remote areas where no better preservation method was available. Fortunately, the bias can be corrected through experiments contrasting an ethanol preservation treatment to a control treatment (Kelly et al., 2006; Barrow et al., 2008; Yoshino et al., 2010). Correa (in press) conducted such an experiment by comparing, for a sample of each species, stable isotope measurements from tissue preserved in ethanol (treatment) versus tissue preserved by freezing for < 5 d and then drying (control). These data were used to develop bias correction factors which corresponded to mean isotopic deviations between the control and treatment groups, adjusting for individual variation (i.e. repeated measures design) within each species. To correct for preservation bias in the full dataset, we then subtracted these bias correction factors from the measured stable isotope values of each ethanol-preserved sample. **Data analyses**

We analysed the stable isotope signatures of 411 fish and 172 molluscs across the 25 lakes (Table 1; Fig. 1). Isotopic calibration baselines were calculated as the average δ^{13} C and δ^{15} N values by mollusc and lake. All snail baselines, and 13 mussel baselines, were calculated directly from the data. However, mussels were absent from 12 lakes and the average baseline values in those cases had to be inferred indirectly. To do so, we regressed $\delta^{13}C_{(mussels)}$ on $\delta^{13}C_{(snails)}$ for the 13 lakes where they co-occurred and the fitted model was used to predict the missing $\delta^{13}C$ values [$\delta^{13}C_{(mussels)} = -16.63 + 0.56 * \delta^{13}C_{(snails)}$, ANOVA, $F_{1,11} = 26.7$, P < 0.001, $R^2 = 0.71$]. Similarly, we regressed $\delta^{15}N_{(mussels)}$ on $\delta^{15}N_{(snails)}$ for the 13 lakes and the fitted model was used to predict missing $\delta^{15}N$ values [$\delta^{15}N_{(mussels)} = 0.80 + 0.68 * \delta^{15}N_{(snails)}$, ANOVA, $F_{1,11} = 10.8$, P = 0.007, $R^2 = 0.50$]. Lake area sometimes explains variation in isotopic calibration baselines (Post 2002) but that was not the case in the present study (results not shown), and so it was ignored when inferring missing values.

The trophic height (TH) of each fish was calculated according to (Post, 2002): TH = λ + ($\delta^{15}N_{(fish)} - [\delta^{15}N_{(snails)} * PL + \delta^{15}N_{(mussels)} * (1- PL)]) / <math>\Delta_n$, where λ is the TH of the molluscs used as isotopic baselines ($\lambda = 2$ for the primary consumers used) and Δ_n is the enrichment in $\delta^{15}N$ per trophic level (assumed to be 3.4‰; Post, 2002). The proportion of littoral carbon (PL, constrained to vary in the range [0, 1]) of each fish was calculated as the proportion of fish mass derived from the littoral (snails) as opposed to the pelagic (mussels) food web (parameter alpha in Post 2002): PL = ($\delta^{13}C_{(fish)} - \delta^{13}C_{(mussels)}$)/($\delta^{13}C_{(snails)} - \delta^{13}C_{(mussels)}$). In our study, $\delta^{15}N$ values were similar between same-lake isotopic baselines (see Results), and so the estimated PL values had little effect on the estimated TH values. Trophic height and PL were treated as response variables separately by species: *G. platei*, *S. trutta*, and *O. mykiss*. Alternative analyses that pooled *S. trutta* and *O. mykiss* reinforced our findings for *S. trutta* by itself (results not shown).

To model variation in TH, we used hierarchical, varying intercept, varying slope, mixed-effect models (Gelman & Hill, 2007). To evaluate our hypotheses, the deterministic portion of the model included CPUE of the relevant species (i.e. trout for a model predicting galaxiid TH and vice versa), individual mass (of the fish whose TH is being predicted), and their interaction, the latter allowing for ontogenetic TH shifts that could vary as a function of fish CPUE. The random portion of the model included the random effect of lake identity (unexplained

between-lake variation), and variation among individual fish (after accounting for mass), which were nested within lakes (residual variation). The (full) model thus can be written as $TH = 1 + CPUE + \ln(g) + CPUE:\ln(g) + (1 + \ln(g) | lake)$, where 1 represent intercepts, CPUE is the relevant lake fish density index (see above), *g* is the mass of individual fish ingrammes, and lake is the identity of the lake (see syntax details in Zuur et al., 2009). The model for each species was fitted by restricted maximum likelihood using the function 'lmer' of the R package 'lme4' (Bates & Maechler, 2010). Predictions were evaluated by testing the significance, and visualizing the effect size, of the interaction term between mass and CPUE. Statistical significance was tested by fitting through maximum likelihood the full model and a reduced model with no interaction term, which were then compared using the likelihood ratio test (LRT) implemented in the R function 'anova' (Zuur et al., 2009; R Development Core Team, 2010).

In contrast to TH, PL is a proportion and so was analysed in a twostage generalized linear model (GLM) (Gelman & Hill, 2007). In the first stage, we fit models to the data (individual mass predicting PL) for each lake and species separately using a GLM with a quasibinomial error structure (logit link) implemented in the R function 'glm' (R Development Core Team, 2010). The quasibinomial was preferred over binomial error structure because the data were often overdispersed (i.e. when using the binomial error structure, residual deviance / df >> 1; Crawley, 2007; P. 573-578). In the second stage, we looked for correlations between the resulting coefficients (slopes and elevations) and the relevant fish density index: i.e., trout CPUE predicting slopes and intercepts for galaxiid PL and *vice versa*). We set the significance level to $\alpha = 0.05$ for all statistical tests. R code is available from the corresponding author.

3.4 Results

In the 13 lakes where both mollusc species were present, the δ^{13} C values of snails were significantly enriched compared to the δ^{13} C values of mussels [one-tailed paired *t*-test, $t_{(12)} = 8.135$, P < 0.001], and fish generally had intermediate δ^{13} C values (Fig. 1). Fish were also substantially enriched in δ^{15} N relative to these

primary consumers, which were similar to each other (Fig. 1; two-tailed paired ttest, $t_{(12)} = 1.208$, P = 0.250). Relative to lighter individuals, heavier fish fed higher in the food web (a greater TH) and sometimes also differed in the proportion of littoral carbon. These results are consistent with general expectations that fish consume a mixture of pelagic and littoral resources, are at a higher trophic position than primary consumers, and show ontogenetic diet shifts.

Ontogenetic shifts in the TH of *G. platei* were related to the presence/absence (Fig. 2) and catch-per-unit effort (CPUE) (Fig. 3) of trout. Small *G. platei* (~ 1 g) had relatively similar TH across the entire gradient of trout-CPUE (Fig. 3a). In contrast, large *G. platei* (~150 g) fed approximately one trophic level higher in lakes where trout were not detected than in lakes with abundant trout (Fig. 3a). Accordingly, the effect of *G. platei* mass on TH (i.e. slope) was negatively correlated with trout-CPUE (Fig. 3b; LRT $\chi^2 = 6.781$, df = 1, P = 0.009). In an information theoretic context, this full model was the best (AIC = 16.63) followed by a reduced model lacking the interaction term (AIC = 21.43).

Similarly, ontogenetic changes in the TH of *S. trutta* were related to the presence/absence (Fig. 4) and CPUE (Fig. 5) of *G. platei*. Small *S. trutta* (~ 10 g) had a relatively similar or slightly lower TH as *G. platei*-CPUE increased (Fig. 5a). In contrast, large *S. trutta* (~1000 g) fed approximately one trophic height higher in lakes with abundant *G. platei* than in lakes where *G. platei* were not detected (Fig. 5a). Accordingly, the effect of *S. trutta* mass on TH (i.e. slope) was positively correlated with *G. platei*-CPUE (Fig. 5b; LRT $\chi^2 = 7.092$, df = 1, P = 0.008). This full model was the best (AIC = 35.95) followed by a reduced model lacking the interaction term (AIC = 41.04).

Oncorhynchus mykiss also showed an ontogenetic increase in TH (see below), but the interaction between *O. mykiss* mass and *G. platei*-CPUE was not significant (LRT $\chi^2 = 3.087$, df = 1, P = 0.079). Nor was there a significant effect of *G. platei*-CPUE without the interaction term in the model (LRT $\chi^2 = 1.785$, df = 1, P = 0.182), which leads to a simplified model involving only the effect of *O. mykiss* mass and the random effect of lake identity (LRT $\chi^2 = 21.004$, df = 1, P < 0.182)

0.001). The simplified model was the best fit (AIC = 15.38) followed by the full model (AIC = 18.29). It must be noted, however, that only seven lakes could be included in the analyses involving *O. mykiss*, and hence statistical inference is tenuous. At the individual level, however, the numbers of fish in lakes where they were present were always large enough to detect the expected ontogenetic TH shifts (i.e. the effect of mass).

In contrast to these clear patterns for TH, ontogenetic shifts in the proportion of littoral carbon (PL) were highly variable and seemingly idiosyncratic for all three species. For *G. platei*, slopes of the generalized linear regressions between ln-mass and PL were positive for six lakes, negative for one, and non-significantly different from zero in the rest of the 20 lakes in the first stage of the analysis (t-tests on slope coefficients). This result suggests a common ontogenetic shift from pelagic larvae to more littoral juveniles and adults. Moreover, the slope coefficients between ln-mass and PL in *G. platei* within lakes were unrelated across lakes to trout-CPUE in the second stage of the analysis (ANOVA, $F_{1,18} = 1.058$, P = 0.317). For *S. trutta* and *O. mykiss*, the slopes of the generalized linear regressions between ln-mass and PL occasionally differed from zero in either direction, and no significant interaction with *G. platei*-CPUE was observed (results not shown).

3.5 Discussion

Trophic height

Our data reveal shifts in trophic height (TH) in co-occurring native galaxiids and invasive salmonids. For the native species, the results suggest that the indirect, non-lethal impacts of invasive trout prevent their normal ontogenetic shift to feeding higher in the food web with increasing body size. For the invader, these results suggest that the reduction they cause in the density of native species (Correa & Hendry, 2012) hampers their own normal ontogenetic shift to feeding higher in the food web as body size increases. These shifts in TH might be caused in at least two (non-exclusive) ways: a) the alteration of available food

(exploitative competition hypothesis) and b) the alteration of feeding behaviour due to predator avoidance (predator avoidance hypothesis).

The outcome of exploitative competition might be affected by ecological differences between *G. platei* and trout. *Galaxias platei* completes its life cycle within lakes (Barriga et al., 2002; Habit et al., 2010; data in Correa & Hendry, 2012) whereas trout spawn in streams where their emerging fry grow for several months or years before entering lakes (Forseth et al., 1999; Arismendi et al., 2011c). This spatial segregation between *G. platei* and trout makes *G. platei* in lakes more vulnerable to predation than salmonids (McDowall, 2006; Milano et al., 2006; Macchi et al., 2007). Indeed, small *G. platei* are frequently found in the diet of large trout and large *G. platei* (cannibalism), whereas small trout are seldom found in these diets (Macchi et al., 1999; Milano et al., 2006; Vigliano et al., 2009; unpublished data). Thus, the TH of trout may be higher in the presence of galaxiids because the latter are a food source of unusually high trophic height, whereas the TH of galaxiids may be lower in the presence of trout because of exploitative competition forcing large galaxiids to feed lower in the food web than they otherwise would.

The predator avoidance hypothesis has received support from the demonstration of trophic niche (TN) shifts in other systems (Crowder & Crawford, 1984; Schmitz & Suttle, 2001; Preisser et al., 2005). For example, many species modify their foraging behaviour according to perceived predation risk (Sih et al., 2010 and references therein). Although galaxiids can certainly sense trout and try to avoid them (Stuart-Smith et al., 2008; Penaluna et al., 2009), the observed TH reductions were actually greatest for the least vulnerable large *G. platei* (150 g, ~225 mm SL) (Keeley & Grant, 2001). An alternative behavioural interpretation that could result in this pattern is progressive learning by galaxiids to avoid trout (with consequences for the diet) in response to previous non-lethal interactions (Lundvall et al., 1999; Kelley & Magurran, 2011). Trout, on the other hand, have little to fear from *G. platei*. Therefore, we conclude that the shifts in TH observed in both native *G. platei* and introduced trout are consistent mainly

with the exploitative competition hypothesis, although other behavioural interactions are also plausible (see also Rechencq et al., 2011).

The shifts in TH observed in our study (about one trophic level) were quite large in relation to similar effects observed in a classical example from Canadian lakes. There, lake trout decreased its TH by about half of a trophic level after the introduction of smallmouth bass (Micropterus dolomieu Lacepède) and rock bass (Ambloplites rupestris Rafinesque) (Vander Zanden et al., 1999). We attribute the especially large effect in our system to one property of the organisms and one property of the environment. With respect to the organisms, both G. platei and S. trutta are generalists (Milano et al., 2006; Casaux & DiPrinzio, 2007; Arismendi et al., 2011a). Upon changes in feeding conditions (food availability or foraging behavior), native generalists can readily engage in potentially adaptive diet shifts. Similarly, invasive generalists can rapidly exploit a variety of food resources across their new range (Clavel et al., 2011). With respect to the environment, the food webs we studied are relatively simple, and "major community effects of invasions are most often observed where the number of species is low" (Moyle & Light 1996). It is thus likely that fishes in the species-poor communities of post-glacial Patagonian lakes are especially susceptible to the direct and indirect effects of invasive predators (see also Elton, 1927; Strong, 1992; Duffy et al., 2007; Sih et al., 2010).

Proportion of littoral carbon

Observed patterns in the proportion of littoral carbon (PL) were rather variable and idiosyncratic. Some of this variation might be the result of methodological issues surrounding the estimation of PL, including heterogeneity in lipid concentration (Post et al., 2007; Fagan et al., 2011), missing baseline values for mussels in some lakes, and inadequate baseline spatio-temporal representation (Guzzo et al., 2011). An even more fundamental issue is that tracing the origins of carbon using one stable isotope (i.e. δ^{13} C) means that only two potential basal sources can be evaluated reliably (reviewed in Boecklen et al., 2011). We based our PL analysis on the assumption that much of the consumer biomass in lakes ultimately comes from the pelagic and littoral food webs (Post,

2002). However, other potentially important sources include allochthonous (i.e. terrestrial) inputs and profundal production (Post, 2002; Cole et al., 2011). Although we cannot assess the contribution of these sources in our study, the last at least seems unlikely to be important given that profundal carbon sources tend to have low δ^{13} C signatures and yet the majority of the fish analysed were enriched in δ^{13} C relative to the pelagic calibration baseline (but see Thompson Lake in Fig. 1). Similarly, the signatures of 191 fish from 25 North American temperate lakes suggest this source is unimportant (Post, 2002).

Assuming the above methodological issues are not too problematic (further discussion in Correa, in press), here we consider ecological factors influencing variation in PL. In six out of seven lakes where *G. platei* showed significant PL shift with body size, the shifts were positive indicating an ontogenetic transition toward greater exploitation of the littoral food web, even in deep, invaded lakes. This finding contrasts with the common contention that *G. platei*, after completing its pelagic larval development (especially in invaded lakes), becomes a deep-benthos dweller (Milano & Vigliano, 1997; Barriga et al., 2002; Cussac et al., 2004; Milano et al., 2006). Indeed, Correa & Hendry (2012) collected juvenile *G. platei* in the shallow littoral zone of every lake where the species was detected, and the same was true for adults in many lakes. Therefore we suggest that the littoral zone might offer food and structural complexity enabling *G. platei* to evade trout predation to some extent (Aigo et al., 2008). It is also possible that some *G. platei* perform extensive diel migrations between the littoral and the profundal zone (Rechencq et al., 2011).

Management implications

Our results are of potential significance for management. Trout decrease the TH of native *G. platei*, particularly of the largest individuals. In principle, this effect could reduce food intake rates, limit growth rates, lower the reproductive potential of adults, and thereby reduce population productivity of the native species (Jonsson et al., 1999; Post, 2003; Vigliano et al., 2009; Britton et al., 2010). Thus, the indirect effects of invasive trout on the native *G. platei* (and presumably other galaxiids) might exacerbate the lethal effects caused by predation (see Sih et al., 2010). Despite these potential fitness costs, trophic plasticity might be necessary to avoid predation (Schmitz & Suttle, 2001). Thus, the observed TH shift in *G. platei* could have profound and contrasting evolutionary implications ranging from accelerated local extinctions to allowance of long-term persistence and adaptation to the invader (Strauss et al., 2006). Invasive trout, on the other hand, feed at a higher TH when native galaxiids are present than when they are absent, and so could experience fitness costs following the collapse of native fish populations (Jonsson et al., 1999; Post, 2003; Vigliano et al., 2009). These costs could include poor condition, small size, smaller size at maturity, slower growth, and slower metabolic rate, especially in oligotrophic lakes. In short, the depletion of native galaxiids is likely to be bad for invading salmonids, pointing directly to a valuable management strategy.

