ON THE IMPORTANCE OF THE STUDY OF PAST CONNECTIVITY TO UNDERSTAND PRESENT AND FUTURE PATTERNS IN A FRESHWATER FISH

by

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When I wake up in the morning I see myself in the mirror and I notice I am older than the day before. Is the reminder of the beginning of a new day and a reminder of the linearity of time. But most of the nights, while I sleep, memories from the past and the future come to me. All possible realities mix in my dreams and the passive linear movement of the time vanishes, until I wake up and I see the linear time is still there. During the night, when I look at the stars, I see all these bright objects as a persistent reminder that the light we see might not be there and that the present is always the past. It is an ironic reminder of the relativity of time and a reminder that as I teenager I wanted to be an astronomer but I never became one. Instead I became a Biologist. But my passion for the study of time never went away and I decided to recover "the light" of past organisms through genetic markers to study the past, the present and predict the future of species. Now I think about it, somehow by studying evolutionary processes, I became an astronomer.

Now I am finishing my PhD and after all these years I can say I did my best to answer the questions I had about the influence of the past in “the present” and the questions about the influence of “the present” in the future. Now I am more convinced that the past influence everything, and our “present” is just an illusion.

Napoleon, in a very autopoietic statement, said that a man creates himself. It is my belief that a man cannot creates himself, because the influence of the past, the landscape, and the people around us shape our minds. Thus I would like to dedicate my thesis to those who have had an influence in my mind and made me the person I am. I would like to dedicate these pages to my parents for giving me the life and support me, to my sister and brother for taking care of me when I was a child, to my grandparents for teaching me to seed, harvest, cook, and enjoy the simple things of life, to my high school math teacher Arturo Nuñez for teaching me that Math was he science of abstraction, to Hugo Moyano for teaching me all he could about Lyell, von Humboldt and Darwin, to Pedro Victoriano for teaching me how to think like a Biologist, to Daniel Ruzzante for giving me the opportunity to make a PhD and support me all these years, to all my friends, especially to Megacephalos, for encouraging me to pursue my goals through these years, to my wife Anahí for her unconditional support and for teaching me that Nietzsche was wrong and that real love was a real feeling, and finally I thank my son Renato (my second PhD) for teaching me how to be a father and how to be a better man.

Iván

Leaves are falling all around
It's time I was on my way
Thanks to you I'm much obliged
for such a pleasant stay
But now it's time for me to go
The autumn moon lights my way…

(Ramble on, Led Zeppelin)

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ABSTRACT

Conceptually, in evolution, the variable "*time*" is fundamental. Extant populations are the result of long- and short-term historical processes affecting them through time. Populations in areas near the poles have been exposed to extreme changes in landscape during the Quaternary glacial cycles. This is the case for *Galaxias platei,* a species that survived the Quaternary in the lakes and rivers of Patagonia, a region that experienced significant changes following the Last Glacial Maximum during deglaciation as well as more recently, as a result of the construction of dams and the introduction of invasive salmonids during the Anthropocene. With these processes in mind I used genetic markers to try to (1) elucidate the effect of long- and short-term historical processes on the genetic diversity and divergence of contemporary *G. platei* populations, and (2) predict what would happen to future populations. I found that while the Quaternary glaciations had a great influence in contemporary populations, most of the configuration of extant populations took place during the Holocene and Anthropocene (Chapters 2, 3, and 4). Dams can have an extremely negative effect, especially for low diversity populations experiencing recent expansion, where population isolation could lead to local extinctions (Chapter 3). The difference in genetic diversity between *G. platei* populations inhabiting lakes with and without salmonids is similar to that observed in marine species between populations that are pristine and those that are threatened by overfishing (Chapter 4). Finally an analysis of simulated populations demonstrated the importance of spatial arrangement in the prediction of the effects of dams (Chapter 5). Thus, by using *G. platei* populations as a model species for the region I revealed the importance of interpreting patterns of diversity and population structure considering processes that act over different temporal scales. I thus used information from the past to understand the present and project what would happen with future populations as consequence of landscape modifications induced by humans.

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CHAPTER 1 INTRODUCTION

1.1 Introduction

The genetic composition of a population as observed with neutral markers is the result of mutation, genetic drift and gene flow acting on individuals over generations (Slatkin 1987). The temporal dimension is inherent to each of these processes but its effects on estimates of population divergence and diversity are however, often overlooked. Consequently, there is often an oversimplification and potential bias in the interpretation of the factors responsible for the observed patterns of population structure and gene flow if only contemporary factors are considered (Dyer et al. 2010).

While classifying biology into subareas facilitates our understanding of the discipline, such classifications also tend to separate the analysis of the observed patterns in a way that may lead to the dismissal of information that can explain these patterns better (Sork & Waits 2010). A case in point is the relationship between phylogeography and landscape genetics. While phylogeography studies the effect of historical processes affecting the evolutionary relationship between and among populations and species, landscape genetics studies the effect of contemporary processes linked to habitat characteristics and their relationship with the genetic diversity of extant populations (Manel et al. 2003; Manel & Holderegger 2013). The resemblance between phylogeography and landscape genetics is high. While the tools used differ, the questions that they address are very similar, differing largely in the temporal scale under analysis and the markers used (Wang 2010; Rissler 2016).

As a general rule, the proper interpretation of diversity patterns should incorporate the temporal dimension to understand the relative importance of historical vs. contemporary processes on genetic diversity. This can be achieved using a vast array of analytical tools at our disposal. A handful of recent studies have done just this (e.g. Hansen et al. 2014; Blair et al. 2015; Woodall et al. 2015; Salisbury et al 2016), including the temporal dimension into the interpretation of patterns of population structure. Nevertheless, it is common to find studies using generalizations that might affect the interpretation of the results. For example, it is commonly unquestioned that software using coalescent approaches would most likely reflect historical processes, and those based on allele diversity should reflect mostly contemporary processes, but this has not always been tested

empirically. A similar situation is often encountered in studies using life history information and assuming the life history trait values are the same across populations and through time. Therefore, it is necessary to review and analyze what has been done up to date in population genetics studies to understand the role of *time* over the main population diversity and divergence patterns.

1.1.1 What are contemporary and what are historical analyses of gene flow and population structure?

Contemporary analyses are those analyses using allele frequencies of extant and recent individuals (i.e. up to a few generations before present) to estimate contemporary diversity, genetic structure and gene flow. On the other hand, historical analyses are those analyses using coalescent approaches, niche modelling and comparisons between samples taken at different periods of time to study the diversity, structure and historical migrations of ancestral populations (e.g. processes related to the anthropogenic changes that may have occurred a few centuries ago and those related to the Quaternary glaciations) based on extant samples and/or archived museum samples (Pearse et al. 2011; Bray et al. 2013; Ploshnitsa et al. 2013; Sharma et al. 2013; Hansen et al. 2014; Inoue et al. 2015)

1.1.2 Genetic markers and samples used to address contemporary and historical questions

One or several organelle DNA sequence markers (i.e. mitochondrial and chloroplast DNA) have been used to analyze historical patterns of populations using coalescent approaches. The relatively low substitution rate ($\mu = 10^{-3}$-10^{-8}) of sequence markers make them suitable for the detection of the traces of historical processes, mostly in the field of phylogeography, with some exceptions in landscape genetics. DNA sequence reliability depends on good sequence alignments, substitution rate variability, nucleotide frequencies and lack of substitution saturation. Substitution saturation can wipe away information of long-term historical processes, nevertheless saturation is rarely tested (e.g. using the method described by Xi et al. 2003), and thus it is likely to find studies misinterpreting results (Xia et al. 2003; Gharib & Robinson-Rechavi 2013). Microsatellite markers, with a faster

evolutionary rate ($\mu = 10^{-3}$–10^{-5}), have been used for the study of both historical and contemporary patterns. However, just like DNA sequences can exhibit substitution saturation, microsatellites can exhibit homoplasy producing the loss of informative characters (Wan et al. 2004; Wang 2010; Bohonak & Vandergast 2011), limiting their utility for detecting long-term historical processes (i.e. those related to the early and middle Pleistocene). Recently, SNPs have shown to be suitable for studying patterns at both temporal scales too, which makes them more likely the marker of choice for future research using a multitemporal perspective. A decade ago, SNPs were used to answer questions regarding recent processes mostly, but soon they proved to be useful for disentangling multitemporal patterns (Brumfield et al. 2003). SNPs are less likely to exhibit homoplasy than microsatellite markers (Morin et al. 2004; McCormack et al. 2013); therefore they can provide information from a wide range of regions of the DNA from highly variable to highly conservative regions ($\mu \approx 10^{-8}$). Overall, a combination of two or more types of markers (e.g. mitochondrial and microsatellite) are most likely to produce credible results for the study of both historical and contemporary patterns. Regardless of the marker used, the number of markers, as well as the sample size, become critical and should always be pre-assessed before moving forward with analyses and interpretation (Landguth et al. 2012; Hoban et al. 2013).

1.1.3 Contemporary population structure

Population structure is key for interpreting the dynamics of extant populations, including how genetic drift and gene flow affect genetic diversity. Population structure can be evaluated with several methods. Three statistics/methods are the most commonly used to assess the number of populations and other inferences. The F_{ST} (Fixation index), which is Wright's *F*-statistic used to measure population differentiation due to genetic structure when divergence is caused by genetic drift (Wright 1949). F_{ST} for diallelic loci ranges from 0, where all populations have equal allele frequencies, to 1 when populations are fixed for different alleles. Although the actual amount of isolation reflected by a given value of F_{ST} is ultimately a function of Ne, F_{ST} estimates in the range of 0-0.05 are generally interpreted to indicate little genetic differentiation, estimates between 0.05-0.15 suggest moderate genetic differentiation and estimates above 0.15 suggest substantial genetic differentiation

(Balloux & Moulin 2002; Frankham et al. 2011). STRUCTURE (Pritchard et al. 2000) is a powerful software used to identify population structure using multi-locus genotype data using Bayesian inference and Markov Chain Monte Carlo estimation, assuming Hardy-Weinberg and linkage equilibrium within genetic clusters. It can also be used to assign individuals to populations and identifying migrants and admixed individuals, among other options. STRUCTURE identifies the most likely number of genetic clusters or populations (K) among a given number of populations to assess ranging from 1 to n + 1, with n = total number of sampled locations. The most likely number of populations is then evaluated using the Evanno method (Evanno *et al*. 2005) and the Log likelihood for each K (Ln P(D) = L(K)). Principal Coordinates Analysis (PCoA), also known as Metric Multidimensional Scale (MDS), is a method that explores similarities and dissimilarities of data by using a distance matrix (e.g. F_{ST} and Nei distances) between populations assigning items in locations along a low-dimensional space. In general, the three methods produce reliable information about the number of populations. Nevertheless, many authors have claimed that all of these methods can under or overestimate the number of populations under certain circumstances. For instance, it has been noticed that the F_{ST}s are not informative of genetic differentiation when the level of genetic diversity is high within populations and that F_{ST}s can be inflated when diversity is low (Jakobsson et al. 2013). For STRUCTURE, it has been found that when the data contains little information (i.e. low sample size, low number of markers, and/or low number of alleles), the software produces imprecise results, and when there is inbreeding or relatedness among sampled individuals, STRUCTURE can overestimate the number of populations (Hubisz et al. 2009).

1.1.4 Contemporary and historical migrations

Migration among populations is one of the main driving forces of evolution (Abdo et al. 2004), with high gene flow blurring population limits, and low gene flow facilitating the differentiation among populations, reducing the likelihood of outbreeding depression and promoting local adaptation on populations (Frankham et al. 2011; Richardson & Urban 2013). The effect of migration on the receiving population depends on parameters such as population size, effective population size (N_e), habitat size, interactions with other species and other ecological and landscape characteristics. All these parameters can fluctuate

through time as a result of the appearance and disappearance of barriers and the effect of genetic drift, therefore historical and contemporary migrations can give some insights on how connectivity changes as response to changes in the physical and biological conditions. Contemporary gene flow considers the proportion of immigrant (individuals originated in nearby populations), into local population where resident (individuals that are part of a focal population) exist. Different software packages are used to estimate contemporary migrations, with BayesAss (Wilson & Rannala 2003) being the most commonly used. BayesAss uses a Bayesian method with a Markov chain Monte Carlo approach to estimate the gene flow among populations from the current generation up to three generations in the past. A limitations of BayesAss lies in the fact that the software assumes that the proportion of migrant individuals cannot exceed 1/3 each generation (migration = 0.33), which occurs when low differentiation exists between populations (Faubet et al. 2007). As a consequence, migration values near 0.33 can indicate an unreal contemporary pattern of migration when low differentiation among populations exists, masking ongoing processes of population divergence (Excoffier & Heckel 2006). This type of error can be minimized by first estimating the number of populations so as to avoid pre-assigned populations based on the geographical distribution of individuals.

Among other software available, BIMr (Faubet & Gaggiotti 2008) appears as a good option to estimate contemporary gene flow when information of the landscape characteristics is available. BIMr uses a Bayesian approach to calculate contemporary gene flow and identify environmental factors influencing migration. Environmental factors are evaluated with a generalized linear model by using a Markov Chain Monte Carlo method to assess the factors list set by the user. Experimental analyses revealed that BIMr provides good estimates of gene flow with a high accuracy to detect a wide range of scenarios (Balkenhol et al. 2009), nevertheless BIMr is less frequently used than BayesAss.

Estimates of historical migrations are usually calculated using Migrate-n (Beerli & Palczewski 2010). Migrate-n, uses a coalescent approach to calculate the cumulative migration among *n* subpopulations up to the most common recent ancestor, assuming asymmetric migration rates and different subpopulation sizes. Migrate-n can calculate the mutation-scaled immigration rate (M), but it can also estimate the number of migrants successfully entering a population per generation (*Nm*). It has been noticed that the

parameter M from Migrate-n can mask recent changes in gene flow if a strong historical migration signal exists (Beerli 2004), and the opposite occurs with the parameter *Nm* (Samarasin et al. 2016).

Several studies have compared contemporary and historical migration patterns using contemporary gene flow (e.g. using BayesAss and BIMr) and mutation-scaled immigration rate (using Migrate-n) respectively (i.e. Smith et al. 2011; Inoue et al. 2015; da Silva Carvalho et al. 2015; Salisbury et al. 2016). These parameters are not as comparable as is frequently assumed. In order to make both parameters comparable it is necessary to convert the historical mutation-scaled immigration rate using the relation M = m μ, with m = the proportion of immigrants among populations per generation and μ = mutation rate. A difficulty arises from the fact that for most species μ is unknown and is thus often assumed to be of a magnitude similar to that estimated for some other species. This uncertainty in the actual value of μ is of major consequence for very small values of the parameter, and this error will be transferred to the estimated m, thus affecting the interpretation of the relationship between historical and contemporary migration rates. Given the fact that μ is unknown for most species, any comparison between historical and contemporary migration should be restricted to an examination of changes in the direction and proportion of the magnitude of the migration (compared against other migrations of the same temporal scale), rather than trying to compare the absolute values of contemporary and historical migrations (see Chiucchi & Gibbs 2010). This problem can be solved by estimating a substitution rate for focal species using ABC methods to compare historical and contemporary migrations (see Converse et al. 2015).

1.1.5 Contemporary and Historical N_es

Effective population size (N_e), the size of a theoretical population affected by genetic drift at the same rate per generation than a population under study (Wright 1931) is a key parameter in evolutionary biology and conservation management that determines the rate of the genetic drift acting over a population, allowing to infer the relative importance of mutation, migration and selection (Waples 2016). In simple terms N_e provides an indication of the health of a population. Consequently, the study of how N_e changes through time is important to understand how species respond to changes in landscape and life history,

being a crucial parameter for conservation. For example, the relationship between N_e and habitat size (i.e. habitable area) can be useful for understanding the role of gene flow and genetic drift over a metapopulation because a strong correlation between N_e and habitat size suggests genetic drift is the main factor responsible for the observed diversity while a lack of correlation suggest that neither genetic drift nor habitat size explain the observed diversity (Castric et al. 2001; Koizumi et al. 2006; McCracken et al. 2013; Salisbury et al. 2016).

Effective population sizes can be estimated directly from demographic data or indirectly from genetic markers. N_e tends to be hard to estimate in nature because natural populations, in contrast to theoretical populations, exhibit demographic fluctuations and changes in their life history characteristics, thus $\widehat{N}_e$s (estimates of N_e) tend to deviate from the actual N_e. Genetic approaches to calculate $\widehat{N}_e$s can solve the problem of estimating N_e, producing reliable estimates of contemporary (short-term) and historical (long-term) N_e (Luikart et al. 2010; Palstra & Fraser 2012). Short-term $\widehat{N}_e$s can be calculated from a single sample using the Linkage disequilibrium (LD) or from two or more points in time using a method based on the temporal changes in allele frequencies (Waples 2016). Depending on the method used to calculate $\widehat{N}_e$ and the life history of the species (i.e. discrete generations and variable age at maturity) $\widehat{N}_e$ can reflect the N_e of the offspring, the parental generation or an average over a number of generations in the recent past. $\widehat{N}_e$s calculated using the LD method provide information of the parental generation for species with discrete generations. For species with variable age at maturity, the LD method can provides $\widehat{N}_e$s of different generations depending on whether juvenile or adult individuals were sampled (Waples 2005). On the other hand, the temporal estimates of $\widehat{N}_e$ provide information of the harmonic mean of the N_es in generations 0 through t-1 for species with discrete generations. For species with variable age at maturity, the temporal method provides more complex results, because the $\widehat{N}_e$s obtained are calculated with individuals from two periods of time, and each period of time presents individuals from different age structures. Consequently, the effective population size is population and life history dependent, making it hard to make general conclusions for several species with different biology.

In contrast to short-term $\widehat{N}_e$s, long-term $\widehat{N}_e$s are estimated using coalescent approaches recovering the average N_e up to the generation of the most common recent ancestor (Hare

et al. 2011). Independently of the methodology used to calculate short and long term $\widehat{N}_e$s, the estimates are always likely to present inaccurate $\widehat{N}_e$s when populations have large census sizes but also when gene flow exists (especially when gene flow is ignored in assumed isolated populations). $\widehat{N}_e$s can also be inaccurate because of methodological problems like low number of loci and sample size used, producing wider confidence intervals (Hare et al. 2011). Therefore, while $\widehat{N}_e$s are useful for conservation, conclusions based in inaccurate estimates must be taken cautiously due to the uncertainty of the results to prevent the development of inappropriate or misguided conservation measures.

Reconstructing the past and the future

1.1.6 Backward-in-time analyses

Computer simulations have significantly enhanced the analyses of the contemporary structure of the populations and also the reconstructions and predictions of the most likely patterns from the past and the future respectively (Hoban et al. 2012). Approximate Bayesian Computation (ABC) methods have risen as important tools for backward-in-time reconstructions allowing simulating and predicting how changes in the connectivity during the past affected the contemporary populations (Marin et al. 2012). ABC methods use genetic data to test biological hypotheses (models). These methods simulate parameters under one or more models proposed and then compare the simulated data with the observed data by calculating their likelihood to determine the most probable model (Beaumont et al. 2002; Csilléry et al. 2010). The reliability of the results depends on the use of real hypotheses based on real data (e.g. geological and/or paleontological data) rather than in suppositions. Otherwise, results can be biased when true models are not included (Beaumont 2010) because ABC methods depend not only on the sets of assumptions for each software, but also on the availability of data related to divergence events and migration flows during specific periods of time to be tested (Hoban et al. 2012).

Studies using ABC have included time scales ranging from Anthropocene to older processes related to the Pleistocene-Holocene glaciations. Such studies have produced reliable evidence to understand how changes in the landscape during the past have affected patterns of genetic divergence in extant populations (Sunnåker et al. 2013). Several software packages using ABC methods have been developed during the last decade, with

three of them being the most commonly used to study contemporary and historical patterns: DIYABC (Cornuet et al. 2014), IMa2 (Hey 2010) and SIMCOAL/FASTSIMCOAL (Laval & Excoffier 2004; Excoffier et al. 2013). These three software packages differ in their approaches with DIYABC being the most user-friendly due to its graphic interface, but also the most restrictive in terms of assumptions because it considers the absence of gene flow after a divergence event. IMa2 and SIMCOAL are less user-friendly but more flexible for gene flow scenarios, with more relaxed assumptions. Instead of assuming zero gene flow among populations after a divergence event assumed (as assumed in DIYABC), IMa2 and SIMCOAL allow for migration between populations after a divergence event, being less restrictive for complex scenarios with changes in connectivity.

1.1.7 Forward-in-time analyses

A multitemporal landscape genetics approach allows to study what occurred with populations in the past, but it also includes the possibility to create simulations to predict what would happen to populations under scenarios that involve the appearance/disappearance of barriers and changes and fluctuations in diversity as a result of bottlenecks, for instance. Thus, it is recommended to use a combination of historical and contemporary analyses with forward-in-time analyses (Aberer & Stamatakis 2013). Forward-in-time analyses are based on simulated models using a set of parameters defined by the user (i.e. mutation, population size, recombination and selection) to test how populations will respond to changes in habitat, connectivity and population sizes (Carvajal-Rodríguez 2010; Scoble & Lowe 2010). Forward-in time analyses producing reliable results are usually highly time consuming: these analyses are individual-based simulations and thus need to simulate population sizes that are similar to the real population being examined (Aberer & Stamatakis 2013). Studies using forward-in-time analyses combined with contemporary and historical analyses are relatively new. As with most model-based simulations they still need to be explored empirically to understand if they can actually predict the genetic patterns of the populations. Software packages used for forward-in-time simulations have been broadly reviewed by Carvajal-Rodríguez (2010) and Hoban et al. (2012), with three software among the most used. These software are CDPOP (Landguth & Cushman 2010), a software package to simulate how complex changes in the landscape

affect diversity and gene flow in a metapopulation, BOTTLESIM (Kuo & Janzen 2003) a software to simulate future bottlenecks given an input file with the allele frequencies for a population and EASYPOP (Balloux 2001) a software that allows to simulate diverse migration and migration models. More flexible software written in python like SLiM (Messer 2013) and SIMUPOP (Peng & Kimmel 2005) have emerged but their pros and cons need to be studied yet. Niche modelling can also be used to look forward and predict the most likely distribution of populations; allowing the inference of scenarios in terms of how changes in the habitability of certain areas will affect migration patterns in a metapopulation (see Metzger et al. 2015).

All of these forward in time software packages can become powerful tools for the assessment of the consequences of environmental modifications including the appearance or disappearance of barriers in the future and finally to define conservation policies based on how a certain species responded to changes in the landscape during the past. For instance, a high number of species are experiencing population reductions and by using these tools we can predict what would happen to future populations facing bottlenecks to know what populations and what areas to prioritize. Here, simulations of the loss of number of alleles can provide useful information about how genetic diversity will change in response to changes in the landscape (Maruyama & Fuerst 1985; Allendorf 1986; Greenbaum et al. 2014).

1.2 A short review of multitemporal analyses

The need for more studies focused on the role of gene flow and genetic drift through time has been highlighted over the last decade (Hall & Beissinger 2014). Empirical studies have shown a strong influence of historical, Pleistocene-Holocene related processes on contemporary genetic patterns (Hickerson et al. 2010). The quaternary was characterised by massive expansions and retreats of glaciers in high altitude and temperate areas worldwide, reducing the suitable habitat available for many species. As a consequence, huge migrations took place, forcing species to survive in refugia, reducing the genetic diversity and in some cases leading to species extinctions (Hewitt 2004). During the Holocene, after the retreat of the ice and the increase in the sea level, several plant and animal species expanded their range and their genetic diversity, leading to the extant

distribution of native species, showing a greater influence of long-term processes linked to the Pleistocene-Holocene over contemporary patterns (Zhao et al. 2013; Yuan et al. 2014; Davis et al. 2015). Although it is commonly accepted that long-term processes had a great influence over extant genetic attributes of populations, some studies analyzing populations at different time scales have started to emphasize that the genetic structure of the populations might be the result of contemporary factors rather than historical factors (Lee-Yaw et al. 2009; Busby et al. 2015).

Despite the recent expansion and pattern of increase in the genetic diversity of the species during the Holocene, recent studies have suggested a shift in this pattern as a consequence of recent processes linked to human activity, remarking the importance of multitemporal analyses (Camargo-Sanabria et al. 2015; Dirzo et al. 2014; McCauley et al. 2015; Thomas 2015). The lack of a multitemporal perspective can hide some demographic processes, as it happens when using a contemporary perspective only in populations where historical processes like founder effect, ancestral or historical habitat fragmentation and population expansion have occurred (Lee-Yaw et al. 2009; da Silva Carvalho et al. 2015). A more complete description of the history of populations appears when analyzing multitemporal patterns. In general, studies show that combined historical and contemporary forces have shaped the observed genetic composition of the populations (Smith et al. 2011; Fontaine et al. 2012; da Silva Carvalho et al. 2015). For example, it has been found that the macro-patterns of the metapopulations of freshwater mussel (*Popenaias popeii*) in North America are the result of the effects of changes in the historical landscape characteristics by the end of the Pleistocene while local structure is the result of recent human intervention in the region (Inoue et al. 2015). A similar patterns can be observed for the gray seal (*Halychoerus grypus*) in the Atlantic where differences in the migration patterns were interpreted as a recent migration from distant populations and historical high connectivity among populations from closer localities (Klimova et al. 2014). Furthermore, recent multitemporal landscape genetics analyses present a wider view of the observed historical and contemporary patterns when taking into account the direct effect of life history, genetic drift and the configuration of the metapopulation. As observed by Salisbury et al. (2016) in a longnose sucker (*Catostomus catostomus*) metapopulation inhabiting a dendritic system, the observed genetic diversity is particular for each population because historical

and contemporary landscape characteristics (e.g. habitat size, physical connectivity, life histories) affect independently the migration-drift equilibrium within each population

Nonetheless, focus on historical processes does not necessarily mean focus on Pleistocene/Holocene temporal scales. For example, the interruption of gene flow has been greatly affected by the appearance of more recent historical processes as medieval dams, as it was observed for *Salmo trutta* in Denmark (Hansen et al. 2014). Furthermore, changes in the landscape and the biological connectivity among populations have also provided information of detectable patterns of the recent loss of the diversity in some species. In some cases, this information comes from comparing the same population at two different times using vouchered samples and direct measures of the loss of alleles (Pearse et al. 2011; Ploshnitsa et al. 2013). In other cases, the information comes from indirect measures using coalescent approaches to estimate changes in population N_es (i.e. Fontaine et al. 2012; Blair et al. 2015) providing a link between the loss of the diversity and processes like human impact, habitat fragmentation and/or introduction of species.

The study of changes in migration patterns ranges from small but significant reductions in gene flow during recent times as observed in the Jerusalem cricket (*Stenopelmatus mahogany*) in southern California (Vandergast et al. 2007) up to high differences in the gene flow with a strong signal of anthropocory (species dispersion mediated by human) increasing the recent gene flow (i.e. the wild carrot *Daucus carota*; Rong et al. 2013). In some cases, the reduction in contemporary gene flow due to physical barriers has isolated populations that were previously connected (Hsieh et al. 2013; Sharma et al. 2013; Vera-Escalona et al. 2015). A few other studies have found little evidence of a difference in migration, as it has been shown for the wild rice (*Oryza rufipogon*) in China, where both contemporary and historical gene flow were low (Zhao et al. 2013).

As it has been remarked, the main goal of multitemporal analyses should be the prediction of future patterns based on contrasting historical and present genetic patterns. For example, analyses based on niche modelling identified that Pleistocene glacial refugia will be unsuitable for the bat species *Plecotus austriacus* in Europe (Razgour et al. 2013) in the future based on predicted changes in habitats and barriers to migration due to future climate change. Nevertheless, there is a wide range of areas to study for future patterns of populations. For instance, forward-in-time analyses can be used for the identification of

priority areas for conservation in the face of global climate change, or to identify the potential effects of habitat fragmentation due to human intervention, as well as to determine species corridors in already threatened areas.

1.3 A brief summary of this thesis

With all these issues in mind I studied populations of the freshwater fish *Galaxias platei* in Patagonia. In Chapter 2 I tried to elucidate the relative influence of long- and short-term historical processes on contemporary genetic patterns in *G. platei* in two river basins in Chile located in North and South Patagonia. ABC methods revealed the footprints of ancestral paleolakes at high latitude and altitude where populations survived during the Pleistocene glaciations. From these paleolakes, populations colonized the lakes where populations are found today. For the population in North Patagonia, I found no evidence of a reduction in the genetic diversity as consequence of the construction of a dam (built 40-50 years ago). In Chapter 3 I then studied historical, contemporary and future genetic and demographic patterns of a *G. platei* metapopulation in Central Patagonia (Puelo drainage) to assess the potential effect of the construction of dams, and see how this multitemporal perspective of landscape genetics could be used in practice to lead to robust, knowledge based conservation measures. This chapter revealed the importance of looking at historical patterns of a population to explain contemporary patterns, and from there identify potential risk of populations since historical patterns suggested low historical genetic diversity and recent population division. Historical patterns revealed that populations in this area will be highly threatened if dams are built in the area, potentially leading to population extinctions. In Chapter 4 I then focused on the effect of other short-term processes (presence of exotic salmonids) that could help understand contemporary patterns for all *G. platei* populations used in this study and that were not studied before. Recent literature has shown that the introduction of salmonids in Patagonia during the last century is one of the biggest threats for native species, but no study exists thus far that examines the effect of salmonids on the genetic composition of native populations. Thus I studied the effect of invasive salmonids on *G. platei* populations during the last century, under the hypothesis that salmonids should reduce the genetic variability of *G. platei* as consequence of predation and or competition. For this purpose I combined several tools to

evaluate what scenario was the most likely to explain the reduction in the genetic diversity of *G. platei* in lakes where the species coexists with introduced salmonids. Using traditional and non-traditional tools (genetic modeling) and a multi-temporal perspective I examined the influence of the presence of salmonids on the genetic diversity of *G. platei*. Finally I created several scenarios to simulate a decrease in connectivity in hierarchical, dendritic systems, as consequence of the construction of dams. The goal was to solve an unanswered question from Chapter 1: Is it possible to observe a change in the genetic diversity in freshwater fish populations after the recent construction (40-50 years) of dams that reduce connectivity? For this purpose I simulated several scenarios with different effective population sizes and gene flow to examine the circumstances under which genetic diversity would decline and what approaches would be the most suitable for answering this question, with the goal of recommending what tools to use for conservation (Chapter 5). Here I found that the probability to detect changes in genetic diversity is very low for recent processes in species with moderate generation time (i.e. 4 years). Further, this probability depends on the effective size and relative position in a dendritic spatial arrangement of the affected populations as well as on the magnitude of the reduction in gene flow. Included in this thesis are two appendices describing the whole mitogenome of *G. platei* and 15 microsattelite genetic markers developed for the species (Appendices 1 and 2).

The thesis culminates with a general discussion of the effect of the variable time on the study of the genetic diversity populations and on how important is it to consider all variables affecting populations for a better understanding how genetic diversity changes through time, as well as how important this information is for the prediction of potential future population changes following habitat alterations.

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CHAPTER 2 ECHOES OF A DISTANT TIME: EFFECTS OF HISTORICAL PROCESSES ON CONTEMPORARY GENETIC PATTERNS IN *Galaxias platei* IN PATAGONIA

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2.1 Abstract

Interpreting the genetic structure of a metapopulation as the outcome of gene flow over a variety of timescales is essential for the proper understanding of how changes in landscape affect biological connectivity. Here we contrast historical and contemporary connectivity in two metapopulations of the freshwater fish *Galaxias platei* in northern and southernmost Patagonia where paleolakes existed during the Holocene and Pleistocene, respectively. Contemporary gene flow was mostly high and asymmetrical in the northern system while extremely reduced in the southernmost system. Historical migration patterns were high and symmetric in the northern system and high and largely asymmetric in the southern system. Both systems showed a moderate structure with a clear pattern of isolation by distance (IBD). Effective population sizes were smaller in populations with low contemporary gene flow. An approximate Bayesian computation (ABC) approach suggests a late Holocene colonization of the lakes in the northern system and recent divergence of the populations from refugial populations from east and west of the Andes. For the southern system, the ABC approach reveals that some of the extant *G. platei* populations most likely derive from an ancestral population inhabiting a large Pleistocene paleolake while the rest derive from a higher-altitude lake. Our results suggest that neither historical nor contemporary processes individually fully explain the observed structure and gene flow patterns and both are necessary for a proper understanding of the factors that affect diversity and its distribution. Our study highlights the importance of a temporal perspective on connectivity to analyse the diversity of spatially complex metapopulations.

2.2 Introduction

2.2.1 Contemporary and historical patterns

An important emerging question in landscape genetics concerns the relative influence of historical vs. contemporary processes on observed patterns of genetic diversity. Until recently, the field of landscape genetics focused almost exclusively on the role of contemporary processes (Manel et al. 2003; Storfer et al. 2010; Manel & Holderegger 2013) with the influence of history most often examined within the framework of phylogeography. It is clear, however, that studies examining the patterns of genetic diversity in either a historical or a contemporary context are likely to provide only a partial and potentially biased view of the biological processes responsible for the observed patterns (Sork & Waits 2010; Wang 2010) and understanding the influence of processes operating over multiple temporal scales is increasingly being recognized as necessary (Dyer et al. 2010). Consideration of both temporal scales in conservation genomics is likely to be important regardless of the taxon and/or region under study, but studies focused on species inhabiting the temperate regions of the world, the regions that experienced the effects of the Quaternary glacial cycles directly (Hewitt 2000; Ruzzante et al. 2008; Ruzzante & Rabassa 2011; Fraser et al. 2012), are most likely to reveal the strongest signals of processes affecting population genetic structure and diversity over both temporal scales. During the Quaternary glacial cycles, large sections of Patagonia were covered by extensive ice sheets (Rabassa et al. 2011), forcing some of the existing flora and fauna to either migrate north and northeast or survive in local refugia (Sersic et al. 2011). While earlier phylogeographic studies have suggested refugia were located on the eastern side of the Andes and along the coasts of Chile and Argentina (e.g. Markgraf et al. 1995), more recent studies have shown evidence of populations that survived in Pleistocene refugia near or within the areas that had been presumed to have been covered by ice (Ruzzante et al. 2008; Xu et al. 2009; Cosacov et al. 2010; Unmack et al. 2012; Vera-Escalona et al. 2012). Large proglacial lakes are known to have existed in some of these refugial areas during and following the last glacial maximum (LGM); some examples are the paleolakes Elpalafquen and Tehuelche in Argentina and Chile, respectively (del Valle et al. 2000; Solari et al. 2012). In most cases, following water-level decline, these proglacial lakes were fragmented

into a number of lakes in existence today resulting in significant changes in connectivity (Garcıa et al. 2014). In other cases, extant lakes had a more recent origin during the Holocene after the retreat of the ice cap and populations inhabiting them are mostly the result of late colonization (Laugenie 1982; Ruzzante & Rabassa 2011; Ruzzante et al. 2011).

Regardless of the strong effects of historical processes, genetic patterns among freshwater populations in Patagonia might also result from contemporary natural processes, like volcanism and earthquakes, but also from human intervention over the course of the last century, like river damming (Meybeck 2003). Such alterations are likely to affect migration patterns, exacerbate asymmetries in gene flow or eliminate gene flow altogether and lead to an increase in differentiation among populations as has been observed elsewhere (e.g. Crispo et al. 2006; Leclerc et al. 2008; Gomez-Uchida et al. 2009).

Our research focused on the genetic structure and diversity of the freshwater fish *Galaxias platei* inhabiting two Chilean drainages differing in their physical connectivity and history, the San Pedro system (Valdivia River Basin) in northern Patagonia (~40°S; Fig. 2.1a) and the Serrano system (~50°S, Fig. 2.1b) in southernmost Chilean Patagonia. The San Pedro system (Fig. 2.1a) comprises eight connected Andean lakes. Five of these lakes (Pellaifa, Pullinque, Panguipulli, Calafquen and Riñihue; 221–196 m above sea level) were part of a post-Pleistocene paleolake hereafter called Huenuhue in existence from the Tardiglacial to the Neoglacial (13 000–2500 year BP; Thomason 1963; Laugenie 1982; Heusser 1995). This paleolake was subsequently fragmented into the extant lakes following the combined effect of ice melting and landslides linked to seismic and volcanic activity (Campos et al. 1980). Contemporary influences of physical connectivity in this system include a fast-flowing river that probably limits upstream migration from Lake Panguipulli to Lake Neltume and the construction of a hydroelectric plant in Lake Pullinque in 1962 (Pullinque Hydroelectric Plant). The Serrano system (Fig. 2.1b) exhibits relatively low physical connectivity (Habit et al. 2012) as it contains three major waterfalls along the river course and several isolated lakes. One of the headwaters, Lake Azul, drains into the main stem of the Serrano River via a very shallow and narrow creek. Moraine studies in the Serrano system indicate the existence of a large late Pleistocene paleolake known as the Great Tehuelche Paleolake formed by several extant connected and isolated lakes (Nordenskjold,

Pehoe, Porteño, Sarmiento and Mellizas among others; 224– 33 m above sea level). The paleolake expanded and contracted during its existence, reaching its maximum extension during the Holocene around 9000–7000 years BP, connecting all extant low-altitude lakes with evidence of physical connectivity until 4000–1000 years BP (Marden 1997; Solari et al. 2012; Fig. 2.1b). All lakes in both systems are inhabited by *Galaxias platei* Steindachner (1898), a species with a geographic distribution that suggests a close relationship with the Quaternary glacial cycles. Phylogeographic studies suggest this species may have survived the glacial periods of the Quaternary *in situ* in local refugia on both sides of the Andes and have indeed shown evidence of drainage reversals and secondary contact zones between genetically divergent lineages during range expansion associated with glacial melting (Zemlak et al. 2008, 2010, 2011). While glacial refugia in the area of the San Pedro system were unlikely due to the recent emergence of the lakes from the melting cap (Tardiglacial), the late Pleistocene origin of the paleolake in the Serrano system suggests that the presence of a glacial refugium for freshwater species was probable in this system.

Here we examine the relative importance of historical vs. contemporary processes on the genetic diversity and structure of the *G. platei* populations inhabiting two systems. In the San Pedro system, we examined the probability of different colonization scenarios, including an origin from an ancestral population inhabiting the paleolake Huenuhue and the likelihood that some populations may have derived from drainage reversals from refugial populations located east of the Andes, as has been shown to have taken place among *G. platei* populations in neighbouring systems (see Zemlak et al. 2008). For the Serrano system, we examined the probability for the extant *G. platei* populations to have derived from a large ancestral population inhabiting the Great Tehuelche Paleolake (Solari et al. 2012; Garcıa et al. 2014). Given the relatively young age of the San Pedro metapopulation, we thus hypothesized there would be smaller differences between historical and contemporary gene flow among *G. platei* populations in this system than among those in the Serrano. Our findings suggest the contemporary landscape structure of the *G. platei* populations in Patagonia is likely best explained through consideration of both historical processes and their timing, as well as contemporary landscape features.

2.3 Methods

2.3.1 Sampling collection

Galaxias platei individuals were collected from two independent drainages located in northern and southern Patagonia, the San Pedro and the Serrano system, respectively. Fish were collected with seine nets and gill nets and by electrofishing and stored in 95% ethanol. The combination of collection methods ensured representation from a range of sizes and thus presumably cohorts (Belk et al. 2014). A total of 363 individuals were collected from five lakes in the San Pedro system (Table 2.1), two of these lakes were located above a dam (Pullinque and Pellaifa; Fig. 2.1a). One of the lakes in the system, Lake Calafquen, was sampled without success. In the Serrano system, 554 individuals were collected from eight lakes (Table 2.1), six of them connected (with low to high physical connectivity), including two lakes (Nordenskjold and Pehoe) with waterfalls above and below them, and two completely isolated lakes (Sarmiento and Mellizas; Fig. 2.1b).

2.3.2 Molecular protocol and genetic diversity

Whole DNA was extracted from a 5- to 10-mg piece of tissue using a glassmilk protocol (Elphinstone et al. 2003). DNA was amplified by PCR using 16 species-specific microsatellite markers (Arias et al. 2012; Vera-Escalona et al. 2014, see Appendix 2) in a 10-ll volume PCR. Two markers were monomorphic in the San Pedro system, reducing the number of markers for this system to 14. The PCR cocktail contained 3 μL of DNA, 1 μL of 10 mM $(NH_4)_2SO_4$, 1 μL of 2 mM $MgCl_2$, 1 μL of 2 mM dNTP, 0.1 μL of each primer, 0.1 μL of m13, 0.1 μL of 0.5 U TSG and 6.5 μL of H_2O. Microsatellite fragment lengths were observed in polyacrylamide gels using LI-COR sequencers (Biosciences, Lincoln, NE, USA) with a molecular ladder of known sizes (65–400 bp). Images were analyzed and the microsatellites were scored with the software SAGA^{TM} (LI-COR). All the following analyzes were made for each system independently.

2.3.3 Genetic variation and differentiation

The presence of null alleles and stutter were assessed with MICROCHECKER 2.2.3 (van Oosterhout et al. 2004). The presence of outliers was evaluated using an F_{ST}-outlier detection method in LOSITAN (Antao et al. 2008) by running $5x10^5$ simulations with confidence interval of 0.95. Allele frequencies, number of alleles, and mean observed and expected heterozygosities were calculated in GENALEX (Peakall & Smouse 2012). Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) tests were assessed in GENEPOP using default values (Raymond & Rousset 1995). The false discovery rate approach was used to correct the results for multiple tests in both the HWE and LD (Benjamini & Hochberg 1995).

2.3.4 Cluster analyses

The presence of populations/clusters was assessed with the software STRUCTURE 2.3.4 (Pritchard et al. 2000; Hubisz et al. 2009) assuming an admixture model and correlated allele frequencies with LOCPRIOR turned off. STRUCTURE was run for K = 1 to K n + 1, where n is the maximum number of lakes sampled for each system. Ten independent runs were conducted for each K, with a $2x10^5$ burn-in period, followed by $2x10^6$ sampling steps for the San Pedro system and $5x10^5$ sampling steps for the Serrano system. The likelihood results were collected and assessed in STRUCTURE HARVESTER (Earl & von Holdt 2012). The Evanno method (Evanno et al. 2005) was used to detect the number of genetic groups. The Greedy algorithm in CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) was used to create a single plot based on the ten replicates, and the final graphic results were obtained with DISTRUCT 1.1 (Rosenberg 2004).

2.3.5 Effective population sizes (N_es) and contemporary migration rates

Contemporary effective population sizes (N_e) were estimated with the linkage disequilibrium method implemented in LDN_e (Waples & Do 2008) using a random mating model, excluding alleles with frequencies <0.02. Recent migration rates were calculated with BAYESASS 3.0 (Wilson & Rannala 2003), identifying the gene flow signature from

the current generation up to two generations in the past. Five replicates with $3x10^6$ iterations were used, sampling every $20x10^3$ samples with burn-in equal to $5x10^6$. The effect of geographic distance on genetic distance was assessed with a Mantel test implemented in GENALEX6.5. F_{ST}s were estimated in GENALEX 6.5 and then linearized ($[F_{ST}/(1\ F_{ST})]$; Rousset 1997). Distance between lakes was measured based upon the stream connections between them. The data were obtained from ARCGIS DESKTOP 10.1 (ESRI 2011) using the data source available for Chile at http://www.diva-gis.org/gdata (inland water and elevation).

2.3.6 Historical migration patterns

Mutation-scaled migration rate (M) estimates were calculated using a Bayesian coalescent approach implemented in MIGRATE-n 3.6 (Beerli 2006) by assessing different migration models based on physical connectivity, as suggested by Carstens et al. (2013). Unlike BAYESASS, MIGRATE-n considers all gene flow between populations from the current generation back to the most recent common ancestor, detecting historical processes. A Brownian motion model was run to estimate M (M = m/l). Our analyses included 3 long chains and 4 replicates. The parameters Θ and M followed an exponential distribution (0–100, mean = 50; 0–1000, mean = 100, respectively). Burn-in was set in $35x10^3$, with a sampling increment of 40 and a total of $25x10^3$ recorded steps. Heating was set with four temperatures (1.0, 1.5, 3.0 and 10^6) with a static scheme. The first model for the San Pedro system assumed equal migration rate among all lakes (M = 100), while the second model considered that the fast-flowing Llanquihue River (Fig. 2.1a), flowing from Lake Neltume into Lake Panguipulli, has historically reduced upstream migration into Lake Neltume (M = 0.01) from the downstream populations. A third model assumed a reduced upstream migration into Lake Neltume (M = 0.01) as well as a reduced upstream migration into Lake Pullinque (M = 0.01), which has been separated from downstream locations since 1962, when the Pullinque Hydroelectric Plant was built.

In the Serrano system, we used two historical migration models to test for the effect of waterfalls on upstream gene flow. The first model included equal migration rates among all lakes; this assumes that waterfalls were not affecting gene flow among populations (M = 100). The second model considered waterfalls as physical barriers reducing upstream

gene flow (M = 0.01) while downstream gene flow was assumed high (M = 100). Posterior probabilities were calculated based on the log marginal likelihood (l mL) of each model using thermodynamic integration with the Bezier implementation from MIGRATE-n and then compared to detect the best fitted model following Beerli & Palczewski (2010).

2.3.7 Historical scenarios: approximate Bayesian computation

An approximate Bayesian computation (ABC) approach was used in the software DIYABC v2.0 (Cornuet et al. 2014) to estimate the likelihood of alternative colonization and divergence scenarios for each system that could explain the historical migration patterns based on the paleohydrology of each system. Four feasible scenarios were simulated for the San Pedro system to detect colonization routes, possible admixture events based on the position of glacial refugia described for *G. platei* in nearby latitudes (Zemlak et al. 2008), population divergence following the fragmentation of the paleolake Huenuhue and a scenario based on the results of STRUCTURE. The first scenario considers two colonization routes, one from populations located west of the San Pedro system, along or near the coast (via Lake Riñihue), and the second located on the eastern side of the Andes (via Lake Neltume) and an admixture event in Lake Panguipulli (Scenario 1; Fig. 2.2a). The second scenario considers the same colonization routes, without an admixture event, following a stepping stone divergence from the lower elevation lakes into the upper lakes, using the available information of the fragmentation of the lakes that were part of the paleolake Huenuhue (Scenario 2; Fig. 2.2b). The third scenario includes a glacial refugium in Lake Pellaifa and assumes that population divergence took place progressively from a high-elevation glacial refugium to the low-elevation lakes (Scenario 3; Fig. 2.2c). Lastly, Scenario 4 (Fig. 2.2d), was based on the results from STRUCTURE, assessing the likelihood of an early differentiation of the populations inhabiting lakes Neltume and Riñihue and the appearance of a late polytomy for the other three populations forming part of the paleolake Huenuhue (Pellaifa, Pullinque and Panguipulli). All four scenarios assume that colonization took place post-Pleistocene based on our knowledge of the first appearance of these lakes (Campos et al. 1980; Laugenie 1982). The analysis was run using four divergence times with prior uniform distribution with time t1 taking place 10–500 generations in the past, times t2 and t3 taking place 200–3000 generations in the past and

time t4 taking place 500–3000 generations in the past. This means $t1 < t2 \leq t3 < t4$, with t1 the most recent divergence and t4 the oldest. Times were based on information available regarding the recent Neoglacial origin of the Lake Pellaifa (t1), the origin of Lake Pullinque (t2) and the formation of the paleolake Huenuhue during the Tardiglacial (t4; Campos et al. 1980; Laugenie 1982). The differentiation of Lake Riñihue is uncertain, so we included this uncertainty in the prior distribution (Table 2.3). Three historical scenarios were evaluated for the Serrano system, assuming that extant populations originated from the Great Tehuelche Paleolake and nearby refugial lakes. In scenarios 1 and 2 (Fig. 2e and 2f), individuals from the five lakes that were once part of the Great Tehuelche Paleolake (Nordenskjold, Pehoe, Mellizas, Sarmiento and Porteño) were considered with an independent origin from the upstream populations (Dickson, Paine and Azul). For both scenarios, we assumed the divergence of the *G. platei* population in Lake Porteño occurring first, at time t4, the divergence of the populations in lake Mellizas, Nordenskjold and Sarmiento occurred next, at time t3, and the divergence of the population in Lake Pehoe occurring third, at time t2 after the retreat of the ice covering the extant area of this lake (Solari et al. 2012). Scenarios 1 and 2 differ in the origin of the populations from lakes Dickson, Paine and Azul. While in Scenario 1 we assumed an independent origin of populations in lakes Dickson and Paine from the population in Lake Azul, in Scenario 2 we considered a recent origin of populations in lakes Dickson and Paine from a refugial population in Lake Azul. Scenario 3 states that the population in Lake Azul has a common origin with the population in the Great Tehuelche Paleolake due to the presence of a river connecting both parts of the system (Fig. 2.2g). For this scenario, we hypothesized that populations in lakes Dickson and Paine had an independent origin. For all scenarios, we used the following prior uniform distribution t1 = 400–2000 generations, t2 = 600–3000 generations, t3 = 800–3000 generations, t4 = 800–5000 generations and t5 = 2200–6000 generations and $t1 < t2 \leq t3 < t4 < t5$, considering generation time for *G. platei* to be 3–3.5 years (Table 2.3).

A total of $5x10^6$ simulations were run for each system. Effective population sizes (N_e) were set to be variable among populations, considering a uniform distribution with a minimum of 10 and maximum of 10^4. Mutation rates were assumed to distribute uniformly, with $1x10^4$ to $1x10^3$ as lower and upper limits and recombination rate with a distribution of

$1x10^4$ to $1x10^1$. The one sample summary statistics used for the analyses were mean number of alleles, mean genetic diversity and mean size variance, and the two-sample statistics were F_{ST}, classification index and $(dl)^2$ distance. All scenarios were pre-evaluated using a principal component analysis (PCA) to test whether the observed data exhibited a similar distribution to the simulated data. Logistic regression analyses using the 1% of the closest simulated data set were performed to evaluate the relative posterior probability of the best explanatory scenario. The posterior distribution of the parameters was calculated under the most likely scenario using linear regression. Bias and precision of the simulations were computed by simulating pseudo-observed data, using the same parameters from the original simulations. New data sets were simulated to evaluate the level of confidence of the chosen scenarios to determine the probability of error type I and the average error type II between the most probable scenario and all the compared scenarios. As suggested by Cornuet et al. (2010), model checking to evaluate the goodness-of-fit of the best scenario was run with two summary statistics that were not used for the model selection. The summary statistics used were mean number of alleles and mean size variance for the two-sample statistics simulating 10000 pseudo-observed data sets.

2.4 Results

2.4.1 Basic statistics

No evidence of null alleles or stutter was observed in any of the microsatellite markers under study. Mean allelic richness ranged from 7.1 to 8.0 for the San Pedro system and from 5.2 to 7.6 for the Serrano system. The number of private alleles ranged from 0.26 to 0.88 for the San Pedro system and from 0.14 to 0.48 for the Serrano system (Table 2.1). No outliers were detected in any of the 14 loci used for the San Pedro system (Fig. S2.1a, Supporting information), and only one of the 16 loci used for the Serrano system departed from the confidence interval for neutral loci (Fig. S2.1b, Supporting information). However, the deviation from neutrality for this locus was marginal and occurred in only one of the systems. Because of the position of the locus and the lack of consistency across systems, the locus was included in all subsequent analyses. Departures from the Hardy–

Weinberg equilibrium (HWE) occurred in nine cases for San Pedro (q = 0.035) system and 17 cases for the Serrano system (q = 0.038); nevertheless, they were neither locus nor population specific. No evidence of linkage disequilibrium was found after applying the false discovery rate approach.

2.4.2 Structure results and isolation by distance

For the San Pedro system, the Evanno method showed that the most likely number of genetic clusters was K = 2 (Figs. S2.2a and S2.3a, Supporting information) where *Galaxias platei* individuals from lakes Pellaifa, Pullinque and Panguipulli (which formed the paleolake Huenuhue) formed a first cluster (Fig. 2.1c), while individuals from lakes Riñihue and Neltume formed a second cluster. Hierarchical analyses showed that individuals from the first cluster were indistinguishable among the three locations (Figs. S2.2b and S2.3b, Supporting information), and those from the second cluster formed two different populations (Figs. S2.2c and S2.3c, Supporting information). In the Serrano system, the most likely number of initial clusters described by the Evanno method was K = 3 (Figs. S2.2d and S2.3d, Supporting information), with lake Mellizas forming one cluster; Dickson, Paine and Nordenskjold forming a second cluster; and the remaining four lakes forming the third well-differentiated cluster (Fig. 2.1d). Subsequent hierarchical analyses showed that individuals from the lakes Nordenskjold, Azul and Pehoe were also distinguishable from each other, while no differentiation was found among individuals from lakes Dickson and Paine, nor among those from lakes Porteño and Sarmiento (Figs. S2.2e–h and S2.3e–h, Supporting information). Both metapopulations of *G. platei* exhibit a well-supported linear relationship between pairwise genetic and geographic distance, indicating a pattern of isolation by distance (IBD; San Pedro, Fig. S2.4a (Supporting information), $r = 0.85$, p-value ≤ 0.04; Serrano, Fig. S2.4b (Supporting information), $r = 0.82$, p-value ≤ 0.02).

2.4.3 Contemporary migration

The five runs in BAYESASS yielded similar results in each system. The results are summarized in Fig. 2.1e and f. Contemporary gene flow in the San Pedro system was mostly asymmetrical, with the population from Lake Panguipulli exhibiting high gene flow

into the downstream Lake Riñihue and the upstream Lake Pullinque. The population in Lake Neltume also presents an asymmetrical pattern with high gene flow towards Lake Panguipulli, but a null number of immigrants. Gene flow between Lake Pullinque and the headwater Lake Pellaifa was mostly nil (Fig. 2.1e). Contemporary gene flow in the Serrano system was mostly nil among all connected lakes, except for the populations from lakes Dickson and Paine where gene flow was highly asymmetrical with high gene flow from Lake Dickson into the downstream Lake Paine, but close to zero in the opposite, upstream direction (Fig. 2.1f). Lake Azul, which was initially considered a second headwater lake, exhibits low gene flow with all other lakes in this system.

2.4.4 Contemporary effective population size (N_e)

The estimates of the effective population size ($\widehat{N}_e$; Table 2.2) for the San Pedro system ranged from 160 in the relatively isolated Lake Pellaifa to 1128 in Lake Neltume, the lake connected to the Argentinean side of the system. Effective population size estimates for populations in the Serrano system (Table 2.2) ranged from 171 in the small and isolated Lake Mellizas to 1601 in the highly connected Lake Paine. Both, the upstream lake Dickson and Lake Pehoe, a lake located between two waterfalls, had moderate N_e (997 and 717, respectively). In general, $\widehat{N}_e$ estimates were lower for lakes with low to null contemporary gene flow and physical connectivity.

2.4.5 Historical migration

The best historical migration model for the San Pedro system assumed equal migration rate (l mL 22.3×10^6; probability = 1). Historical migration, based on coalescent reconstructions, appears to have been moderate and symmetric among four of five lakes examined in the San Pedro system. These results slightly contrast with the asymmetric nature of contemporary gene flow in this system (Fig. 2.1e). Only one case was observed of asymmetry in historical migration, with higher migration from Lake Panguipulli into the downstream Lake Riñihue, a similar pattern to contemporary gene flow between these lakes. Unlike the results for the San Pedro system, the most likely migration model for the Serrano system exhibited high downstream and low upstream migration rate (l mL 29.0×10^6; probability = 1). Historical migration in the Serrano system was in general

considerably higher than contemporary gene flow, although there was variation among lakes (Fig. 2.1f). While historical migration between lakes Nordenskjold and Pehoe and between Pehoe and Porteño was high and asymmetric, contemporary gene flow is mostly null in either direction. Historical migration estimates among the subset of lakes that formed part of the Great Tehuelche Paleolake (Mellizas, Nordenskjold, Pehoe and Porteño) are mostly moderate to high. This includes the currently isolated lake Mellizas, which exhibits similar values to those found between lakes Pehoe and Porteño (Fig. 2.1f).

2.4.6 Approximate Bayesian Computation

For the San Pedro system, the scenario that explains the contemporary metapopulation pattern with the highest probability (0.56 of probability; Fig. S2.5a, Supporting information) assumes colonization events from two refugia (Scenario 2; Fig. 2.2b), one located west of the system (via Lake Riñihue) and the other located on the eastern side of the Andes (via Neltume) and subsequent stepping stone divergence following the fragmentation of the paleolake Huenuhue. The divergence of these populations is recent, with means of 240 generations (720–840 years BP) for the population in Lake Pellaifa, 280 generations (840–980 years BP) for the population in Lake Pullinque, 340 generations (1020–1190 years BP) for the population in Lake Panguipulli and 680 (2040–2380 years BP) generations for the populations in lakes Neltume and Riñihue (Table 2.3).

In the Serrano system, the scenario with the highest probability was Scenario 2 (0.99 of probability; Fig. S2.5b, Supporting information), the one assuming an independent origin of the populations inhabiting lakes Azul, Dickson and Paine from the populations forming part of the Great Tehuelche Paleolake (Fig. 2.2f). Following this model and the posterior probabilities estimated with DIYABC, the colonization of the upper lakes in this system (Dickson and Paine) occurred 480 generations ago (1400–1700 years BP; Table 2.3), the separation from the populations inhabiting lakes Pehoe and Nordenskjold occurred 890 generations ago (2700–3100 years BP; Table 2.3), the first differentiation of individuals forming part of the Great Tehuelche Paleolake took place 1800 generations ago (5400–6300 years BP; Table 2.3), and the divergence of the populations forming the Great Tehuelche Paleolake from the one at Lake Azul occurred 2600 generations ago (7800–9100). Type I error and the average type II error for the most likely scenarios were low for

both systems (San Pedro: 0.020 and 0.004, respectively; Serrano: 0.040 and 0.005, respectively). The model checking for the most likely scenario for each system showed low probability values when the most likely scenarios were checked (Tables S2.1 and S2.2), concluding that the selected scenarios for each system fitted the observed data well.

2.5 Discussion

We have examined the patterns of historical and contemporary gene flow and structure among *Galaxias platei* populations inhabiting two Patagonian systems that differ in origin and history. *Galaxias platei* populations in the San Pedro system in northern Chilean Patagonia have diverged relatively recently, and their differentiation is linked to geological events and recent contact between refugial populations located in the coastal areas of Chile and eastern Andes. Populations in the Serrano system in southernmost Patagonia derive, in part, from a late Pleistocene paleolake that was subsequently fragmented following water-level declines during the Holocene. The biological connectivity patterns reflect this history: historical gene flow appears to have been high and symmetric, whereas contemporary gene flow is highly limited. Regardless of history, *G. platei* populations exhibit a clear pattern of isolation by distance (IBD) in both systems suggesting a process of isolation-by-dispersal limitation (Orsini et al. 2013) and habitat fragmentation. Below, we discuss the evolutionary implications of these findings emphasizing the importance of considering a multitemporal scale to understand the factors responsible for observed patterns of contemporary population structure.

2.5.1 Historical and contemporary patterns

San Pedro system. Paleolakes were not unusual in Patagonia (Caldenius 1932; Clapperton 1994; Clapperton et al. 1995; Bell 2008; Hein et al. 2010); while their extension ranged from a few tens to hundreds of kilometres (Bell 2008), their biological significance and potential role in the distribution of freshwater species has only recently begun to be disentangled (Ruzzante et al. 2006, 2008; Barber et al. 2012). Paleolakes acted as glacial refugia allowing the in situ survival of cold-adapted aquatic species during the Quaternary glacial cycles and their subsequent expansion into new areas through colonization and

recolonization processes (Zemlak et al. 2008; Xu et al. 2009). For *G. platei* for instance, glacial refugia are known to have been located in northern Patagonia, east of the Andes and in small relict lakes west of the Andes (Zemlak et al. 2008). Here we describe a colonization process in the western Andes in the San Pedro system and the differentiation process of a large population into fragmented populations. Geological studies in the San Pedro system show that four of the lakes under study (Riñihue, Panguipulli, Pullinque and Pellaifa) formed part of the paleolake Huenuhue, in existence from the Tardiglacial (13 000–10 000 years BP) to the Neoglacial (4700–2500 years BP; Laugenie 1982). The most likely scenario using the ABC approach is consistent with this history, supporting an early differentiation of the populations from lakes Neltume and Riñihue and a subsequent, more recent divergence of the populations inhabiting the lakes in the other upstream branch following a stepping stone model from the low- to the high-elevation lakes. Because no glacial refugia existed in the area of the extant lakes, the most probable colonization is likely to have taken place from coastal and eastern Andean refugia as it has been suggested on the basis of mitochondrial DNA polymorphisms for a nearby system (Zemlak et al. 2008). One refugial population was presumably located in the coastal areas of Chile, colonizing via Lake Riñihue upstream to Lake Pellaifa (the lake at highest elevation). The second refugial population is likely to have been east of the Andes (extant Lake Lacar, Argentina) colonizing the stem via the upstream Lake Neltume. Contemporary gene flow appears to be ongoing from Lake Neltume into Lake Panguipulli.

Historical migration in this system appears to have been moderate and mostly symmetrical, suggesting higher biological connectivity in the past than during contemporary timescales. The high historical connectivity is consistent with the presence of the paleolake Huenuhue, which is thought to have been in existence up to the end of the Neoglacial (4700–2500 years BP). No geological information is available about the time of the separation of the extant Lake Panguipulli from the paleolake after the end of the Neoglacial, but the results suggesting that the population inhabiting this lake diverged between 1020 and 1190 years BP indicate that the separation probably occurred at least 1100 years BP. Lake Pullinque appeared during the Holocene as a result of landslides taking place after a megathrust earthquake in the area, splitting the Lake Calafquen into one major and one minor lake (Calafquen and Pullinque, respectively), and the divergence of the population inhabiting

this lake took place most likely between 840 and 980 years BP (Campos et al. 1980). Lake Pellaifa has a similar origin; it was originally part of Lake Calafquen, but became separated from it after the eruption of the Villarrica volcano during the recent Neoglacial (Campos et al. 1980) and their populations diverged 720–840 years BP. While according to DIYABC the populations in the upstream lakes (Pellaifa, Pullinque and Panguipulli) diverged during the last two thousand years, the results from STRUCTURE and BAYESASS indicate that an increase in gene flow during recent times decreased the differentiation among the populations in lakes Pullinque and Panguipulli. No records of fish transfers in the regions exist that could explain this pattern; therefore, this pattern is most likely explained by an upstream migration. The similarity in the patterns described by both approaches does not occur with the population in Lake Pellaifa. The results from STRUCTURE show no differences between individuals from lakes Pellaifa and Pullinque; BAYESASS detected an extremely reduced gene flow between individuals from these two populations. A reduction in recent gene flow between both populations can be the result of recent volcanic and seismic activity affecting their physical connectivity (Wright & Mella 1963).

The particular case of the divergence of the Lake Neltume population can best be explained by both historical processes and contemporary landscape characteristics. Lake Neltume was not connected to the paleolake Huenuhue, and while at present it is connected to Lake Panguipulli through the fast-flowing Llanquihue River, the high gradient of this river appears to be limiting upstream migration as suggested by the low contemporary gene flow detected. Thus, the low biological connectivity detected over both temporal scales probably led to the high differentiation of this population.

Despite the presence of the Pullinque Hydroelectric Plant in Lake Pullinque, no clear effect was observed on the upstream migration from Lake Panguipulli, except for a lower gene flow than observed in other cases. The absence of an expected reduction in upstream gene flow from Lake Panguipulli into Lake Pullinque despite a dam construction can be explained by the low probability of fully detecting a recent (50 years ago, 15 *G. platei* generations) gene flow interruption (Landguth et al. 2010).