Patagonia is a world-class destination for trout sport fishing and the economic benefits are great (Arismendi & Nahuelhual, 2007; Vigliano et al., 2007; Pascual et al., 2009; Vigliano et al., 2009) – and angler satisfaction increases with fish size (Loomis, 2006). Arismendi *et al.* (2011b) suggested that some lakes in Northern Chilean Patagonia are overpopulated with salmonids, resulting in food shortage and poor body condition of *O. mykiss*. Similarly, our results suggest that overpopulation of trout and the associated decline of *G. platei* probably cause suboptimal ontogenetic trajectories of TH in *S. trutta*. The likely reduction in trout growth would thus compromise the value of the fishery. Therefore an excellent management strategy for Patagonia might be to reduce trout population density. The resulting increase in native fish density would simultaneously help to conserve a rare endemic group while also enhancing economic opportunities provided by the invader (see also Arismendi et al., 2011b; Correa & Hendry, 2012).

3.6 References

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3.7 Tables

Table 1: Lake descriptors and sample sizes^a of the molluscs and fish analysed.

ID	Lake	Latitude (°S)	Longitude (°W)	Invasion	Area (ha)	Shore. dev. index ^b	Altitude (m)	Order ^c	Depth (m)	Spec. cond. (µS cm ⁻¹)	Deforestation (%) ^d	D. chilensis	<i>Chilina</i> sp.	G. platei	S. trutta	O. mykiss
1	Alta	45.5347	72.7086	Uninvaded	89.4	1.38	80	2	55	26.9	9	5	4	8	0	0
2	Barroso	45.7396	72.2451	Invaded	56.4	1.87	334	2	43	32.9	55	10	10	13	13	0
3	Brava	45.0997	71.8272	Invaded	77.5	1.59	840	1	41	50.7	68	0	4	0	9	0
4	Burgos	45.7128	72.2185	Invaded	22.9	2.04	375	2	30	58.8	93	3	3	0	2	8
5	Castor	45.6003	71.7815	Invaded	432.3	2.67	696	2	52	78.7	49	5	5	24	0	9
6	Cea	45.6744	72.2378	Invaded	23.1	1.50	289	2	11	31.8	40	5	5	18	15	0
7	Chacano	46.1728	72.1933	Invaded	15.4	1.87	510	2	14	112.1	64	0	3	0	12	5
8	Escondida	45.5324	71.8259	Uninvaded	74.2	2.04	695	1	17	99.0	29	0	5	9	0	0
9	Espejo	45.7407	72.204	Invaded	9.6	1.18	336	2	21	75.5	84	0	1	0	11	0
10	Frio	45.6675	71.9438	Invaded	396.7	1.58	513	4	15	73.5	47	5	5	9	0	12
11	Gemela	45.5463	71.8162	Uninvaded	25.7	1.54	759	0	14	92.9	57	0	5	12	0	0
12	Juncos	45.6336	71.8509	Uninvaded	6.9	1.15	748	0	3	56.9	44	0	5	11	0	0
13	Leon	45.6161	71.8405	Uninvaded	29.3	1.85	756	0	8	61.6	57	0	2	11	0	0
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14	Mercedes	45.7361	72.2173	Invaded	18.9	1.53	349	1	22	75.0	76	0	4	0	10	0
15	Monreal1	45.6369	71.8696	Uninvaded	34.2	1.54	753	0	14	48.8	50	5	5	9	0	0
16	Monreal2	45.8725	72.0209	Invaded	232.2	1.90	603	2	51	52.8	37	0	5	6	17	8
17	Palos	45.3209	72.7027	Invaded	539.5	1.62	0	3	60	23.6	10	4	5	2	4	0
18	Pollux	45.6763	71.8627	Invaded	872.3	2.52	679	3	55	71.6	42	5	5	17	0	24
19	Portales	45.5353	72.5904	Invaded	571.0	2.13	53	3	150	24.2	30	5	5	12	8	0
20	Quijada	45.7177	71.911	Invaded	32.3	1.24	727	0	10	65.4	63	0	3	11	2	0
21	Rengifo	45.6176	72.5113	Invaded	187.2	1.69	110	1	20	24.9	40	3	4	3	8	0
22	Thompson	45.6467	71.7929	Invaded	111.0	2.12	748	1	15	72.3	44	3	4	8	0	6
23	Toro	45.5299	71.8535	Uninvaded	27.3	1.06	686	0	na	na	na	0	2	10	0	0
24	Yulton	45.118	72.9022	Uninvaded	6111.1	2.60	488	3	170	25.0	4	0	5	18	0	0
25	Zenteno	45.5921	72.5482	Invaded	656.1	2.47	95	2	50	25.3	41	5	5	15	2	0

Notes: Lake descriptors follow Correa & Hendry (2012). (^a) Sample sizes are after the removal of nine isotopic outliers distributed among fish and molluscs in six lakes. Lake Sepulveda (Correa and Hendry 2012) was also excluded due to uncertainty in isotopic baselines. (^b) Shoreline development index quantifies the development of the littoral zone relative to open waters. It is defined as the perimeter of a lake divided by the perimeter of a circle of equal area. (^c) Lake order describes lake position in a river network, and is measured as Strahler stream order at the lake outlet (endorheic lakes were indexed as Order = 0). (^d) Deforestation is the proportion of lake basin deforested during the twentieth century.

3.8 Figures

Figure 1: Stable isotope data for each lake (sorted alphabetically). The snail *Chilina* sp. (\Box) is taken to represent the isotopic baseline for the littoral food web and the mussel *Diplodon chilensis* (\circ) is taken to represent the same for the pelagic food web (means and inferred values, used to calculate fish trophic height and proportion of littoral carbon, are shown with larger, darker symbols). The remaining symbols represent individuals of *Galaxias platei* (\bullet), *Oncorhynchus mykiss* (+), and *Salmo trutta* (×). Values were corrected for preservation bias (see Methods for details).

Figure 2: Ontogenetic shifts in trophic height for the native *Galaxias platei* in the 20 lakes where they were found. Lines represent the fitted model for height on lnmass in lakes with (dark lines) and without (light lines) invasive trout. Points represent the values for individual fish from all the lakes (for clarity, individual lake membership is not shown). Numbers associated with the fitted lines correspond to the alphabetical order of lakes in Fig. 1 and Table 1, and model coefficients are displayed in Fig. 3.

Figure 3: Multi-level model coefficients for the trophic height of *Galaxias platei* as a function of trout-catch-per-unit-effort (trout CPUE) (from model in Fig. 2). a) Elevation represents the trophic height evaluated at a fish mass of 1 g (filled symbols) and 150 g (open symbols) in each lake. b) Slope represents coefficients for the effect of ln-mass on trophic height in each lake. Vertical lines represent 95% confident intervals. Coefficients corresponding to CPUE = 0 were slightly dispersed along the x-axis for increased clarity.

Figure 4: Ontogenetic shifts in trophic height for the invasive *Salmo trutta* in the 13 lakes where they were found. Lines represent the fitted model for height on lnmass in lakes with (dark lines) and without (light lines) native *Galaxias platei*. See Fig. 2 for additional details.

Figure 5: Multilevel model coefficients for the trophic height of *Salmo trutta* as a function of *Galaxias platei* catch-per-unit-effort (CPUE) (from model in Figure 4). a) Elevation represents the trophic height evaluated at a fish mass of 10 g (filled symbols) and 1000 g (open symbols) in each lake. b) Slope represents coefficients for the effect of log-mass on TH in each lake. See Fig. 3 for additional details.





Figure 2



Figure 3



Figure 4



Figure 5



Understanding ecosystem-wide consequences

In the previous chapters (§ 2 & 3) I have shown how invasive trout can alter the galaxiid community in chronic and catastrophic ways, yet it remains unclear if and how these effects are relevant to lake ecosystems. The possibilities range from a (partial or complete) taxonomic replacement with little ecosystem consequences, to the substitution of functional groups and alteration of ecosystem functions. One way to address this question is by reference to the zooplankton community, asking how native galaxiids and invasive trout affect zooplankton individuals, populations and communities. I hypothesize fundamentally different processes and outcomes depending on the dominant fish species. For galaxiids, direct top-down control mechanisms would favour smaller zooplanktonic individuals and species, and evasive behaviour against fish predation. For trout, cascading top-down control mechanisms would favour larger zooplanktonic individuals and species, and bolder behaviour. In the next chapter (\S 4) I test these hypotheses using a combination of approaches involving contemporary pelagic zooplankton surveys - along the native-invasive fish countergradient -, and paleolimnological zooplankton reconstructions in a single invaded lake.

4. Disruption of top-down control of zooplankton by invasive salmonids

Correa, C., I. Gregory-Eaves, and A.P. Hendry (in preparation). Disruption of topdown control of zooplankton by invasive salmonids.

4.1 Abstract

Ecological disruptions caused by biological invasions can be used as large-scale experiments to understand the functioning of natural ecosystems. We here evaluate the effects of invasive salmonids to understand the role of native galaxiids in structuring the zooplankton community in Patagonian lakes. Our study took place in a region (western Patagonia, ~46°S) where the native fish community is dominated by the most widespread Patagonian-endemic fish, the puye grange Galaxias platei. G. platei might exert top-down control of the zooplankton community through planktivory by larva and juveniles, yet this hypothesis remains largely untested. The results of zooplankton surveys in a set of 21 lakes analyzed as a function of a strong G. platei-to-trout abundance countergradient showed a marked dominance of small cladocera (Bosmina) only at high G. platei abundance. At the opposite extreme, lakes with abundant trout had zooplankton assemblages dominated by calanoid copepods (Boeckella) and, in a few lakes, large cladocera (Daphnia). Furthermore, the body size of two cladocerans that are widely distributed across lakes (Bosmina and Ceriodaphnia) increased from G. platei-dominated to trout-dominated lakes. A paleolimnological study confirmed some of these findings, and suggested that a change in invertebrate predation intensity (as inferred from Bosmina's morphology) could also be associated with the salmonid invasion. Overall, our results promote the importance of the top-down control hypothesis on natural Patagonian lake ecosystem functioning. Finally, we suggest that trout invasions can disrupt galaxiids' top-down control of zooplankton through a cascading interaction and thus may have profound ecosystem-wide consequences.

4.2 Introduction

In comparison to temperate lakes of the North Hemisphere that have been heavily studied, Patagonian lakes feature a peculiar zooplankton community characterized by relatively low species richness, high endemism, predominance of calanoid copepods and small cladocerans, and absence of chaoborus and leptodora that are important invertebrate predators (Soto & Zuñiga, 1991; Modenutti et al., 1998). These properties have been explained in terms of physical factors such as climate, lake morphometry, nutrient concentrations, trophic status, and geographic isolation (Soto & De los Ríos, 2006; De los Ríos & Soto, 2007a, 2007b). For example, the oligotrophic state of most Patagonian lakes has been regarded as a key constraint to primary productivity (Steinhart et al., 1999), zooplankton richness, and daphnid abundance (Balseiro et al., 2007; De los Ríos & Soto, 2007a). In addition, it has been proposed that lake morphometry interacts with climate to limit primary productivity, whereby intense winds coming from the South Pacific cause a deepening of the mixing zone to a point where phytoplankton is limited by light intensity and quality rather than by nutrients (Soto, 2002). Under these condition, starvation-tolerant calanoids are competitively superior over other zooplankton (Soto, 2002). This body of work has highlighted the importance of abiotic drivers as predictors of zooplankton abundance and composition (Soto, 2002; Soto & De los Ríos, 2006; De los Ríos & Soto, 2007a), but the relative role of top-down control, which has been shown to be important in temperate lakes in many parts of the world (Carpenter et al., 1985; Jeppesen et al., 1997a), has rarely been assessed in Patagonian lakes. A few notable exceptions include Modenutti et al.'s (1993) study of a small lake where the native galaxiid showed strong top down control. Several others have also shown that fish can suppress very large (> 1 mm) zooplankton taxa that are otherwise typical of fishless Patagonian ponds and occasionally occur in lakes with fish (e.g., Reissig et al., 2006; Soto & De los Ríos, 2006; Balseiro et al., 2007). Based on comparative inference with small New Zealand lakes, which have similar biogeographical and ecological affinities to Patagonian lakes (Soto & Zuñiga, 1991; Bayly, 1995; McDowall, 2006), we predict that planktivorous fish

will play a strong role in shaping zooplankton communities in Patagonian lakes (Jeppesen et al., 1997b; Jeppesen et al., 2000). However, the studies from New Zealand are complicated by the inclusion of several additional fish taxa of local importance (Jeppesen et al., 1997b).

The native fish communities in Patagonian lakes, and more generally in cool-temperate lakes of the southern hemisphere, are characterized by low species richness and a paucity of highly piscivorous fishes (McDowall, 2006; Aigo et al., 2008; Habit et al., 2012). The family Galaxiidae, with its pan-austral distribution (Burridge et al., 2012), is a clear candidate potentially shaping zooplankton communities (Brooks & Dodson, 1965; Modenutti et al., 1993; Jeppesen et al., 1997b), especially in postglacial lakes devoid of other native fish (Zemlak et al., 2008; Correa & Hendry, 2012). For example, many galaxiids reproduce within lakes and their pelagic larva eats zooplankton before metamorphosing and switching to larger littoral or benthic prey (Barriga et al., 2002; McDowall, 2006; Milano et al., 2006; Casaux & DiPrinzio, 2007). Data for G. maculatus larva (which are behaviorally and morphologically-similar to the dominant galaxiid, G. platei, in our study system) show that galaxiid larva undergo ontogenetic diet shifts encompassing a broad variety of phyto- and zoo-plankton prey (Cervellini et al., 1993; Modenutti et al., 1993). In fact, the predation intensity of galaxiid larva is such that it caused the failure of the summer cohort of the calanoid copepod *Boeckella gracilipes* (through predation on nauplii), and the virtual disappearance of Ceriodaphnia dubia in the summer plankton of a small lake (Modenutti et al., 1993).

Triggered by deliberate introductions beginning in the late 19th century, salmonids (native to the northern hemisphere) begun a period of great range expansion in austral environs (Elton, 1958). A century later, salmonids were nearly ubiquitous in the galaxiids's range (Pascual et al., 2002; McDowall, 2006; Habit et al., 2012). As a result, these salmonids could play a novel role in the (former) realm of galaxiids, although the mechanisms might be fundamentally different. For example, in contrast with some galaxiids, introduced trout leave lakes to spawn in streams, and juvenile trout spend several months to years

feeding in streams before returning to lakes (Forseth et al., 1999; Arismendi et al., 2011b). Lake-dwelling trout are generalist predators, typically consuming benthic macro-invertebrates (and occasionally large zooplankton), and often become piscivorous if fodder fish are present (Vigliano et al., 2009; Arismendi et al., 2011a). Therefore, invasive trout are less likely to exert a strong control on lake zooplankton compared with galaxiids, and if trout had any effect, it would be on the very large (> 1 mm) zooplankton prey which are rare in the realm of galaxiids (Galbraith, 1967; Kitchell & Kitchell, 1980; Biro et al., 2003; Reissig et al., 2006; Fisk et al., 2007). Nevertheless, salmonids might have cascading effects through negative effects on galaxiids (Carpenter et al., 1985).