Serrano system. Glacial refugia in southernmost Patagonia have been inferred for several steppe species of Gramineae and Tubuliflorae, as well for tree species of the genus *Nothofagus* and lizards from the genus *Liolaemus* (Heusser et al. 2000; Cosacov et al. 2010;

Sersic et al. 2011; Vidal-Russell et al. 2011; Breitman et al. 2012; Premoli et al. 2012;). Such an observation has, however, not been previously made for any freshwater species yet. Here we provide the first genetic evidence that a paleolake known to have existed in southernmost Chilean Patagonia (the Great Tehuelche paleolake; Solari et al. 2012) acted as a glacial refugium for *G. platei*, indicating that glacial refugia existed for freshwater species even at the high latitudes of southernmost Patagonia (~50°L S). Two of the lakes that are presently part of the Serrano system (lakes Nordenskjold and Pehoe) and two that are geographically close but are partially or totally isolated (lakes Porteño and Mellizas, respectively) exhibit contrasting patterns of connectivity over different timescales, with relatively high historical, but low or null contemporary biological connectivity. We argue the high historical connectivity reflects the existence of this late Pleistocene–Holocene Great Tehuelche Paleolake, comprising lakes Pehoe, Nordenskjold, Mellizas, Sarmiento, Porteño, del Toro and several small isolated lakes in an area surrounded by the glacial mass and the topographic rise of the Serrano system (Solari et al. 2012; Garcıa et al. 2014). The historical signal includes a high migration from the currently isolated Lake Mellizas into three of the lakes in the lower part of the Serrano system (Nordenskjold, Pehoe and Porteño). After the reduction in the connectivity of the Serrano system with Lake Mellizas, a high effect of the genetic drift occurred on the population inhabiting the lake due to its isolation and small area (0.3 km^2). The most probable scenario suggested by the ABC method thus includes a large historical population of *G. platei* inhabiting the lakes that were once part of the Great Tehuelche Paleolake. This paleolake reached its maximum extent *ca*. 9000 years BP (Solari et al. 2012), a time that was followed by a period of increased fragmentation and reduced gene flow. The DIYABC analyses suggest that the populations in the Serrano system most likely diverged after the maximum extension of this paleolake. The contemporary pattern of population structure in the Serrano metapopulation appears therefore to be largely the result of habitat fragmentation of the Great Tehuelche Paleolake and not necessarily of population expansion. The ABC analysis also suggests a second refugium in the upper part of the Serrano system, with an independent origin of the population in Lake Azul and a relatively recent differentiation of populations inhabiting the upper lakes Dickson and Paine from a refugial population located in Lake Azul. The independent origin of a *G. platei* population inhabiting Lake

Azul from lakes forming part of the Great Tehuelche Paleolake is consistent with the known origin of this lake following the deglaciation of the area (14700–12600 years BP; Garcıa et al. 2014). The subsequent or more recent colonization of lakes Paine and Dickson is also consistent with the more recent deglaciation events around these lakes during the Holocene (2400–2000 years BP; Marden 1997), which might have been boosted by the lack of physical barriers among lakes Dickson and Paine. *Galaxias platei* populations inhabiting lakes Dickson and Paine also exhibited changes in migration patterns as a function of temporal scale. While historical coalescent migration appears to have been highly symmetric, contemporary gene flow estimates are highly asymmetric with high downstream but reduced upstream gene flow. Although this asymmetry may be related to the altitudinal differences between these lakes (39 m), it may also be the result of a recent (1982–1983) glacial outburst flood episode from Lake Dickson into Lake Paine (Peña & Escobar 1983; Dussaillant et al. 2010).

ABC methods are increasingly being used to assess the likelihood of alternate colonization scenarios where model parameters are estimated via simulations based on prior information, and observed and simulated data are compared via summary statistics (Beaumont et al. 2002; Sunnaker et al. 2013). Here we used DIYABC (Cornuet et al. 2014), a widely used user-friendly software that assumes no gene flow among populations after their divergence, an assumption slightly violated in the Serrano system where contemporary gene flow is generally low, but more significantly violated in the San Pedro system where contemporary gene flow appears moderate. Despite this violation, the results in the San Pedro system are highly congruent with the geological history of the area, suggesting reliable historical results may still be obtained in the presence of recent gene flow.

2.5.2 Isolation by distance

Despite the fact that there are differences in connectivity across the two systems, *G. platei* populations in both systems exhibit a pattern of IBD. Isolation by distance is not an unusual pattern in freshwater systems (Pearse et al. 2011; Petrou et al. 2014), especially for species exhibiting low vagility as *G. platei* (Piedra et al. 2012). *Galaxias platei* females spawn their eggs on vegetated sandy bottoms near the littoral areas of lakes (Ortubay Wegrzyn

1991), with eggs most likely attaching to the substrate (as occurs in other galaxiids; Charteris et al. 2003; O'Connor & Koehn 1998; Montoya et al. 2012). Eggs are thus unlikely to be dispersed across lakes. Larvae generally reside in the limnetic zones, and adults are generally found most abundantly near the benthos from where they move into shallow areas to feed on macroinvertebrates, eggs and small fish, including *G. platei* individuals (Barriga et al. 2002; Belk et al. 2014). The IBD pattern we have observed in *G. platei* is thus most likely explained by a process of isolation by dispersal limitation (IBDL, Orsini et al. 2013). However, an artificial IBD pattern can take place in species inhabiting regions affected by the Pleistocene glaciations. A history of colonization from multiple divergent lineages can also contribute to and inflate an IBD signal (Castric & Bernatchez 2003; Rey & Turgeon 2007). Given that we found evidence of colonization from two divergent lineages in both systems, the IBD patterns observed are thus unlikely to be solely the result of dispersal limitation; history is likely to also be partly responsible for this pattern.

2.5.3 Effective population sizes

Local $\widehat{N}_e$ estimates were higher for well-connected populations than for populations with relatively low contemporary connectivity, and this is true even for lakes with high historical connectivity. These results are consistent with the Death Valley model of population connectivity proposed by Meffe & Vrijenhoek (1988) where systems exhibiting high connectivity during the Late Pleistocene turn into fragmented subpopulations during the Late Holocene (Meffe & Vrijenhoek 1988). Thus, the lowest $\widehat{N}_e$ in these lakes reflects the effect of genetic drift on populations experiencing reduced or nil gene flow as a result of habitat fragmentation (Meffe & Vrijenhoek 1988). Although our $\widehat{N}_e$ estimates in lakes with low contemporary connectivity were low, they were not as critically low as expected for populations experiencing high drift (see also Hansen et al. 2014). This is true in particular for the Lake Mellizas, an isolated lake occupying a small area with a long history of physical isolation.

2.5.4 A temporal perspective on gene flow: Concluding remarks

We have shown that in landscape genetics studies, the exclusive consideration of contemporary processes, to the detriment of history, is likely to lead to misinterpretation of the processes responsible for the observed genetic patterns. History matters and processes taking place at disparate timescales need to be considered to avoid the over- or underestimation of the relative importance of the factors responsible for the generation and maintenance of genetic diversity (Pearse et al. 2011; He et al. 2013; Hansen et al. 2014). However, while consideration of both temporal scales has increased over the last decade (Paun et al. 2008; Chiucchi & Gibbs 2010; Graham & Burg 2012; Portnoy et al. 2014), the practice is not yet standard. While the absence of geological or glaciological information may sometimes limit the extent to which history can be incorporated in such studies, ABC methods can still be applied as exploratory analyses to examine the relative merits of alternate prior assumptions and can indeed be used to assist in our understanding and predictions of how populations are likely to change under potential future environmental change. Thus, by looking to the past to explain the present, we are better able to make predictions about the future.

2.6 Tables

Table 2.1 Collection site details for individuals of *Galaxias platei* from the San Pedro and Serrano systems including lake name, sampling year, location (Lat and Long), altitude, area (km^2), physical connectivity (U = upstream, D = downstream, H = high, L = low, N = null, I = isolated), sample size (N), allelic richness (A_r), private allelic richness (A_p), observed heterozygosity (H_o), expected heterozygosity (H_e).

Lake	Year	Lat	Long	Altitude	Connectivity	Area	N	Ar	Ap	Ho	He
San Pedro											
Pellaifa	2010-2012	39° 36' 38.0" S	71° 56' 00.7" W	221	U (N), D (H)	7.2	58	7.8	0.88	0.539	0.525
Pullinque	2010-2012	39° 34' 30.1" S	72° 10' 22.8" W	204	U (H), D (L)	5.8	98	7.4	0.47	0.520	0.530
Panguipulli	2012	39° 43' 08.3" S	72° 12' 06.3" W	130	U (L), D (H)	117	78	7.3	0.26	0.530	0.523
Riñihue	2012	39° 47' 22.7" S	72° 26' 21.4" W	106	U (H), D (H)	82.5	47	7.1	0.59	0.571	0.551
Neltume	2010-2012	39° 48' 17.1" S	71° 58' 49.7" W	196	U (L), D (H)	12	82	8.0	0.7	0.569	0.575
Serrano											
Dickson	2011	50° 52' 42.9" S	73° 04' 25.4" W	202	U (N), D (H)	17.3	98	6.9	0.25	0.612	0.607
Paine	2009-2011	50° 51' 00.0" S	72° 57' 00.0" W	163	U (H), D (H)	4.06	65	6.9	0.23	0.650	0.603
Azul	2009-2010-2011	50° 52' 17.2" S	72° 44' 49.9" W	224	U (N), D (L)	6.07	112	7.6	0.38	0.648	0.647
Nordenskjold	2009-2011	51° 02' 33.7" S	72° 55' 46.4" W	65	U (L), D (H)	28.0	83	6.9	0.14	0.627	0.598
Pehoe	2009-2011	51° 06' 31.2" S	72° 59' 18.3" W	33	U (L), D (H)	22.0	79	7.4	0.27	0.601	0.592
Porteño	2009-2010	51° 20' 18.7" S	72° 47' 43.1" W	35	U (L), D (N)	22.0	39	7.2	0.48	0.639	0.657
Sarmiento	2009	51° 03' 02.6" S	72° 54' 33.7" W	75	I	86.2	15	6.5	0.14	0.576	0.572
Mellizas	2008	51° 03' 39.1" S	72° 58' 12.7" W	91	I	0.3	63	5.2	0.27	0.579	0.534

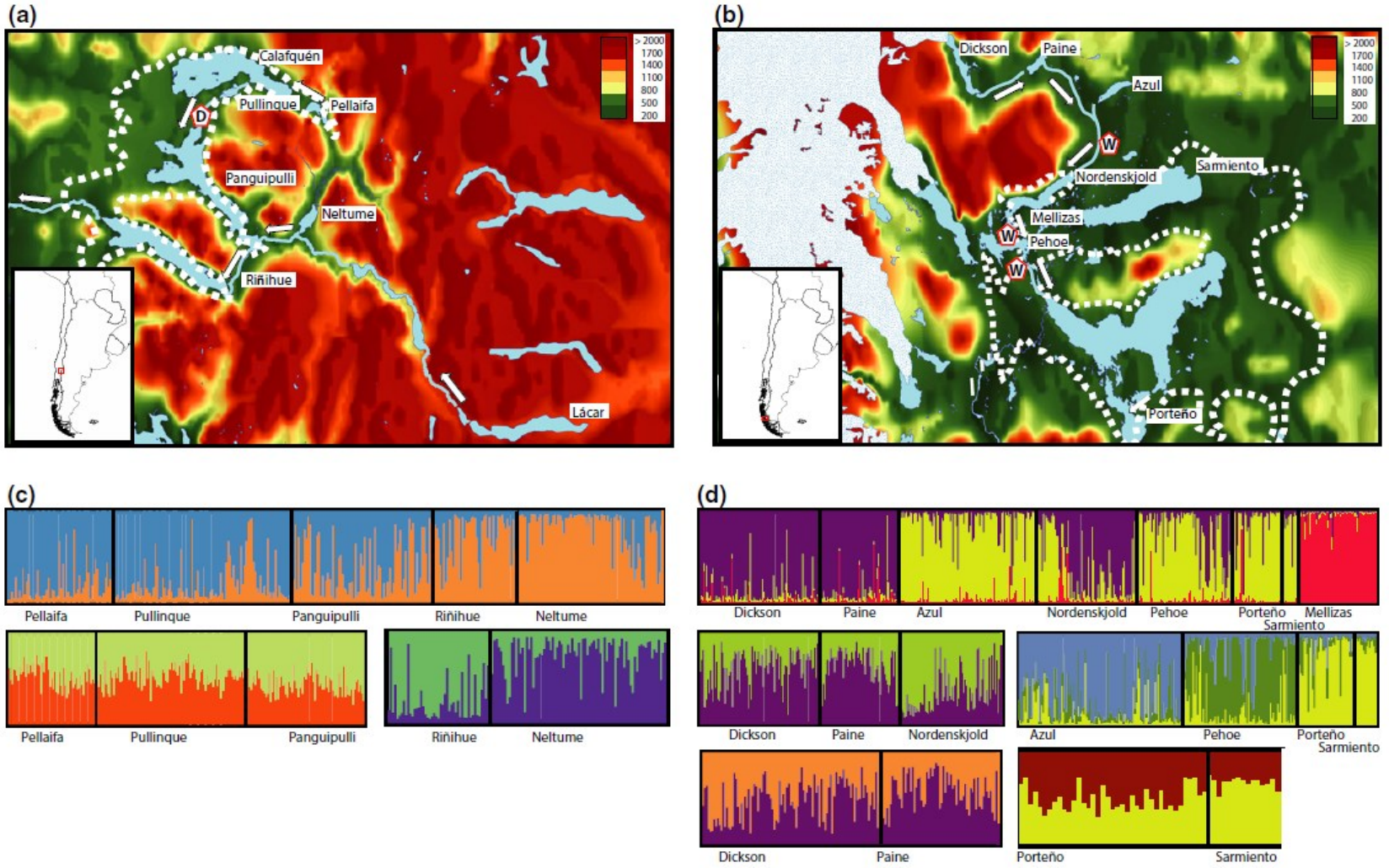
Table 2.2 Contemporary effective population size estimates (N_e) of *Galaxias platei* populations for the San Pedro and Serrano systems using LDN_e, including the 95% confidence interval, n = sample size.

Lake	n	Contemporary $\widehat{N}_e$	95% CI
San Pedro			
Pellaifa	58	160	98 – 371
Pullinque	98	369	217 – 1036
Panguipulli	78	307	162 – 231
Riñihue	47	214	110 – 1497
Neltume	82	1128	313 – ∞
Serrano			
Dickson	98	997	413 – ∞
Paine	65	1601	354 – ∞
Azul	112	191	151 – 254
Nordenskjold	79	244	164 – 444
Pehoe	83	717	313 – ∞
Sarmiento	15	96	280 – ∞
Porteño	39	250	130 – 1803
Mellizas	63	171	104 – 405

Table 2.3 Minimum and maximum values for the prior distribution of parameters and mean posterior values (including 0.025–0.975 quantiles) for the parameters estimated in DIYABC for two metapopulations of *Galaxias platei*. Parameters were obtained for the best-supported scenario for each metapopulation. N = effective population size, t = time (generations), r = recombination rate and l = mutation rate

	San Pedro		**Serrano**	
Parameter	Prior	Mean prior ($Q_{0.05} - Q_{0.95}$)	Prior	Mean posterior ($Q_{0.05} - Q_{0.95}$)
N1	$10 - 15x10^3$	$7.7x10^3$ ($4.0x10^3$ - $9.9x10^3$)	$10 - 15x10^3$	$8.3x10^3$ ($6.5x10^3$ - $9.7x10^3$)
N2	$10 - 15x10^3$	$6.2x10^3$ ($2.8x10^3$ - $9.7x10^3$)	$10 - 15x10^3$	$6.1x10^3$ ($3.4x10^3$ - $9.1x10^3$)
N3	$10 - 15x10^3$	$7.3x10^3$ ($4.6x10^3$ - $9.8x10^3$)	$10 - 15x10^3$	$9.1x10^3$ ($8.0x10^3$ - $9.9x10^3$)
N4	$10 - 15x10^3$	$3.4x10^3$ ($1.3x10^3$ - $7.5x10^3$)	$10 - 15x10^3$	$8.6x10^3$ ($7.6x10^3$ - $9.8x10^3$)
N5	$10 - 15x10^3$	$7.6x10^3$ ($4.3x10^3$ - $9.8x10^3$)	$10 - 15x10^3$	$8.5x10^3$ ($6.9x10^3$ - $9.8x10^3$)
N6			$10 - 15x10^3$	$7.5x10^3$ ($5.5x10^3$ - $9.0x10^3$)
N7			$10 - 15x10^3$	$5.3x10^3$ ($3.1x10^3$ - $7.8x10^3$)
N8			$10 - 15x10^3$	$2.5x10^3$ ($8.3x10^2$ - $6.0x10^3$)
t1	$1x10^1$ - $5x10^2$	$2.4x10^2$ ($4.1x10^1$ - $4.5x10^2$)	$4.0x10^2$ - $2x10^3$	$4.8x10^2$ ($4.0x10^2$ - $6.3x10^2$)
t2	$2x10^2$ - $3x10^3$	$2.8x10^2$ ($2.1x10^2$ - $4.2x10^2$)	$6.0x10^2$ - $3x10^3$	$8.9x10^2$ ($6.6x10^2$ - $1.3x10^3$)
t3	$2x10^2$ - $3x10^3$	$3.4x10^2$ ($2.3x10^2$ - $5.9x10^2$)	$8.0x10^2$ - $3x10^3$	$1.3x10^3$ ($8.9x10^2$ - $2.3x10^3$)
t4	$5x10^2$ - $4x10^3$	$6.8x10^2$ ($6.1x10^2$ - $9.1x10^2$)	$8.0x10^2$ - $5x10^3$	$1.8x10^3$ ($1.1x10^3$ - $3.4x10^3$)
t5			$2.2x10^3$ - $6x10^3$	$2.6x10^3$ ($2.3x10^3$ - $3.0x10^3$)
r1	$1.0x10^{-4} – 1.0$	$2.8x10^{-1}$ ($1.9x10^{-1}$ - $3.0x10^{-1}$)	$1.0x10^{-4} – 1.0$	$2.4x10^{-1}$ ($1.4x10^{-1}$ - $3.0x10^{-1}$)
μ	$1.0x10^{-4}$ - $1x10^{-3}$	$3.9x10^{-4}$ ($1.6x10^{-4}$ - $7.4x10^{-4}$)	$1.0x10^{-4}$ - $1x10^{-3}$	$3.3x10^{-4}$ ($1.7x10^{-4} – 5.6x10^{-4}$)

2.7 Figures


(a)
Calafquén
Pullinque
Pellaifa
D
Panguipulli
Neltume
Riñihue
Lácar
> 2000
1700
1400
1100
800
500
200
(b)
Dickson
Paine
Azul
W
Nordenskjold
Sarmiento
Mellizas
Pehoe
W
W
Porteño
> 2000
1700
1400
1100
800
500
200
(c)
Pellaifa
Pullinque
Panguipulli
Riñihue
Neltume
Pellaifa
Pullinque
Panguipulli
Riñihue
Neltume
(d)
Dickson
Paine
Azul
Nordenskjold
Pehoe
Porteño
Mellizas
Sarmiento
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Sarmiento

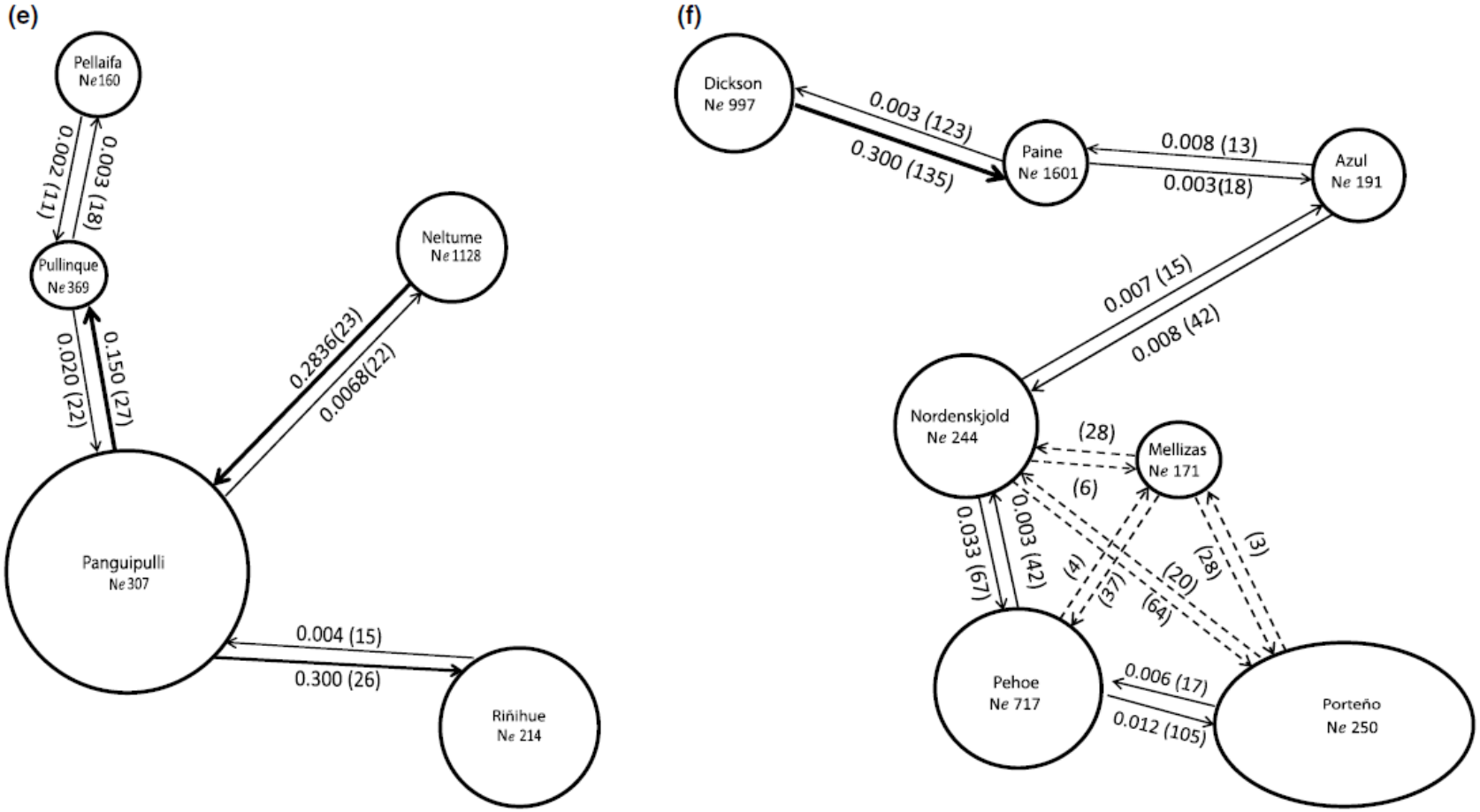
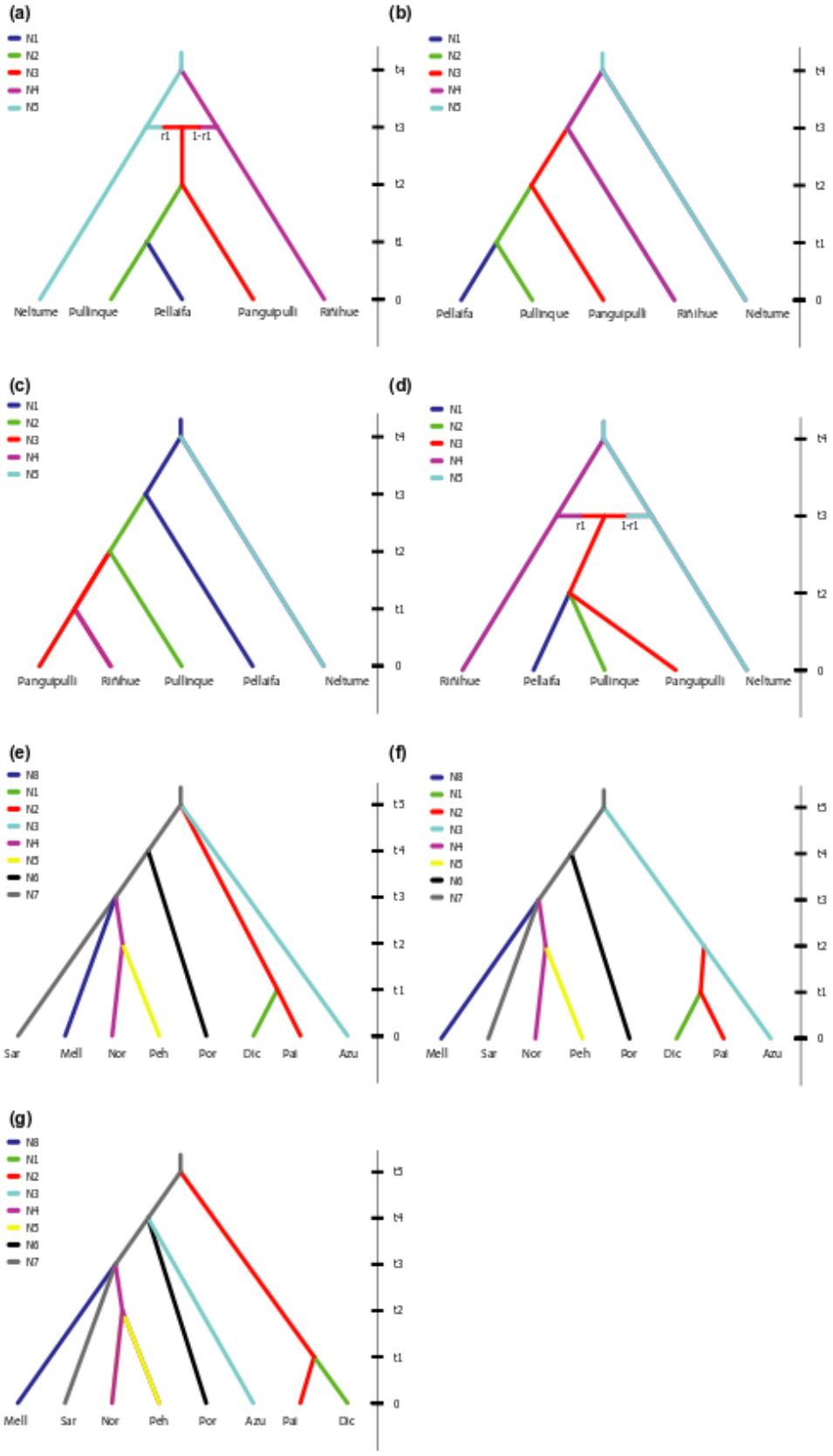


Figure 2.1. The San Pedro system (a) located in Northern Patagonia, and the Serrano system (b) located in Southern Patagonia. Arrows show the direction of the water flow, showing the maximum range of the paleolake Huenuhue (Fig. 2.1a) and the Great Tehuelche Paleolake (Fig. 2.1b). D = Dam, W = waterfall. Hierarchical population analysis using STRUCTURE 2.3.4 based on 14 loci for *Galaxias platei* individuals collected from 5 lakes in the San Pedro system with K = 2 for the first level group, K = 2 for the first subgroup

(Pellaifa, Pullinque-Panguipulli) and K = 2 for the second subgroup (Riñihue-Neltume) (c) and 16 loci for individuals from 8 lakes in the Serrano system with K = 3 for the first level group, K = 2 for the first subgroup (Dickson-Paine-Nordenskjold) and K = 3 for the second subgroup (Azul-Pehoe-Porteño-Sarmiento) in the second level group, and K = 2 for the two remaining subgroups of the third level (Dickson-Paine; Porteño-Sarmiento). (d). Vertical lines show individual admixture coefficients (Q). Contemporary gene flow estimates using BayesAss and Historical migration estimates using Migrate (in parentheses) as mutation-scaled mutation rate (M=m/μ) for the San Pedro (d) and the Serrano (e) systems. $\widehat{N}e$ estimates are shown between circles. Continuous lines show where connectivity is still present between lakes and dashed lines show were connectivity is non-existent. Lake Sarmiento was not included for the contemporary/historical scheme because of the low sample size.


(a)
N1
N2
N3
N4
N5
r1
1-r1
t4
t3
t2
t1
0
Neltume
Pullinque
Pellaifa
Panguipulli
Rinihue
(b)
N1
N2
N3
N4
N5
t4
t3
t2
t1
0
Pellaifa
Pullinque
Panguipulli
Rinihue
Neltume
(c)
N1
N2
N3
N4
N5
t4
t3
t2
t1
0
Panguipulli
Rinihue
Pullinque
Pellaifa
Neltume
(d)
N1
N2
N3
N4
N5
r1
1-r1
t4
t3
t2
0
Rinihue
Pellaifa
Pullinque
Panguipulli
Neltume
(e)
N8
N1
N2
N3
N4
N5
N6
N7
t5
t4
t3
t2
t1
0
Sar
Mell
Nor
Peh
Por
Dic
Pal
Azu
(f)
N8
N1
N2
N3
N4
N5
N6
N7
t5
t4
t3
t2
t1
0
Mell
Sar
Nor
Peh
Por
Dic
Pal
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(g)
N8
N1
N2
N3
N4
N5
N6
N7
t5
t4
t3
t2
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0
Mell
Sar
Nor
Peh
Por
Azu
Pal
Dic

Figure 2.2 Scenarios tested on DIYABC for populations of *Galaxias platei* in the San Pedro system, (a) Scenario 1, a colonization scheme from two refugia via Neltume and Riñihue Lake with an admixture event, Scenario 2 (b) two refugia without admixture, Scenario 3 (c) a high-elevation (*in situ*) glacial refugia scenario and Scenario 4 (d) STRUCTURE scenario; scenarios tested for the populations in the Ser-rano system. (e) Scenario 1, a common origin for five of the lakes forming part of the Great Tehuelche Paleolake and independent origin of the upstream populations in lakes Azul and Paine with a late differentiation of the Dickson population, (f) Scenario 2, a common origin for five of the lakes forming part of the Great Tehuelche Paleolake and the appearance of the populations in lakes Paine and Dickson from a refugial population in Lake Azul, (g) Scenario 3, a common origin for six lakes (including Lake Azul) forming part of the Great Tehuelche Paleolake and independent origin from the populations on Paine and Lake Dickson. Sar = Sarmiento, Mell = Mellizas, Peh = Pehoe, Nor = Nordenskjold, Por = Porteño, Azu = Azul, Dick = Dickson, and Pai = Paine.

2.8 Supporting Information

Table S2.1. Model checking results showing the deviation of summary statistics for the observed data from the distribution of the posterior parameters for the most probable colonization and divergence of the *Galaxias platei* populations in the San Pedro system in Northern Patagonia. N2P = mean number of alleles for the two sample statistics, H2P = mean genic diversity for the two sample statistics and V2P = mean size variance for the two sample statistics

Summary statistics	Observed value	Probability (simulated < observed)	Significance
N2P_1_1&2	10.0714	0.728	
N2P_1_1&3	9.8571	0.6697	
N2P_1_1&4	9.6429	0.741	
N2P_1_1&5	10.4286	0.6712	
N2P_1_2&3	9.2857	0.4941	
N2P_1_2&4	9.2857	0.6047	
N2P_1_2&5	10.1429	0.5702	
N2P_1_3&4	9.1429	0.5793	
N2P_1_3&5	9.7143	0.4792	
N2P_1_4&5	9.7143	0.5991	
V2P_1_1&2	11.2949	0.8097	
V2P_1_1&3	9.7572	0.744	
V2P_1_1&4	11.3883	0.8142	
V2P_1_1&5	12.5857	0.8433	
V2P_1_2&3	9.6718	0.7401	
V2P_1_2&4	10.7205	0.7893	
V2P_1_2&5	12.0469	0.8295	
V2P_1_3&4	8.9148	0.702	
V2P_1_3&5	10.7652	0.7789	
V2P_1_4&5	12.3009	0.8345	

Table S2.2. Model checking results showing the deviation of summary statistics for the observed data from the distribution of the posterior parameters for the most probable colonization and divergence of the *Galaxias platei* populations in the Serrano system in Southern Patagonia. MGW = N2P = mean number of alleles for the two sample statistics and V2P = mean size variance for the two sample statistics.

Summary statistics	Observed value	Probability (simulated < observed)	Significance
MGW_1_1	0.585	0	(***)
MGW_1_2	0.7049	0.0616	
MGW_1_3	0.7162	0.0608	
MGW_1_4	0.6651	0.0175	(*)
MGW_1_5	0.7222	0.0695	
MGW_1_6	0.6531	0.0504	
MGW_1_7	0.6139	0.1205	
MGW_1_8	0.6688	0.1215	
N2P_1_1&2	10	0.7023	
N2P_1_1&3	11.625	0.8143	
N2P_1_1&4	11.125	0.6892	
N2P_1_1&5	11.6875	0.7844	
N2P_1_1&6	10.6875	0.6759	
N2P_1_1&7	10.1875	0.7214	
N2P_1_1&8	10.375	0.6797	
N2P_1_2&3	11.125	0.7766	
N2P_1_2&4	10.125	0.5296	
N2P_1_2&5	10.625	0.6395	
N2P_1_2&6	10.125	0.6185	
N2P_1_2&7	9	0.5178	
N2P_1_2&8	9.25	0.484	
N2P_1_3&4	11.5	0.7248	
N2P_1_3&5	11.625	0.7422	
N2P_1_3&6	11.125	0.7192	
N2P_1_3&7	10.625	0.7513	
N2P_1_3&8	10.9375	0.741	
N2P_1_4&5	10.9375	0.7782	
N2P_1_4&6	10.625	0.7279	
N2P_1_4&7	9.5	0.6712	
N2P_1_4&8	9.875	0.6668	
N2P_1_5&6	11	0.7891	
N2P_1_5&7	10.0625	0.7813	
N2P_1_5&8	10.4375	0.7732	
N2P_1_6&7	8.8125	0.5782	
N2P_1_6&8	9.5625	0.6649	
N2P_1_7&8	8.6875	0.7139	
V2P_1_1&2	9.5095	0.8358	
V2P_1_1&3	10.94	0.8714	
V2P_1_1&4	9.8625	0.7976	
V2P_1_1&5	10.2005	0.8087	
V2P_1_1&6	10.2774	0.8253	
V2P_1_1&7	10.1093	0.8425	
V2P_1_1&8	10.6615	0.8393	
V2P_1_2&3	10.7621	0.865	

V2P_1_2&4	9.5222	0.7819
V2P_1_2&5	9.8343	0.7949
V2P_1_2&6	9.7473	0.7932
V2P_1_2&7	9.6208	0.812
V2P_1_2&8	10.3238	0.8299
V2P_1_3&4	10.633	0.8285
V2P_1_3&5	10.728	0.8311
V2P_1_3&6	10.8116	0.8447
V2P_1_3&7	10.9728	0.8685
V2P_1_3&8	12.2324	0.8891
V2P_1_4&5	9.7149	0.8406
V2P_1_4&6	9.8052	0.8302
V2P_1_4&7	9.7965	0.8548
V2P_1_4&8	10.5824	0.8746
V2P_1_5&6	9.7855	0.8225
V2P_1_5&7	9.7519	0.8445
V2P_1_5&8	11.1593	0.8881
V2P_1_6&7	9.1253	0.799
V2P_1_6&8	10.9719	0.8811
V2P_1_7&8	10.9923	0.9082

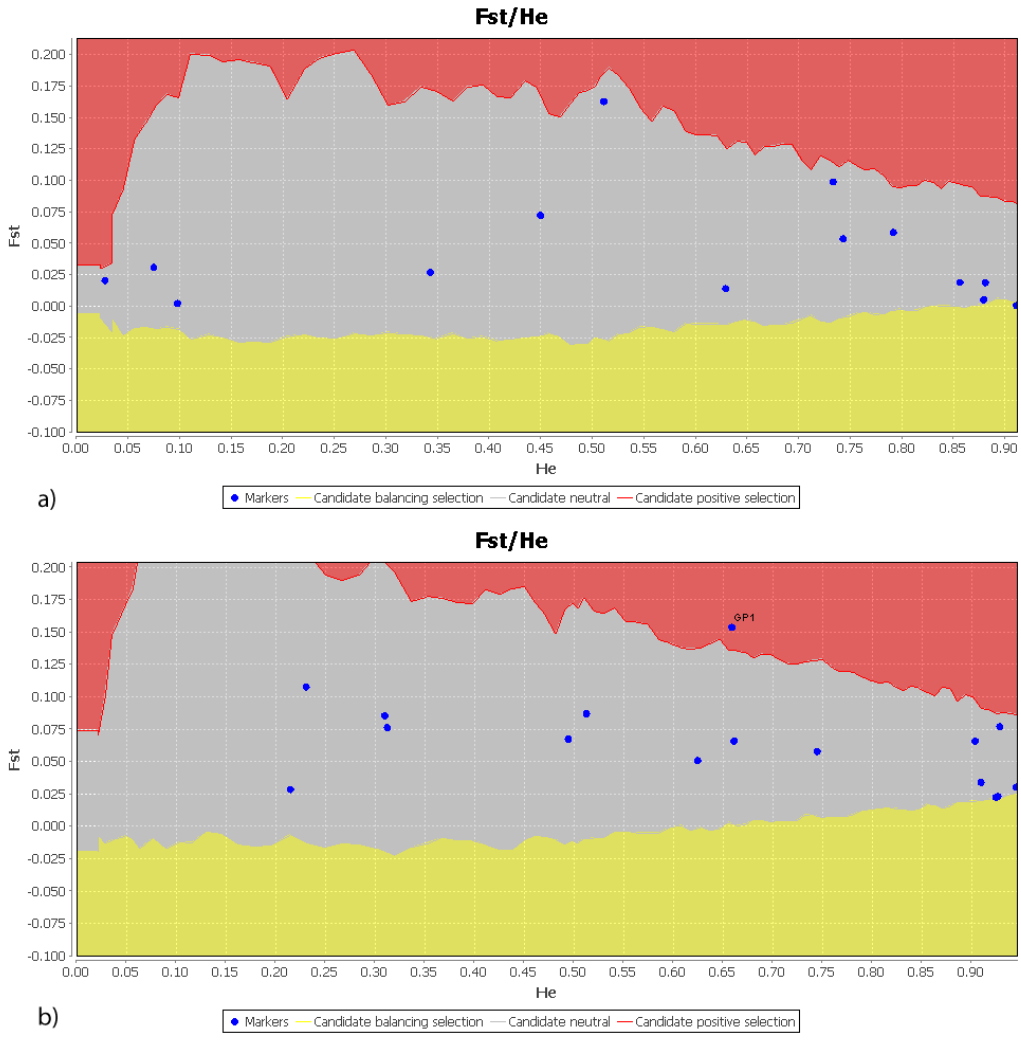


Figure S2.1. Outlier markers detection using LOSITAN for 14 microsatellite markers from San Pedro system (a) and for 16 microsatellite markers of the freshwater fish *Galaxias platei*. Yellow area shows candidate markers under balancing selection, gray areas show candidate markers under neutral selection and red areas show candidate markers under positive selection.

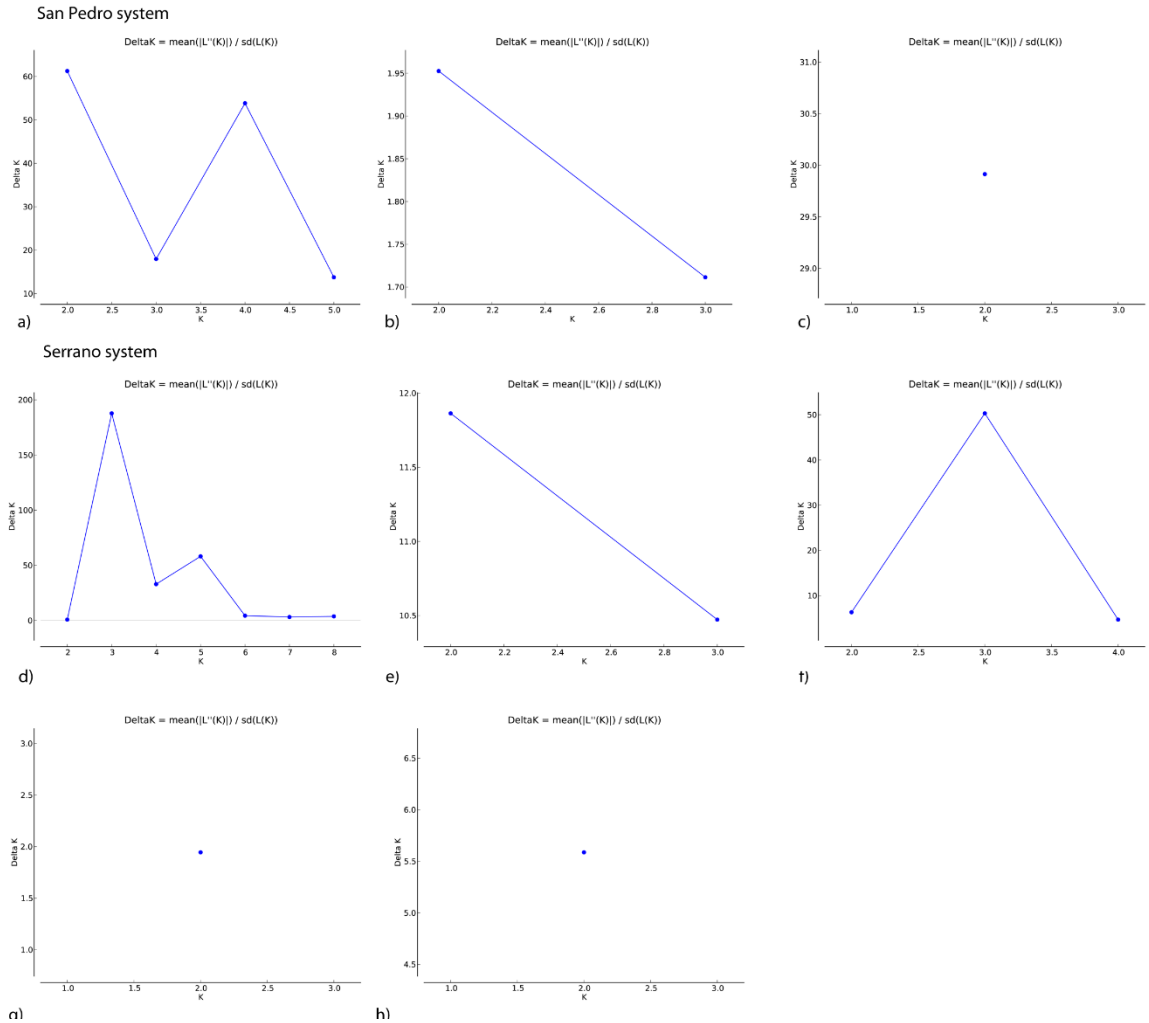


Figure S2.2. Most likely number of genetic clusters using the Evanno method for *Galaxias platei* individuals using 14 microsatellite markers from (a) all the localities in the San Pedro system in Northern Patagonia (n = 5, K = 2), (b) the Pellaifa-Pullinque-Panguipulli subgroup (n = 3, K = 2) and (c) the Riñihue-Neltume subgroup (n=2, K = 2); and (d) 16 markers for all the localities the Serrano system in Southern Patagonia (n = 8, K = 3), (e) the subgroup Dickson-Paine-Nordenskjold (n = 3, K = 2), (f) the subgroup Azul-Pehoe-Porteño-Sarmiento (n = 4, K = 3), (g) the subgroup Dickson-Paine (n = 2, K = 2) and ((h) the subgroup Porteño-Sarmiento (n = 2, K =2); Mean of estimated –Ln probability v/s K for the San Pedro system (a) and the Serrano system (b). n = number of populations tested and K = most likely number of genetic clusters selected by the Evanno method.

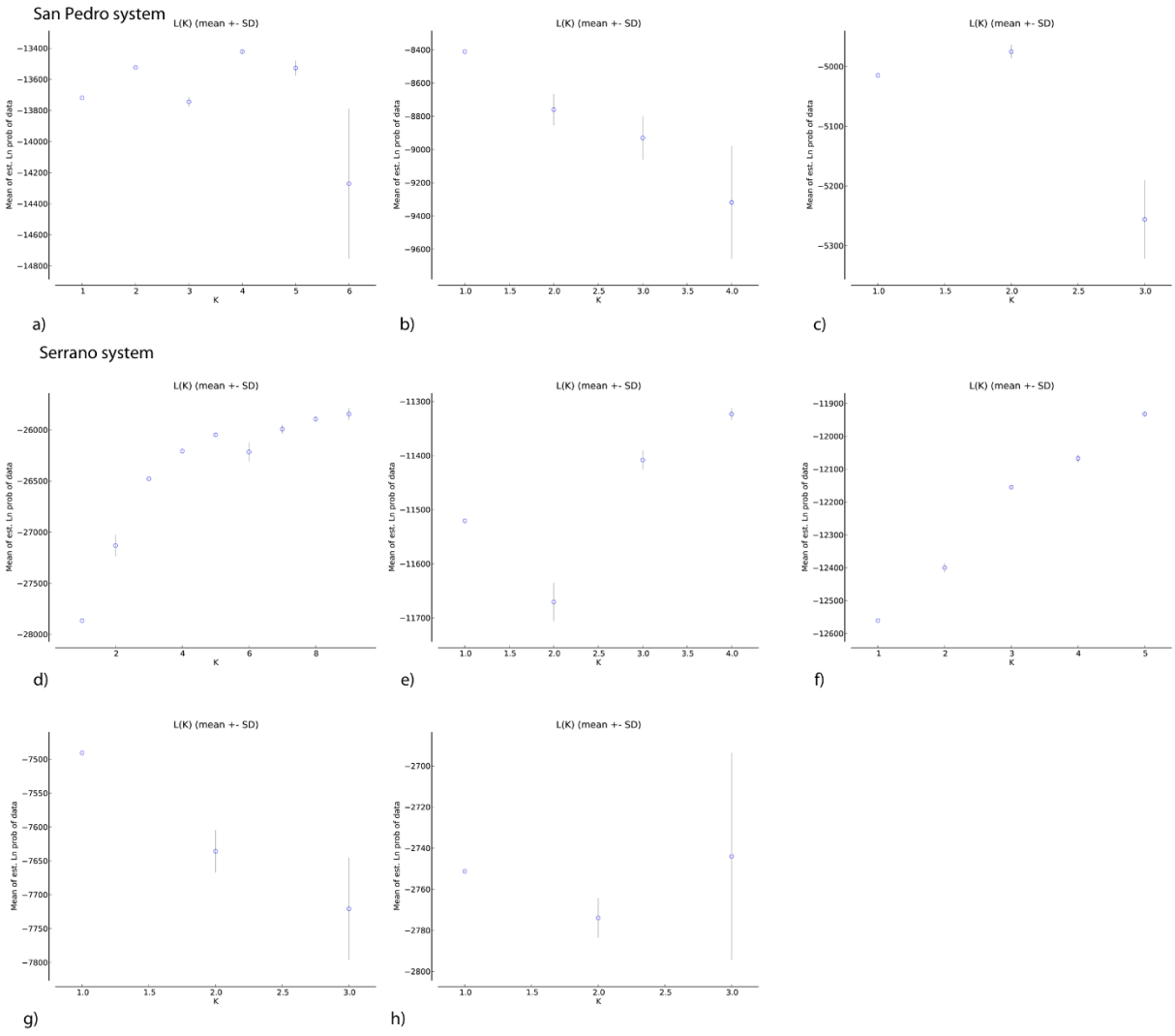
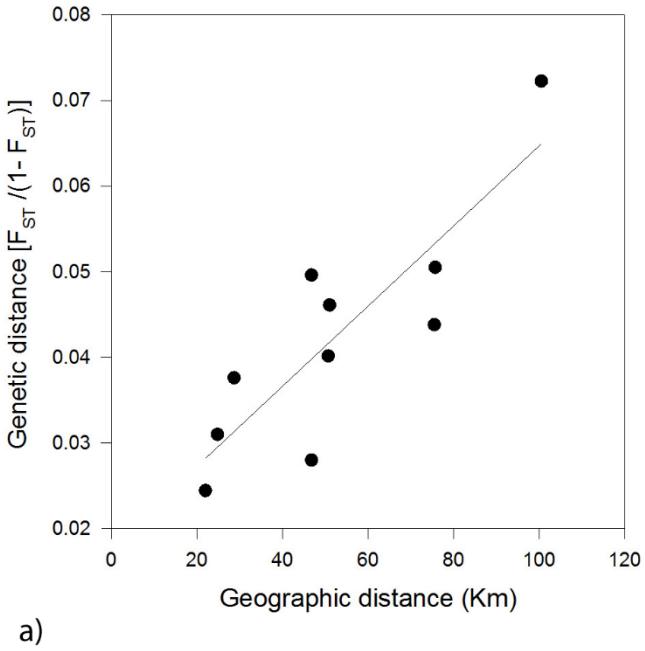


Figure S2.3. Most likely number of genetic clusters using the Pritchard method for individuals of *Galaxias platei* using 14 microsatellite markers from (a) all the localities in the San Pedro system in Northern Patagonia, (b) the Pellaifa-Pullinque-Panguipulli subgroup and (c) the Riñihue-Neltume subgroup; and (d) and 16 markers for all the localities the Serrano system in Southern Patagonia, (e) the subgroup Dickson-Paine-Nordenskjold, (f) the subgroup Azul-Pehoe-Porteño-Sarmiento, (g) the subgroup Dickson-Paine and ((h) the subgroup Porteño-Sarmiento



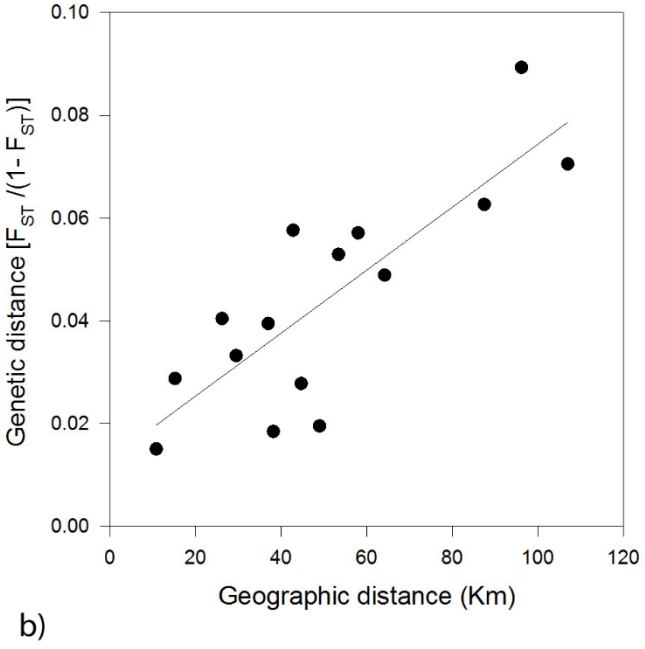


Figure S2.4. Isolation by distance analyses for two metapopulations of *Galaxias platei* showing the correlation between linearized F_{ST} and geographic distance (km) among five connected lakes from (a) the San Pedro system in Northern Patagonia (r = 0.85, p-value = 0.04) and six connected lakes on (b) the Serrano system in Southern Patagonia (r = 0.82, p-value = 0.02).

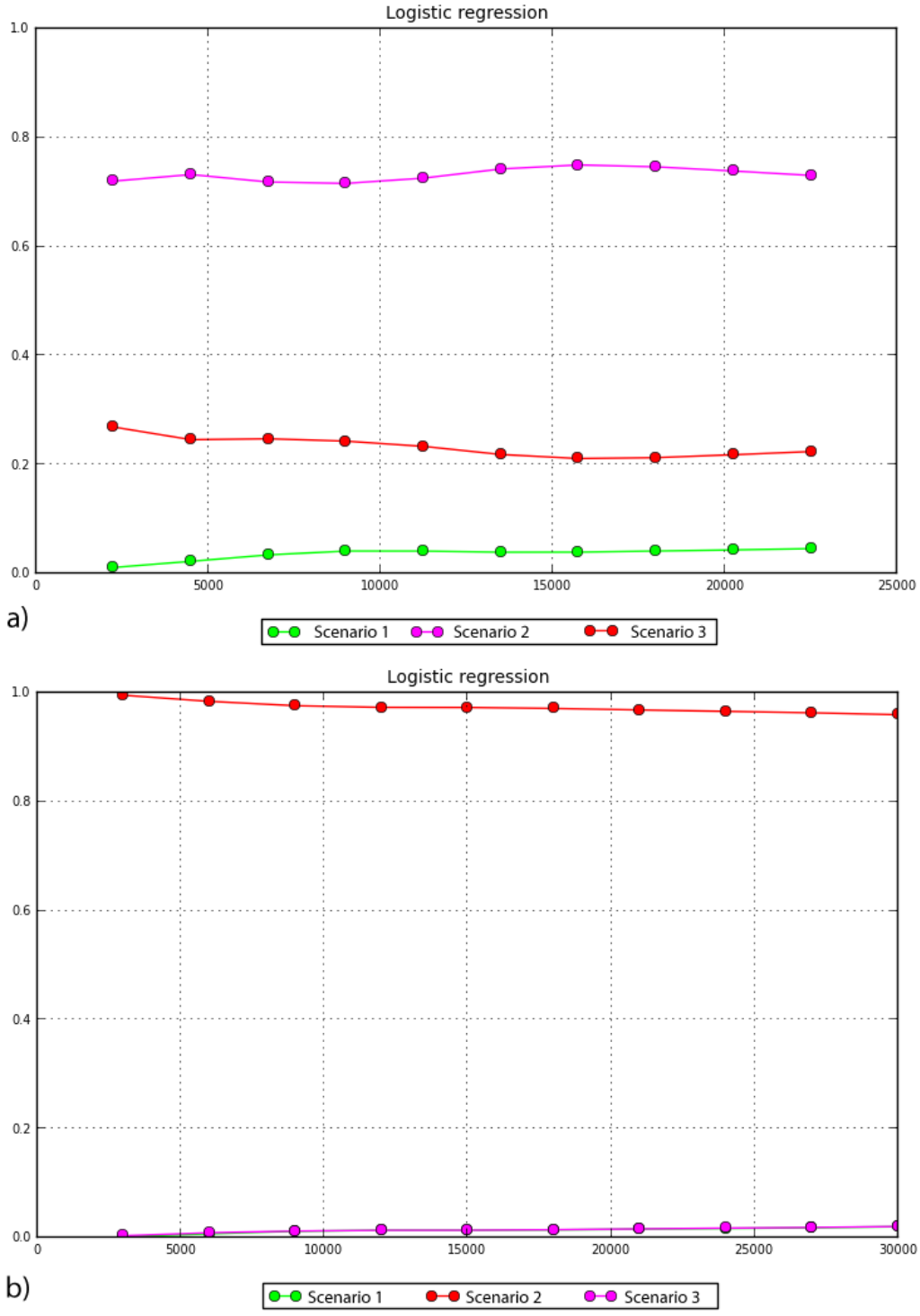


Figure S2.5 a). Analysis of the posterior distribution of scenarios tested in DIYABC for individuals of *Galaxias platei* in the San Pedro system in Northern Patagonia b) the Serrano system in Southern Patagonia using 14 and 16 microsatellite markers respectively.

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CHAPTER 3 PAST, PRESENT, AND FUTURE OF A FRESHWATER FISH METAPOPULATION IN A THREATENED LANDSCAPE

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3.1 Abstract

It is well documented that hydropower plants can affect the dynamics of fish populations through landscape alterations and the creation of new barriers. Less emphasis has been placed on the examination of the genetic consequences for fish populations of the construction of dams. The relatively few studies that focus on genetics often do not consider colonization history and even fewer tend to use this information for conservation purposes. As a case study, we used a 3-pronged approach to study the influence of historical processes, contemporary landscape features, and potential future anthropogenic changes in landscape on the genetic diversity of a fish metapopulation. Our goal was to identify the metapopulation's main attributes, detect priority areas for conservation, and assess the consequences of the construction of hydropower plants for the persistence of the metapopulation. We used microsatellite markers and coalescent approaches to examine historical colonization processes, traditional population genetics, and simulations of future populations under alternate scenarios of population size reduction and gene flow. Historical gene flow appeared to have declined relatively recently and contemporary populations appeared highly susceptible to changes in landscape. Gene flow is critical for population persistence. We found that hydropower plants could lead to a rapid reduction in number of alleles and to population extirpation 50–80 years after their construction. More generally, our 3-pronged approach for the analyses of empirical genetic data can provide policy makers with information on the potential impacts of landscape changes and thus lead to more robust conservation efforts.

3.2 Introduction

Freshwater biodiversity has decreased over the last century at a faster rate than marine and terrestrial biodiversity, due mostly to the effects of human activity (Dudgeon et al. 2006; Strayer & Dudgeon 2010). Hydropower plants have seriously affected freshwater biodiversity, reducing the number of species and the genetic diversity of metapopulations (Poff et al. 2007; Liermann et al. 2012). Freshwater species live in lakes and river systems, and their ability to move between river drainages is limited by physical connectivity (Dias et al. 2014). Thus, physical connectivity is key to the exchange of individuals among populations and ultimately to the preservation of diversity through gene flow (Baguette et al. 2013). The study of the genetic consequences of landscape changes is thus critical for the conservation of biodiversity (Balkenhol et al. 2015).

A proper understanding of the drivers affecting diversity in extant metapopulations requires consideration of both historical and contemporary processes and their interactions (Epps & Keyghobadi 2015). Understanding the role of past demographic processes facilitates the assessment of the extent to which patterns observed in the present are influenced by historical and contemporary events. This information in turn provides evidence of how individuals are likely to respond to future landscape changes, including changes that affect physical connectivity. A multitemporal approach that examines the influence of historical processes, based on coalescent approaches; contemporary genetic patterns and gene flow, based on classical genetic analyses; and potential future conditions, based on simulations constitute a powerful 3-pronged approach to identifying priority areas for conservation and the population attributes that can help predict the outcome of landscape-level changes.

Glacial and postglacial refugia and the subsequent colonization of adjacent areas are expected to have had great impact on the genetic diversity of species in temperate areas (Hewitt 2000). In Patagonia refugia were located mostly in ice-free areas along the Pacific Coast and east of the Andes, although some refugia were also located along the western side of the Andes (e.g., Xu et al. 2009; Zemlak et al. 2008, 2010). The identification of refugial populations and historical migration patterns for species inhabiting temperate regions affected by glaciation (e.g., Patagonia) show that contemporary spatial population structure is the result of a complex interaction between historical and recent processes (Zemlak et al. 2008, 2011; Vera-Escalona et al. 2015; Salisbury et al. 2016). The

identification of refugial populations and determination of how historical populations became differentiated improves the understanding of historical and contemporary diversity patterns. Refugial populations often occur in biodiversity hotspots and thus they are of interest for the conservation of future populations (Hampe & Petit 2005).

We evaluated the power of multitemporal analyses in a case study of a freshwater fish, *Galaxias platei*, in the Puelo River Basin in northwestern Patagonia. The Puelo River Basin is a trans-Andean system, with its headwaters east of the Andes in Lake Puelo, Argentina. The river crosses the political divide into Chile and drains into the Pacific Ocean (Fig. 3.1a). Long-term historical studies of the mitochondrial DNA of *G. platei* from Lake Puelo show this population is more closely related to *G. platei* from other Atlantic drainage systems than they are to other Pacific drainage systems (Zemlak et al. 2008). Recent analyses of two metapopulations of Patagonia demonstrated that metapopulations of *G. platei* have a complex pattern of colonization that, although different among systems, indicates a strong influence of historical processes on its contemporary patterns of genetic diversity (Vera-Escalona et al. 2015). Few researchers have described the genetic composition of a metapopulation based on an approach that includes the influence of historical processes, contemporary landscape features, and potential future changes resulting from anthropogenically induced changes in the landscape. We attempted such a 3-pronged approach in an examination of the influence of historical and contemporary landscape features on the diversity of a *G. platei* metapopulation in the Puelo River Basin. We then modeled the system's potential response to planned changes in landscape. Hydropower generation plans are being developed for the Puelo River Basin. A hydropower plant in the lower area of the basin was recently approved and plans exist for several hydropower plants in upstream areas. Hydropower is generated with dams or run-of-the-river plants that restrict and regulate stream flow, respectively. Empirical data reveal that dams reduce freshwater biodiversity by 85–95% (Pringle et al. 2000; Ugedal et al. 2008) during their standard 50–100 year lifetime. Most of this reduction in biodiversity follows a decline in connectivity resulting from the construction of concrete barriers. But gene flow is also reduced by changes in flooding regimes that affect downstream egg-deposition areas (e.g., littoral areas, alluvial plains) and by changes in water chemistry and physical properties (e.g., temperature, salinity, turbidity). Simulations of future conditions

under different scenarios can help one to infer future patterns of genetic diversity and inform conservation planning. We conducted a standard analysis of population structure and examined population-differentiation scenarios to determine the extent to which short-term historical processes influenced the genetic composition of extant populations. Subsequently, we used empirical data to simulate how changes in connectivity (i.e., as a result of the construction of hydropower plants) are likely to influence genetic diversity and gene flow of future populations. We considered the potential role of these analyses for design of robust conservation strategies for species worldwide.

3.3 Methods

3.3.1 Sampling

Galaxias platei individuals were collected from 2005 to 2008 from 5 locations in the Puelo River Basin (Table 3.1 & Fig. 3.1a) with gill nets, seine nets, and electrofishing. Sampling locations were distributed through-out the river basin, from its headwaters east of the Andes in Argentina to downstream environments near the Pacific Coast (Chile). In a sixth sampled location (Lake Tagua Tagua), no *G. platei* were found. Collected individuals were stored in 95% ethanol. A tissue sample was taken from each individual for DNA extraction. We followed the guidelines specified in the Law 18.755 of the Agricultural and Livestock Service of Chile for the care and use of animals for scientific purposes.

3.3.2 Laboratory Protocols

We extracted DNA with a glass milk protocol from a 5 to 10 mg piece of tissue (Elphinstone et al. 2003). The extracted DNA was amplified by polymerase chain reaction (PCR) (10 μL volume reaction) with 14 microsatellite markers developed by Arias et al. (2012) and Vera-Escalona et al. (2014; See Appendix 2). The PCR mix contained 1 μL of 10 mM $(NH_4)_2SO_4$, 1 μL of 2 mM $MgCl_2$, 1 μL of 2 mM dNTP, 0.1 μL of each primer, 0.1 μL of m13 (700 and 800), 0.1 μL of 0.5 U TSG, 6.5 μL of H_2O, and 3 μL of DNA. We used LI-COR sequencers (Biosciences, Lincoln, Nebraska) and a molecular ladder of 65–400 bp to detect amplified microsatellite fragment lengths in polyacrylamide gels. We

analyzed images from LI-COR and scored microsatellites with SAGA (Biosciences, Lincoln, Nebraska). We used MICROCHECKER version 2.2.3 (van Oosterhout et al. 2004) to detect scoring errors in population allele frequencies due to null alleles or stutter bands. All data files are available in Figshare (https://figshare.com/s/18ea7af0218afde0abf1). We used POWSIM version 4.1 (Ryman & Palm 2006) to evaluate the power of the microsatellite markers set to detect genetic differentiation at different levels of divergence. Simulations were carried out for 500 replicates, effective population sizes (N_e) were 1000, and t (generations of drift before sampling) varied to yield F_{ST} values of 0.001 to 0.005.

3.3.3 Contemporary Parameters

Allele richness, number of alleles, and mean observed and expected heterozygosities were estimated in GENALEX (Peakall & Smouse 2012). We evaluated Hardy–Weinberg equilibrium (HWE) and link-age disequilibrium (LD) in GENEPOP on the web (genepop.curtin.edu.au) with default values (Raymond & Rousset 1995). To correct for multiple comparisons for the HWE and LD tests, we used the false discovery rate (FDR) approach (Benjamini & Hochberg 1995). Presence of populations or population clusters was evaluated in STRUCTURE version 2.3.4 (Pritchard et al. 2000; Hubisz et al. 2009). We assumed an admixture model with correlated allele frequencies. Analyses were conducted for $K = 1$ to $K = n + 1$, where K is the number of clusters and n is the total number of locations sampled in the Puelo River Basin ($n = 5$). Ten replicates were conducted for each K, burn-in period was 5×10^4, and there were 2.5×10^5 sampling steps. We collected and assessed the results in Structure Harvester (Earl & von Holdt 2012) and used the Evanno method (Evanno et al. 2005) to determine the most likely number of genetic groups. A summarized plot based on the 10 replicates was created with the greedy algorithm in CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007). Final plots were obtained with Distruct version 1.1 (Rosenberg 2004). Recent migration rates were estimated with BayesAss version 3.0 (Wilson & Rannala 2003). Five replicates with 3×10^6 iterations were run, samples were taken every 10^3 iteration, and the burn-in period was 5×10^6. Contemporary estimated $\widehat{N}_e$ was estimated in NeEstimator version 2.1 (Do et al. 2014) through a random-mating model that excluded alleles with frequencies <0.02.

3.3.4 Historical Migration Patterns and Location of the Most Recent Common Ancestor

We used MIGRATE-n version 3.6 (Beerli 2006) in the CIPRES Science Gateway version 3.3 clusters (https:// www.phylo.org) to estimate mutation-scaled migration rates (M) based on $M = m/\mu$ (m, probability of a lineage immigrating per generation; μ, mutation rate). Two models were evaluated to identify the most likely migration model that explains the migration patterns of *G. platei*, a full migration model, and a model based on the observed patterns from BayesAss (see Table S3.1 and Table S3.2, Supporting Information). The 2 models were compared with the Bezier implementation in Migrate-n based on the log marginal likelihood of the posterior probabilities following Beerli and Palczewski (2010).