To explicitly test how both native galaxiids and invasive trout affect zooplankton communities in Patagonian lakes, we conducted both a crosssectional lake survey and longitudinal study. Overall, our study sites represent a collection of comparable lakes that span a steep countergradient of trout and galaxiid abundance (Correa & Hendry, 2012) and embody the well-known galaxiid conservation crisis (McDowall, 2006). The galaxiids represented in our sites were solely the puye grande G. platei, the most widespread Patagonianendemic fish (Zemlak et al., 2008; Habit et al., 2012). The salmonids are represented by rainbow trout O. mykiss and brown tout S. trutta, amongst the most disruptive, cosmopolitan aquatic invaders (Cambray, 2003; Casal, 2006). Based on the literature, we developed specific predictions concerning fish top-down control on zooplankton in Patagonia lakes. Firstly, we expect a gradient in community composition with relatively small zooplankters dominating at high G. platei and low trout abundance, and large zooplankters dominating at low G. *platei* and high trout abundance (Brooks & Dodson, 1965; Modenutti et al., 1993; Jeppesen et al., 1997b). Second, the body sizes of widely distributed zooplankters that are susceptible to fish predation are expected to increase along the G. plateito-trout gradient (Galbraith, 1967; Jeppesen et al., 2002). Third, the diel vertical migration pattern should also reflect the fish predation risk, and hence we expect stronger migration, away from the epilimnetic zone during daylight, in lakes with abundant G. platei (Gliwicz, 1986; Cousyn et al., 2001). We tested these

predictions by surveying the epilimnetic zooplankton in many lakes along the fish gradient (neolimnological, cross-sectional approach), and we further examine the first two predictions by studying cladoceran resting eggs (ephippia) from historic sediment deposits in one invaded lake (paleolimnological, longitudinal approach; Korhola & Rautio, 2001; Jeppesen et al., 2002; Mergeay et al., 2004).

4.3 Methods

Study area & fish variables

Our study includes 21 lakes from the region of Aysén, Chilean Patagonia $(\sim 46^{\circ}S)$, varying in altitude, size, shape, etc. (Table 1). [For a complete description of the study lakes and a map, see Correa and Hendry (2012)]. Our original survey involved 25 lakes, but two lakes were excluded for the lack of quantitative samples (Juncos and Thompson), and two lakes were excluded because these were the only lakes where galaxiids other than G. platei were present (Alta and Palos). Correa and Hendry (2012) recently showed a strong negative association between the abundances of trout and G. platei in these lakes, and proposed a few instances of galaxiid extirpations due to the negative effects of invasive trout. These findings opened the possibility of studying the cascading effects of invasive trout on the zooplankton community through a survey approach, which is made possible because very few environmental variables were found to covary with the fish gradient (Correa & Hendry, 2012), thus limiting the potentially confounding effect of other factors. Stream order at lake outlet (lake order), however, was found to be positively related to trout abundance and negatively related to G. platei abundance. Because there is sustantial evidence for a causal pathway between these variables, whereby higher lake order results in higher trout abundance, and higher trout abundance results in lower G. platei abundance (Correa & Hendry, 2012), we decided to leave out lake order from all analyses described below.

In the present study, we needed an index of the fish community. For this we used fish catch-per-unit-effort (CPUE) data published earlier (Correa & Hendry, 2012). Those data comprise CPUE estimates derived from multi-pannel

survey gillnets (G, used as suffix), electric fishing (E), and (minnow) trapping (T). The first two methods yielded CPUE for trout (TG, TE) and *G. platei* (PG, PE), whereas trapping was only effective for quantifying *G. platei* (PT). [PT was originally expressed as $\ln(0.01 + \text{mean-catch}*\text{trap}^{-1})$ and herein as $\ln(1 + \text{mean-catch}*\text{trap}^{-1})$]. These indicators of fish abundance were used as explanatory variables or as the basis to calculate explanatory variables (see below).

Neolimnology - plankton

Pelagic zooplankton collections took place in a narrow seasonal window during the austral summer of 2007 or 2009 [Jan 17th through Mar 18th; with most (n = 13) lakes sampled in Feb]. This schedule minimized potential biases associated with seasonal species turnover in Patagonian lakes while also facilitating the effects of high spring-summer fish predation intensity (Modenutti et al., 1993; Modenutti et al., 1998). Samples were collected by hauling a plankton net vertically (20 cm diameter; 150 μ m pore size; estimated volume filtered ~314 L⁻¹) from a depth of 10m to the surface (from 5m in Leon Lake that is 8m deep), and the catch was preserved in ~5% formalin solution. Two samples per lake were collected within 24h of each other, one at midnight and one at midday (± 2h). These paired samples allowed us to estimate diel vertical migration (see below).

In the lab, the samples were split repeatedly using a Folsom splitter until we obtained an aliquot of 200-500 microcrustacean individuals. One sample of the last split pair was examined under the microscope and individual microcrustaceans were classified and enumerated. Specifically, we identified taxa as calanoid copepods of the genus *Boeckella*, cyclopoid copepods, bosminid cladocerans of the genus *Bosmina* (*Neobosmina*); or the daphniid cladocerans *Ceriodaphnia dubia*, and *Daphnia curvirostris* (henceforth we only use the genera names for short). Organisms sparsely represented, immature stages, or non-zooplankton were excluded (e.g., Chydoridae, Acari, Chironomidae, nauplii, copepodid larva, and phytoplankton). Densities were obtained by multiplying the counts by the dilution factor, such that counts are expressed as individuals * 314L⁻¹. We also measured the body length of *Bosmina* (17 lakes; median[range]

number of individuals, 36[31-39]) and *Ceriodaphnia* (17; 36[21-43]) from subsample of individuals when they were present in sufficient densities. Specifically, aliquots of plankton were deposited on a gridded Sedgwick rafter counting cell and individuals were photographed using a digital camera attached to a compound microscope set to 100X magnification. Measurements were made from the tip of the head shield to the base of the posterior carapace process (mucro; *Bosmina*) or posterior-dorsal margin the carapace (*Ceriodaphnia*).

Analysis of community composition

We used multivariate approaches to study the top-down effects of fish abundance on zooplankton community composition. We chose two fundamentally different statistical approaches to illustrate how robust our general conclusions are; other possible analytical approaches were omitted for space limitations. We first adopted an unconstrained, non-eigenvalue, parametric-assumptions-free, ordination technique to clearly visualize lake similarities (differences) in the relative abundance of zooplankters, and to overlay environmental information. Specifically, we conducted a two-dimensional non-metric, multi-dimensional scaling (NMDS) on a Bray-Curtis distance matrix calculated from the forth-roottransformed zooplankton density data, and plotting the NMDS-scores in 2dordination space (Clarke, 1993; Borcard et al., 2011). The distances among lakes in the resulting plot reflected, as best as possible, their ranked multivariate distances in zooplankton composition. Furthermore, we regressed the fish-CPUE variables on the two axes of the NMDS biplot to obtain vectors representing fish abundances. The same approach was used to represent one prominent multivariate environmental gradient of lakes' physical attributes: scores of the first principal component (PC1; scaling =2) of principal component analysis (PCA) conducted on the correlation matrix of lakes' shoreline development index, altitude (m), specific conductance (μ S *cm⁻¹), Secchi depth (m), watershed deforestation (%), area [ln(m)], and maximum depth [ln(m)] (Correa & Hendry, 2012). PC1 represented 48.3% of the variance of environmental variables. Because PC2 represented substantially less variance (<20%) and the broken stick model only promoted PC1, we only used PC1 to define the environmental gradient (Borcard

et al., 2011). Statistical significance of the R^2 of the vectors fitted to the NMDS was tested by permutation test (10⁴ permutations). We used the R functions 'metaMDS' and 'envfit' (package vegan) to conduct the NMDS, and analyze the vectors, respectively (Oksanen et al., 2011).

For the second approach, we adopted a constrained, eigenvalue, linear, and multivariate-normal technique to formally test the predictive value of the fish and environmental-gradient variables on the zooplankton community composition matrix. Specifically, we conducted transformation-based redundancy analyses (tb-RDA) on the Hellinger-transformed (Legendre & Gallagher, 2001) zooplankton density data (response matrix), and a table containing the fish CPUE data and the PC1-environmental-gradient (explanatory matrix). The main procedures involved fitting a 'global' tb-RDA model, testing its overall fit and the fit of each canonical axis (permutation test; 10^4 permutations; $\alpha = 0.1$), computing the adjusted coefficient of determination R²_{adi} (Peres-Neto et al., 2006), and then repeating these steps after simplifying the model by forward selection (with doublestopping criterion; Blanchet et al., 2008). We used the R functions 'rda', 'anova.cca', and 'RsquareAdj' of package vegan (Oksanen et al., 2011), and 'forward.sel' of package packfor (Stephane Dray with contributions of Pierre Legendre and Guillaume Blanchet, 2011). The results were illustrated using scaling-2 triplots that simultaneously displayed fitted lake scores (i.e., "lc" type scores), response variables, and explanatory variables (for more details see Borcard et al., 2011).

Analysis of population size structure

We analyzed the cladoceran length data (*Bosmina* and *Ceriodaphnia*) using generalized linear mixed effect models, which in their 'full' or most complex form can be written as:

 $ln(length) \sim species + PT + species:PT + (1|lake) + f$

where 'length' is the body length (defined above), 'species' is the fixed effect of species membership, 'PT' is the fixed effect of the CPUE of *G. platei* (defined above), 'species:PT' is the interaction term, '(1|lake)' is the random effect of lake membership, and *f* is a power residual-variance-function of the fitted values to

allow for heteroscedasticity. The latter variance structure was chosen from among several other options on the basis of the minimum small-sample size-corrected Akaike information criterion (AICc), keeping the above fixed structure unchanged, and using restricted maximum likelihood estimation (REML) (for further details on the variance functions, see Zuur et al., 2009, P. 74-80). The statistical significance of the model's fixed structure was studied by sequentially dropping (fixed) terms and comparing nested models, fitted by maximum likelihood (ML), using likelihood ratio test (LRT; Zuur et al., 2009). We also ranked the set of (nested) models based on increasing AICc (Gelman & Hill, 2007; Zuur et al., 2009).

Analysis of diel vertical migration

Predation by planktivorous fish could select for enhanced zooplankton diel vertical migration. If this prediction holds in our study system, then the probability of obtaining denser superficial zooplankton samples during the day than during the night should be lower in lakes with abundant *G. platei* and few trout. We measured the probability of obtaining a denser diurnal sample (Pr-day) by species and lake using the formula:

Pr-day = d-day / (d-day + d-night)

where *d* stands for density as defined above. (Alternative indices of diel vertical migration require stratified information of the whole water column and could not be used: e.g., Leech et al., 2005). Note that our Pr-day measure could only be effective if the depth range of the plankton samples (10 m) was (1) shallow enough to capture fish-predation avoidance, and (2) deep enough to exclude the confounding effect of ultraviolet-radiation (UVR) avoidance (Moore, 1912; Hansson et al., 2007). Admittedly, an optimal depth for predation and UV avoidance likely varies from lake to lake, but our Secchi depth measurements together with experimental and field observations suggest that zooplankton diel vertical migrations in Patagonian lakes generally traverse the depth of 10m (Morris et al., 1995; Zagarese et al., 1998; Alonso et al., 2004; Marinone et al., 2006; Balseiro et al., 2007; Rechencq et al., 2011).

We analyzed Pr-day as a function of the general fish assemblage gradient. Specifically, we conducted a PCA on the scaled fish CPUE data. PC1 represented 62.2% of the variance in fish CPUE, and correlated positively with the *G. platei*-CPUE variables and negatively with the trout-CPUE variables (loadings: PT = 1.18, PE = 1.15, PG = 0.92, TE = -0.99, TG = -1.30). Hence, the scaling 2 site scores of PC1 were indexed as an explanatory variable (i.e., fish-gradient). PC2 was discarded because it represented substantially less variance (16.6%) and the broken stick model only promoted PC1 (Borcard et al., 2011). We then modeled Pr-day using the following 'global' linear mixed model:

logit(Pr-day) ~ fish-gradient + taxon + fish-gradient* taxon + (1|lake)

where the last two terms allow for a taxon-specific response to the fish gradient and a random effect of lake identity, respectively. To test the importance of lake, the model was first fitted by REML and compared with a reduced model devoid of the random term using LRT (Zuur et al., 2009). Subsequently, we based our conclusions on comparisons of the five possible submodels that resulted from dropping the fixed effects while keeping the random effect. These models were fitted with ML, ranked on the basis of increasing AICc, and tested for statistical significance using LRT. In order to avoid a disproportionate influence of uncertain Pr-day that were based on sparse counts, lakes were only included in the analysis if we counted at least 50 individuals in total (day and night).

Paleolimnology – history, chronostratigraphy & plankton

To complement our neo-limnological study, we conducted a paleolimnological, longitudinal study of Pollux Lake, one of the salmonid-invaded lakes with relatively good historical records. Lago Pollux is a medium-size (879ha, 55m deep), elongated, second order lake, located downstream from lakes Castor and Thompson (Correa & Hendry, 2012). The terrestrial native vegetation above the lake's altitude (679 m) in the region is dominated by sub-Antarctic deciduous Nothofagus forest, although the Patagonian steppe flora dominates close to mountain summits (Gajardo, 1994). Beginning in the 1930s and likely becoming more extensively in the 1940s and 1950s, the forest was burnt for pasture (Butland, 1957; Yarrow & Torres, 2009). These activities resulted in ~40% loss of the original forest within the watershed (Correa & Hendry, 2012).

Salmonids were first introduced into Lake Pollux in 1968, when 78,000 *O. mykiss* fingerlings (unreported age/size) were stocked and quickly established (Sakai, 1989). Later, in the period 1985-1988, 207,000 *O. masou* parr and smolts were stocked (0+ and 1+ year classes; 4.9–11.2 cm mean fork length). Although survival and growth of these stocked *O. masou* may have been favorable in subsequent years, recruitment seemed compromised at least in 1988 (Sakai, 1989) and today they are, at best, present in relatively low density. Recent surveys only detected *O. mykiss* and *G. platei* in this lake. The CPUE of *O. mykiss* was high relative to that of any salmonid in most other lakes studied, and much higher than that of *G. platei* in Pollux lake (Correa & Hendry, 2012). Although no historical records are available, it seems likely that *G. platei* thrived in this lake before the introduction of salmonids given their known predominance in other nearby lakes where salmonids are rare or absent (Correa & Hendry, 2012).

Coring and chronostratigraphy

A core of lake sediments was retrieved in February11th, 2007, by means of a gravity corer deployed near the deepest part of the lake's basin at 47m of depth (45°40'30"S, 71°51'50"W). The core (44.6 cm long) was sectioned in the field (see sectioning scheme in Appendix A), and the sediment samples were stored in plastic whirl bags. Back in the lab, the sediment samples were lyophilized, and aliquots of sediment from 16 strata spanning the core length (but mainly concentrated in the upper half) were prepared for radiometric dating. The radioisotope activities of 210Pb, 214Pb, and 214Bi from a known mass (0.4 to 1 g) and volume of freeze-dried sediment were measured for 24 h using a Canberra well-detector gamma ray spectrometer. Sediment ages were calculated using the constant-rate-of-supply (CRS) model (Binford, 1990). To confirm the exactitude of the CRS model dating, we measured magnetic susceptibility (MS) in every sample from the core and identified the depth of the peak that is coincident with a tephra layer deposited during the 1991 Hudson Volcano eruption (i.e. an eruption that is known to have covered the region with volcanic ash; Naranjo & Stern, 1998; Kratzmann et al., 2010). To measure MS, dry sediment samples were placed in 10 mL plastic vials and measured with a Bartington MS2B meter, calibrated with a standard of known MS.

Recovery of subfossil remains

The sediments were then used to reconstruct some features of the lake's invertebrate community that might have been influenced by the introduction of salmonids and the subsequent decline of G. platei. Previous paleolimnological work has documented that changes in fish predation pressure alters the ratio small-bodied to large-bodied zooplankton species, and affect individual body size (Jeppesen et al., 2002; Amsinck et al., 2007). As a result of our neo-limnological study of Pollux Lake (Results: Population size structure), we expect that the two dominant pelagic cladocerans show changes in their relative abundance because Ceriodaphnia is generally larger than Bosmina and presumably experienced stronger predation pressure from planktivorous galaxiids (Modenutti et al., 1993). Given that ephippia of both *Ceriodaphnia* and *Bosmina* are relatively easy to recover and identify in sediments and can provide valuable information on the size structure and morphology of mature females (Korhola & Rautio, 2001; Amsinck et al., 2007), we chose ephippia as proxy indicators for relative abundance and morphometrics of these species. In addition, we quantified the postabdomens of a large benthic cladoceran, Alona affinis, because it is a likely prey of small galaxiids in the littoral zone. Despite the specific habitat preferences (littoral-benthic for Alona and planktonic for Ceriodaphnia and Bosmina; Korhola et al., 2000; Nevalainen, 2011), examination of remains recovered from the sediment at one off-shore location in the bottom of a lake's basin is generally sufficient because transport and mixing of the remains before deposition generally ensure an integrated sample across habitats (Korhola & Rautio, 2001).