3.3.5 Approximate Bayesian Computation of Population Differentiation

We used an approximate Bayesian approach in DIYABC version 2.1.0 (Cornuet et al. 2014) to evaluate 4 scenarios of population differentiation for five *G. platei* populations from the Puelo River Basin. Scenario 1 (Fig. 3.3a) was based on the location of the most recent common ancestor and had early differentiation of populations from lakes Azul and Las Rocas and later differentiation of populations from the lower Puelo River, Lake Inferior, and Lake Puelo. Scenario 2 (Fig. 3.3b) had population differentiation from the eastern Andes (Lake Puelo and Lake Inferior), followed differentiation of the populations in the Lower Puelo River, Lake Azul, and finally Lake Las Rocas. Scenario 3 (Fig. 3.3c) was based on the STRUCTURE plots. The oldest populations were in the eastern (Lake Puelo) and western Andes (Lake Las Rocas), a later differentiation of populations occurred at Lake Azul and the Lower Puelo River, and the appearance of *G. platei* at Lake Inferior was recent. In scenario 4 (Fig. 3.3d), population differentiation occurred from west to east, from the Pacific Coast (Lower Puelo River) to the Andes following the existent connections in the Puelo River. Details of these simulations are described in the Supplementary methods from the Supporting Information.

3.3.6 Simulations of Future Populations

We conducted our simulations in NEMO version 2.3.46 (Guillaume & Rougemont 2006) to assess the effects of hydropower development on *G. platei* population size, population survival, number of alleles, and observed heterozygosity in the 4 sampled lakes for 100 years following dam construction (from year 0 to beyond 100 years). Individuals from Lower Puelo River were removed from the analysis because of the low sample size. In scenario 1, the landscape was unchanged and the same migration scheme was that obtained from BayesAss. In scenario 2, hydropower plants completely isolated all populations and population size was reduced by 90%. In scenario 3, all populations were isolated by hydropower plants but population size was not reduced. In scenario 4, hydropower plants reduced population size by 90% and mitigation measures (e.g., through fishways or translocation) maintained gene flow at 1% (m = 0.01 [approximately one-quarter of the mean estimated contemporary gene flow, m = 0.038]). In scenario 5, hydropower plants with mitigation measures (i.e., maintaining gene flow at m = 0.01) did not result in a reduction in population size. The 0% and 90% levels of population size reduction represented 2 extreme scenarios. For these analyses, we used the present genotypic data, a carrying capacity of 500 individuals/km^2, as suggested for *G. platei* populations from Chile (Sobenes et al. 2013), mean fecundity of 20,000 (Zama 1986), and parameters detailed in Table S3.3, Supporting Information.

3.4 Results

There were no null alleles or stutter bands. Only 1 locus in 1 population (Las Rocas) deviated from the HWE. Four of 91 comparisons deviated from equilibrium; no single locus pair appeared out of equilibrium in more than a single population. Allelic richness ranged from 3.41 in Lake Azul to 5.01 in the Lower Puelo River, and private alleles fluctuated from 0.24 to 1.40 in these locations (Table 3.1). Observed heterozygosities ranged from 0.37 to 0.53 in the Lower Puelo River (Table 3.1). All populations had higher observed than expected heterozygosities. For 14 microsatellite markers, we detected changes in F_{ST} of 0.0015 3 generations after a divergence event with a probability of 0.645 and changes in F_{ST} of 0.0035 7 generations after a divergence event with a probability of 1 (Fig. S3.1, Supporting Information). Assuming a generation time for *G. platei* of 3.5 years,

our results were consistent with a 100% probability for detecting changes in the F_{ST} 0.0035, which would cause population differentiation approximately 25 years (7 generations) after a divergence event. Thus, our data were well suited for detection of small changes in genetic differentiation among subpopulations that may have occurred as recently as 25 years in the past.

3.4.1 Number of Populations and Population Clusters

The Evanno method revealed 3 clusters (Fig. S3.2, Supporting Information), and hierarchical analyses indicated that the final number of clusters was 4. Individuals from lakes Puelo and Inferior formed a single genetic group (Fig. 3.1b), which is consistent with the short geographic distance separating these two lakes (0.5 km) and the high contemporary gene flow (Fig. 3.2a). The populations at Lower Puelo River and Lake Azul were more differentiated from the other populations, and individuals from Lake Las Rocas exhibited higher admixture than other populations. Hierarchical analyses confirmed the absence of differentiation between individuals from lakes Puelo and Inferior (Fig. 3.1b).

3.4.2 Contemporary Gene Flow

Contemporary gene flow from the population inhabiting Lake Inferior to the upstream population in Lake Puelo was high (Fig. 3.2a). We also found high contemporary gene flow from the Lake Azul population to the down-stream population at the Lower Puelo River. Gene flow estimates over 1% were common in the system, except to Lake Azul, where estimates were close to nil with the exception of that from Lake Las Rocas (Table S3.4, Supporting Information).

3.4.3 Effective Population Sizes

In general, all populations in the Puelo River Basin exhibited low to moderate $\widehat{N}_e$. The $\widehat{N}_e$s were very low for the upstream populations in lakes Puelo and Inferior ($\widehat{N}_e$ 40; CI, 19–235; and $\widehat{N}_e$ 137; CI 82–330, respectively) and high for the population in Lake Las Rocas ($\widehat{N}_e$ 601, CI 244–infinite). Estimates for the populations at lower Puelo River and Lake Azul were infinite and thus were not included in subsequent analyses. Because 2 $\widehat{N}_e$s were

infinite (Lower Puelo River and Lake Azul), we also focused on the lower limit of $\widehat{N}_e$, which can provide some insights into the tendency of the estimates. The lowest $\widehat{N}_e$s were found for Lake Puelo and lower Puelo River, whereas the highest $\widehat{N}_e$s were found for Lakes Azul and Las Rocas (Table 3.1).

3.4.4 Historical Migrations

The full migration model showed the highest likelihood (-5.6×10^6 vs. -6.2×10^6) and was thus the most likely model (among 2 tested models) to reflect historical patterns. Unlike the pattern observed with contemporary gene flow, historical scalar migrations were moderate to high from Lakes Azul and Las Rocas to the other 3 locations, whereas very little historical migration was observed to the Lower Puelo River near the Pacific (Fig. 3.2b; Table S3.5, Supporting Information) or to Lake Las Rocas.

3.4.5 Population Differentiation Scenarios

Scenario 3 (Fig. 3.5b; Fig. S3.3, Supporting Information) provided the most likely explanation of observed patterns of genetic differentiation in the Puelo River Basin. This scenario included refugial populations in Lake Puelo and later differentiation in the Lake Las Rocas population followed by differentiation from the other lakes. Time estimates suggest that most of the differentiation in the system occurred from 420 to 1050 years BP (120–300 generations) (Fig. S3.4, Supporting Information). Model checking revealed high support for all 4 scenarios evaluated (Fig. S3.5, Supporting Information).

3.4.6 Simulations of Effects of Hydropower Development on Future Populations

Two scenarios with population isolation and the one with mitigation and population reduction led to a dramatic reduction in population sizes (Fig. 3.4a). The scenario with an unchanged landscape was the best for maintenance of genetic diversity (Fig. 3.4c). Among the other four scenarios, the one with mitigation (i.e., 1% gene flow among populations) and no reduction in population size had the lowest demographic and genetic impact (Fig. 3.4b– d). Both scenarios with population isolation (with and without population reduction)

highlighted the dramatic consequences likely to follow a hydropower plant construction: extirpation of 2 of 4 populations 50 years after gene flow interruption and extirpation of 3 of 4 populations after 70–80 years (Fig. 3.4b). In these scenarios, due to the reduction in the connectivity, only the population from the largest lake (Lake Puelo) (Fig. 3.4b) persisted in each simulation after 100 years. Although loss of heterozygosity appeared not to vary with population size (Fig. 3.4d), the number of alleles was more affected when population size decreased (Fig. 3.4c).

3.5 Discussion

We used a multitemporal approach to examine the potential consequences of landscape changes on the genetic diversity of a metapopulation. We examined the extent to which the genetic composition of this metapopulation was explained by historical processes versus contemporary landscape features and how anthropogenic changes in landscape features affected genetic diversity in the future. By contrasting short-term historical migrations with contemporary gene flow, we found an apparent decline in connectivity following population differentiation. These results complement earlier studies of long-term historical processes (Zemlak et al. 2008) and illustrate the importance of considering alternate historical scales. Most populations had low to moderate N_es, which suggests individuals in the system are vulnerable to landscape alterations and historical and contemporary gene flow are both important factors in contemporary diversity. Evaluation of the consequences of building hydropower plants in the river basin revealed that the likelihood of population extirpation increased as isolation increased and that number of alleles declined faster as population size declined.

3.5.1 Historical and Contemporary Patterns

The advance and retreat of ice during the Quaternary glaciations had a great impact on Andean regions in southern South America (Rabassa et al. 2011). Zemlak et al. (2008), using mitochondrial DNA sequences to study long-term historical processes, found that *G. platei* individuals from the Puelo River Basin survived in refugial areas east of the Andes during glaciations (see also Zemlak et al. 2010, 2011). Our analyses of short-term historical processes complemented their results and suggest that during the Holocene and after the

retreat of the ice a paleolake drained toward the western Andes into Lake Las Rocas (Fig. 3.5b). A new population established there and diverged around 1050 years BP (300 generations). After this, *G. platei* individuals moved downstream from Lakes Las Rocas into Lake Azul and later into the Lower Puelo River. Similarly, the population from Lake Puelo expanded into Lake Inferior (420 years BP). Historical migrations suggest the ancestral system in the Puelo River Basin was highly connected (i.e., MIGRATE-n results, including the selection of a full migration model). This high historical migration contrasts with the connectivity decline observed in the contemporary analyses of gene flow and the shift in the direction of gene flow. Although historical gene flow in general took place in every population, contemporary gene flow occurred mostly among the western Andean Lake Azul upstream to Lake Inferior and from Lake Inferior upstream to Lake Puelo. Together, these results reveal that colonization and contemporary gene flow shaped the genetic patterns of extant populations and the importance of combining analyses of historical and contemporary patterns of structure and gene flow.

3.5.2 Multitemporal Data and the Study of a Threatened Population

Effective population size lower bounds provide useful information for conservation because they indicate the plausible lower limits of $\widehat{N}_e$ (Waples & Do 2010). Because N_es were infinite in 2 populations, we based some of our $\widehat{N}_e$ inferences on $\widehat{N}_e$ lower bounds. In general, $\widehat{N}_e$ mean and $\widehat{N}_e$ lower bounds observed in the Puelo River Basin were low to moderate. As suggested by Frankham et al. (2013, 2014), populations with low N_e (100) are likely to be affected by landscape changes that lead to bottlenecks. Our results therefore suggest that the metapopulation in the Puelo River Basin is highly sensitive to changes in the landscape that reduce connectivity, which in turn will lead to a significant loss of genetic diversity (Whitlock & Barton 1997). The building of barriers inhibiting connectivity with-out mitigation measures presents a serious threat to population dynamics (Duguay & Lacey 2015; Pracheil et al. 2015) that can lead to a decline in genetic diversity and likely to extirpations. Connectivity can also be indirectly affected by the building of wall dams and run-of-the river hydropower plants because of changes (increases or de-creases) in water level due to regulation of water flow. *G. platei* reproduce in littoral areas

(Ortubay & Wegrzyn 1991); thus, egg and juvenile survival are affected by changes in littoral water levels.

Hydropower projects in the Puelo River Basin present a serious threat to *G. platei* metapopulations. They can lead to population fragmentation, to reduction in feeding, breeding, and sheltering areas, and to population extirpation (Horreo et al. 2011; Brown et al. 2013; Fuller et al. 2015). Simulations of future population conditions showed that 40% of the alleles could vanish 100 years after any landscape intervention that resulted in large declines in population size. Even under a scenario with mitigation number of alleles would decline considerably if the population size is reduced. Furthermore, the complete isolation of populations (with or without population size reduction) would likely lead to the extirpation of 3 to 4 sampled populations 70–80 years after the construction of hydropower plants. The only surviving population was in Lake Puelo (the largest lake and the one with the largest assumed population size). Maintaining connectivity is thus key for the survival of populations, especially small populations. Consequently, conservation programs should focus on maintaining connectivity among populations to avoid their extirpation. In our scenarios, we considered mitigation measures that maintained 1% of gene flow ($m = 0.01$) among populations (25% of the extant gene flow estimated with BayesAss). A 1% gene flow is a realistic amount as estimated from empirical data (Oldani & Baigún 2002; Noonan et al. 2012; Harris et al. 2017). Thus, our results suggest mitigation may not be sufficient for the maintenance of genetic variability if it focuses only on maintaining a percentage of connectivity and not on both percentage of connectivity and population size. A decline in the number of alleles affects a population's potential for adaptation to future changes in the environment, and a decline in heterozygosity can lead to decreases in population fitness (Allendorf 1986; Caballero & García-Dorado 2013). Although number of alleles and heterozygosity will respond over time following changes in connectivity, our results are consistent with the results of previous studies that indicate number of alleles more accurately reflects changes in population size than heterozygosity (Maruyama & Fuerst 1985; Allendorf 1986; Greenbaum et al. 2014). Thus, we suggest the use of simulations focusing on number of alleles for the assessment of the effects of landscape changes on genetic diversity.

3.5.3 A Three-Pronged Approach

For a system of interconnected fish populations, we used a 3-pronged approach based on genetic methods that can be applied to assessments of the consequences of landscape intervention and used for the identification of priority areas for conservation (Fig. 3.5a). We described historical population differentiation and historical migrations and then compared these results with contemporary patterns to infer the most likely factors (historical processes, current environmental factors) shaping observed contemporary genetic patterns of diversity and structure. With data from the extant populations, we simulated the effects of landscape intervention plans. We suggest conservation programs focus on maintaining connectivity, especially among small populations to avoid local population extinctions, and avoid declines in population size to avoid the loss of genetic diversity. This approach can easily be extended to questions about the role of human intervention and climate change on species. For instance, they could be used to evaluate the effect of sea-level rise on coastal populations (Leonard et al. 2017), of desertification on land populations (Whitford 1997), and of competition between introduced and native species (Jeschke 2014). This 3-pronged approach can be considered an attempt to learn from the past to understand the present and predict potential future diversity patterns following landscape alterations, and it can be used in the design of robust conservation policies (Manel et al. 2010; Sork & Waits 2010).

3.6 Tables

Table 3.1. Description of sites where *Galaxias platei* were collected in the Puelo River Basin of Patagonia. Allelic richness (Ar), private allelic richness (Ap), observed heterozygosity (Ho), expected heterozygosity (He), effective population size (N_e) including lower and upper limits. inf = infinite.

Location	Year	Latitude	Longitude	Elevation (masl)	Area (km^2)	*n*	Ar	Ap	H_o	H_e	N_e (range)	
Lower Puelo River	2008	41°38′35″S	72°16′44″W	8	-	14	5.01	1.40	0.53	0.47	inf	(68-inf)
Lake Azul	2008	41°57′05″S	71°50′39″W	217	14.0	72	3.41	0.24	0.37	0.34	inf	(758-inf)
Lake Las Rocas	2008	42°02′50″S	71°49′25″W	300	10.8	128	4.03	0.48	0.43	0.41	601	(244-inf)
Lake Inferior	2008	42°05′42″S	71°45′33″W	190	6.5	80	4.02	0.66	0.40	0.39	137	(82-330)
Lake Puelo	2005	42°09′55″S	71°38′13″W	192	48.0	32	3.53	0.44	0.37	0.35	40	(19-235)

*Abbreviations: Ar, allelic richness; Ap, private allelic richness; H_o, observed heterozygosity; H_e expected heterozygosity; N_e , effective population size (N_e); masl, meters above sea level; inf, infinite.

3.7 Figures

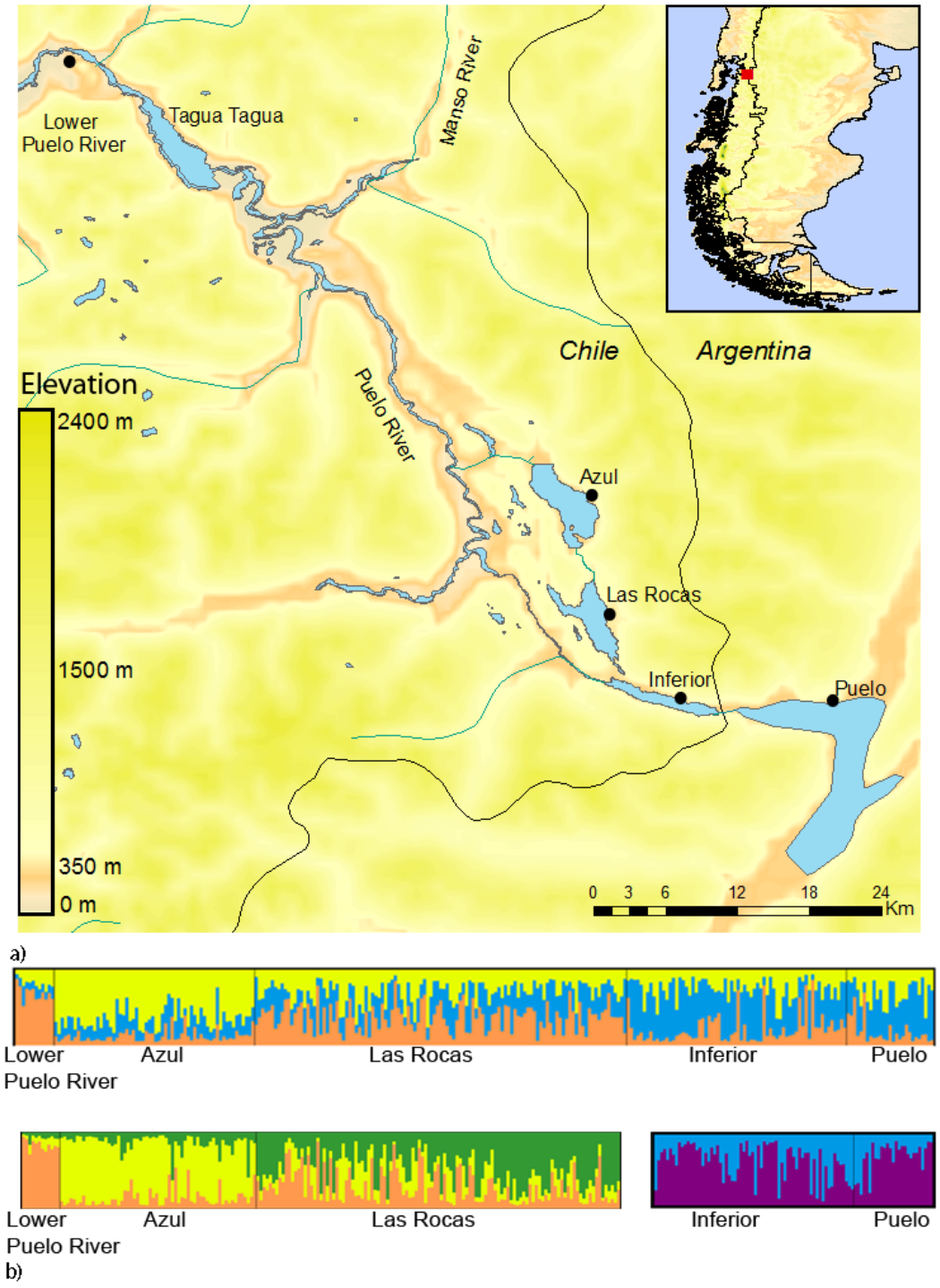

Figure 3.1. (a) Puelo River in northwestern Patagonia (black circles, sampling sites). (b) STRUCTURE plots of the most likely relative number of populations genetic clusters (populations) of *Galaxias platei* shown in (a). Black vertical lines represent individual admixture coefficients.

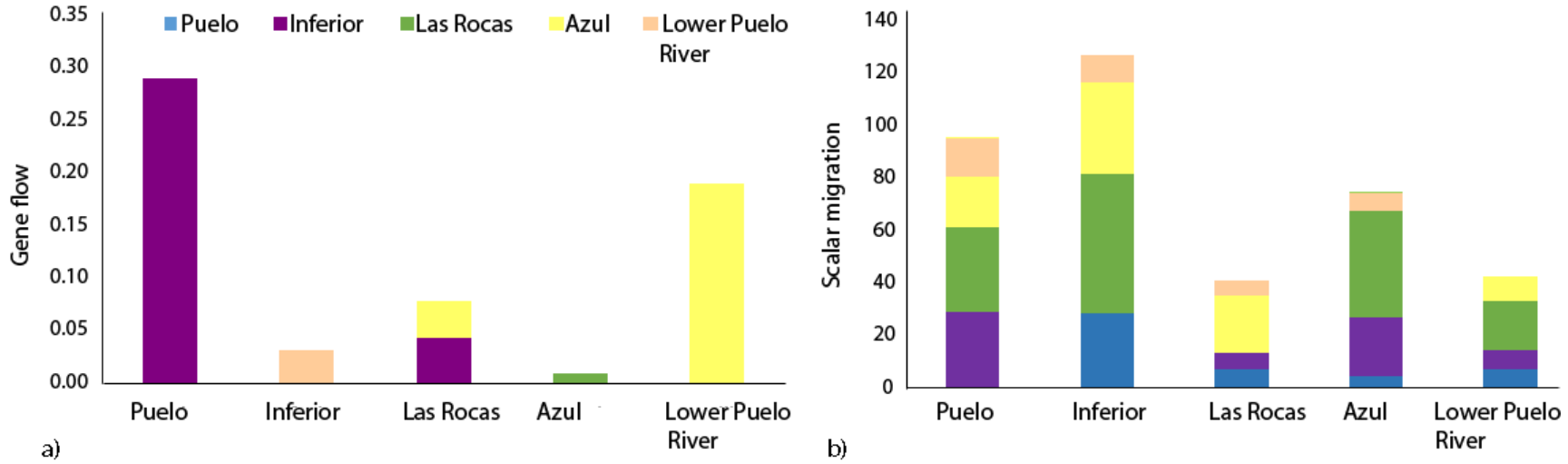

Figure 3.2. (a) Contemporary gene flow (BayesAss) and (b) historical scalar migration (Migrate-n) in 5 populations of *Galaxias platei* in northwestern Patagonia (x-axis, receiving populations).

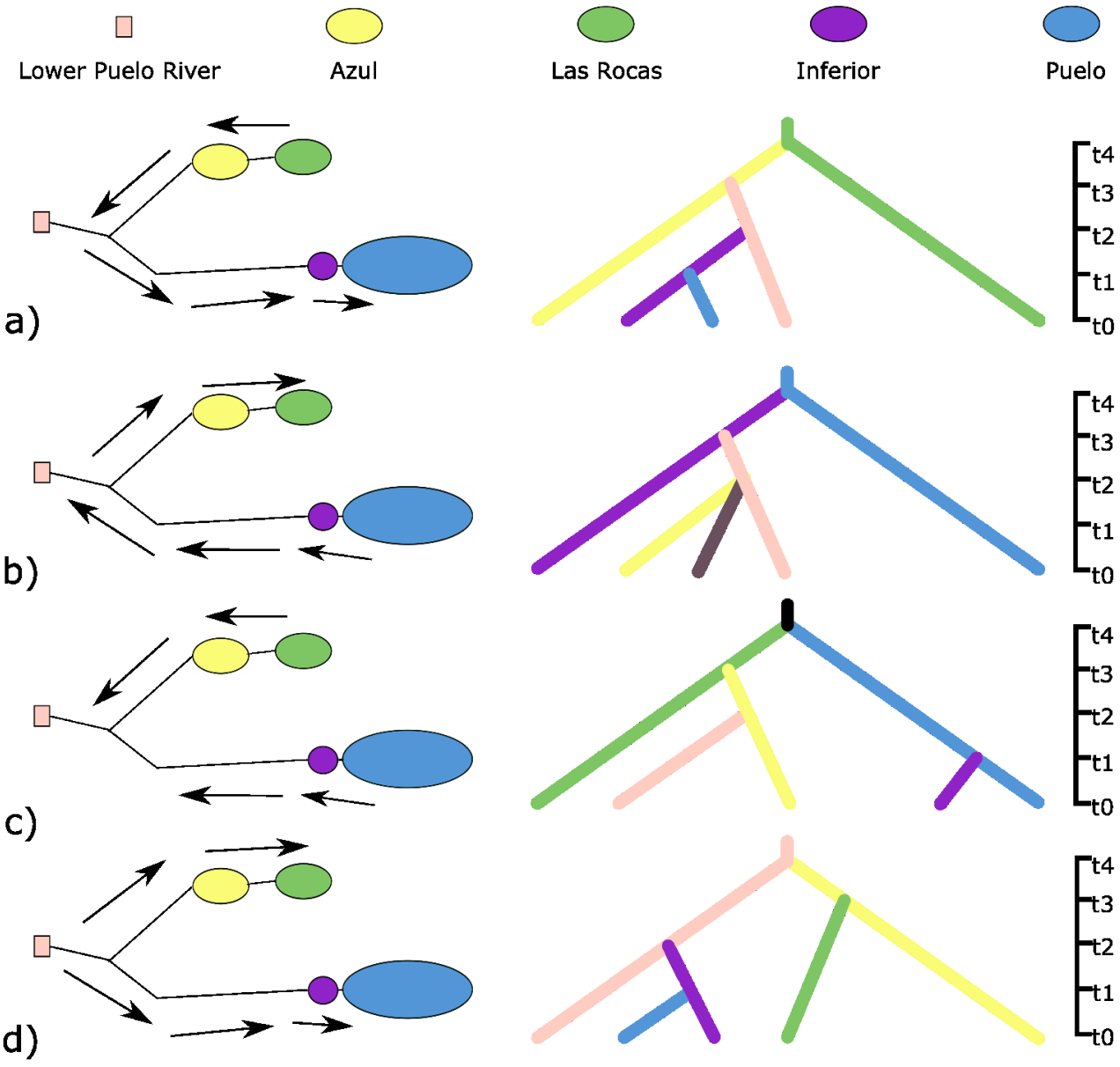

Figure 3.3. Four scenarios of population differentiation for 5 populations of *Galaxias platei* from the Puelo River Basin in northwestern Patagonia: (a) scenario 1, differentiation from western to eastern Andes; (b) scenario 2, differentiation from eastern to western Andes; (c) scenario 3, differentiation based on STRUCTURE plots; (d) scenario 4, differentiation from the Pacific to the Andean lakes. All scenarios followed the existent river connections in the Puelo River Basin.

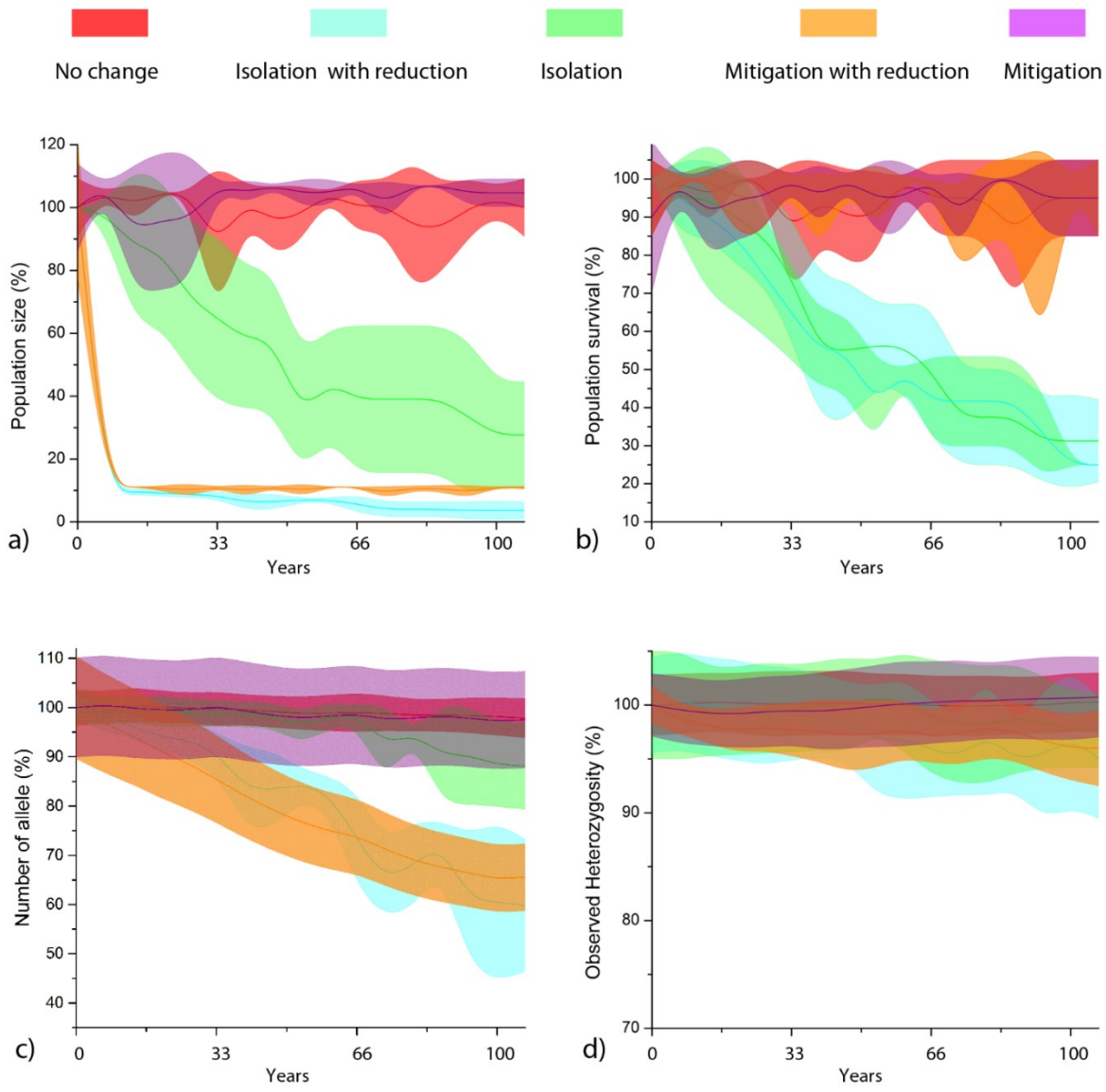

Figure 3.4. Over 100 years, mean (line) (SD, shading)) *Galaxias platei* (a) percentage of individuals surviving in each generation, (b) percentage of populations surviving in each generation, (c) percentage of number of alleles persisting in each generation, and (d) observed heterozygosity in 5 population-connectivity scenarios: no change, connectivity remains the same over time; isolation with reduction, population reduced to 10% of original size at generation 1 after dam construction; isolation, population isolated due to dam construction but population does not decline; mitigation with reduction, dam constructed and mitigation measures taken to maintain 1% connectivity among populations and 90% reduction in population ; and mitigation, dam construction with mitigation measures to maintain a 1% of connectivity among populations with

no population reduction. Simulations are for a metapopulation of *Galaxias platei* composed of 4 populations from northwestern Patagonia.

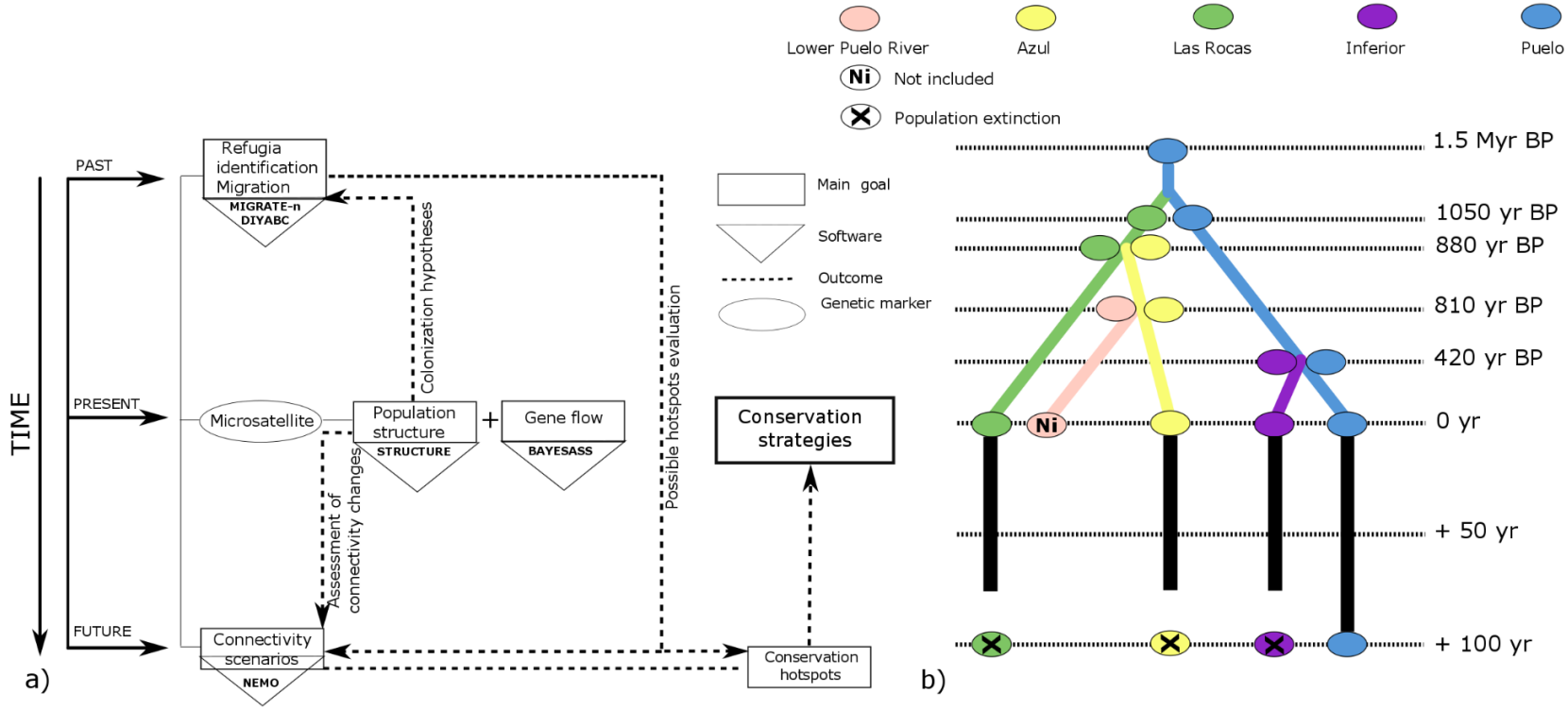

Figure 3.5. a) Summary of the software packages used for a multitemporal perspective including main goals and outcomes for historical, contemporary and future patterns of population structure and genetic diversity, and the use of the main outcomes for conservation strategies b) Most likely colonization and population differentiation scenario for *Galaxias platei*. The ancestral population in Lake Puelo (1.5 Myr BP) expands and colonizes Lake Las Rocas 1050 years BP, appears in Lake Azul 880 years BP and the lower Puelo River 810 years BP, and finally the contemporary population's structure appears (0 yr) in Lake Inferior 420 years BP. Future populations are shown from 0 year to + 100 year. yr = years.

3.8 Supporting Information

Supplementary Methods

Backward-in-time analyses using DIYABC were run for 20 million iterations using a mean mutation ranging from $1x10^{-5}$-$1x10^{-2}$, recombination rate ranging from 10^{-3} to $9.9x10^{-1}$, with prior distribution of parameters as shown in Table S33., calculating the one sample summary statistics mean number of alleles, mean genic diversity, mean Garza-Williamson's M and two-sample summary statistics mean number of alleles, mean genic diversity and F_{ST}. Scenarios were pre-evaluated with a Principal Component Analysis (PCA). The posterior distributions of the parameters were observed to detect anomalies in their distribution. Pseudo-observed data were computed to estimate the bias and precision of the simulations using the same parameters as for the original simulations. Scenarios and prior parameters were evaluated with the model checking option using a PCA, redefining summary statistics, using the statistics that were not used in the previous analysis. Posterior probabilities were obtained using the logistic regression. New datasets were simulated to evaluate the levels of confidence for the chosen scenario to estimate the probability of error types I and II.

Table S3.1: Migration model used in MIGRATE-n based on contemporary gene flow estimates from BAYESASS for a metapopulations of the freshwater fish *Galaxias platei* in NW Patagonia. Values in tables are mutation scaled migrations (M). Receiving population are in the top.

	Lower Puelo River	**Azul**	**Las Rocas**	**Inferior**	**Puelo**
Lower Puelo River	0	0.1	0.1	100	0.1
Azul	1000	0	100	0.1	100
Las Rocas	100	0.1	0	100	100
Inferior	100	100	100	0	1000
Puelo	100	0.1	0.1	0.1	0

Table S3.2: Parameters used in MIGRATE-n for two migration models (full migration model and a model based on the results from BAYESASS) for the freshwater fish *Galaxias platei* in NW Patagonia. Θ = mutation-scaled effective population size, M = mutation-scaled migration

Parameter	**value**
Model	Brownian
Long chains	1
Replicates	5
Θ	0-100 (exponential)
M	0-1000 (exponential)
Heating	1.0, 1.5, 3.0, 1000000

Table S3.3: Parameters used in NEMO for five simulated scenarios of a metapopulation of the freshwater fish *Galaxias platei* in NW Patagonia.

	Parameter	**Value**
Initialization	Carrying capacity	500
	Gene flow	BAYESASS results
	Number of generations	10
Forward-in-time simulations	Carrying capacity	500
	Fecundity	20000
	Number of microsatellite	14
	Maximum number of alleles	26
	Mutation rate	0.00005
	Mutation model	Single step mutation
	Number of generations	30

Table S3.4: Contemporary gene flow estimated with the software BAYESASS for a metapopulation of the freshwater fish *Galaxias platei* from five locations in NW Patagonia. Receiving population are in the top.

	Lower Puelo River	**Azul**	**Las Rocas**	**Inferior**	**Puelo**
Lower Puelo River	0.7285(+/- 0.0312)	0.0050(+/- 0.0048)	0.0064(+/- 0.0044)	0.0316(+/- 0.0114)	0.0089(+/- 0.0086)
Azul	0.1898(+/- 0.0483)	0.9647(+/- 0.0156)	0.0356(+/- 0.0206)	0.0104(+/- 0.0091)	0.0131(+/- 0.0125)
Las Rocas	0.0389(+/- 0.0351)	0.0096(+/- 0.0085)	0.9121(+/- 0.0297)	0.0143(+/- 0.0110)	0.0118(+/- 0.0115)
Inferior	0.0250(+/- 0.0233)	0.0158(+/- 0.0121)	0.0431(+/- 0.0229)	0.9388(+/- 0.0178)	0.2887(+/- 0.0209)
Puelo	0.0177(+/- 0.0171)	0.0050(+/- 0.0049)	0.0028(+/- 0.0028)	0.0049(+/- 0.0048)	0.6775(+/- 0.0104)

Table S3.5: Historical mutation-scaled migrations (calculated in MIGRATE-n) for a metapopulation of the freshwater fish *Galaxias platei* in NW Patagonia.

	Lower Puelo River	**Azul**	**Las Rocas**	**Inferior**	**Puelo**
Lower Puelo River	-	6.4	4.9	9.2	13.9
Azul	8.6	-	21.1	33.7	18.7
Las Rocas	17.6	39.4	-	51.0	30.8
Inferior	7.3	21.2	6.0	-	27.9
Puelo	6.8	4.4	6.7	27.4	-

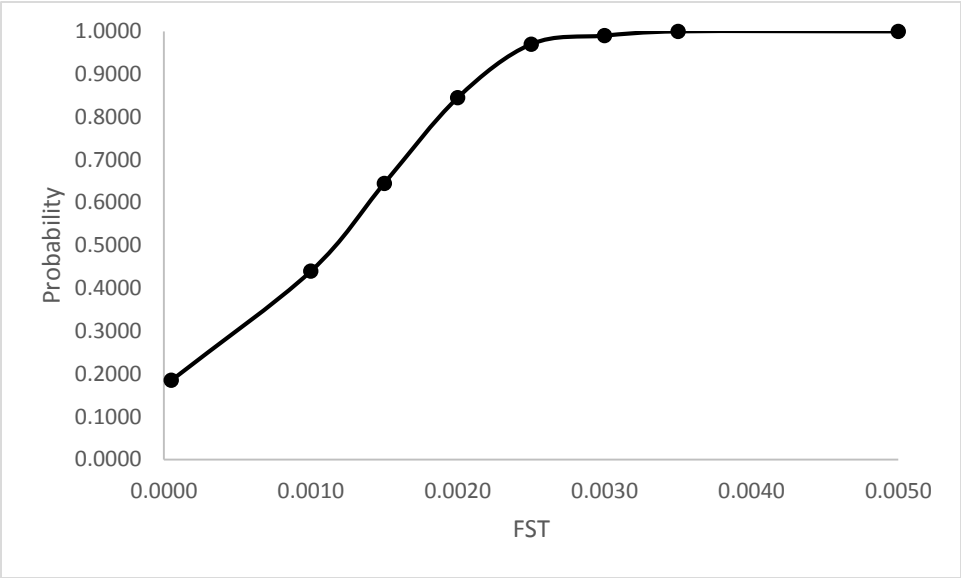

Figure S3.1: Power analysis obtained with POWSIM. Y-axis shows the probability (0-1) of 14 microsatellite markers to detect an F_{ST} value indicated in the x-axis. The maximum probability is reached when F*ST* is 0.0035 at t = 7 generations before present.

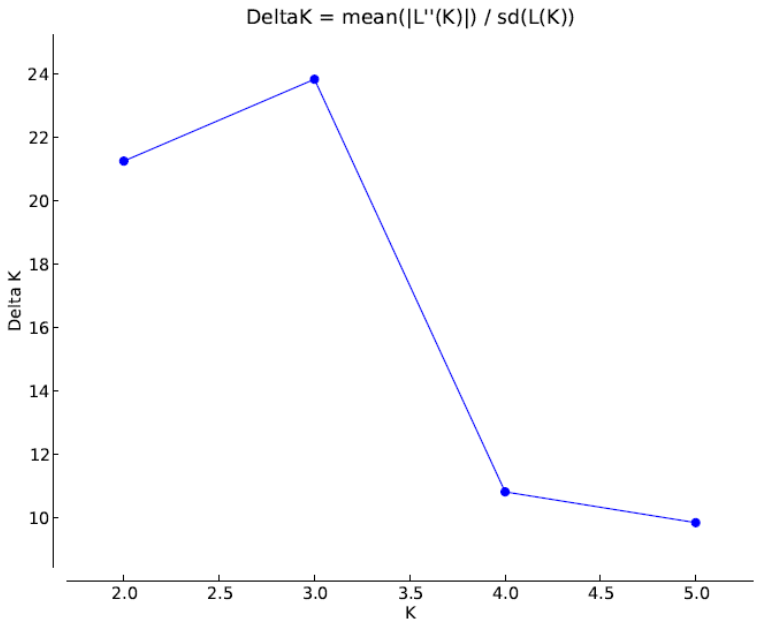

Figure S3.2: Most likely number of clusters/populations (using the software STRUCTURE) for a metapopulation of the freshwater fish *Galaxias platei* in NW Patagonia. Results were calculated using the Evanno method.

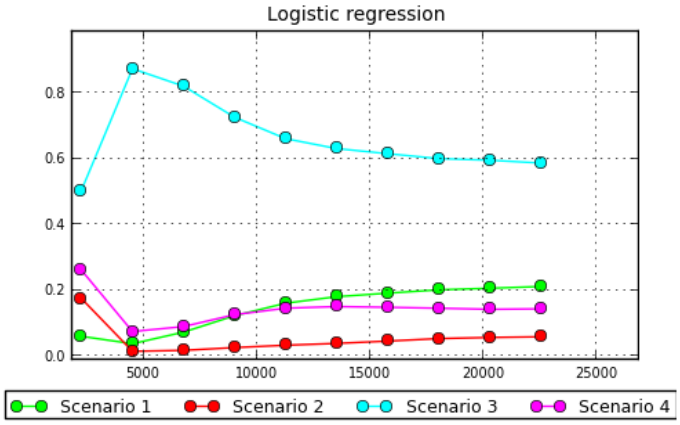

Figure S3.3: Most likely colonization scenario (Scenario 3; population differentiation of Lake Las Rocas from Lake Puelo and subsequent differentiation of all other three populations) obtained with the software DIYABC for a metapopulation of the freshwater fish *Galaxias platei* in NW Patagonia.

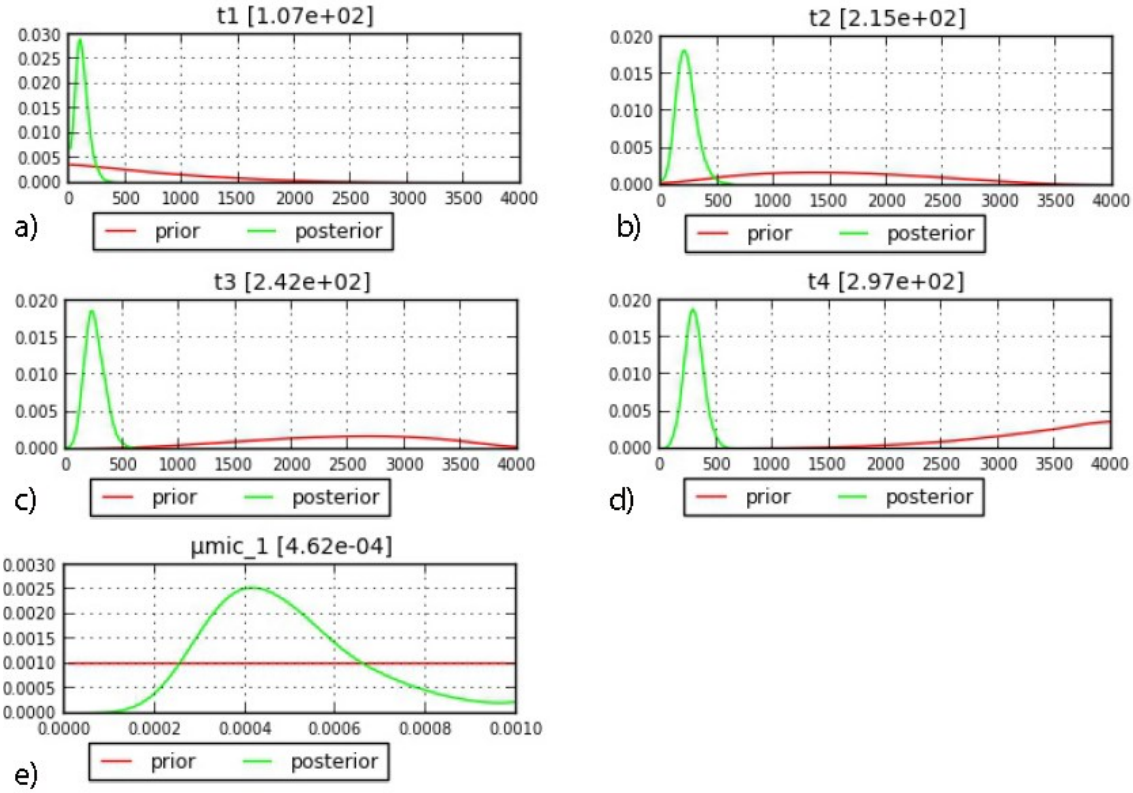

Figure S3.4: Posterior distribution of t1 (time 1; Figure S3.4a), t2 (time2; Figure S3.4b), t3 (time 3; Figure S3.4c), and t4 (time 4; Figure S3.4d), and µmic_1 (mutation rate; Figure S3.4d), calculated with DIYABC for a *Galaxias platei* population in NW Patagonia, based

on the most likely scenario Scenario 3; population differentiation of Lake Las Rocas from Lake Puelo and subsequent differentiation of all other three populations.

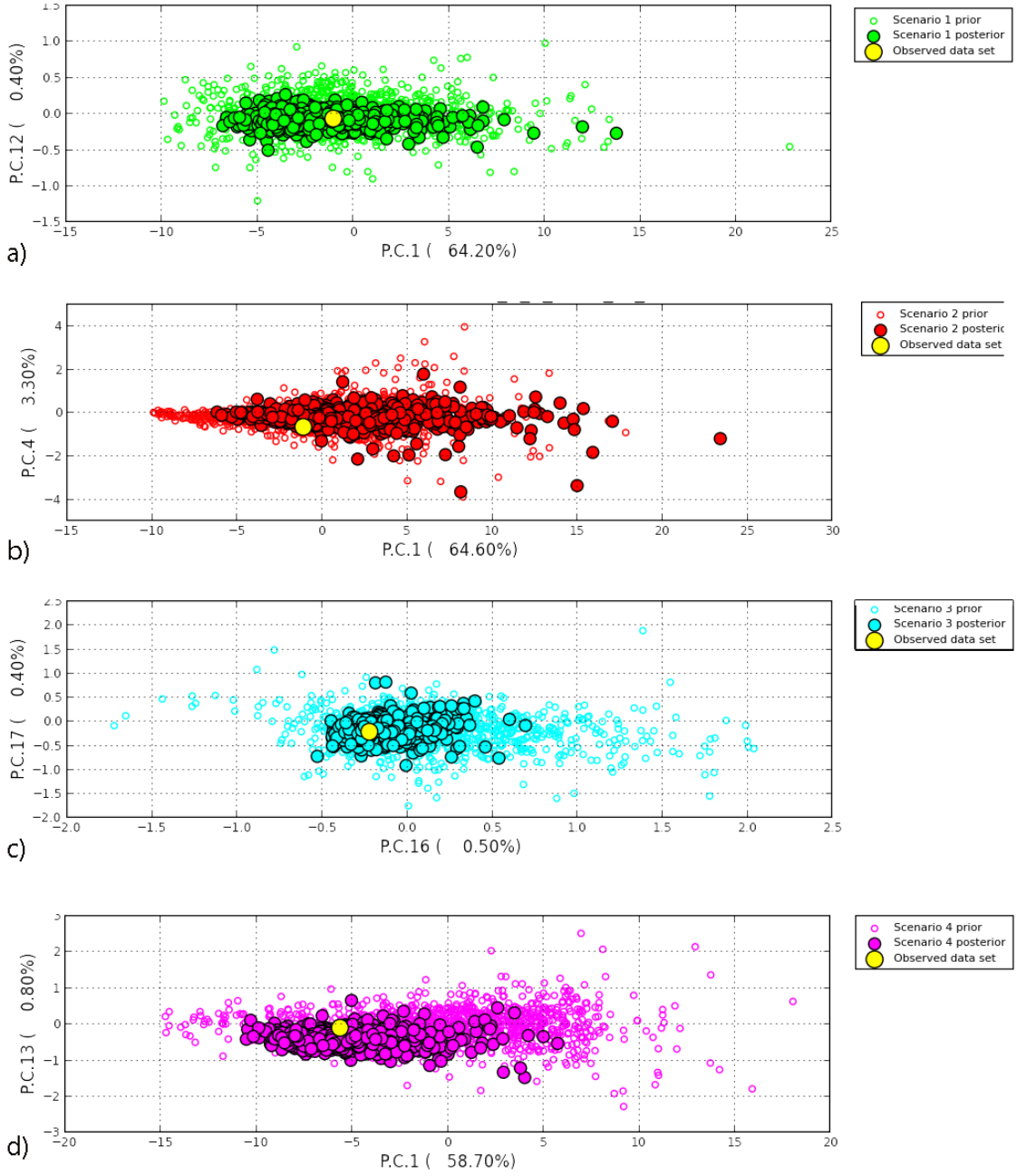

Figure S3.5: Model checking for the four scenarios (Scenarios 1-4, Figure 54a-d) evaluated with DIYABC for a *Galaxias platei* metapopulation in NW Patagonia.

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CHAPTER 4 INVASIVE SPECIES AND POSTGLACIAL COLONIZATION: THEIR EFFECTS ON THE GENETIC DIVERSITY OF A PATAGONIAN FISH.

4.1 Abstract

Freshwater biodiversity has decreased at local scales during the Anthropocene largely as a consequence of climate change, habitat/landscape modifications and of the introduction of invasive species. Thus, human-induced pressure on native species has dramatically increased, even in areas considered to be relatively removed from highly populated areas of the world, such as Patagonia. Patagonian species present a complex history explained by Pleistocene refugial populations that expanded their distribution range during the Holocene, occupying areas that were previously covered by ice. One of the major threats for Patagonian populations during the last century has been the introduction of salmonid species for recreation and fish farming. Salmonid species have increased their presence, occupying most rivers and lakes in Patagonia, where they prey on, and compete with native species, including the fish *Galaxias platei*. Here we studied how long-term processes (i.e. population differentiation during the Holocene) and short-term historical processes (salmonid introduction) have shaped the genetic diversity (N_a and H_o) of 21 *G. platei* populations using microsatellite markers. We found that N_a is negatively affected by salmonid presence with *G. platei* populations in lakes with salmonids exhibiting ~10% fewer alleles than populations from lakes without salmonids. H_o is instead, affected by a combination of salmonid presence, divergence time and distance to refugia. Simulations (100 years backwards) showed that this difference in the genetic diversity can be explained by a 99% reduction in the population size. Our analysis reveals the magnitude of the damage potentially caused by invasive on native species and demonstrates how a combination of genetic statistics and simulations can help to understand the demographic changes that have likely taken place with *G. platei* populations during the last century.

4.2 Introduction

Global biodiversity is thought to have declined sharply over the last century (Dirzo & Haven 2003; Ceballos et al. 2015, González et al. 2016). Although actions have been taken to slow down the rate of species loss, such actions have largely been insufficient (Butchart et al. 2010). Much of the recent biodiversity loss has taken place as a consequence of human expansion, the consequences of which have been compared to the previous mass extinctions on Earth (Barnosky et al. 2011). Human modifications of the environment have reached such magnitude that scientists have proposed a new geological epoch named Anthropocene, that begins with human expansion as a driving force for global change (Crutzen & Steffen 2003; Smith & Zeder 2013; Lewis & Maslin 2015). During the Anthropocene, animal biodiversity has been seriously affected by human intervention of the landscape, including the introduction of exotic (nonnative) species to regions outside their range, some of which become invasive affecting the abundance and distribution of native species (Dirzo et al. 2014). The expansion of invasive species into exotic ranges has often been thought to cause not just declines in the population sizes of native species but also declines in their heterozygosity and effective population sizes as well as inbreeding depression (Gasc et al. 2010; Handley et al. 2011).

Compared to terrestrial and marine ecosystems, freshwater ecosystems present the highest biodiversity per surface area (Dudgeon et al. 2006; Balian et al. 2008). Freshwater biodiversity has constantly been threatened by the introduction of invasive species (Simberloff 2013), reducing the presence of native species and their local populations (Havel et al. 2015; Thomaz et al. 2015). From an ecological viewpoint, invasive species can transform habitat (e.g. increasing the eutrophication in freshwater systems), affect the biotic interaction among species (predation, competition and grazing), and finally decrease the overall diversity (Jones & Closs 2015; Gallardo et al. 2016).

Although far from most highly populated areas worldwide, Patagonia exhibits a high number of introduced species, including a number of salmonid fishes. Salmonid fishes have been introduced into Patagonian lakes and rivers starting in the early 20th century (Basulto 2003), with some species currently exhibiting naturalized populations (see Ruzzante et al 2003; Ciancio et al. 2015; Canales-Aguirre et al. 2018). The presence of these invasive species in Patagonia has resulted in significant impacts in lakes and river populations,

where salmonid fishes have displaced native freshwater fishes, especially from streams (Pascual & Ciancio 2007; Vigliano & Alonso 2007; Arismendi et al. 2009; Habit et al. 2012, Cussac et al. 2014). Salmonids are known to prey on widely distributed native fish, which has facilitated their success throughout Patagonia (Macchi et al. 2007). One of these prey species is the freshwater fish *Galaxias platei* found throughout Patagonia. Salmonids have been shown to actively prey on *G. platei* individuals, affect their habitat preferences, and act as stream barriers for their dispersal (Correa & Hendry 2012; Habit et al. 2012). Nevertheless, the extent to which the presence of salmonids affects the size and genetic diversity of *G. platei* populations remains unknown. Indeed, very few studies are available that show an impact of invasive species on the genetic composition of native species (e.g. Gasc et al. 2010; Iwai & Shoda-Kagaya 2012; Rowe & Zanatta 2015; Palmer et al. 2016), revealing the need for more studies in this area.

Aside from the short-term temporal processes affecting the genetic diversity of populations such as those induced by the presence of invasive species, long-term historical processes have also revealed an important role in the genetic composition of species (e.g. Epps & Keyghobadi 2015; Hernawan et al. 2017). Most of the long-term historical processes explaining the genetic diversity of Patagonian species have been linked to refugial areas and postglacial colonization routes (Vera-Escalona et al. 2015; Fasanella et al. 2017). This is especially true for *G. platei* where several authors have shown the importance of these processes (Zemlak et al. 2008, 2010, 2011; Vera-Escalona et al. 2015). Nevertheless, the relative magnitude of the effects of long-term historical processes (i.e., those related to Quaternary glacial cycles) vs. that of short term processes (i.e., invasive species) remains largely unexplored. This information is likely to be useful for the design of robust conservation policies, especially for those areas where invasive species could be affecting the diversity of freshwater fish populations that survived the Quaternary glaciations in glacial refugia.

Here we examined the genetic diversity in *Galaxias platei* populations inhabiting 21 lakes in Patagonia in an effort to elucidate the roles of long-term vs. short-term (e.g., invasive salmonids) historical processes on this species diversity.

4.3 Methods

4.3.1 Sampling collection and molecular protocols

Galaxias platei (N=1801) were collected from a total of 21 lakes distributed from 39°S to 50°S in the south in Chile and Argentina (Table 4.1, Fig. 4.1). Individuals collected from the isolated lakes Yulton, Thompson and Belgrano, as well as those collected from two other (connected) lakes, Pollux and Del Mie are used for the first time in this study. The remaining samples have been used in previous studies (Vera-Escalona et al 2015; 2018; see Table 4.1). The five new collections were obtained as before, using seine nets, gill nets and by electrofishing. Tissue collections were then stored in 95% ethanol Eppendorf tubes. Whole DNA was extracted using a glass milk protocol (Elphinstone et al. 2003) and amplified by PCR using 10 species-specific microsatellite markers (Arias *et al.,* 2012; Vera-Escalona *et al.,* 2014; see Appendix 2), 8 of which have been used in our previous studies dealing with the Valdivia, Puelo and Serrano river systems (Vera-Escalona *et al.,* 2015, 2018). The PCR contained 3 µl of DNA, 1 µl of 10-mM $(NH_4)_2SO_4$, 1 µl of 2-mM $MgCl_2$, 1 µl of 2-mM dNTP, 0.1 µl of each primer; 0.1 µl of m13, 0.1 µl of 0.5U Tsg and 6.5 µl of H_2O was used. Amplified microsatellite fragments were observed in polyacrylamide gels with LI-COR® sequencers (Biosciences, Lincoln, NE, USA) and a molecular ladder of 65-400 bp. Images were then analyzed with the software SAGA™ (LI-COR®) for microsatellite scoring.

Eleven out of 21 lacustrine *G. platei* populations examined in the present study coexist with invasive salmonids, while the remaining 10 populations originate from salmonid-free lakes (Table 4.1). Five of the 21 collections originate from populations inhabiting isolated lakes or lakes with nil connectivity with downstream environments, while the remaining 16 collections originate from populations inhabiting lakes with at least some degree of connectivity with other *G. platei* populations in their respective river systems (Table 4.1).

4.3.2 Descriptive analyses

Hardy-Weinberg Equilibrium (HWE) and Linkage Disequilibrium (LD) tests were assessed in Genepop on the web (http://genepop.curtin.edu.au/; Raymond & Rousset 1995). The False Discovery Rate approach was used to account for multiple tests in both the HWE and LD using FDR (Benjamini & Hochberg 1995).

Observed heterozygosity (H_o), number of alleles (N_a), and the Fixation Index (F, = 1 - (H_o / H_e)) were calculated with Genalex 6.5 (Peakall and Smouse 2012). Data on presence/absence of introduced salmonids in Chile was retrieved from Ortiz-Sandoval et al. (2016) and Habit et al. (2012). Divergence times were obtained from Vera-Escalona et al. (2015; 2018), as well as estimated here for lakes Thompson, Pollux, Belgrano and Del Mie in DIYABC (Cornuet et al. 2014) following the same methodology from Vera-Escalona et al. (2015). Lake area and distance to refugial population were calculated with ArcGIS 10.3.1 (ESRI 2011). Distances to refugia were based on the minimum distance between a sampled lake and refugia (estimated with DIYABC) following the river flow.

4.3.3 Statistical tests

Forward stepwise regression analyses were conducted to examine the influence of latitude, lake area, divergence time, gene flow, presence/absence of salmonids, and distance to refugial populations on dependent variables N_a, H_o, and F. Independent variables were added or removed one at a time, using a forward stepwise with F-to-enter 3.8416 and F-to-remove 2.7056. Because no information for distance to refugia or for divergence time existed for the five isolated lakes, these were not considered when examining the roles of distance to refugia and divergence time. The remaining 16 populations (Valdivia, Puelo, and Serrano basins), shared all 10 microsatellite markers, thus the regression analyses involving these 16 populations included genotypic data for all 10 markers while those involving all 21 populations were restricted to the eight markers that were common to all collections.

Two-sample methods were used to quantify the effect of salmonids on the genetic variables H_o and N_a. This analysis was also conducted with both data sets, the first involving just 16 populations and 10 microsatellite markers and the second involving all 21 populations with

8 microsatellite markers. T-tests were used for N_a while Mann-Whitney U tests were used for H_o because the equal variance test failed for the analysis with 16 and 21 populations. All statistical analyses were run in STATISTICA 7.0 (Statsoft. Inc 2004).

To control for a potential effect of lake area (habitat size) on genetic parameters H_o and N_a, the two-sample analyses were repeated using the residuals of the regression of these parameters on lake area. Although the regression coefficients were non-significant, the residuals were still used to compare *G. platei* populations from lakes with and without salmonids.

4.3.4 Simulations

Simulations were performed in NEMO 2.3.46 (Guillaume 2006) to evaluate which of a number of population size reduction scenarios can lead to at least a 10% decline in N_a within the timeframe of 100 years from an initial reduction in population size. The genotypic data for simulations was created from the *G. platei* population from Lake Thompson, a population inhabiting a salmonid-free and isolated lake with a number of alleles similar to the average over all ten salmonid-free lakes. We used the genotypic data from this population to simulate populations with initial size $N = 100000$ and final size $N = 50000$, 25000, 10000, and 1000 (corresponding to 50%, 25%, 10%, and 1% of the original population size). An initial population size of $N = 100000$ was chosen as this is the expected maximum size of a *G. platei* population inhabiting a lake with an área of 10000 m^2 (i.e., maximum expected density: 10 individuals per m^2, Sobenes et al. 2013). We thus simulated a typical *G. platei* population inhabiting a salmonid free lake. We then plotted the percentage of the initial number of alleles still remaining in the population over a 100 year period. Simulations were run using 10 replicates, mean fecundity = 10000, reducing population sizes every 3 generations (10.5 years), according to Fig. 4.3a. All inputs were uploaded to https://figshare.com/s/15378b944339ed847a19.

4.4 Results

4.4.1 Descriptive analyses

There were 21 departures from the Hardy-Weinberg equilibrium (HWE) with FDR = 0.2, but they were neither locus nor population specific. No evidence of linkage disequilibrium was found after applying the False Discovery Rate approach. With 10 microsatellite markers, the mean number of alleles within populations ranged from 6.3 in Lake Puelo (Puelo River basin) to 13.3 in Lake Azul (Serrano River basin) and from 0.474 in Lake Puelo (Puelo River Basin) to 0.762 in Lake Pehoe (Serrano River Basin), while F ranged from -0.092 in Lake Azul (Puelo River Basin) to 0.054 in Lake Pullinque (Valdivia River Basin). And with 10 microsatellite markers, the mean number of alleles within populations ranged from 7.250 in Lake Puelo (Puelo River Basin) to 15.750 in Lake Azul (Serrano River Basin), the mean observed heterozygosity within populations ranged from 0.476 in Lake Puelo (Puelo River Basin) to 0.856 in lakes Paine and Nordenskjold (both in the Serrano River Basin). In general, we observed lower mean number of alleles, observed heterozygosities and inbreeding coefficients when using 10 microsatellite markers, because the two extra markers available for populations from the Valdivia, Puelo and Serrano River were significantly less variable then the other eight markers that were common among the 21 populations.