To identify and enumerate the ephippia and postabdomens, we followed standard procedures (Jeppesen et al., 1996; Korhola & Rautio, 2001; Smol et al., 2001) with minor modifications. Basically, \sim 1 g of dry sediment was mixed in a beaker with \sim 100 mL of 10% KOH solution, heated to 70-80°C for 45min while stirring occasionally with a glass rod, and then washed, over a 37 µm sieve, with a

gentle tap-water jet for at least 5min. The retained fraction was transferred back to the beaker with ~20 mL of distilled water, and 4 drops of safranin-glycerin-water solution were added to stain the remains. The following day, the samples were rinsed with tap-water over 150 μ m and 37 μ m nested sieves, and the retained fractions were transferred to 15 mL centrifuge vials with some water. We analyzed the >150 μ m fraction by sequentially scanning under the microscope (100X magnification) 1 mL aliquots on a Sedgewick rafter counting cell gridded every 1 mm until completing the sample. Upon encountering an ephippium, ephippium remain, or postabdomen of the selected species, a digital picture was taken after switching to higher magnification (200X). To correct for fragmented ephippia (*Ceriodaphnia*), we counted complete ephippia and added the minimum number of ephippia that could have generated the sample of fragmented ephippia.

Proportion Ceriodaphnia

With the counts of pelagic cladocera, we calculated the proportion of *Ceriodaphina* relative to the sum of *Ceriodaphnia* and *Bosmina*, hereafter Pr(Ceriodaphnia) (Kitchell & Kitchell, 1980; Amsinck et al., 2003; Jeppesen et al., 2003; Amsinck et al., 2007). We also used the counts of the three cladocera species to calculate fluxes (or accumulation rates) to gain a better understanding of temporal variations in net abundance (Kerfoot, 1974; Amsinck et al., 2007). Fluxes were calculated by multiplying concentration [relative to volume of wet mud analyzed (i.e., $\# * \text{ cm}^{-3}$)] by sedimentation rate (i.e., cm * year⁻¹) inferred using the CRS model. Hence cladocera fluxes were expressed in number-per-unit-area-per-year ($\# * \text{cm}^{2}*y^{-1}$).

Ephippia morphology

Additionally, the morphometry of ephippia was also investigated making measurements on the images with a digital caliper calibrated to the counting cell grid. In the case of *Ceriodaphnia*, ephippium length was measured along the longest anteroposterior axis of the ephippium (n = 325 structures measured). In cases where only the internal egg membrane was encountered (n = 122), the length of the ephippium was estimated from the equation EL = 0.0668 + 1.2217 * EGL, where EL and EGL are the ephippium and egg length (mm), respectively,

and the equation was the result of regressing EL on EGL using the pooled data of intact ephippia ($R^2 = 0.76$, F(1, 131) = 405.1, P < 0.001). If only the external shells were encountered, EL was directly measured on them (n = 70). Heavily damaged fragments were not measured. For *Bosmina*, ephippium length was measured from the anterodorsal extreme of the shell to the base of the mucro (n = 489), and the mucro was measured from base to tip (n=453). To remove the effect of ephippium length on mucro length (*Bosmina*), the latter was transformed to residuals after being regressed nonlinearly to ephippium length, using all pooled available data from complete ephippia (n = 453). The fitted model had sigmoid shape and is defined by the equation ML = 0.0742 - 0.0349 / ($1 + (EL / 0.4425)^{23.5541}$), where ML and EL are mucro and ephippium length (mm), respectively.

Data analyses

To analyze temporal trends without imposing pre-conceived fixed structures (e.g., linear), we adopted a general additive modeling approach (GAM; Zuur et al., 2009). This approach allowed, for example, the identification of a positive linear trend in addition to a non-linear smoother function (the latter modeling the residuals of the linear term). By using LRT, we were able to test statistically the contribution of linear or non-linear model terms to overall model deviance (Zuur et al., 2009). Non-significant terms were subsequently dropped. In the case of individual data nested within sediment strata (i.e., morphometric data) we used a similar approach yet implemented in a general additive mixed model framework (GAMM). Models were fit in R (version 2.11.1; R Development Core Team, 2010) using the 'gam' function in the case of non-nested data, or the 'gamm' function in the case of nested data ('mgcv' package; Wood, 2006), and we conducted the LRTs using the R function 'anova'. As a further reassurance of model selection, or when comparing non-nested models, we used the AIC implemented in the R function 'AIC' (Zuur et al., 2009; R Development Core Team, 2010). Data transformations and specific residual error structures were adopted as needed to eliminate patterns in standardized residual plots (Zuur et al., 2009). The best residual error structure was selected on the basis of AIC using

REML estimation (Zuur et al., 2009). Follows a brief description of the specific models selected.

For all of our analyses, we decided to model all response variables as a function of the sediment depth because the chronostratigraphic dating estimates are only really accurate within the top portion of the core (Appendix A). Thus to model fluxes of cladoceran remains, we used species as an explanatory variable in addition to sediment depth, where the full GAM model can be written as:

```
ln(1 + flux) \sim species + depth + species*depth + s(depth) + e
```

where 'flux' has been log-transformed, 'species' and 'depth' are categorical and continuous explanatory variables, 'species*depth' is an interaction term, 's(depth)' is an smoothing function of depth, and 'e' is the residual error modeled as a gamma distribution on log-residuals.

For ephippia morphometrics, we used GAMM by species with the following general model structure:

```
length ~ depth + s(depth) + (1|StratumID) + f
```

where 'length' is the response variable in question, 'depth' and s(depth) as above, '(1|StratumID)' is a random intercept per sediment stratum that are treated as categorical, and 'f' is a (Gaussian) power (ephippium length-*Ceriodaphnia*), or exponential (ephippium length-*Bosmina*), residual function of the fitted values or sediment-depth, respectively (i.e., functions 'varPower' and 'varExp'; Zuur et al., 2009, P. 78). In the case of mucro-length the chosen model was homoscedastic (i.e., f was a normal random variable).

Finally, the Pr(*Ceriodaphnia*) was estimated using the Binomial distribution in a Bayesian framework to obtain posterior probabilities and 95% credible intervals (CrI; for details see McCarthy, 2007, P. 36).

4.4 Results

Community composition

The non-metric, multidimensional scaling (NMDS) ordination showed that the zooplankton communities are heterogeneous among lakes and that this variation was correlated to changes in the fish community (Figure 1). In particular, we found that lakes with higher abundance of G. platei (e.g., higher mean catch-per-trap) were characterized by a dominance of the small cladoceran (Bosmina) whereas lakes with lower abundance of G. platei had greater densities of large cladocera (Daphnia) or calanoid copepods (Boeckella). These qualitative observations were supported by *a posteriori* statistical analyses. For example, the NMDS ordination scores explained a significant proportion (\sim 35%) of the mean catch-per-trap of G. platei (PT), and this was represented by a vector pointing on the direction of expected higher fish abundance, based on the plankton composition (i.e., relatively more Bosmina). We also found that the trout's CPUEvectors were marginally significant and were associated with Daphnia and Boeckella. The relative abundance of cyclopoid copepods and Ceriodaphnia seemed to be most associated with environmental variables summarized as PC1, whereby smaller, shallower, rounder, more deforested, more conductive, and higher altitude lakes had higher PC1 loadings. However, the PC1 vector was not significant predictor of the overall zooplankton assemblage (Figure 1).

The constrained canonical ordination identified patterns that were consistent with those identified above. The transformation-based redundancy analysis (tb-RDA) using all six variables was marginally significant (permutation test, P = 0.0991) so we ventured to interpretation. All fish and the environmental variable explained 41.6% of the total variance in zooplankton community composition ($R^2_{adj} = 0.166$), and the first constrained axis (RDA1) was the only significant explanatory axis and responsible for most (72.6%) explained variation (permutation test, P = 0.0024). PT was the variable that loaded heavier in RDA1, whereas the least influential was the environmental variable (PC1). RDA1, in turn, was positively correlated to *Bosmina*, and negatively correlated to the other species, particularly *Boeckella* (Figure 2a). Using a forward selection procedure, we found that the only variable that entered the model was PT, and that the model fit ($R^2_{adj} = 0.22$; P = 0.0026) was superior to the model where all possible predictors were included. We also found that PT was positively correlated to *Bosmina* densities, and negatively correlated to the other species, particularly

Boeckella (Figure 2b). We further investigated the *Bosmina* dominance among cladocerans by conducting logistic regression. We found a strong positive effect (Figure 3): *Bosmina* tend to become the dominant cladoceran as soon as one individual *G. platei* fish was caught for every two traps set overnight [Pr(*Bosmina* is dominant) = logit⁻¹(-1.1403 + 2.5062 * PT); LRT χ^2 = 9.5014, df = 1, *P* = 0.0021].

Population size structure

Based on linear mixed effect modeling, we found that PT had a negative effect on cladoceran body length (BL) and that this effect is stronger in the larger species. These results, however, were obtained through a series of iterative modeling steps. First, we evaluated model heteroscedacity and found that by allowing the residual variance of log-BL to increase as a power function of the fitted values (AICc = -441.27; model probability = 0.44) we significantly reduced the deviance of the homoscedastic model (LRT, L = 84.2740, df = 1, P < 0.0001). With regard to the random effect, the variance due lake membership was important; it captured 12-39% of the random variance in the fitted model (i.e., intraclass correlation evaluated at the highest and smallest fitted value; Gelman & Hill, 2007, P. 258). Consequently, the random effect of lake membership, while controlling for PT and species, was highly significant (LRT, L = 219.7444, df = 1, P < 0.0001). In addition, we found that including an interaction between the effect of species and PT was by far the best AICc model (AICc = -462.54; model probability = 1.00). Ceriodaphia was generally larger than *Bosmina* yet with considerable overlap (Figure 4; LRT, L = 601.4716, df = 1, P < 0.0001). Once accounting for the effect of species, PT had a negative effect on log-BL (LRT, L = 5.1868, df = 1, P < 0.0228), yet the latter effect was stronger in *Ceriodaphnia* (Figure 4; LRT, L = 15.1790, df = 1, P < 0.0001).

Diel vertical migration

Zooplankton diel vertical migration varied substantially among lakes (Figure 5). The random effect of lake significantly enhanced the model fit (LRT, L = 15.6875, df = 1, P = 0.0001), yet the effects of zooplankton taxon and the *G*. *platei*-trout fish countergradient were not important. In fact, the best AIC-model

was the model devoid of all fixed effect (AICc = 111.64; model probability = 0.70). Lake membership alone was able to capture about 80% of the total variance (intraclass correlation = 0.8). The second best model included the negative effect of fish-gradient (AICc = 113.87; model probability = 0.23), but this term was not statistically significant (LRT, L = 0.0607, df = 1, P = 0.8054). The high importance of lake relative to species-membership suggests that all taxa are responding similarly to environmental cues that differ between lakes, but whether the fish assemblage is related to these cues remains unclear.

Historical change reconstruction

The chronostratigraphy of Lake Pollux's sediment core suggested that the depth of the sediment that marks the transition from an uninvaded- to a salmonidinvaded lake is ~15 cm of depth (Figure 6a; the chronostratigraphic results are detailed in Appendix A). The uncertainty associated with the CRS model at this depth ($\pm \sim 15$ years) prevents us from pinpointing the exact date, but the consistent match between the 1991 Hudson tephra (as detected by our magnetic susceptibility analyses – see Appendix A) and the estimated CRS dates give us some confidence on the aging model. The three cladoceran taxa showed a general trend of an increase in flux from the bottom to the top of the core (i.e., from preto post-invasion times). However, this trend was complicated by non-linear relationships and species-specific effects (Figure 6b). The ephippia fluxes of the planktonic Ceriodaphnia and Bosmina showed similar fluctuations throughout the core-depth, with flux minima below 20 cm, and maxima between 2-8 cm. The postabdomen flux of the littoral-benthic *Alona* showed a more monotonic increase from the bottom to the top of the core, yet all three species had a relatively low flux in the topmost sediment layer. Consequently, the model that best fitted the data had a species-depth interaction term (LRT, deviance = 8.89, df = 4.00, P = 0.003), a nonlinear smoothing function (LRT, deviance = 8.38, df = 4.22, P =0.003), and a monotonic negative effect of sediment depth [slope(SE) = -0.0669(0.0140); ANOVA, df = 1, F = 22.925, P < 0.0001]. This model explained 69% of the deviance.

Considering only the pelagic cladocera, we found that the proportion of *Ceriodaphnia* relative the sum of *Ceriodaphnia* and *Bosmina* [Pr(*Ceriodaphnia*)] fluctuated greatly through the sediment profile (Figure 6c). The greatest proportions were recorded at a depth of 15-22 cm when Pr(Ceriodaphnia) reached >50%, significantly higher than the proportions observed either at the bottom or the top of the profile (i.e., non-overlapping 95%CrI; Figure 6c). No clear (linear) trend encompassed the overall depth-range.

The size structure analyses of ephippium length of the pelagic cladocerans showed that the different taxa elicited different responses. With regard to the mean ephippium length of Ceriodaphnia, sediment depth did not have a significant effect (Figure 6d). The model that best described the data was a mixed model including the random effect of stratum-id, a negligible linear effect of sediment depth [ANOVA, F(1) = 0.9100, P = 0.3410], and a significant, positive effect of sediment-depth on the residual variance (LRT, L = 27.3589, df = 1, P < 10000.0001). The non-linear appearance of sample means was absorbed by the model's random factor (stratum-id) because the non-linear smoother did not reduce the deviance significantly (LRT, L = 0.2083, df = 1, P = 0.6481). In contrast, the mean ephippium length of *Bosmina* tended to increase from the bottom to the top of the core (Figure 6e). The model that best descried the data was a linear mixed model including the random effect of stratum-id, a significant, negative linear effect of sediment depth (slope(SE) = -0.0028(0.0006); LRT, L = 16.91033, df = 1, P < 0.0001), and a significant, negative effect of sediment-depth on the residual variance (LRT, L = 36.5536, df = 1, P < 0.0001). Interestingly, if we only included samples where at least five individuals were considered, and one outlier was excluded, we found that the mean ephippia length of Ceriodaphnia was correlated with that of Bosmina (r = 0.84, t = 4.0406(7), P =0.0049).

We also conducted analyses of *Bosmina*'s mucro length (size-adjusted) and found that this metric showed a similar pattern to ephippium length data (Figure 6f). The model that best described the *Bosmina* mucro data was a linear mixed model including the random effect of stratum-id, and a significant,

negative linear effect of sediment depth on mean adjusted mucro length (slope(SE) = -0.0002(0.0001); LRT, L = 3.9208, df = 1, P = 0.0477).

4.5 Discussion

This is the first study showing the top-down effects of *G. platei* in replicated lakes without the confounding effects of several fish species. Among cladocerans, there is a stark range in vulnerability to fish predation, whereby larger species are generally more vulnerable than smaller species (i.e., size-efficiency hypothesis; Brooks & Dodson, 1965). Our results and those of others (Modenutti et al., 1993) suggest that Patagonian lakes conform to this theory, particularly in the small to medium size lakes that are both strongly represented in our dataset and in many regions around the world (Downing et al., 2006). Furthermore, we show that a single fish species that is only strictly planktivorous during early life is sufficient to exert strong top-down zooplankton control.

Shifts in community structure

Cladocerans were particularly informative in detecting the top-down control. Consistent with one of our predictions, for example, we found that *Bosmina*, a small pelagic taxon, was dominant in the pelagic samples from lakes where G. platei were abundant. This observation does not indicate a lack of predation of galaxiids on bosminids (Modenutti et al., 1993; Barriga et al., 2012) but rather point to how resilient bosminids are to fish predation (Brooks & Dodson, 1965; Modenutti et al., 1993; Jeppesen et al., 2003). The relative success of bosminids despite planktivory is (partly) due to size-selective predation and earlier collapses of larger, more vulnerable cladocerans that otherwise hold foraging competitive advantage (Brooks & Dodson, 1965). One such competitor is Ceriodaphnia (Modenutti et al., 1998). This medium-size cladoceran is abundant and even dominant in many Patagonian lakes (Modenutti et al., 1998; Balseiro et al., 2007), but has been shown to be severely affected by galaxiid predation in one small lake (Modenutti et al., 1993). Thus, it is not surprising that Ceriodaphnia rarely dominates in lakes with abundant G. platei. On the predation-vulnerability axis, Daphnia exceeds all other pelagic cladocerans

(Brooks & Dodson, 1965). Due to its large size (>1 mm), *Daphnia* is a profitable prey not only for specialized planktivorous fish, but also for generalists such as trout and large galaxiids (Galbraith, 1967; Kitchell & Kitchell, 1980; Jeppesen et al., 1997b; Biro et al., 2003; Reissig et al., 2006; Fisk et al., 2007). Our results are consistent with earlier surveys of Patagonia lakes, where *Daphnia* tended to occur in lakes with little or no fish predation risk (Reissig et al., 2006; Soto & De los Ríos, 2006; Balseiro et al., 2007).

Calanoid copepods of the genus Boeckella also presented a strong distributional pattern, yet dominating more often in lakes with few G. platei and abundant trout. This might seem counter-intuitive considering the lower visibility and higher evasion rate of calanoid prey (cf. Cladocera; Wright & O'Brien, 1984; Butler & Burns, 1991). However, *Boeckella* can be heavily preved upon by G. maculatus at the nauplii (Modenutti et al., 1993) or at the nauplii and adult stages (Barriga et al., 2012), and so is most likely also preyed upon by G. platei. Modenutti et al (1993) interpreted the failure of the *Boeckella* summer cohort in a shallow Patagonian lake as a consequence of predation on nauplii. These antecedents, together with our results, suggest strong top-down control on *Boeckella*. An alternative hypothesis is that *Boeckella* is a competitive grazer at low phytoplankton concentration (i.e., sensitive to bottom-up control), typical of deep oligotrophic Patagonian lakes (Soto, 2002). To better discriminate between these two hypotheses we fit two simple linear statistical models representing the bottom-up and the top-down control hypotheses. For bottom-up we used Secchi depth as a surrogate for phytoplankton dilution, and for top-down we used G. platei-CPUE by trapping as a surrogate for predator abundance. The slopes of the regressions of the relative abundance of Boeckella (% over all taxa) on the (standardized) explanatory variables took the expected directions, positive for the bottom-up (marginally significant) and negative for the top-down (significant) [bottom-up, slope(SE) = 12.413 (6.444), t = 1.926(19), P = 0.07, $R^2_{adi} = 0.12$; topdown, slope(SE) = -14.316(6.233), t = -2.297(19), P = 0.03, $R_{adi}^2 = 0.18$]. In addition, the top-down model was slightly better based on information-theoretic ground (delta-AICc = 1.4, conditional model probability of (1) = 0.67; Anderson,

2008). Furthermore, if the effects of phytoplankton dilution are accounted for by regressing the residuals of that model on the response variable of *G. platei*-CPUE, the negative slope of the resulting regression is still negative yet non-significant [slope(SE) = -9.407 (6.072), t = 1.549(19), P = 0.14, $R^2_{adj} = 0.07$]. Our results and these *a posteriori* data explorations support the top-down hypothesis, at least as an additional mechanism affecting *Boeckella*'s fitness.

Another alternative is that *Boeckella* have few resources to counteract the damaging effects of ultraviolet radiation aside from migrating deep into the water column and thus would be rare or absent in shallow lakes, regardless of fish predation (Alonso et al., 2004; Marinone et al., 2006). We doubt this mechanism can explain the patterns observed, however, because only very shallow lakes would be severely compromised and these were rare in our sample (two lakes \leq 10 m deep; more about this below). This constraint is less stringent in species with photorecovery (e.g., *Ceriodaphnia*) or protective pigments (Zagarese et al., 1998; Marinone et al., 2006).

We attempted to confirm some of the community composition results by reconstructing these changes historically in a lake invaded by salmonids. Based on our neolimnological results, as well as previous paleolimnological studies (Jeppesen et al., 2003), we thought that the ratio of Ceriodaphnia to Ceriodaphnia + Bosmina [Pr(Ceriodaphnia)] would be an adequate index to infer planktivorous fish abundance changes in a single lake. However, we did not find the expected increase in Pr(Ceriodaphnia) based on ephippia counts after the introduction of salmonids. Pr(*Ceriodaphnia*) fluctuated greatly, yet was not strongly related to historical fish introduction events. We interpret this negative result with great caution, given two potential pitfalls of our proxy. First, although generally the abundance of ephippia in the sediments from European lakes significantly reflects the abundance of these cladoceran in the water column, the relationship for Ceriodaphnia is weak (Jeppesen et al., 2003). Second, the rate of ephippia production, at least in Bosmina, can vary greatly depending on environmental conditions (Jeppesen et al., 2003), and therefore Pr(Ceriodaphnia) would be very sensitive to differential responses between species. In future studies, we

recommend calculating Pr(*Ceriodaphnia*) based on counts of somatic structure such as carapaces or headshields (*Bosmina*) and postabdominal claws (*Ceriodaphnia*). With regards to the cladoceran fluxes, they might present similar problems except for *Alona*'s which were based on postabdomen counts. It is noteworthy that all three cladoceran showed a marked general increase in fluxes after salmonids introductions, which agrees with the hypotheses of reduced fish predation in that period. Yet the fluxes begun to increase arguably earlier, perhaps as a consequence of deforestation and increase in lake productivity due to increased nutrient runoff from the watershed (Kerfoot, 1974).

Shifts in morphology

The analysis of individual body lengths for Bosmina and Ceriodaphnia collected in the pelagia of lakes during the day further supported the top-down control and the size-efficiency hypothesis (Brooks & Dodson, 1965), but the paleolimnological data were less clear. For the neolimnological samples, the observed patterns closely matched our predictions; the average length of both species declines as the abundance of G. platei increases, and this effect was stronger for Ceriodaphnia, the larger species. For our paleolimnological study, we were only able to confirm an increase in Bosmina body length, which was presumably related to the introduction of salmonids and drastic decline of G. platei (Correa & Hendry, 2012). The lack of a clear temporal trend with Ceriodaphnia ephippia size, however, could be due to a number of causes including invertebrate predation (Galbraith, 1967; Zaret & Kerfoot, 1975; Kitchell & Kitchell, 1980; Jeppesen et al., 2002; Sweetman & Finney, 2003). To explore whether invertebrate predation varied over the sequence of our record, we conducted a historical reconstruction of *Bosmina* morphology, indexed as the length of posterior carapace processes (mucrones; singular, mucro), which has been shown to reflect invertebrate predation regimes. Mucrones are part of Bosmina's defence mechanisms against invertebrate predators. For example, they can frustrate prey handling by predatory cyclopoid copepods (Kerfoot, 1977; Post et al., 1995). In Pollux Lake, paleolimnological samples indicate a proportional mucro length increase towards recent years suggesting increased selection in response to invertebrate predation, possibly due to the relaxation of pelagic fish planktivory (Post et al., 1995; Sweetman & Finney, 2003). Hence, the selection regime leading to larger Bosmina might correspond to a combination of decreased fish predation pressure (favouring smaller individuals) and increased invertebrate predation (favouring larger individuals; O'Brien et al., 1979; Sweetman & Finney, 2003). Unfortunately, it will not be possible to confirm an historical increase in predatory copepods in Pollux Lake because copepods are generally not preserved in the sediment record (Davidson et al., 2007). The contemporary sample, on the other hand, is of limited use to elucidate this mechanism since the pelagos of Lake Pollux at the time of sampling was strongly dominated by Boeckella and Ceriodaphnia, whereas Bosmina and predatory cyclopoids were uncommon (combined, 4% of density). Predatory calanoids were absent. In sum, we have shown historical morphological changes in Bosmina that are in concert with our main predictions, although invertebrate predation likely played a role which is hard to factor out. Clearly, the functional morphology of *Bosmina* is complex and there are other candidate traits that are potentially important in predator-prey interactions (Zaret & Kerfoot, 1975; Hessen, 1985; Hellsten et al., 1999).

Diel vertical migration

An additional approach to evaluate top-down control of zooplankton assemblages was to measure diel vertical migration. Traditionally this has been interpreted as a response to food and visual fish predation but there is mounting evidence promoting alternative causes (De Meester et al., 1999; Williamson et al., 2011). The leading alternative is avoidance of DNA damage due to epilimnetic ultraviolet radiation (Moore, 1912), especially in clear lakes and when organisms lack photoprotective pigmentation or reparation mechanisms (Leech et al., 2005; Hansson et al., 2007). We propose that in our study system this photoreactive behaviour likely obscured the expected response to the fish gradient, particularly in very clear lakes, and especially for sensitive taxa like *Boeckella* (Zagarese et al., 1998; Alonso et al., 2004). The detection of diel vertical migration in lightabsorbent lakes, on the other extreme, might have also hidden the response to planktivory because water clarity is often correlated to zooplankton migration amplitude (i.e., low amplitude in eutrophic lakes; Dodson, 1990). This could partially explain the outlier from Sepulveda Lake (i.e., 'Sep' in Figure 5) that had the lowest Secchi depth (1.8 m). These interpretations are supported by *post hoc* exploratory analyses; if the analyses are re-done but only including the lakes with shallower Secchi depth (i.e., \leq 7.5 m; Table 1) and excluding Sepulveda Lake, which had an extremely low Secchi depth (see quartiles in Table 1), the effect of the fish gradient on diel vertical migration (Pr-day) is highly significant and the best model only included this effect in the expected direction (slope(SE) = -1.13(0.26); LRT = 8.0411, df = 1, *P* = 0.0046). In summary, it is very possible that the fish gradient is important but alternative drivers must be carefully considered. Williamson (2011) has recently proposed the transparency-regulator hypothesis that can be used as an integrated framework for the design of more sensitive surveys in this regard.

Conclusion

We have presented evidence suggesting that a single galaxiid – *G. platei* – is able to exert strong top-down control on the zooplankton community, and that invasive trout is able to uplift this control through cascading effects. Specifically, our results show, with varying degrees of support, that the *G. platei*-trout abundance countergradient is related to changes in zooplankton presence-absence, relative abundance, and morphology. Thus, the fish community changes can have far reaching implications in whole ecosystems, ranging from altering nutrient recycling rates, inducing phytoplankton changes related both to herbivory and nutrients quantity or quality, and changing water transparency (Carpenter et al., 1985; Jeppesen et al., 1997a; Vanni & Layne, 1997; Reissig et al., 2003; Balseiro et al., 2007). In addition, the morphological responses are indicative of changes in the selective regimes that can lead to rapid adaptive evolution (Cousyn et al., 2001; Fisk et al., 2007). Salmonid invasions in austral environs provide an outstanding experiment to understand the natural functioning of local ecosystems, and the far reaching consequences of invasions by top-predators.

4.6 References

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4.7 Tables

	0%	25%	50%	75%	100%
area (ha)	9.7	25.1	67.9	424.2	6103.6
shoreline	1.2	1.5	1.9	2.0	2.7
altitude (m)	53	336	510	696	840
order	0	1	2	2	4
depth max. (m)	8	14	21	50	170
conductance (μS)	24.2	32.9	61.6	75.5	112.1
Secchi depth (m)	1.8	5.0	7.5	10.0	14.5

Table 1: Quartiles of lake variables.

Notes: Twenty-one lakes were included. Shoreline, corresponds to the shoreline development index (Bain & Stevenson, 1999); order, to Strahler stream order at lake outlet; and conductance, to specific conductance. Data from Correa and Hendry (2012).

4.8 Figures

Figure 1: Non-metric, multidimensional scaling (NMDS) plot of zooplankton composition (stress = 0.14). Lakes are labelled with a three-letter-code that corresponds to the initial letters of lake's names, and black circles whose size is proportional to the mean catch-per-trap of G. platei [ln(mean-catch * trap⁻¹ +1)]. Lakes where G. platei was not detected by any of the three fishing gears are marked with an X. The ordination of zooplankton taxa is shown by three-letter codes (in larger font) which correspond to the cladocerans *Bosmina* (*Neobosmina*) spp. (Bos), Ceriodaphnia dubia (Cer), and Daphnia curvirostris (Dap); calanoid copepods of the genus *Boekella* (Boe); and cyclopoid copepods (Cyc). The vectors (arrows) are linear representations of variables fitted a posteriori where arrows in black indicate variables significant at $\alpha = 0.1$ (or gray if $\alpha > 0.1$). Vectors are also labelled with two-letter codes that correspond to fish-catch-perunit-effort: trout captured by gillnetting (TG) or electrofishing (TE), and G. platei captured by gillnetting (PG), electrofishing (PE) or trapping (PT). Finally, PC1 corresponds to the vector derived from the PC1-scores defining the environmental gradient. The NMDS ordination explained ~35% of the variance of PT (permutation test, P = 0.0210), ~28% of TE (P = 0.0544), ~23% of TG (P =0.1000), and it was more loosely related to the other vectors.

Figure 2: Redundancy analyses (tb-RDA) scaling-2 plots showing the fitted lake scores, species scores, and explanatory variables. a) Model including all six explanatory variables. b) Simplified model after forward variable selection. The RDA1 in both models was statistically significant (see text for details). Acronyms as in Figure 1 [the *y*-axis in (b) corresponds to PC1 scores of residuals and not to the environmental gradient displayed elsewhere].

Figure 3: *Bosmina* dominance as a function of overnight mean catch per trap of G. *platei*. Bars represent number of lakes in which *Bosmina* was (top) or not (bottom) dominant. The black line corresponds to the fitted logistic regression

(see *Results*: *Community composition*). In each lake, *Bosmina* was considered dominant if *Bosmina* density / cladoceran density > 0.5.

Figure 4: Body length of two pelagic cladoceran species as a function of *G. platei* catch-per-unit effort. Individual measurements are shown in gray (jittered in the *x*-axis); lake-coefficient estimates and (multi-level) regression lines, in black.

Figure 5: Diel vertical migration in 21 lakes of Aysén. Top quadrants within each panel include lakes where plankton in the upper 10m of the water column was more abundant during the day, and vise versa. From left to right, the *x*-axis represents the fish assemblage gradient with trout-dominated lakes to *G. platei*-dominated lakes. Note that although the *y*-axis is labelled with proportions, it is scaled to the proportion's logits. Acronyms as in Figure 1.

Figure 6: Results of the paleolinmological study of Lake Pollux. (A) Summary of the chronostratigraphic analysis and relevant historical records. The years in the *y*-axis correspond to the constant rate supply model (Appendix A). (B-F) Results of the analyses conducted on cladoceran remains recovered from the sediment core. Small numbers within the panels are number of individuals measured. For further details please see *Historical change reconstruction* sections in *Methods* and *Results*.

Figure 1



Figure 2



Figure 3



Figure 4



Figure 5







4.9 Appendix A

Chronostratigraphy of Lake Pollux core

The chronostratigraphic analysis of Lake Pollux sediment using the constant rate supply (CRS) model suggested that the time of the first salmonid introduction (i.e., 1968) coincided with a depth of ~15 cm in our sediment core. Examination of the standard errors of the CRS model, however, suggests this is not a clear-cut threshold and interpretations should be made with caution, particularly below 20 cm of depth where the ²¹⁰Pb activity was too low to make strong inferences. An independent chronostratigraphic piece of evidence supported the CRS model, at least for relatively recent years. The CRS date of the sharp peak in magnetic susceptibility (~9 cm of depth; Figure A1) matched the 1991 Hudson Volcano eruption, and the tephra-fall might explain the peak (e.g., Bertrand et al., 2005). Hudson Vo. is locates 85Km SW of Pollux lake, and during the 1991 eruption, ash plumes covered vast territories including Lake Pollux (Kratzmann et al., 2010). Unfortunately, or perhaps fortunately, the anthropogenic radioisotopic activity of ¹³⁷Cs did not show the typically clear 1963 peak associated with the climax in atmospheric testing of nuclear weapons during the Cold War (Appleby, 2001), and hence cannot be used as a temporal landmark. The ¹³⁷Cs activity showed a moderate and diffused increment after c.a. 1950, which agrees with the developments of the atomic era (Figure A1).



Figure A1: Chronostratigraphy of short sediment core retrieved from Lake Pollux in Feb 2007. From left to right, (1) magnetic susceptibility profile showing a clear peak likely caused by the 1991 Hudson's Vo. eruption. The sectioning scheme of the core is shown as a broken line. (2) Natural activity of total 210Pb. (3) Anthropogenic activity of 137Cs. Finally, (4) fitted constant rate supply (CRS) model and estimated dates [years A.D.(SE)] of samples analysed for radioisotopic activity. *Note*: The magnetic susceptibility of samples <8 cm deep was measured on <10 mL of sediment, and standardized to 10 mL of sediment by extrapolation using linear models.