4.4.2 Multiple Linear Regressions

Of all variables examined in the stepwise regression approach, only the presence of salmonids was identified as significantly (p = 0.014; Table 4.2) decreasing the number of alleles (N_a). Observed heterozygosity (H_o) was instead affected by three variables: divergence time (p = 0.038), distance to refugia (p = 0.028) and presence of salmonids (p = 0.009; Table 4.2). Finally, the fixation index (F; Table 4.2) was affected by the variable "distance to refugia" with a p-value close to, but somewhat above the acceptance limit (p = 0.076). Thus, while the number of alleles and heterozygosity both declined with the presence of salmonids, heterozygosity was also affected by divergence time (older populations have higher heterozygosities), and by distance to refugia (populations near

refugial areas showed lower heterozygosities). Finally, the fixation index increased with distance to refugia.

4.4.3 Lakes with and without salmonids

Galaxias platei populations coexisting with salmonids exhibited fewer alleles (lower N_a) than populations living in salmonid-free lakes (mean N_a = 9.380 +/- 1.427 vs. mean N_a =11.617 +/- 1.733; 16 populations, 10 microsatellites, *t*-test, Fig. 4.2a,). Similar results were obtained when considering all 21 populations and 8 microsatellites: lakes with salmonids mean N_a = 10.705 +/- 1.675 vs. salmonid-free lakes mean N_a = 12.968 +/- 2.242; Fig. 4.2b.Among the *G. platei* populations experiencing gene flow those coexisting with salmonids exhibited lower heterozygosities than those inhabiting salmonid-free lakes [lakes with salmonids (N=11) median *Ho*=0.606 (0.523-0.687) vs. salmonid-free lakes (N=10) median *Ho*= 0.726 (0.700-0.738); Mann-Withney U test]. A similar pattern was observed when considering all lakes, including those that are connected and those that harbor relatively isolated populations [lakes with salmonids, median H_o= 0.733 (0.530-0.769) vs. salmonid-free lakes, median H_o = 0.815 (0.763-0.838); Fig. 4.2.c-d]. Genetic diversity as measured by N_a or H_o appears to have declined by more than 10% among *Galaxias platei* populations living in lakes with salmonids relative to *G. platei* populations inhabiting salmonid-free lakes.

Linear regression analysis between the genetic variables N_a and H_o with lake area (as a proxy for population size) were non-significant (Table S4.1, Supporting Information). The residuals of these regressions exhibited a pattern with regard to the presence/absence of salmonids that was similar to that observed above where lake area was not considered (Fig. S41, Supporting Information) suggesting the declines in N_a and H_o among *G. platei* populations when salmonids are present are not an artefact of variation in population size.

4.4.4 Simulations

The simulations using NEMO indicate that a decline in the number of alleles approximately equivalent to 10% of the initial number occurs only under the scenario where the population is reduced by two orders of magnitude, to 1% of its original size by declining from an initial size of $N=10^5$ to a final size of $N=10^3$ (Fig. 4.3a-b).

4.5 Discussion

Quaternary glaciations left a strong imprint on contemporary populations as a consequence of the changes in population sizes and connectivity that took place during the glacial cycles. (Hewitt 2000; Stewart et al. 2010). Patagonian freshwater species are no exception as they experienced contractions and expansions in population size that affected their connectivity, and exposed some to new areas and to other processes including secondary contact during the Holocene. Altogether these expansions were accompanied by increases in population size and genetic diversity (i.e. Stewart et al. 2010; Ruzzante et al. 2006, 2008; Zemlak et al. 2008; Cosacov et al. 2010; Zemlak et al. 2010; Vera-Escalona et al. 2012, 2015). More recently though, species worldwide have been affected by landscape/habitat changes, by changes in climate and by the introduction of invasive species, all mediated by humans. All these processes, at global scale, form part of the new geological epoch called the Anthropocene. In Patagonia, the introduction of invasive species from the Northern Hemisphere (i.e. beavers and salmonids) has seriously affected the population dynamic of freshwater species, including the reduction of population sizes and local extinctions (Iriarte et al. 2005; Nuñez et al. 2012; Anderson et al. 2014). Here we studied the effects of Pleistocene glaciations and the introduction of invasive salmonids over a Patagonian native fish. We showed that the number of alleles (N_a) and observed heterozygosity (H_o) of *G. platei* populations in Patagonia were 10% lower in lakes with salmonids than in salmonid free lakes, irrespective of lake area (as a correlate of population size). Salmonid presence negatively correlated with N_a, and the same pattern was observed for H_o. Observed heterozygosity was also affected by of divergence time and by distance to glacial refugial. Finally, we showed that the observed decrease in the number of alleles (10%) in *G. platei* populations inhabiting salmonid-invaded lakes relative to salmonid-free lakes within a 100-yr time frame can only be explained by a 99% reduction in population size, revealing the magnitude of the negative effects of salmonids at population and genetic level. Below we discuss the conservation implications of these results.

4.5.1 Long-term historical processes

The effects of the Pleistocene glaciations on contemporary genetic diversity of populations has been widely discussed in general (i.e. Hewitt 2000; Hewitt 2011). Nevertheless, the question of the extent to which different genetic parameters are affected by long-term historical processes like those related to the Pleistocene glaciations vs. short-term historical processes remains largely unexplored. Here we showed that divergence time and distance to refugia affected H_o, though this relationship was minimal (regression coefficients: $0.52x10^{-4}$ and $7.35x10^{-4}$, respectively), compared to the effect of the presence of salmonids (regression coefficient: -0.1119). The positive effect of divergence time on H_o might be explained by the effect of mutation rate on heterozygosity explained by Nei et al. (1976), while the positive effect of distance to refugia on H_o can be explained by the position of the sampled lakes. It is known that genetic variability of lake populations increases in populations where two or more headwaters converge, as in the case of dendritic populations (Morrissey &de Kerchove 2009). Dendritic systems were common among sampled populations, and thus it is likely that populations far from the refugia (refugia were usually located in the headwaters) presented higher levels of heterozygosity due to their nodal position connecting two or more lakes. Two other variables were expected to correlate with genetic parameters, these were lake area as a measure of habitat (i.e., population) size and latitude (Martin & McKay 2004; Vanoberbeke et al. 2007; Ehrich et al. 2009; Neville et al. 2009). Our results suggested that neither variable significantly affected the genetic diversity of *G. platei*. Contrary to other examples in the literature (i.e. Ehrich et al. 2009; Neville et al. 2009), lake area might not properly estimate habitat size for this species because of the species preferential use of littoral and deep benthic areas (Habit et al. 2010, 2012). Finally, genetic diversity in *G. platei* is not related to latitude, likely because on a local scale (50-200 km), the glacial history of the region is more closely related to longitude [i.e., distance west (or east) of the Andean peaks] than to latitude. This explanation is also supported by the effect of distance to refugia (usually located in the Andean zones).

4.5.2 Short-term historical processes

The effect of long-term versus short-term events over the number of alleles and heterozygosity have been widely discussed (Maruyama & Fuerst 1985; Allendorf 1986).

Rare alleles are lost at a faster rate than the decline in heterozygosity following a bottleneck (Fuerst & Maruyama 1985). Recent bottlenecks are thus more easily detected examining the number of alleles rather than heterozygosity. We showed with the stepwise regression analyses that presence of salmonids explains the changes in N_a across *G. platei* populations, while a combination of variables including salmonid presence, divergence time, and distance to refugia explain the variation in H_o. Thus, our results support the hypothesis that the presence of salmonids affects negatively the genetic composition of native *Galaxias platei* populations (Pascual et al. 2007; Soto et al. 2009; Habit et al. 2012).

Invasive species are known to modify the environment, lead to increased predation and competition intensities as well as to decreases in overall native diversity (Gallardo et al. 2016) though the effects of invasive species on diversity is hotly debated and may be a function of the spatial scale of analysis. On the other hand, there is little controversy in the assertion that invasive species affect the spatial distribution of native species (Simberloff 2013; Havel et al. 2015; Thomaz et al. 2015). Here we showed that the introduction of salmonids in Patagonia, which began in the early 20th century has likely reduced the genetic diversity of *G. platei* populations by >10% when measured by the number of alleles N_a. Our simulations indicate that such a decline in the number of alleles can best be explained by a 99% reduction in population size. These results are in line with other studies analyzing the genetic consequences of population size decline in marine species due to overfishing, which found that overfished populations exhibit a 12% decline in allelic richness (Pinsky & Palumbi 2014). Although our simulations show a dramatic effect in terms of population size and genetic reduction they should be taken cautiously due to the low number of microsatellite markers (8-10) and perhaps also the initial sample size of the population from Lake Thompson ($N = 52$) used for the simulations that could be affecting the results.

To our knowledge this is the first study examining the genetic consequences of invasive salmonids on the genetic diversity of a native Patagonian fish, and thus should open a wider discussion on the effects of invasive salmonids, not limited to the effect of population sizes, but also to effect on genetic diversity. Moreover, considering that the introduction of salmonids in Patagonia took place over different periods of time, first 100 years ago when salmonids were first released in lakes, and then 40-50 years ago when salmonids were grown in hatcheries in the sea and then escaped to the rivers and lakes (Table S4.2), we

can expect that the effect of salmonids on native species will only increase. Among the salmonids species introduced in Patagonia, two of them have been very successful, *Salmo trutta* and *Oncorhynchus mykiss*, which are the most abundant species in 10 of the 11 lakes studied (Table S4.2). Nevertheless, at least four more salmonid species can be found in one of the sampled lakes, Lake Puelo (Table 4.1; Ruzzante et al 2003; Soto et al. 2007; Aigo et al. 2008; Correa & Gross 2008), which might have synergistic effects on the genetic diversity of the *G. platei* population in this lake.

While we have demonstrated a decline in the genetic diversity of *G. platei* populations inhabiting salmonid-invaded lakes, which simulations show can be explained by a population size reduction of two orders of magnitude (99% population size reduction), we also observe increases in the population sizes of invasive salmonids (Macchi et al. 1999; Pascual et al. 2007; Arismendi et al. 2009; Habit et al. 2012). Introduced species like *Salmo trutta* and *Oncorhynchus mykiss* have successfully invaded different places of the world like Patagonia (Ibarra et al. 2011), and while in some regions these species have problems surviving and are even cataloged as endangered, they have shown to be very successful in various regions of the southern hemisphere. Thus, what is bad for native species may be good for introduced species, exemplifying a case of the so called biodiversity paradox (Vellend 2017), where a reduction of the local diversity results in the increase of the diversity of successful invasive species. The similar conditions of Patagonian lakes to the natural habitats in Northern hemisphere from where introduced salmonids were introduced, the pristine conditions free of pollulants, and as shown in our results, the apparently high amount of fish to prey on, helped to the successful introduction of invasive salmonids.

4.5.3 Conservation implications

The introduction of salmonids in Patagonia for recreation purposes and production in the early 1900s and subsequent additional introductions and releases from local regional aquaculture initiatives as well as escapes of farmed salmonids from hatcheries starting in the 1970s have seriously threatened native populations (Basulto 2003; Soto et al. 2007; Sepúlveda et al. 2013; Arismendi et al. 2014). Although several studies have examined the ecological consequences of salmonid species in lake and river fish species (e.g. Correa & Gross 2008; Correa & Hendry 2012; Habit et al. 2010, 2012; Macchi & Vigliano 2014),

no study has evaluated the genetic consequences of invasive salmonids in native species from Patagonia. Here we studied *Galaxias platei*, a galaxiid freshwater fish living in lakes with and without salmonids. Co-occurrence of galaxiid fishes with invasive salmonids has been widely documented, with authors finding that fast life-history traits species like *G. platei* are more prone to co-occur with salmonids (Jones & Closs 2015). From a conservation management perspective, *Galaxias platei* is only considered of special concern because of its wide geographic distribution and the fact that it is still commonly found in salmonid-free lakes where it tends to be found in high numbers (unpublished). Other Patagonian threatened fishes have been seriously affected by salmonids, reducing their presence in lakes and rivers, and even disappearing from certain areas where salmonids have depleted native species, and are thus of a higher conservation importance (Habit et al. 2010, 2012). Our results reveal that simply considering the presence of individuals of a given species is likely to hide the species' real conservation status. Moreover, our results suggest that invasive salmonids are likely to effect the evolutionary potential of *G, platei,* especially if the *G. platei* populations remain small as consequence of the salmonids or of any other landscape modification. We here show that genetic tools can and perhaps should routinely be used as a monitoring tool for the quantification of the impact of invasive species on native diversity. Furthermore, we show that the use of simulations could be useful to evaluate the effects of short-term processes when no historical samples are available.

4.6 Tables

Table 4.1 Sampled *Galaxias platei* lake populations including information of latitude, lake area (Km^2), divergence time (estimated with DIYABC), distance to refugia (based on the results from DIYABC), contemporary gene flow (incoming gene flow estimated with BAYESASS), absence (0) and presence (1) of invasive salmonids, observed heterozygosity (Ho), number of alleles (Na), and Fixation index (*F*). Genetic parameters were obtained through GENALEX. -10 = genetic parameters estimated with 10 microsatellite markers (16 populations), -8 = genetic parameters estimated with 8 microsatellite markers (21 populations).

Basin	Lake	N	Year	Lat	Area	Time	Distance	Gene flow	Salmonids	Na-10	Ho-10	*F*-10	Na-8	Ho-8
Valdivia	Pellaifa	58	2010-2012	39	7.2	180[1]	97[1]	0.155[1]	1[3]	9.8	0.579	-0.001	11.375	0.685
	Pullinque	98	2010-2012	39	5.8	240[1]	132[1]	0.088[1]	1[3]	10.1	0.627	0.054	11.750	0.746
	Panguipulli	78	2012	39	117	300[1]	152[1]	0.084[1]	1[3]	10.1	0.638	-0.021	11.625	0.733
	Riñihue	47	2012	39	82.5	600[1]	88[1]	0.327[1]	1[3]	8.6	0.687	-0.019	10.125	0.776
	Neltume	82	2010-2012	39	12	600[1]	47[1]	0.075[1]	1[3]	10.8	0.687	0.031	12.750	0.781
Puelo	Puelo	32	2005	41	48	23[2]	2	0.325[2]	1[5]	6.3	0.474	-0.018	7.250	0.476
	Inferior	80	2008	41	6.5	15[2]	1.1[2]	0.068[2]	1[3.4]	9.9	0.523	-0.022	10.750	0.507
	Las Rocas	128	2008	41	11	91[2]	3.2[2]	0.127[2]	1[3.4]	10.7	0.586	-0.026	12.375	0.598
	Azul	72	2008	41	14	91[2]	2	0.061[2]	1[3.4]	7.7	0.501	-0.092	8.750	0.490
Cuervo	Yulton	95	2009	45	60	-	-	0[+]	0[3.4]	-	-	-	9.625	0.573
Aysén	Thompson	52	2006	45	1	1240[*]	-	0	0[3.4]	-	-	-	11.625	0.763
	Pollux	29	2006	45	8	1240[*]	-	-	1[3]	-	-	-	9.375	0.735
Nansen	Belgrano	319	2001-2014	47	50	1700[*]	-	-	0[5]	-	-	-	15.083	0.763
Chico	Del Mie	92	2014	47	0.2	1700[*]	-	-	0[5]	-	-	-	11.375	0.806
Serrano	Dickson	98	2011	50	17.3	480[1]	28[1]	0.022[1]	0[3.4]	11.7	0.700	0.027	13.625	0.821
	Paine	65	2009-2011	50	4.1	890[1]	16[1]	0.329[1]	0[3.4]	11.2	0.738	-0.067	13.125	0.856
	Azul	112	2009-2011	50	6.1	2600[1]	0[1]	0[1]	0[3.4]	13.3	0.725	0.010	15.750	0.823
	Mellizas	63	2008	50	0.3	1300[1]	0[1]	0[1]	0[3.4]	8.5	0.674	-0.072	9.875	0.808
	Nordenskjold	83	2009-2011	50	28	1300[1]	2[1]	0.294[1]	0[3.4]	11.9	0.727	-0.049	14.000	0.856
	Pehoe	79	2009-2011	50	22	890[1]	2[1]	0.105[1]	0[3.4]	13.1	0.762	-0.053	15.500	0.838
	Porteño	39	2009-2010	50	22	1800[1]	39[1]	0.129[1]	1[3.4]	9.8	0.695	0.048	11.625	0.816

[1] Vera-Escalona et al. 2015, [2] Vera-Escalona et al. 2018, [3] Habit et al. 2012, [4] Ortiz-Sandoval et al. 2016., [5] Aigo et al. 2008, [*] values estimated in this manuscript, [+] isolated lake.

Table 4.2. Stepwise selection regression models predicting the number of alleles (N_a), observed heterozygosities (H_o) and Fixation Index (*F*) of *Galaxias* populations from Patagonia with n = 16 (10 microsatellite markers) using a forward stepwise regression. SE Beta = Standard error of Beta

Na				
Term	Beta	SE Beta	t-value	p-value
Intercept	11.617	0.6300	18.44	0
Salmonids	-2.237	0.7970	-2.81	0.014

Ho				
Term	Beta	SE Beta	t-value	p-value
Intercept	0.6498	0.0352	18.48	0
Time	0.000052	0.0000	2.33	0.038
Distance	0.000735	0.0003	2.49	0.028
Salmonids	-0.1119	0.0358	-3.13	0.009

F				
Term	Beta	SE Beta	t-value	p-value
Intercept	-0.0316	0.0126	-2.51	0.025
Distance	0.000385	0.0002	1.92	0.076

Data are shown only for variables that remain in the final model with a significance threshold of 0.05.

4.7 Figures

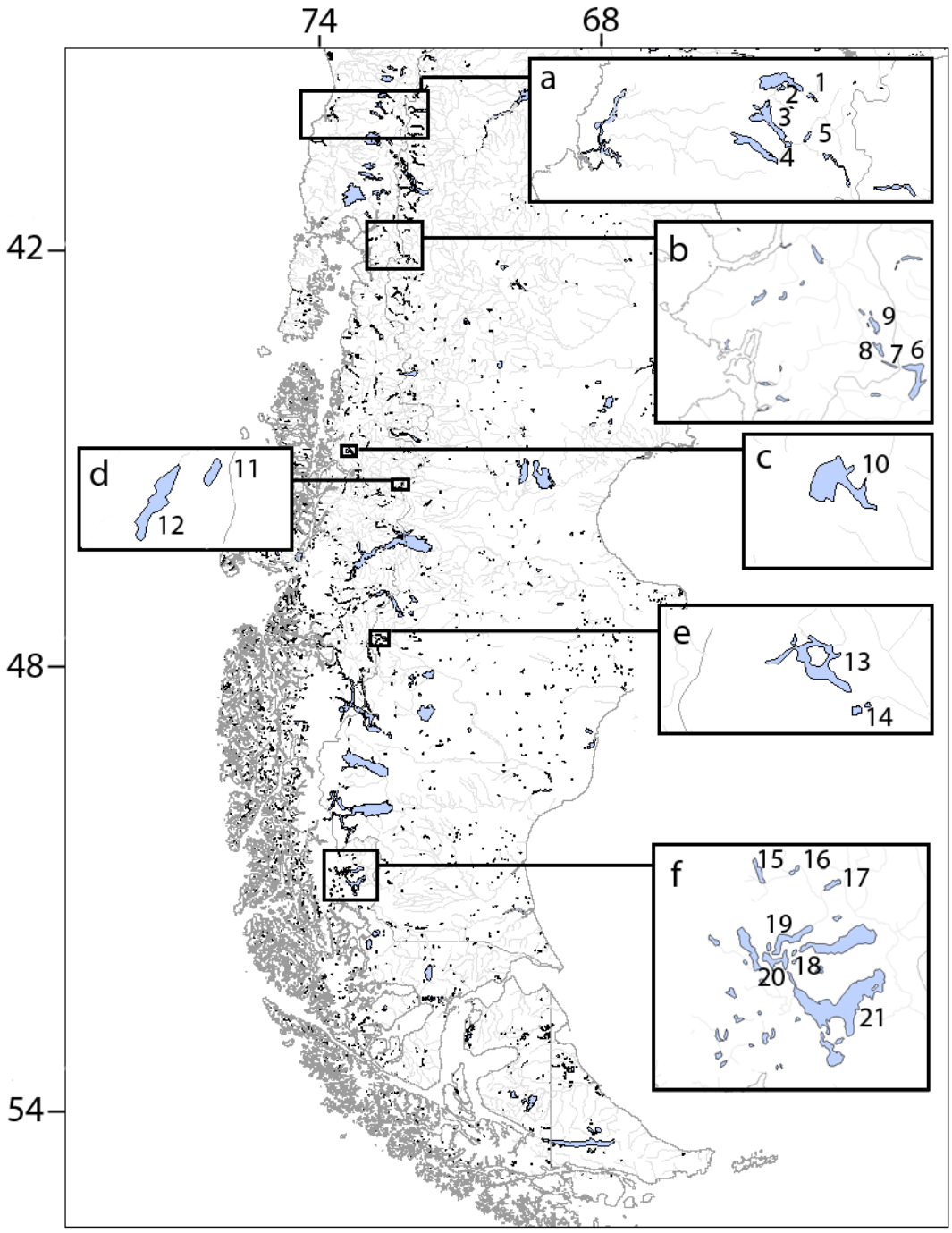


Figure 4.1. *Galaxias platei* collection locations (lakes and river basins in Chile and Argentina). a) Valdivia River Basin; 1: Lake Pellaifa, 2: Lake Pullinque, 3: Panguipulli, 4: Riñihue, 5: Neltume (Data from Vera-Escalona et al. 2015), b) Puelo River Basin; 6: Puelo, 7: Inferior, 8: Las Rocas, 9: Azul (Data from Vera-Escalona et al. 2018), c) Cuervo River Basin; 10: Lake Yulton, d) Aysén River Basin; 11: Lake Thompson, 12:

Lake Pollux, e) Nansen (13: Lake Belgrano) and Chico (14: Lake Del Mie) River Basin, and f) Serrano River Basin; 15: Lake Dickson, 16: Lake Paine, 17: Lake Azul, 18: Lake Mellizas, 19: Lake Nordenskjold, 20: Lake Pehoe, 21: Lake Porteño (Data from Vera-Escalona et al. 2015).

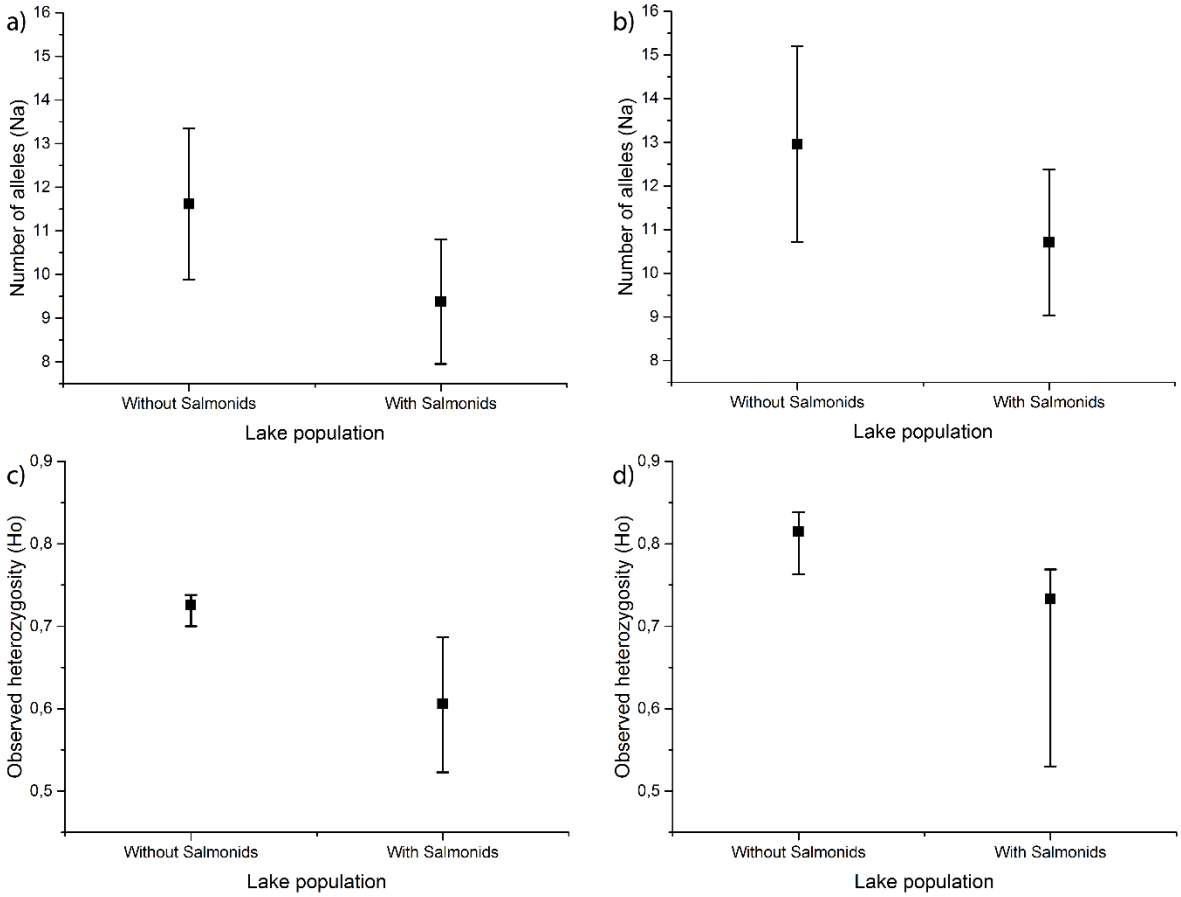


Figure 4.2. Number of alleles calculated as mean of the mean number of alleles and confidence interval for *Galaxias platei* populations in lakes with and without salmonids. (A) 16 connected lakes (t-test = -2.807, p 0.014), (B) 21 connected and isolated lakes (t-test = -2.626, p 0.017) Observed heterozygosity for *Galaxias platei* populations in (C) 16 connected lakes with and without salmonids (Mann Whitney U = 3.000, p = 0.004), and in (D) 21 connected and isolated lakes with and without salmonids (Mann Whitney test U = 16.000, p = 0.007).

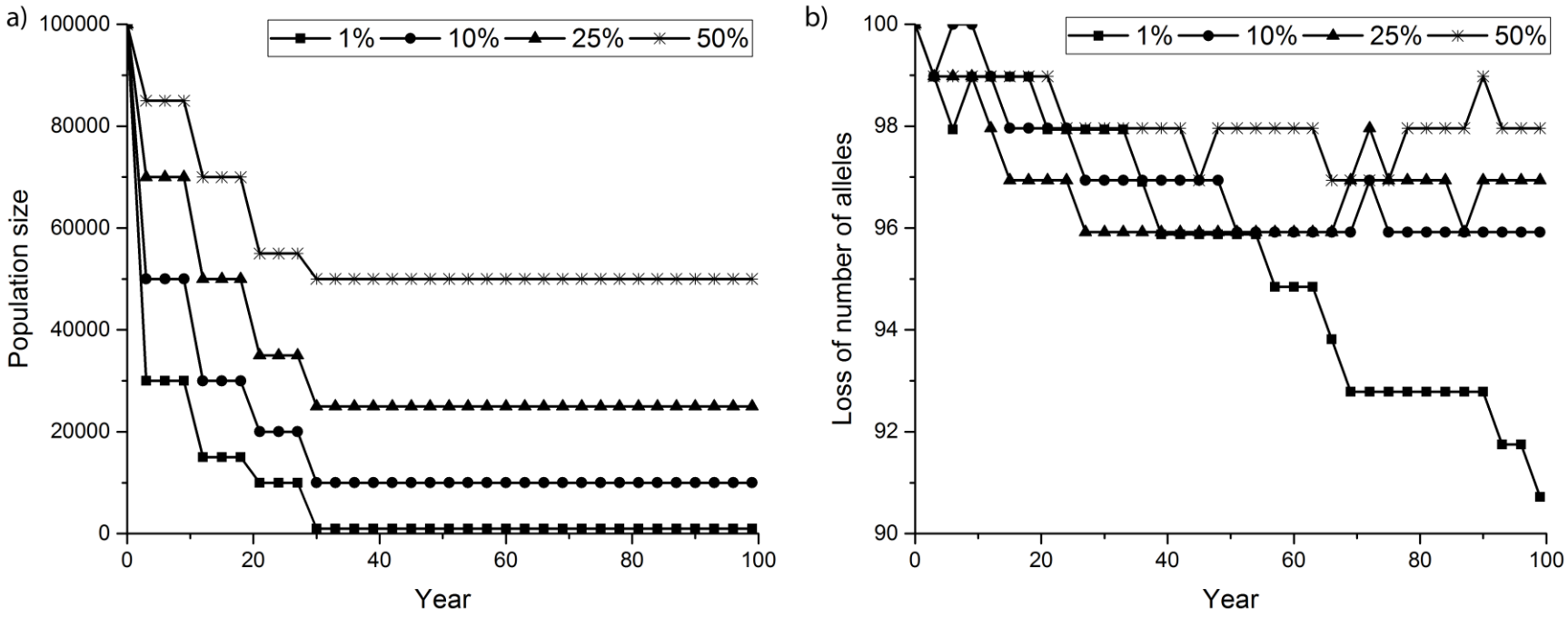


Figure 4.3. a) Simulated *Galaxias platei* populations size (N) reduced from an initial population of N = 100000 to a final population of 1% (N = 1000), 10% (N=10000), 25% (N=25000), and 50% (N=50000) of the original population). b) Loss of number of alleles measured as percentage of the initial number of alleles still remaining in the population under a simulated population size reduction.

4.8 Supporting Information

Table S4.1. Regression coefficients from linear regressions using for the number of alleles (N_a) and observed heterozygosities (H_o) with lake area of *Galaxias platei* populations from Patagonia. Coef = Coefficient, SE Coef = Standard error of the coefficient, VIF = Variance inflation factor.

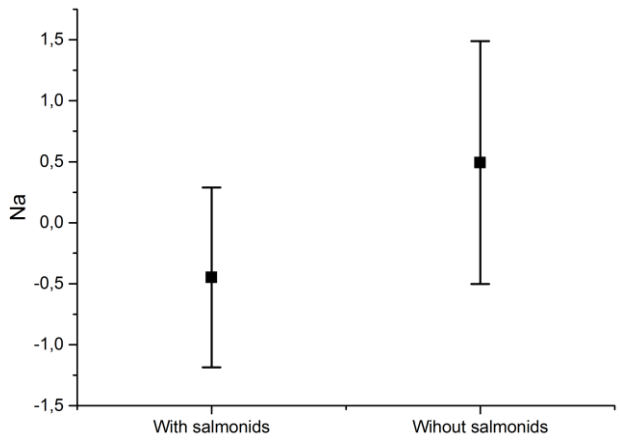
Na				
Term	Coef	SE Coef	t-value	p-value
Constant	10.574	0.604	17.501	6.513E-11
Lake area	-0.014	0.015	-0.929	0.369

Ho				
Term	Coef	SE Coef	t-value	p-value
Constant	0.647	0.029	21.832	3.268E-12
Lake area	-8.891E-5	7.429E-4	-0.119	0.906

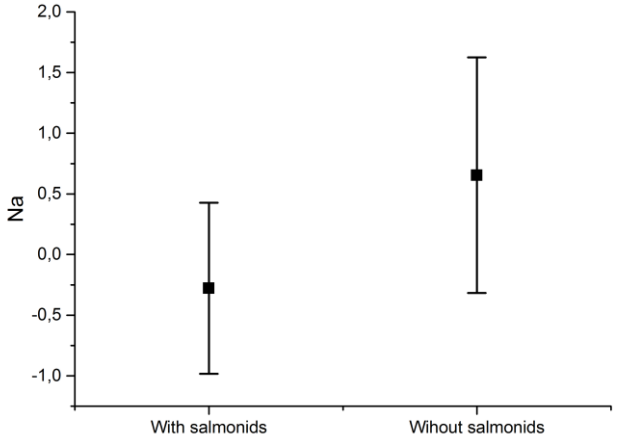
Table S4.2. Introduced salmonids species in 21 Patagonian lakes including year of introduction.