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5. GENERAL CONCLUSIONS

Lakes offer well-defined, replicated model systems for studying the effects of introduced species, yet research has only glimpsed the extent to which invasions have transformed these ecosystems (Ricciardi & Macisaac, 2011). My thesis is a rare example of a study that simultaneously addresses the impacts of an invasive top-predator at multiple levels of ecological organization (i.e., individual, population and community) thereby providing a unique, applied perspective on freshwater invasion ecology (Simon & Townsend, 2003).

From the evidence conveyed, it is concluded that invasive trout cause pervasive impacts on Patagonian lakes. This adds to the more mature literature on the ecological impacts on invasive trout on streams (Townsend & Crowl, 1991; Crowl et al., 1992; Simon & Townsend, 2003; Townsend, 2003; Soto et al., 2006; Young et al., 2010). To my knowledge this is the first virtually unequivocal demonstration of the negative individual- and population-level impacts of trout on any galaxiid in Patagonian lakes. In Chapter 2 (§ 2) I showed that the single best predictor for high G. platei abundance was a rarity or absence of trout, especially brown trout. Similar findings have been reported for stream habitats in Patagonia and New Zealand (Crowl et al., 1992; Young et al., 2010). The stronger negative effect of brown trout is in concert with the theory that predation is more disruptive than exploitative competition (Moyle & Light, 1996) because brown trout is generally more piscivorous than rainbow trout (Arismendi et al., 2011a). Like in streams (Crowl et al., 1992; Young et al., 2010), I found that the detrimental effects of trout can cause galaxiid population decline to the point of extirpation (undetection). This finding suggests that the common assertion that lakes provide ecological refugia for native fishes against the negative impact of salmonids must be assessed more rigorously (Milano & Vigliano, 1997; Habit et al., 2010).

Central issues in invasion ecology and conservation biology is to understand the factors controlling invasiveness and survival of native species upon disruptive invasions (Elton, 1958; Moyle & Light, 1996; Ricciardi & Macisaac, 2011). I have made some interesting contributions in these regards. For example, I found that salmonids were abundant and *G. platei* rare in high-order lakes, and through structural equation modeling I concluded that this pattern is best explained by the limited success of trout in lakes with little or no superficial connectivity to river network (i.e., low order lakes). Thus, I demonstrated that lake order is a convenient and inexpensive variable that can be widely used to measure the risk of trout invasion, and hence the risks of threats to native biota. To my knowledge this is the first time this application has been formalized and applied to different regions of the world (Patagonian and Canadian lakes), although the concepts and mechanisms are well known (Donald, 1987; Townsend, 1996; Habit et al., 2012). One important implication stemming from these findings is that there are natural ecological refugia for at least some galaxiids occurring in headwater lakes, yet possibly not for others that are more restricted to lower reaches (e.g., requiring sea connectivity). Examples of the latter seem to be endangered *Aplochiton* spp. (Appendix 1).

Trout invasions are changing the adaptive landscape of surviving galaxiids worldwide, and those capable of adapting have greater chanced to endure (Mooney & Cleland, 2001; Strauss et al., 2006; Carroll, 2011). In § 3 I addressed one aspect of this problem - trophic niche shifts. I showed that the natural ontogenetic trophic height increase of G. platei is severely compromised upon trout invasion. Similar effects have previously been described for other fish taxa in North America (Vander Zanden et al., 1999), but the effect size I detected is surprisingly high. At high tout abundance, large individual G. platei fed one trophic level below the expected with no trout. Furthermore, individuals in invaded lakes barely improved or even decreased their trophic height throughout their ontogeny. This effect adds to the population-level negative effects documented in § 2. Nevertheless, the remarkable capacity of G. platei to adjust its trophic niche likely pays off in terms of enhanced resilience to the invasion. Likewise, other adaptations that have been proposed as important to the survival of G. platei to severe tectonic and climate-change events (Milano & Vigliano, 1997; Cussac et al., 2004; Ruzzante et al., 2008), might also aid in surviving the current trout-driven crisis. Future work is granted on the specific mechanisms

causing the impoverished trophic niche, as well as on the associated fitness costs (tradeoffs).

From the point of view of the invader, the coexistence with the native fish was positive. I found no evidence of biological resistance to the trout invasions. Galaxiids are ill-equipped to defend themselves from efficient piscivorous fish such as trout (McDowall, 2006), and this reflects the lack of evolutionary history (Cox & Lima, 2006; Sih et al., 2010) with such predators in cold-temperate Austral regions. On the contrary, I show evidence that the presence of galaxiids is a facilitator for trout invasions. Specifically, in § 3 I show that large brown trout fed one trophic level higher in lakes with abundant G. platei than in lakes where G. platei were not detected. Combining these results with those of § 2 I propose a chain of events whereby trout, say brown trout, somehow colonizes a pristine lake with a healthy galaxiid population that opposes no ecological resistance and, on the contrary, provides invigorating fodder for piscivorous trout. If the abiotic conditions of the lake are also benign, and particularly if the lake is superficially connected to the river network, tout thrives and population size increases. Galaxiid mortality increases too, particularly among recruits, while large galaxiids forcibly switch to an impoverished diet. The increasing ecological pressures on galaxiids lead to their population collapse. Trout nutrition is compromised by the crash of the galaxiid population but endure thanks to their ability to exploit other food resources such as benthic and allochtonous invertebrates (the chain of events will continue).

The impacts of trout invasions in Patagonia surpass native fish species replacements. It is apparent from the results of every chapter that invasive trout represent a new functional group in the lakes studied, and possibly in most cold-temperate lakes in Austral countries (Pascual et al., 2002; McDowall, 2006; Macchi et al., 2007). As such, it is not surprising to find the kind of disruptions documented herein (Moyle & Light, 1996; Cox & Lima, 2006; Ricciardi & Macisaac, 2011). Particularly, in § 4 I showed opposing roles of *G. platei* and trout in the way they affect individuals, populations and communities of planktonic crustaceans. Firstly, the invasion served as a large-scale correlative

experiment to demonstrate, for the first time, the high importance of top-down control for zooplankton imposed by the most widespread Patagonian-endemic fish - G. platei. The proposed mechanism is direct predation on zooplankton by small G. platei, resulting in predictable responses such as smaller individuals and smaller species dominating (Brooks & Dodson, 1965). I now resume the chain of events incorporating the lessons from § 4. The replacement of G. platei by trout results in dramatic changes to the zooplankton community. Formerly suppressed zooplankters are now released through the cascading, positive effects of trout. The zooplankton, once dominated by small cladocerans, is now dominated by relatively large cladocerans and/or calanoid copepods. Although the consequences of this species turnover were not studied, they can have far-reaching ecosystemwide implications including an alteration of nutrient recycling rates, induction of phytoplankton changes related to both herbivory and nutrients quantity or quality, and changes in water transparency (Carpenter et al., 1985; Jeppesen et al., 1997; Vanni & Layne, 1997; Reissig et al., 2003; Balseiro et al., 2007). Interestingly, the cascading effect of invasive trout has been demonstrated experimentally in streams where trout prey and inhibit invertebrate grazers, thus increasing benthic primary production (Flecker & Townsend, 1994). The results of § 4 point in another direction - trout has indirect, positive effects on planktonic grazers and thus potentially suppresses phytoplankton in lakes. These fundamental differences emphasize the context-dependencies of the ecological impacts of invasive predators (Ricciardi & Macisaac, 2011).

Finally, I advocate management programs aiming to promote galaxiidtrout coexistence in sites where trout is already present at high densities. This could be achieved by reducing the population abundance of trout through catch quotas or fishing weirs (see also Arismendi et al., 2011b). From a conservation viewpoint, manipulations of this kind would allow a much better understanding of the ecological interactions among invasive and native fish, as well as the native fish resilience upon trout invasions. Also, the assisted coexistence would facilitate adaptive responses to salmonid invasions and long term maintenance of biodiversity (see e.g. of adaptive responses to invaders in Crowder & Crawford, 1984; Strauss et al., 2006). From the societal viewpoint, trout culling in overpopulated lakes would reduce trout intraspecific competition and increase their foraging base, which would help enhance the quality of the fishery through faster growth and larger fish. Furthermore, a reduction of the intensity of the trout-driven trophic cascade would result in higher standing crop, enhancing the overall productivity of oligotrophic Patagonian lakes. In short, such manipulation could aid native fish and ecosystem recovery while maintaining the social benefits of trout sport fishery in a way that is reversible and modifiable (Pascual et al., 2009; Arismendi et al., 2011b; Carroll, 2011). Finally, trout should be kept away from rare unspoiled relicts of biodiversity, particularly from pristine high order lakes.

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Appenxix 1: Diversity of the endangered *Aplochiton* (Galaxiidea) in Patagonia: insights from mtDNA

D. Alò*, C. Correa* and L. Cárdenas (in preparation). Diversity of the endangered *Aplochiton* (Galaxiidea) in Patagonia: insights from mtDNA.
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A1.1 Abstract

Aplochiton is a genus of endangered freshwater and diadromous fishes endemic to Patagonia and Falkland Islands whose taxonomy is still unclear. Barcoding suggested a phylogenetic split within the group that does not coincide with the prevailing taxonomic hypothesis that is based solely on morphology. It is urgent to elucidate *Aplochiton* diversity before anthropogenic activities continue to inadvertently spoil habitat critical for the species conservation.

Aplochiton (Galaxiidae: Aplochitoninae) is a small and distinct genus within the galaxiids, a family of widespread cold-temperate austral freshwater and diadromous fishes derived from amongst the lower euteleosteans (Johnson & Patterson, 1996; McDowall, 2006). Unlike most galaxiids, *Aplochiton* are troutlike in body form and relatively large (360 mm max. Total Length; McDowall, 2006). Three species have been described for the genus; two undisputed and one that has long been controversial. Henceforth these will be referred to as 'morphospecies' because their descriptions were based solely on morphology. This contribution provides the first phylogenetic tree of the group based on the 'barcode gene' [i.e., mitochondrial DNA (mtDNA), cytochrome oxidase subunit I (COI)]. Paradoxically, results show that the two undisputed morphospecies do not separate in different COI clades, whereas the controversial morphospecies corresponds to the most distinctive clade.

The officially recognized morphospecies are *Aplochiton taeniatus* Jenyns 1842, endemic to Patagonia, and *Aplochiton zebra* Jenyns 1842, native to Patagonia and the Falkland (Malvinas) Islands (Fig. 1, insert). Both occur in

freshwater and are considered in danger of extinction (Campos *et al.*, 1998; Habit *et al.*, 2006; Comisión Nacional del Medio Ambiente, 2009). The controversial morphospecies is *Aplochiton marinus* Eigenmann 1928, which appears to be related to estuaries and inner seas, although it has been recorded only sporadically (Fig. 1, insert; Eigenmann, 1927; McDowall, 1971; McDowall & Nakaya, 1988). *Aplochiton marinus* specimens have been suggested to be breeding adults of diadromous *A. taeniatus*, and hence McDowall (1971) has reduced the taxon to synonymy with *A. taeniatus*. This synonymy, however, is arguably premature because life history and ontogenetic development of *Aplochiton* have never been studied in sufficient detail. *Aplochiton marinus* is catalogued as "data deficient" in the IUCN Redlist (World Conservation Monitoring Centre, 1996; Campos *et al.*, 1998) and several authors continue to list *A. marinus* as a good species (Dyer, 2000; Aigo *et al.*, 2008; Habit *et al.*, 2010).

To date, three morphological characters, relative head depth, stomach shape, and colour patterns, have mostly been used to provide a distinction between morphospecies, yet none provide clear-cut diagnosis (McDowall, 1971; McDowall and Nakaya, 1988). The paucity of useful morphological diagnostic characters is due, at least in part, to interspecific similarities and substantial intraspecific variation associated to allometric growth, alternative life histories, and ecology (e.g., lacustrine vs. non-lacustrine existence, and potential facultative diadromy; McDowall, 1971; McDowall & Nakaya, 1988; McDowall, 2005; McDowall & Pankhurst, 2005; Lattuca *et al.*, 2007).

The aim of this study was to test the validity of the morphospecies using molecular data. To this end, the commonly used barcoding gene, COI, was used to generate a phylogenetic tree, and subsequently, the morphological diagnostic characters were mapped onto the tree to assess the extent of agreement. COI barcoding has been shown to be extremely effective for species level identification in several groups of animals (Hebert *et al.*, 2004a; Hebert *et al.*, 2004b; Ball *et al.*, 2005; Saunders, 2005; Cywinska *et al.*, 2006; Clare *et al.*, 2007). Studies on marine and fresh-water fishes have shown that sequence diversity of COI can help distinguish species boundaries efficiently (Ward *et al.*,

2005; Hubert et al., 2008; Ward et al., 2009).

Between 2004 and 2010, *Aplochiton spp.* were collected using various net types from six locations along the Chilean Patagonia, trying to encompass a broad geographical area within the distribution range of the species (Fig. 1). At each location, fish specimens were euthanized by an overdose of anaesthetic solution (Tricaine Methanesulfonate, MS 222 or clove oil), set on a scaled board and photographed using a digital camera (lateral view). Additionally, a small fin clip was removed and preserved in 95% ethanol for DNA analysis. Voucher specimens were preserved in a 5-10% formalin solution, and a subset was deposited in the Museo Nacional de Historia Natural, Santiago, Chile. A total of 39 highly polymorphic individuals were chosen for the current analyses. It is important to note that in only one location, the morphospecies *A. zebra* and *A. taeniatus* were collected in sympatry (Caro Lake).

Morphospecies were diagnosed using the traditional characters. Head depth measurements were made on the digital pictures adapting methods from McDowall (1971). Head depth was measured close to the occiput but since it was difficult to precisely locate the occiput on fish photos, head depth was also measured along a transversal axis passing through the posterior margin of the fish ocular orbit. Head-depth-to-head-length ratios (HD ratios; %) were also calculated following McDowall (1971). Individuals with relatively low HD ratio (i.e. slender heads) generally belong to *A. taeniatus* or *A. marinus*; whereas individuals with deeper bodies to *A. zebra* (McDowall, 1971). The critical HD ratio thresholds were estimated *ad hoc* as 53% (post-orbital) and 59% (occiput) by inspecting bimodality on the frequency distributions of HD ratios. Since the same conclusions were reached using both methods, only HD ratio at occiput will be discussed. Stomach morphology was recorded following McDowall & Nakaya (1988) as bulbous or elongated. Colour patterns were recorded from photos of fresh fish and coded as chevron bands, spots, or none.

Our results suggested that diagnosis based on these morphological characters may lead to ambiguous identification of morphospecies. The use of HD ratio alone was insufficient because this character, though bimodal, showed a continuous distribution (Fig. 2). Likewise, colour was useful but insufficient; in particular, the presence of spots was distinctive of *A. marinus*, at least in large individuals (small *A. marinus* were not available); whereas the presence of chevron blotches was distinctive of *A. zebra* but some individuals with high HD ratio lacked these characteristic markings suggesting that the lack of colour patterns may not be a unique trait of *A. taeniatus* (Fig. 2). Stomach morphology revealed two groups; *A. zebra* and *A. taeniatus* showed bulbous stomachs, whereas *A. marinus* stomach was elongated, which might reflect an adaptation to piscivory (McDowall & Nakaya 1988). Although McDowall & Nakaya (1988) associated the elongated stomach to *A. taeniatus* have a bulbous stomach similar to *A. zebra*. In their stomach shape analysis McDowall & Nakaya (1988) likely examined individuals of *A. marinus* but treated these individuals as *A. taeniatus* due to the aforementioned controversial synonymy.

Barcoding was used to identify phylogenetic lineages and assess whether these correspond to the above diagnostic characters and morphospecies. DNA extractions were performed using EZNA Tissue DNA Kit D3396-02 (Omega Biotek, Inc., USA). Polymerase chain reaction (PCR) was employed to amplify a 739 base-pair fragment of mtDNA using COI universal primers LCO1490 and HCO2198 (Folmer et al., 1994). PCR products were purified using EZNA Cycle-Pure kit D6493-02 (OMEGA bio-tek) and sequenced using Macrogen custom sequencing service (Macrogen, Seoul, Korea). Bi-directional sequences were processed by eye and aligned with ClustalW multiple alignment option within the software BioEdit (Hall et al., 2011). Genetic distances, detailed sequencing methods, and Genbank accession numbers (JQ048547-JQ048552) as linked to the specific haplotypes are provided in the Appendix. Haplotype diversity was calculated for each morphospecies by locality using Arlequin version 3.5 (Excoffier & Lischer, 2010). A neighbour joining phylogenetic tree and nucleotide sequence divergence (% base substitutions) were calculated using Kimura-2 parameter distance model (Kimura, 1980) with 1000 bootstrap samples using MEGA version 5 (Tamura et al., 2011). Galaxias maculatus and Galaxias

platei (both Galaxiidae) were included in the analysis as outgroups because of their close relationship to *Aplochiton* (Waters *et al.*, 2000; Burridge *et al.*, 2011).

The observed COI phylogeny conflicted with the prevailing *Aplochiton* morphospecies because only *A. marinus* was monophyletic (Fig. 2). *Aplochiton marinus* sequences differed between 1.39–1.47 % from other *Aplochiton*. This genetic distinctiveness suggests – if monophyly is confirmed in further studies – that *Aplochiton marinus* is a good species, since COI is generally considered distinctive of a species when at least 1% of its sequence is unique, As for example, 96% of Canadian freshwater fishes and 93% of Australian marine fishes fall within this criterion (Hubert *et al.*, 2008; Ward *et al.*, 2005).

Paradoxically, the undisputed morphospecies (*A. zebra* and *A. taeniatus*) were virtually indistinguishable from each other by barcoding. The population of *A. taeniatus* from Caro Lake shared the same haplotype (0% sequence divergence) with most allopatric *A. zebra* populations across a great latitudinal gradient (Fig. 1). Interestingly, only in sympatry, *A. taeniatus* did differ substantially (0.93% sequence divergence) from *A. zebra* (Fig. 2, note 11 small individuals from Caro Lake unambiguously identified). To put these evolutionary distances in perspective within galaxiids, all *Aplochiton* sequences differed within a range of 2.1–3.38% from the *Galaxias* outgroups.

According to these results, morphospecies *A. taeniatus* and *A. zebra* could therefore represent extreme ecomorphs in a morphological continuum, rather than monophyletic groups. As Figure 2 suggests, individuals with different haplotypes might express the same ecomorph, and individuals within the same clade can develop into dissimilar phenotypes. Many fish species show variable ecomorphs which can result from adaptation to contrasting environmental conditions such as benthic, pelagic or littoral habitats, still versus swift water flow, or predation against no predation (Robinson, 2000; Ehlinger & Wilson, 1988; Langerhans, 2008; Robinson *et al.*, 2008). In *Aplochiton*, some studies have suggested that differences in trophic strategies or environment can lead to morphological diversification, as for example water turbidity has been found to affect the typical negative eye size allometry exhibited by *A. zebra* (McDowall, 2005; McDowall & Pankhurst, 2005).

Future work should confirm the monophyly of A. marinus by analyzing additional samples and localities, although, this might prove difficult because of the unknown current distribution of the species. It is unclear if the paucity of records for A. marinus is due to true rarity or to lack of targeted surveys. Given the relatively high allelic diversity found in A. marinus, it is unlikely that its monophyly could be generated by a bottleneck event. Furthermore, a geographical isolation hypothesis seems unlikely, since A. marinus has always been recorded in estuaries or fjords where gene flow through marine dispersal is probably frequent. Akin to high marine dispersal (Ward et al., 1994), this population showed the highest haplotype diversity. (h = 0.67; Fig. 1). Additionally, the lineage that included A. zebra from various locations did not show strong phylogeographic patterns, despite that isolated freshwater populations are more likely to face barriers to gene flow. In conclusion, there is compelling genetic and morphological evidence supporting the high distinctiveness of A. marinus, which, unless new evidence shows otherwise, should continue to be considered a good species. New insights from mtDNA also suggest that A. taeniatus and A. zebra, might be ecomorphs of a single species; unless, the overlap observed in mtDNA may be explained either by a recent speciation event or by hybridization between the pair (Hubert et al., 2008) which should be investigated further targeting rapidly evolving nuclear markers that underlie phenotypic differences (Kocher, 2004).

Deficient understanding of *Aplochiton* diversity, biology, ecology and distribution compromises its conservation. Rapid anthropogenic changes will continue to spoil the habitat of these endangered and poorly known fishes unless action is undertaken. For example, the *A. marinus* population studied herein might be at imminent threat. The largest hydroelectric project in Chile's history (Hidroaysén), involving the construction of two dams in the Baker River and three in the nearby Pascua River (Vince, 2010), has been approved in May, 2011. Unfortunately, *A. marinus and A. taeniatus* were neglected in the project's environmental impact assessment – let alone the ecological needs for the species.
Many galaxiids use littoral habitat to spawn (Campos, 1969; Ortubay & Wegrzyn, 1991; Barriga *et al.*, 2002; McDowall, 2006; Lattuca *et al.*, 2008), and one of the potential impacts of the projected hydropeaking (abrupt caudal changes due to dam operation) is the degradation of galaxiid reproductive habitat. Dam implementation has also been associated with the loss of longitudinal hydrological connectivity, especially in migratory fishes that move between marine and freshwater environments, but also in obligate riverine fishes where anthropogenic barriers to water flow are likely to disrupt population processes (Fullerton *et al.*, 2010). Various other factors, such as the negative effect of invasive species (Young *et al.*, 2010) may pose additional and continued threats to *Aplochiton*. Resolving the taxonomy of the genus is an important necessary step to support research, awareness, and the decisions of managers responsible for the protection of biodiversity in Patagonia.

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A1.3 Figures

Figure 1: Distribution range of *Aplochiton* (insert), collection sites, and COI haplotypes. For each site, numbers correspond to sample sizes, and letters to haplotypes. Abbreviations: AT, *Aplochiton taeniatus*; AM, *Aplochiton marinus;*, AZ, *Aplochiton zebra*; SAN, Santo Domingo River, Valdivia; RIÑ, Riñihue Lake; PAL, Palos Lake; CAR, Caro Lake; BAK, Baker River; QUE, Quetru Lake.

Figure 2: Phylogeny and morphology of *Aplochiton*. Each leaf of the tree represents one individual labeled with an ID number (n = 39 + 2 outgroups). Site refers to the sampling locations; Std. Length is standard length in cm; Stomach displays either the bulbous (B) or the elongated (E) morphology; HD ratio is relative head-depth (%) measured at the occiput (the dashed line indicate the *ad hoc* 59% criterion); Pattern refers to coloration categories which were chevron blotches (\blacktriangle), spots (\bullet) and no clear pattern (\circ). Morph corresponds to the resulting morphospecies after evaluating the previous list of diagnostic characters—*A. zebra* (Z), *A. taeniatus* (T) and *A. marinus* (M).





Figure 2



A1.4 Appendix

Genbank accession numbers for MtDNA COI sequences of *Aplochiton* spp. and outgroups.

Haplotype A	JQ048547
Haplotype B	JQ048548
Haplotype C	JQ048549
Haplotype D	JQ048550
Haplotype E	JQ048551
Haplotype F	JQ048552
Galaxias maculatus	AP004104
Galaxias platei	FJ178355

DNA extractions were performed using E.Z.N.A. Tissue DNA Kit D3396-02 (Omega Bio-tek, Inc., USA). Polymerase chain reactions (PCR) was employed to amplify a 739 basepair (bp) fragment of mitochondrial DNA using 1/10 dilutions of DNA extracts in 25µl PCR reactions using 0.625 units of Taq (MBI Fermentas), 2.5 µl 10X buffer, 3 µl MgCl2 (25mM), 1 µl each of both COI LCO1490: 5'universal primers $(10 \text{pm/}\mu\text{l})$ GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3', 0.5µl of dNTP mix (10mM), 1.5µl BSA (10mg/ml) and dH2O with initial 95°C denaturation for 5 minutes, 40 cycles of 45seconds denaturation at 95°C, 45 seconds annealing at 50°C, 1 minute extension at 72°C, and a final 10 minutes hold at 72°C. PCR products were verified on 1.5% agarose gels stained with EtBr, purified using E.Z.N.A. Cycle-Pure kit D6493-02 (OMEGA bio-tek) and sequenced using Macrogen custom sequencing service (Macrogen, Seoul, Korea).

Estimates of net evolutionary divergence between groups of sequences: The analysis involved 41 nucleotide sequences. The number of base substitutions per site from estimation of net average between groups of sequences are shown below the diagonal; analyses were conducted using the Kimura 2-parameter model. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The number of base differences per sequence from averaging over all sequence pairs between groups are shown above the diagonal. Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. There were a total of 162 informative nucleotide positions in the final dataset. Evolutionary analyses were conducted in MEGA5. AZ, AT, and AM refer to the morphotypes identified in the study and respectively assigned to *Aplochiton zebra*, *A. taeniatus*, and *A. marinus*. Numbers within first column refer to sample sizes.

Sto. Domingo R. (AZ, 6)		0.1	18.1	0.1	13.1	0.1	13.1	28.1	41.0
Caro Lake (AT, 11)	0.000		18.2	0.0	13.0	0.0	13.0	28.0	41.0
Baker Channel (AM, 9)	0.014	0.014		18.2	19.2	18.2	19.2	30.2	39.2
Palos Lake (AZ, 5)	0.000	0.000	0.014		13.0	0.0	13.0	28.0	41.0
Caro Lake (AZ, 5)	0.009	0.009	0.015	0.009		13.0	0.0	27.0	37.0
Quetru (AZ, 1)	0.000	0.000	0.014	0.000	0.009		13.0	28.0	41.0
Riñihue (AZ, 1)	0.009	0.009	0.015	0.009	0.000	0.009		27.0	37.0
G. platei (1)	0.023	0.023	0.026	0.023	0.022	0.023	0.022		34.0
G. maculatus (1)	0.041	0.041	0.038	0.041	0.034	0.041	0.034	0.031	

Appenxix 2: Tissue preservation biases in stable isotopes of fishes and molluscs from Patagonian lakes

Correa, C. (in press) Tissue preservation biases in stable isotopes of fishes and molluses from Patagonian lakes. *Journal of Fish Biology*

A2.1 Abstract

Field work commonly involves preserving samples for later use; however, most preservation methods distort stable isotope (SI) signatures of interest to ecologists. Although preservation of muscle samples with table salt and rubbing alcohol affected the SI (δ^{13} C & δ^{15} N) of important consumers in Patagonian lakes (molluscs and fishes), variation among individuals and lakes generally exceeded that among preservation treatments. Mathematical corrections for these preservation biases are provided, and potentially bias-free preservation by air-drying is suggested.

Naturally occurring stable carbon and nitrogen isotopic signatures (i.e., δ^{13} C and δ^{15} N) have proven extremely enlightening to ecologists tracking trophic interactions (Newsome et al., 2007; Winemiller, 2007), and the ratio of carbon-tonitrogen by mass (C:N) has also gained substantial interest due to its close relation to lipid concentration (Post et al., 2007; Fagan et al., 2011). However, effects of sample preservation methods used in stable isotope (SI) research are still under scrutiny (Arrington & Winemiller, 2002; Sweeting et al., 2004; Kelly et al., 2006; Xu et al., 2011). Many studies avoid potential preservation issues by quickly transferring collected samples to a laboratory where they are oven-dried, lyophilized, or frozen (though freezing can distort the isotopic signatures of certain organisms; Syväranta et al., 2011). Nevertheless, these resource-intensive methods are not always possible or practical. Hence there is a growing body of literature addressing biases due to preservatives [reviewed in Barrow (2008) and Fanelli *et al.*, (2010)]. These studies commonly demonstrate that SI biases can be mathematically corrected if correction factors are known, but cast little light on the magnitude of preservation biases relative to other sources of ecological variation such as variation among individuals and ecosystems. Two potentially convenient preservation methods are sample immersion in ethanol or salt-drying (Arrington & Winemiller, 2002; Barrow et al., 2008; Xu et al., 2011). Both are available worldwide in the form of rubbing alcohol and table salt. The aim of this study was to quantify alcohol- and salt-tissue preservation bias of two important groups of consumers from Patagonian lakes, and compare preservation-induced variation to variation among observations, individuals, and lakes.

The first group of organisms comprised two mollusc primary consumers: the snail *Chilina* sp. Gray 1828 (Basommatophora: Chilinidae) and the mussel *Diplodon chilensis* (Gray, 1828) (Unionacea: Hyriidae). These primary consumers could become instrumental in SI studies if they prove effective as isotopic calibration baselines, that is standards permitting estimation of trophic level, and comparison across food webs and across lakes (Vander Zanden & Rasmussen, 1999; Post, 2002; Vander Zanden et al., 2005; Vuorio et al., 2007). In accordance with criteria reviewed in Post (2002), these molluscs are ideal candidates. They are abundant and widely distributed in Patagonia (Parada & Peredo, 2006; Valdovinos, 2006), they frequently co-occur (e.g. 16 out of 25 lakes searched in the region of Aysén; Correa et al., 2012), they consume pelagic (mussels) and littoral (snails) primary producers (Estebenet et al., 2002; Lara et al., 2002), and they are long-lived compared to most other primary consumers (>30 years for *D. chilensis* and 1 year for *Chilina sp.*, Parada et al., 1989; Bosnia et al., 1990; Valdovinos & Pedreros, 2007).

The second group comprised native and invasive fishes. The puye grande *Galaxias platei* Steindachner 1898 (Galaxiidea) is the most widespread native fish endemic to Patagonia (Zemlak et al., 2008), whereas brown trout *Salmo trutta* L. 1758 and rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) (both Salmonidae) are the most widespread invasive fish predators in the same region (Aigo et al., 2008; Habit et al., 2010). In general, Patagonian lakes host a low diversity of fishes, and many small lakes host one or a combination of these species (Milano et al., 2006; Correa & Hendry, 2012). Due to their strong top-

down effects on lake ecosystems, these fishes can be considered keystone species of high ecological importance in Patagonian lakes (Modenutti et al., 1993; Balseiro et al., 2007; Correa & Hendry, 2012).

Fishes and molluscs were collected between 30 October and the thirth of November 2009, in three lakes within the watershed of Río Aysén in western Patagonia (45°S; Table I). Fish were collected with a backpack electrofisher, euthanized with an overdose of clove oil solution, and immediately transferred to the lab located within 1 h driving distance from all sampling sites [Centro de Investigaciones Patagónicas (CIEP), Coyhaique], where the fish were frozen at - 20° C until all collections were completed within five days (freezing did not affect the SI signatures of other fishes; Kaehler & Pakhomov, 2001; Sweeting et al., 2004). Molluscs were collected manually from the littoral zone, transferred to the lab in 1 l bottles half filled with lake water, then held overnight before being processed.

Tissue sampling and preservation were performed as soon as collections were completed. Frozen fish were thawed at room temperature (except for O. *mykiss* which were used fresh), and a small portion (about 3-5 mm cross-section and 1 cm in length) of muscle and skin was dissected from the anterodorsal region of the fish. For mussels, samples were obtained from the foot. Individual snails were preserved after their shells were crushed, or their foot was dissected out. Individual muscle samples were split into three, and each sub-sample was randomly assigned to each of three treatments: denatured 95% ethanol (Alcohol), salted-dried (Salt), and oven-dried (Control; more details in Supporting Information). Because small organisms have little tissue, whole individuals of a few snails were randomly assigned to each of the three treatments. After preservation, samples were stored for nine-weeks to ensure that most of the effects of the preservatives on the SI of our samples had occurred (Kaehler & Pakhomov, 2001; Sweeting et al., 2004; Syväranta et al., 2011; Xu et al., 2011). The samples were then packed for elemental analysis (Supporting Information), and sent to the Stable Isotope Facility of University of California, Davis, for ¹⁵N and ¹³C analysis. The procedure involved a PDZ Europa ANCA-GSL elemental

analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., www.sercongroup.com). SI values were delivered by the facility in δ notation: $\delta^{15}N$ or $\delta^{13}C = [(R_{sample} * R_{standard}^{-1}) - 1]1000$, where *R* is ${}^{13}C * {}^{12}C^{-1}$ or ${}^{15}N * {}^{14}N^{-1}$ relative to international standards (Vienna PeeDee Belemnite for carbon, and atmospheric air for nitrogen).

A set of univariate, linear, hierarchical, mixed-effect models were used (Gelman & Hill, 2007) to evaluate the effect of sample preservation on δ^{13} C and δ^{15} N while controlling for the effect of individual- and lake-variation (if applicable). The data from each species and response variable were analyzed separately. The datasets of G. platei and Chilina sp. were analyzed using a model that included the effect of lake whereas the datasets of S. trutta, O. mykiss and D. *chilensis* were analyzed with a model that lacked the effect of lake because these species were only present in one lake (Table I). 'Treatment' was treated as a fixed factor, and the oven-dry treatment (i.e., Control) was set as the reference group. 'Lake' (G. platei and Chilina sp.) was also treated as a fixed factor both because the lakes were deliberately chosen to represent a wide range in altitude, area, and hydrological position (Table I), and because only three lakes were sampled (Gelman & Hill, 2007). Finally, 'individual' and 'e' were random and normally distributed factors included to account for variation across individuals (nested in lakes) and observations (most nested in individuals and corresponding to the residual error). Usually each individual was measured three times (three treatments), but snails from Pollux and Toro lakes had only one measurement (Table I). This affects the precision of parameter estimates for Chilina sp., particularly those for residual variation and treatment effects, but should not introduce biases. The statistical significance of the effect of treatment was tested using likelihood ratio tests (LRT; Zuur et al., 2009). All models were fit using maximum likelihood in the R package lme4 (Bates & Maechler, 2010), and the change in deviance from these 'full' models to 'reduced' models - with the treatment effect removed – was compared to a null χ^2 reference distribution, as implemented by the function anova of the stats R base package (Zuur et al., 2009; R Development Core Team, 2010). The full models were then re-fitted (R

package lme4) using restricted maximum likelihood in order to illustrate the effect of the preservatives and obtain correction factors to be used for adjusting δ values of preserved samples.

The results revealed a significant effect of tissue preservation on δ^{13} C and δ^{15} N for each of the species analyzed (LRT, d.f. = 2, *P*<0.05 in all 10 cases). With regards to the treatment effects on δ^{15} N, salt and alcohol had similar effects on samples from all fish species, introducing a significant positive bias [i.e., 95% C.I. (i.e., the mean ± 2 * S.E. does not encompass zero; Fig. 1, left panel). These preservatives had smaller effect size (positive or negative; sometimes significant) on the δ^{15} N of molluscs. Because δ^{15} N-biases were higher for fishes than for molluscs, studies comparing these two groups must be particularly cautious. For example, one method to estimate a fish's trophic level requires the difference in δ^{15} N between molluscs and the fish (Post, 2002), and failure to correct for preservation biases could result, for example, in overestimation of fish trophic position by an average of about +0.1 to +0.2 trophic levels (assuming a δ^{15} N enrichment of 3.4‰ per trophic level), and even more if δ^{15} N of mussels preserved in salt are used as calibration baseline, because this latter group had an unusual negative bias (Barrow et al., 2008).

With regard to δ^{13} C, salt had no significant effect, or a small significant negative effect in all species but snails. This contrasts to the alcohol treatment in all species and the salt treatment in snails, which always had a significant (or marginally significant) positive effect on δ^{13} C values (Fig. 1, right panel). A stronger, positive effect of alcohol is consistent with previous work (Kelly et al., 2006; Xu et al., 2011) and suggests lipid loss due to dissolution and extraction during storage (see below). Nevertheless, correction factors for δ^{13} C and δ^{15} N (Fig. 1) can be subtracted from SI of preserved samples to achieve an effective mathematical bias correction (e.g., Correa et al., 2012).

How does the SI variation due to different sample treatments compare to other important sources of ecological SI variation? To answer this question, a set of models mimicking the structure of the aforementioned models were implemented in a Bayesian framework, and the posterior distributions of parameters describing variation due to the different effects were compared. The models were implemented in R and WinBUGS (Lunn, 2000; Sturtz et al., 2005) and uninformative priors were used such that the data dominated the results [i.e., dnorm(0, 1E-6) for fixed effects and, and dgamma(0.01,0.01) for the precision of random effects]. The posterior distributions of S.D. of lake (when applicable), individual, treatment, and observation (i.e., residual) effects were sampled after model convergence. Due to the small number of levels for some factors in the models, finite-population S.D. estimates were used to assess variation across lakes and treatments, and superpopulation S.D. estimates to assess variation across individuals and observations (Gelman & Hill, 2007; Supporting Information: R and WinBUGS code).

Variation in δ values due to treatments was substantially lower than variation due to lake membership, and lower than individual variation, except for *G. platei*- δ^{15} N, *S. trutta*- δ^{13} C and *D. chileansis*- δ^{13} C. The residual variation was of similar magnitude, or slightly lower, than the treatment variation (Fig. 2). These findings suggest that the isotopic signature of processes at the individual (e.g., ontogenetic diet shifts) and ecosystem levels (e.g., isotopic variability of nutrients available for uptake) would be generally robust to methodological differences in sample preservation, and especially if appropriate corrections are made to account for biases (Arrington & Winemiller, 2002; Barrow et al., 2008).

To analyze the effect of preservatives on C:N, the same statistical approach as for δ^{13} C and δ^{15} N was used (i.e., LRT; four outliers were excluded). Furthermore, the C:N of the control samples was used to infer lipid concentration following Post *et al.* (2007) because heterogeneity in lipid concentration among organisms can introduce biases to ecological studies using δ^{13} C.

The C:N ratio of untreated (control) samples was always lower than 4.4, which, when applying Post's *et al.* (2007) formula, corresponds to 11% lipid content. Heterogeneity was present in these untreated samples, however: fishes had similar and lower C:N ratios (with lipid content generally <6%) than molluscs (6% < lipids < 11%), and the snail *Chilina* sp. had the highest values (Fig. 3, left panel). The effect of tissue treatment on C:N ratio was significant for all five

species (LRT, d.f. = 2, P < 0.05 in all five cases). Samples preserved in alcohol almost always had significantly lower C:N ratios (with mean biases ranging from -0.2 to -0.4), whereas samples preserved in salt either did not show an effect, or showed significantly increased C:N ratios (0 to 0.8; Fig. 3, right panel).

The analyses of δ^{13} C and C:N strongly suggest lipid loss due to dissolution and extraction during the alcohol treatment. The lipid loss is revealed by the reductions in C:N ratio (Post et al., 2007). Because lipids are depleted in δ^{13} C relative to proteins and carbohydrates, the alcohol preserved samples show higher δ^{13} C values than dried or salted samples (Sweeting et al., 2004; Kelly et al., 2006). This bias, however, is only weakly related to the estimated lipid content (following Post's et al. 2007 formula), which indicates that other factors must be involved (Kelly et al., 2006). For example, dried molluscs had relatively high C:N ratios (hence high lipids) compared to fishes and yet the alcohol- δ^{13} C biases for these molluses were not higher than those for the two salmonid species (Figs. 1 & 3). One likely explanation is that alcohol removed only a fraction of the lipids present in the samples, and the magnitude of this fraction depends on a number of physical and biochemical attributes of the sample, storage conditions, and differential removal of soluble acids or sugars (Kelly et al., 2006). Salt, on the other hand, had little effect on δ^{13} C and C:N, except for snails. Thus the method proved superior to alcohol in maintaining the lipid integrity of fish and mussel samples. It is unclear why snail muscle responded differently to the addition of salt, but it could be related to the excretion and loss of mucus upon salt addition.

Differences in lipid concentration between organisms or tissues may introduce considerable bias into SI analyses that use δ^{13} C, and, therefore, proper *a priori* chemical lipid extraction, or *a posteriori* mathematical normalization, have been proposed (Post et al., 2007; Fagan et al., 2011). Unfortunately, no chemical lipid extraction was used (Folch et al., 1957), and no mathematical normalization was possible in the treated samples because it is still unknown how the C:N-tolipid-content relationship was affected by the treatments. Admittedly, this is probably the major drawback of our study—the effect of the preservatives on δ^{13} C can be corrected, but the bias of the treated samples due to the initial heterogeneity in organisms' lipid content cannot. Using Post's *et al.* (2007) mathematical normalization on the control samples, however, it is possible to get a rough estimation of the magnitude of this latter bias. Because the fishes in our study had relatively low C:N ratios (<3.6; equivalent to <6% lipid content), the potential biases in δ^{13} C are small, if any (Post et al., 2007). However, the molluscs showed higher C:N ratios (<4.4; 11% lipids) and this can lead to biases in δ^{13} C values of up to +1‰ in the worse case scenario, but typically <0.8‰ (i.e., 3.32 + 0.99 * C:N; Table 1 in Post et al. 2007). This could, for example, lead to generally <15% but occasionally much larger error in the estimation of source importance using two-end-member mixing models (Supporting Information; see also Post et al., 2007).

Stable isotopes are labile to all sorts of chemical treatments and sometimes, even to the non-chemical preservation by freezing (Barrow et al., 2008; Fanelli et al., 2010). Although systematic biases are sometimes easy to correct mathematically using specific factors, the optimal preservation means is undoubtedly to dry (or lyophilize) the fresh tissue (Xu et al., 2011). Drying is a necessary step before mass spectrometry and drying right after sampling guarantees sample integrity in terms of SIs, C:N, and lipids. Research is needed to validate simple drying techniques. For example, it might be sufficient to sun- or air-dry bits of tissue in a mesh-covered tray, and then samples could be stored in small paper envelopes inside a sealed container with desiccant (silica gel or perhaps rice). The development of such simple – and potentially bias-free – methods would greatly facilitate SI research in remote locations, and would decrease the need for chemical preservation and the associated caveats.

A2.2 References

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A2.3 Tables

Lake	Barroso	Pollux	Toro	Total
Latitude S	45° 44'	45° 40'	45° 31'	
Longitude W	72° 14'	71° 51'	71° 51'	
Altitude (m)	334	679	~695	
Area (ha)	50	879	30	
Strahler' order	2	3	0	
<i>Chilina</i> sp.	5	6*	6^*	17(54)
D. chilensis	5	0	0	5(30)
G. platei	4	4	4	12(72)
O. mykiss	0	7	0	7(42)
S. trutta	5	0	0	5(30)
Total	19	17	10	46(228)

Table I: Lake environmental descriptors and sample sizes for the experiment.

Notes: For each species, the number of individuals sampled is provided. The muscle sample of each individual was split in three—two treatments plus one control—except for the cases indicated with an asterisk, which had one observation per individual. In the right column, the total number of stable isotope measurements is provided in parentheses (i.e., number of subjects × number of treatments per subject × number of stable isotopes measured).

A2.4 Figures

Figure 1: Preservation bias of δ values of tissue samples treated with alcohol (filled) and salt (open symbols). Biases are deviations relative to the control group which had no chemical preservation. Horizontal lines indicate 95% C.I. (mean ± 2 S.E.).

Figure 2: Components of variation of δ^{13} C and δ^{15} N values for each species. Standard deviation values are presented for variation across lakes (lak), individuals (ind), treatments (tr) and observations (obs). The dashed lines represent the average treatment variation to facilitate comparison to other sources of variation.

Figure 3: Inter-specific variation in C:N ratios and effect of preservatives. Left: C:N ratios (bottom scale) and estimated % lipid content (top scale; see text) in the reference group for each species. Reference groups correspond to control groups in single-lake models and the control group in Barroso Lake in multi-lake models. Right: deviations in C:N relative to the reference group due to tissue preservation in alcohol (filled) and salt (open symbols). Horizontal lines indicate 95% C.I. (mean ± 2 S.E.).

Figure 1



Figure 2



Figure 3



A2.5 Supporting Information

This supplement includes:

- Additional details about treatments and sample preparation for elemental analysis
- Impact of lipid-related δ^{13} C bias on two-end-member mixing model
- Data, R & WinBUGS code [this lengthy supplement is available upon request]

Additional details about treatments and sample preparation for elemental analysis

Salt: tissue samples were dipped in iodized table-salt (commercial brand Lobos) and stored in business-card paper envelopes. Batches of envelopes were loosely packed and left to dry in a warm, dry place, such as indoors or on the dashboard of the field vehicle (which warms as a greenhouse). After the storage period and before SI analysis, the salted-dried samples were transferred to 25 mL scintillation vials which were filled with distilled water in order to dissolve and remove the salt. The water-based saltremoval process lasted for four hours, and the water was replaced every hour. The solution was gently agitated several times throughout the process. Samples were then transferred to the drying oven. Alcohol: The tissue samples were preserved and stored in 95% denatured alcohol (denaturing agent: 0.02% diethyl phthalate, Cruz Verde). Individual samples were kept in 1.5 mL Eppendorph tubes. Some samples from several invertebrates of different species were kept together in 25 mL scintillation vials. After the storage period and before SI analysis, the samples were removed from the alcohol solution and transferred to the drying oven. Control: The tissue samples were dried in a drying oven at 50°C for 24 h at CIEP, and subsequently stored in dry Eppendorph tubes. Samples were flown to McGill University, Montreal, where they completed the storage period. Before SI analysis, samples were dried again along with the salted and alcoholpreserved samples at 50°C degrees for 24 h.

Finally, each sample was sub-sampled by weighing 1.0 - 1.4 mg of tissue using an analytical balance (0.1 mg resolution, Mettler Toledo AB104). The right amount of tissue was extracted from the dry tissue with scalpel and forceps, carefully avoiding nonmuscle tissue. The resulting samples were then encapsulated in tin-capsules for stable isotope analyses (Elemental microanalysis D1002) and sent to the Stable Isotope Facility of University of California, Davis, for ¹⁵N and ¹³C analysis. The procedure involved a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Stable isotope values were delivered by the facility in δ notation: δ^{15} N or δ^{13} C = [(R_{sample} * R_{standard}⁻¹) – 1]1000, where R is ¹³C * ¹²C⁻¹ or ¹⁵N * ¹⁴N⁻¹ relative to international standards (Vienna PeeDee Belemnite for carbon, and atmospheric air for nitrogen).

Impact of lipid-related $\delta 13C$ bias on two-end-member mixing model

Biases due to heterogeneity in lipid concentration among samples, and particularly between fishes and invertebrates used as isotopic calibration baselines, could potentially affect trophic position estimates. In our experiments no chemical lipid extraction was conducted before stable C¹³ analysis (Folch et al., 1957), and no mathematical normalization was possible for the preserved samples. Using Post's et al. (2007) mathematical normalization on the control samples, however, it is possible get a rough estimation of the magnitude of biases due to heterogeneity in lipid concentration. Because the fishes in our study had relatively low C:N ratios (<3.6; equivalent to <6% lipid content), the potential biases in δ^{13} C are small, if any (Post et al., 2007). However, the molluscs showed higher C:N ratios (<4.4; 11% lipids) and this can lead to biases in δ^{13} C values of up to +1‰ in the worse case scenario, but typically <0.8‰ (i.e., 3.32 + 0.99 * C:N; Table 1 in Post et al. 2007). The significance of this bias is illustrated by assessing what would be the impact on inferences regarding the proportion of a consumer's mass that is derived from the pelagic food web as opposed to the littoral food web of a lake. Specifically, this is the parameter alpha in Post's (2002) two-end-member mixing model, where the consumer is represented by δ^{13} C-fish, the pelagic food web by δ^{13} C-mussel, and the littoral food web by δ^{13} C-snail. In a δ^{13} C axis, the fish lies in between the mussel (to the left) and the snail (to the right) because the fish can incorporate nutrients from both the littoral and pelagic food webs, and because the littoral food web is enriched in δ^{13} C compared to the pelagic food web (Vander Zanden & Rasmussen, 1999; Post, 2002; this study). A positive bias in the signature of both molluscs would make the fish look closer to the pelagic food web (assuming unbiased δ^{13} C-fish), and the magnitude of this effect is inversely related to the difference between the two molluses. The mean of the latter difference (i.e., $\delta^{13}C_{\text{snails}}$ - $\delta^{13}C_{\text{mussels}}$) from a

sample of 13 Patagonian lakes is 6.84 but there is substantial variation (S.D.=3.03; Correa and Hendry, in rev.). Taking this mean difference between the molluscs, and an extreme lipid-induced δ^{13} C bias of +1‰, the proportion of interest (alpha) could be underestimated in ca. 15%. If the difference is 1, 1.5, or 2 S.D. smaller than the mean difference, the underestimation increases to 26, 44 or 128%. These are unlikely worstcase-scenarios (generally the overestimations will be <15%). However, they make the point that the results of studies using tissues with different lipid concentrations must be interpreted with caution, particularly when differences in isotopic signatures between baselines are small and variation in lipid content among organisms is high (Post et al., 2007; Fagan et al., 2011).

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