Basin	Lake	Species	Year
Valdivia	Pellaifa	*Salmo trutta*	1912[1]
		Oncorhynchus mykiss	1907[1]
	Pullinque	*Salmo trutta*	1912[1]
		Oncorhynchus mykiss	1907[1]
	Panguipulli	*Salmo trutta*	1912[1], 1972[1]
		Oncorhynchus mykiss	1907[1]
	Riñihue	*Salmo trutta*	1912[1]
		Oncorhynchus mykiss	1907[1]
	Neltume	*Salmo trutta*	1912[1]
		Oncorhynchus mykiss	1907[1]
Puelo	Puelo	*Salmo trutta*	1927[1]
		Oncorhynchus mykiss	1927[1]
		Oncorhynchus tshawystcha	1924[12]
		Oncorhynchus kisutch	1961[1]
		Salvelinus fontinalis	1932[1]
		Salmo salar	1927[1]
	Inferior	*Salmo trutta*	1927[1]
		Oncorhynchus mykiss	1927[1]
	Las Rocas	*Oncorhynchus mykiss*	1927[1]
	Azul	*Oncorhynchus mykiss*	1927[1]
Cuervo	Yulton	None	
Aysén	Thompson	*Oncorhynchus mykiss*	2002[3]
	Pollux	*Salmo trutta*	n.i
Nansen	Belgrano	None	
Chico	Del Mie	None	
Serrano	Dickson	None	
	Paine	None	
	Azul	None	
	Mellizas	None	
	Nordenskjold	None	
	Pehoe	none	
	Porteño	*Salmo trutta*	1927[1]

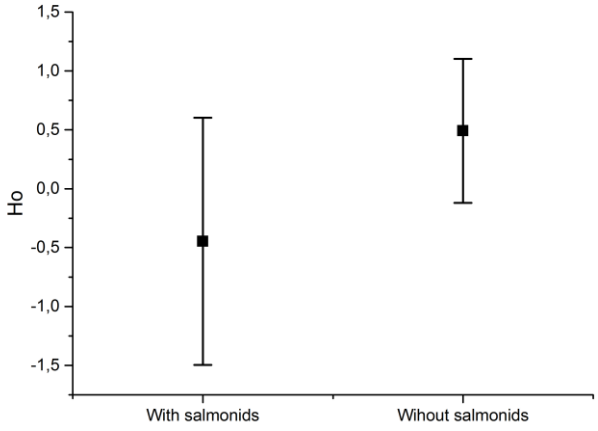
[1] Basulto 2003, [2] Soto et al. 2007, [3] Evelyn Habit pers.comm. n.i. no information available



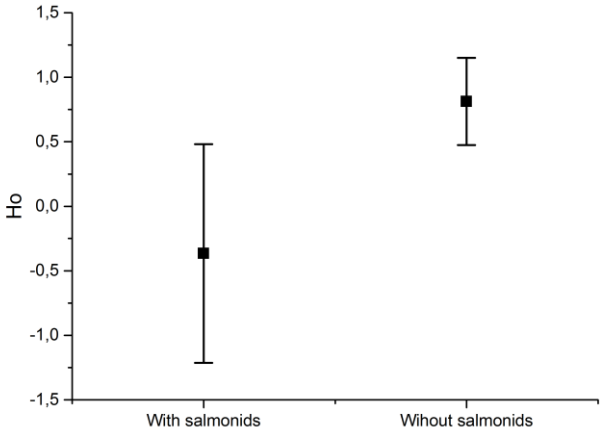
a) Na (16 populations), p = 0.049



b) Na (21 populations), p = 0.022



c) Ho (16 populations), p = 0.023



d) Ho (21 populations), p = 0.007

Figure S4.1. t-test analyses showing the mean number of alleles a-b) and observed heterozygosity (c-d) with standard deviation for *Galaxias platei* populations in lakes with and without salmonids including 16 connected lakes (a and c), and 21 connected and isolated lakes (b and d). t-tests analyses were made using the standardized residuals between the genetic variable (Na and Ho) and area with the presence and absence of salmonids.

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CHAPTER 5 THE ORDER OF THE POPULATIONS DOES ALTER THE GENETIC DIVERSITY

5.1 Abstract

Dams reduce connectivity in riverine environments and are thus considered one of the main threats for the survival of riverine fish species. They can interrupt gene flow among populations and thus change their genetic diversity. Such changes in genetic diversity can however, take several generations before they manifest themselves. While some studies have focused on the number of generations required before changes in genetic diversity can be detected following a change in connectivity, others have focused on the effect of population spatial arrangement on the maintenance of genetic diversity. To our knowledge however, no study has focused on the combined effect of both topics. Here we studied how gene flow reduction and population spatial arrangement combined with variation in effective size can affect genetic diversity differentially following a change in connectivity. We show that after a decline in upstream gene flow dendritic systems with headwater populations exhibiting relatively low N_es show a higher decline in genetic diversity, with increased inbreeding and more highly differentiated populations than when effective sizes in headwater populations are relatively high. Indeed, dendritic systems with headwaters with relatively high N_es show little changes in genetic diversity, inbreeding and population differentiation after a reduction in upstream gene flow. Population spatial arrangement is thus key to understanding the temporal consequences of habitat modification; dendritic systems with lower genetic diversity in headwaters should be of major concern for conservation policies.

5.2 Introduction

Changes in genetic diversity patterns following an event that reduces connectivity are likely to take several generations before they manifest themselves (Keyghobadi et al. 2005; Holzhauer et al. 2006). This time-lag for the detection of changes in diversity patterns is dependent on the magnitude of the changes in gene flow, on effective population size which influences genetic drift, and on the census population size (Landguth et al. 2010). Simulations have shown that such changes can take from 10 to 80 generations to be noticed depending on the population characteristics and the statistics used. For example, changes in the heterozygosities take longer to be detected than changes in number of alleles (Maruyama & Fuerst 1985; Allendorf 1986; Greenbaum et al. 2014). As a result, statistics based on heterozygosities as in the case for F_{ST} ($F_{ST} = [H_T\text{-}H_S]/H_T$), are affected by this time-lag more than statistics based on number of alleles (Maruyama & Fuerst 1985).

Researchers have studied the time-lag for the detection of changes in genetic diversity and gene flow (e.g. Epps & Keyghobadi 2015; Samarasin et al. 2017). However, less attention has been paid to the question of how the spatial arrangement of population sizes affects this time-lag. Spatial structure can strongly affect population dynamics and the distribution of genetic diversity (e.g., Fagan 2002; Morrissey & de Kerckhove 2010; Carrara et al. 2013; Salisbury et al. 2016). For instance, modeling approaches indicate that population complexes inhabiting simple dendritic systems exhibit higher genetic diversity than those living in linear systems (Morrissey & de Kerckhove 2010; Thomaz et al. 2016). Nevertheless, some studies have shown that these theoretical results might not always occur in nature (i.e. Salisbury et al. 2016).

Freshwater ecosystems are among the most transformed ecosystems by anthropogenic sources (Benejam et al. 2015). Several types of river systems occur in nature, including linear and dendritic systems. Freshwater species living in dendritic systems have been seriously affected by habitat modifications and the building of dams over the last century or two (Anderson et al. 2006; Vörösmarty et al. 2010; Lynch et al. 2011). Dams tend to isolate populations, by decreasing connectivity, and increasing the genetic differentiation between populations (Crispo et al. 2006; Pearse et al. 2009; Waters et al. 2015). Population survival following the construction of a dam depends, among other variables, on the maintenance of gene flow, the genetic diversity previous to the construction of the dams,

and the population spatial arrangement (Morrissey & de Kerckhove 2010; Vera-Escalona et al. 2018). Several studies have focused on the question of the time lag to detect changes in genetic patterns (Epps & Keyghobadi 2015; Samarasin et al. 2017).), while others have focused on the effect of the spatial arrangement of populations for the total metapopulation diversity (Labonne et al. 2008; Morrissey & de Kerckhove 2010; Carrara et al. 2013; Altermatt et al. 2018). No study has thus far focused on the importance of the interaction between population spatial arrangements in hierarchical systems with the time lag to detect these changes. In other words, no study has focused yet on how genetic parameters vary as a function of population spatial configuration and their relative effective sizes (N_es) when subject to a reduction in gene flow as occurs with the construction of dams (Benejam et al. 2016For instance Vera-Escalona et al. (2015) studying a Patagonian freshwater fish found that the construction of a small dams restricting upstream gene flow had no major effect yet in population structure nor in gene flow in a hierarchical system with moderate to large N_es in upstream populations compared to downstream populations. These kinds of studies suggest there will be a time lag before changes in genetic diversity and divergence following the interruption or reduction in gene flow are fully manifest and both the time lag and the magnitude of the changes may be a function of the effective sizes of headwater and downstream populations. To answer these questions we simulated 12 gene flow and population size models with different population size arrangements and gene flow differences (Fig. 5.1) in order to understand how different genetic parameters change through time. Our hypothesis was that models with lower N_es in headwater populations and nil upstream gene flow should be more prone to increases in population differentiation and the loss of genetic diversity than systems with relatively large effective sizes in headwater populations. Thus, we expect to observe the importance of the combined effects of population spatial arrangements and gene flow to predict changes in the genetic diversity and population differentiation.

5.3 Methods

5.3.1 Forward-in-time simulations

Twelve dendritic models that differed in the spatial arrangement of population size and gene flow were generated to assess changes in genetic parameters that can result from the decline in connectivity following the construction of dams in a freshwater system. Models were generated with the software package NEMO 2.3.46 (Guillaume & Rougemont 2006) and assumed different effective population sizes (N_es) that remained constant through time and asymmetric gene flow. Each metapopulation included seven populations arranged dendritically: 4 headwaters, 2 central nodes and 1 terminal node (Fig. 5.1). We considered three spatial arrangements for effective population size: (1) N_e downstream-increase (N_es increase from headwaters to terminal populations), N_e downstream-decrease (N_es decrease from headwaters to terminal populations), and N_e 250 constant (all populations remain with a constant N_e = 250). Gene flow was initially symmetric, and two values were considered m=0.1 and m=0.05 (Models 1-6 and 7-12, respectively; Fig. 5.1), until parameter F_{ST} stabilized. Population parameters became stable (when they stopped having significant changes) at generation 30 for all models. At generation 0, when F_{ST} had reached stability a change in gene flow was introduced for 20 generations (Fig. 5.1), where upstream gene flow declined from 0.1 to 0.01 (Models 1-3), from 0.1 to 0 (Models 4-6), from 0.05 to .01 (Models 7-9), and from 0.05 to 0 (Models 10-12). The models thus reflect the range of gene flow asymmetry observed in freshwater systems exposed to barriers of anthropogenic origin like dams built for hydropower generation.

Simulations were run assuming ideal populations, i.e., discrete generations, random mating, equal sex ratios, and stable carrying capacities. We assumed a fecundity in the range of values observed in freshwater fish (fecundity = 10000) (Zama 1986). Individuals were assumed to have been genotyped at 40 microsatellite markers, each with 12 alleles and an average mutation rate of $5x10^{-5}$ (Wan et al. 2004; Shaikhaev & Zhivotovsky 2014). Each model was run with 5 replicates. These forward-in-time computations were conducted on the Supercomputer Mammoth-MP2 from Université de Sherbrooke.

5.3.2 Descriptive statistics, fixation methods and allelic differentiation methods

For each generation and model we estimated number of alleles (N_a), observed heterozygosity (H_o), and inbreeding coefficient (F_{IS}) with the software package NEMO 2.3.46 (Guillaume & Rougemont 2006). Each variable was then plotted as the mean of the five replicates through time. Jost *et al*. (2018) classified genetic methods for estimating population differentiation in two families, fixation methods and allelic differentiation methods. Based on this, we used the two most widely used fixation methods, F_{ST} and G_{ST}, and one allelic differentiation method, Jost's D. Estimates of F_{ST} were obtained with NEMO 2.3.46 (Guillaume & Rougemont 2006), while estimates of G'_{ST} and Jost's D were obtained with GENALEX with 999 permutations and 999 bootstraps.

In order to understand the importance of the N_es of population at different hierarchical positions (i.e. headwaters, nodal populations and terminal populations) we used the statistic *assign.per.pop* obtained from a Discriminant Analysis of Principal Components (DAPC). DAPC is a non-model-based method that looks for linear combinations of alleles that show differences between groups as best as possible while minimizing variation within clusters without relying on the presence of linkage disequilibrium or on the assumption of Hardy Weinberg Equilibrium. The analyses were run in ADEGENET server (Jombart 2008) to obtain the statistic *assign.per.pop,* the proportion of successful reassignment of individuals to their original clusters. This parameter can be used to assess the power of the package for the detection of admixture at hierarchical level (*assign.per.pop*) by analyzing average *assign.per.pop* in the headwaters (level 1), first nodal populations (level 2) and terminal nodal populations (level 3). Large values (i.e. 1) indicate well defined genetic clusters with low admixture, while small values (i.e. 0) indicate a high admixture.

5.4 Results

All models exhibited a similar value of N_a at generation 0, the generation before the change in gene flow. For parameters H_o and F_{IS} all models showed small differences in their values at time 0 (before the change in gene flow), explained by the combined effect of differences in the N_es during the initial conditions as well as in the magnitude of the symmetric gene flow (Fig. 5.2). In general, models showed a 1-15% decrease in N_a, and 1-3% decrease in

H_o at generation 20. Nevertheless models 1, 4, 7, and 10 (all N_e downstream-increase, i.e. small N_e in headwaters) showed the highest decrease in N_a and H_o (Fig. 5.2a, b, d, and e). All other model were mostly indistinct for these three descriptive statistics. Among the four N_e downstream-increase models, models 4 and 10, those with upstream gene flow = 0 among those with small N_e in headwaters, showed the highest loss of alleles. The decrease in N_a was less noticeable in models with larger N_e in the headwaters, compared to those with smaller N_e in the headwaters where 10-15% of the alleles were lost after 20 generations (Fig. 5.2a). A similar trend was observed for H_o, with a lower decrease in the parameter through time. F_{IS} presented negative values (a signal of inbreeding) in all N_e downstream-increase models (Fig. 5.2c, f), reaching down up to -0.02-0.03 and overall values near 0 for 8 of 12 models (all N_e downstream-decrease and N_e 250 constant models). Population differentiation was more pronounced in models 1, 4, 7, and 10 (N_e downstream-increase) than in any other model as assessed by all fixation methods. At generation 10, only N_e downstream-increase models showed a significant increase in the F_{ST} with most of other models showing little noticeable increase in the F_{ST} despite the imposed reduction in the gene flow (Fig. 5.3a, 5.3d). After 20 generations, only two models, models 4 and 10 (N_e downstream-increase and gene flow = 0.01 (downstream) and 0 (upstream)) exhibited $\hat{F}_{ST} > 0.05$ a signal of moderate population differentiation, while the other two N_e downstream-increase models exhibited $0.03 < \hat{F}_{ST} < 0.04$. The G_{ST} statistics exhibited similar patterns, where Figures 3b, and 3e reveal the importance of gene flow conditions previous to the reduction in gene flow. Thus, N_e downstream-increase models with lower gene flow before the reduction in the gene flow (gene flow = 0.05) presented higher G_{ST} values with a maximum of 0.04-0.05 after 20 generations, while in N_e downstream-increase models with higher gene flow before the reduction in the gene flow (gene flow = 0.1) presented G_{ST} values ranging between 0.03-0.04 after 20 generations. The same pattern is observed with Jost's D. Jost's D ranged from 0.05 to 0.40, with an average of 0.15 for 8 models at generation 20 (Fig. 5.3c, 5.3f). The only models escaping from this tendency were N_e downstream-increase models, coinciding with the observed patterns in the two statistics from the fixation methods.

The parameter *assign.per.pop*, the proportion of successful reassignment of individuals to their original clusters, ranged from 0.6-0.9 among level 1 populations (headwaters), with

N_e downstream-decrease models, those with higher N_e (N_e = 500) showing the highest admixture, and N_e downstream-increase models, those with the lowest N_e (N_e = 100) presenting the lowest admixture (Fig. 5.4a, 5.4d). The *assign.per.pop* values were lower among level 2 populations, ranging from 0.4-0.6 with the higher values found among models 4, 6, 10, and 12, those models with N_e downstream-increase and N_e constant 250 with upstream gene flow = 0 (Fig. 5.4b, 5.4e). The highest admixture (i.e. lower *assign.per.pop)* values were found among populations from models 2, 5, 8, and 11, those with N_e downstream-decrease. All populations at level 2 had the same N_es (250), revealing that the admixture levels were a result of gene flow and N_e arrangement scheme. At level 3 it is observed the opposite from level 1 (Fig. 5.4c, 5.4f). Here N_e downstream-decrease populations, with the lowest N_e (N_e = 100) showed the highest admixture, and those with a N_e downstream-increase scheme, with the highest N_e (N_e = 500) showed the lowest admixture.

5.5 Discussion

We studied how the genetic diversity of a dendritic metapopulation system was affected by changes in asymmetric gene flow combined with differences in the hierarchical arrangement of effective population sizes: We examined the effects of four changes in upstream gene flow combined with three effective population size arrangements on the detection of changes in genetic diversity and population differentiation with a focus on time-lag to detection. Our goal was to predict the consequence of the construction of dams in dendritic river basins and understand how long does it take to observe any genetic change in populations with different arrangements and why some studies do not reflect any important change in the genetic diversity after the construction of dams. We show that genetic diversity declines at a faster rate in dendritic systems with headwater populations exhibiting relatively low N_e (N_e downstream-increase), and that population arrangement is key to understand the temporal consequences of habitat modification. Consequently, dendritic systems with a N_e downstream-increase model where N_e is relatively high in downstream populations present an increase in the inbreeding creating more differentiated populations.

5.5.1 Main results

The study of the loss of genetic diversity (number of alleles and observed heterozygosity) showed that models with higher N_e in headwater lose less the genetic diversity, especially in number of alleles, than models with lower N_e in the headwaters. In these latter models, the decrease in gene flow has serious consequences, with the loss of 10-15% of the alleles and 2-3% of H_o after 20 generations, with a constant increase in the inbreeding through time. The opposite occurs with N_e downstream-decrease, where only a 1% of the alleles and 0.5-1.0% of H_o were lost after 20 generations. This effect in the genetic diversity is also reflected in statistics used to identify population differentiation (F_{ST}, G_{ST}, and Jost's D). Consequently hierarchical metapopulation systems that differ in the position of the populations with the highest diversities will differ in their response to changes in gene flow. Hierarchical analyses reveal that admixture levels depend mostly on individual N_es in headwaters and terminal positions (level 1 and 3 respectively). All this reveals that the time lag to detect changes in the genetic diversity and differentiation of populations is not only related to gene flow and effective population sizes, but also to population arrangement in hierarchical systems. Therefore, the order of the population arrangement affects the detection of changes in genetic diversity and differentiation. These results have important consequences in terms of the interpretation of what is so called "contemporary populations". For instance, freshwater systems have been highly affected by the construction of dams disrupting intradrainage connectivity during the last century (Bednarek 2001; Anderson et al. 2006; Oliveira et al. 2018; Stevens et al. 2018). Species differing in generation time and effective size will also differ in the time-lag before genetic changes can be observed. For instance, most dams have a life expectancy of 50-100 years. If changes in the genetic diversity can be observed only after at least 10 generations then for a species with a generation time = 1 year, these changes will be observed 10 years after the dam construction but for a species with a generation time of 4 years and similar effective size it will take 40 years before changes become evident. Thus, only by the end of the life of a dam we might be able to detect a considerable effect over the F_{ST}, G_{ST} and Jost's D statistics for species with a generation time = 4 years living in dendritic river basin with higher N_e in the headwaters (N_e downstream-decrease models). This might be too late

for the implementation of timely and robust conservation measures, revealing the importance of the temporal dimension in modeling and empirical studies of the consequences of habitat modification on diversity and its conservation.

These results reveal that methods based on these statistics may not be reflecting the disruption in the gene flow caused by human activity during part of the last century in population differentiation as measured by F_{ST}, G_{ST} and Jost's D statistics and different genetic diversity statistics. If this is true, consequences of human activity are likely to be underestimated in studies analyzing species with similar or longer generation times to the ones used in our example. It is also possible that significant changes in diversity and divergence may not be taking place or may be lower in magnitude despite the reduction in gene flow simply because the headwater populations are the largest and most diverse in the system. Therefore, in the absence of more studies analyzing the effect of population arrangements in the genetic parameters of populations after the construction of dams, we should be cautious about the overinterpretation of these results for populations with larger N_es in headwaters. Nevertheless, a few studies show that the main observations of these simulations occur empirically and theoretically. For instance the effects of dams in northern Patagonia populations with large N_e in headwaters compared to nodal and terminal populations are not visible despite the fact that dams were built 40-50 years ago (approximately 20 generations, Vera-Escalona et al. 2015). The opposite is observed with forward-in-time simulations, which reveal that lake populations with relatively small N_es in headwaters will exhibit changes in diversity and divergence 40-50 years (approximately 20 generations; Vera-Escalona et al. 2018) after the construction of dams.

5.5.2 Model choices and caveats

Initial conditions influence the conditions at generation 0, and therefore not all populations begin with the same value for all genetic parameters, except for N_a. Nevertheless these differences do not appear to affect the changes that occur after the change in gene flow. An important caveat with our simulations is that effective sizes remained constant through the entire simulations despite the changes in connectivity. While this may not be fully realistic, keeping N_e constant reduced the variables for consideration facilitating the focus on the effect of changes in the gene flow and population arrangements. If 20 generations were

enough to change N_es we could expect to see these changes first in headwater, where the nil upstream gene flow would mean no new individuals introducing new alleles. This assumption also considers that in 20 generations the effect of mutation are insignificant. Under these circumstances, we could expect to observe a higher fixation of alleles in headwaters due to a higher effect of genetic drift, and thus a higher loss of global genetic diversity and higher population differentiation. Another caveat to our results lies in the particular N_es chosen for simulations. Value were considered to represent the lower N_e for viable populations (N_e = 100) and limit inbreeding depression, up to moderate N_e for populations to reduce significantly the effect of drift (N_e = 500; Frankham et al. 2014). Because we were considering two extremes, scenarios with equal N_es considered N_e=250, thus it should be a representation of an average N_e. We should also point out that we assumed symmetric gene flow before the change in the connectivity. In nature, some populations present both symmetric and asymmetric gene flow (Schaefer et al. 2001; Fraser et al. 2004; Vuilleumier and Possingham 2006). Symmetric gene flow is more common than asymmetric gene flow, which is more related to species living in areas with significant differences in elevation (Toju 2008). By considering symmetry in our models we reflect the most common patterns found in nature and we also simulate idealized populations, which then allows us to then focus on changes in the two variables of interest: changes in gene flow and population spatial arrangement. Another caveat with our simulations is that dams were assumed to reduce only upstream gene flow. In reality, however, downstream gene flow is also likely to be affected by dams. Nevertheless because downstream connectivity is mainly related to the probability of fish survival in dams and the presence of channels and pipelines (Rincón et al. 2017) and these conditions usually exist, we assumed that downstream connectivity should be kept in all models.

The study of the effects of population spatial arrangement on genetic diversity after the construction of dams is relatively new and our simulations help solve some of the questions. There are still however, plenty of questions that remain open. For instance, lake systems with lower N_es in headwaters seem to be more sensitive to the construction of dams, and they should be a priority for conservation programs and continuous monitoring in areas where dams have been built during the last decades. On the other hand, lakes with headwater with higher N_es should also be studied to understand whether they are less

affected by the construction of dams reducing upstream connectivity and to what extant these results can be projected to populations with lower N_es.

5.6 Figures

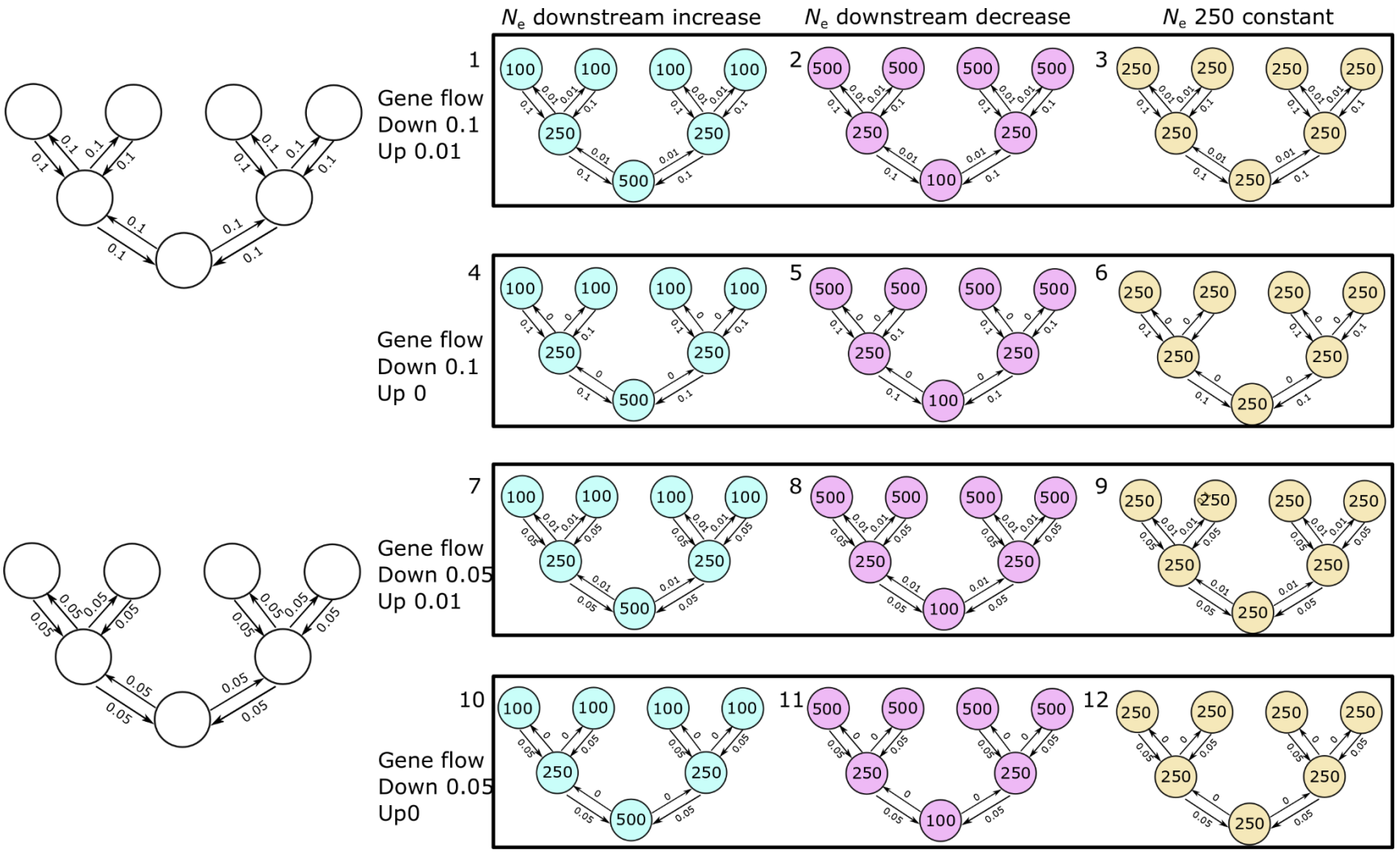
N_e downstream increase
N_e downstream decrease
N_e 250 constant
1
2
3
4
5
6
7
8
9
10
11
12
Gene flow
Down 0.1
Up 0.01
Gene flow
Down 0.1
Up 0
Gene flow
Down 0.05
Up 0.01
Gene flow
Down 0.05
Up0

Figure 5.1. Twelve models of idealized dendritic freshwater fish populations simulating the construction of dams reducing the upstream gene flow (from the initial gene flow conditions showed at the left of every model) producing asymmetric gene flow 0.1 and 0.01 (Models 1-3; Fig. 5.1.1-3), asymmetric gene flow 0.1 and 0 (Models 4-6; Fig. 5.1.4-6), asymmetric gene flow 0.05 and 0.01 (Models 7-9; Fig. 5.1.7-9), and asymmetric gene flow 0.05 and 0 (Models 10-12; Fig. 5.1.10-l2). Models 1, 4, 7, and 10 (Fig. 5.1.1, 1.4, 1.7, and 1.10 respectively) show and N_e downstream-increase scheme (N_e increases from the headwaters to the terminal node), Models 2, 5, 8, and 11 (Fig. 5.1.2, 5.1.5, 5.1.8, and 5.1.11 respectively) show and N_e downstream-decrease scheme (N_e decreases from the headwaters to the terminal node), and Models 3, 6, 9, and 12 (Fig. 5.1.3, 5.1.6, 5.1.9, and 5.1.12 respectively) show and N_e 250 constant scheme (all populations have the same $N_e = 250$),

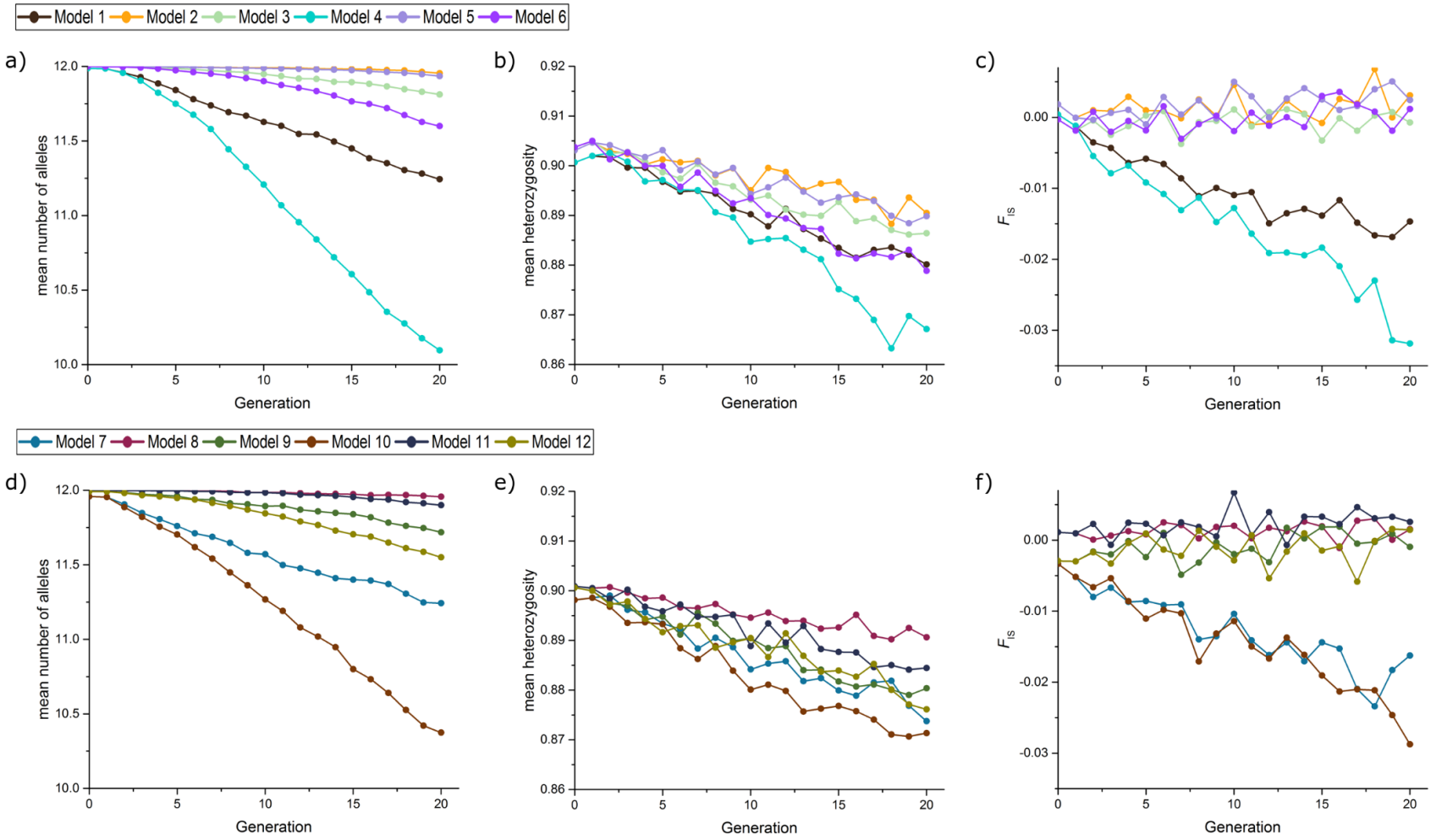

Figure 5.2. Changes in the allele diversity (N_a; Fig. 5.2a, 2d), observed heterozygosity, (H_o; Fig. 5.2b, 2e) and inbreeding coefficient (F_{IS}; Fig. 5.2c, 2f) after the construction of dams, assuming symmetric 0.1 and 0.05 (Figures 2a-c, and 2d-f respectively) at generation 0 and reduction in the upstream gene flow from generation 1-20. N_e downstream-increase (models with higher N_e in headwater lakes) showed the higher decrease in the number of alleles and increase of the inbreeding (negative values).

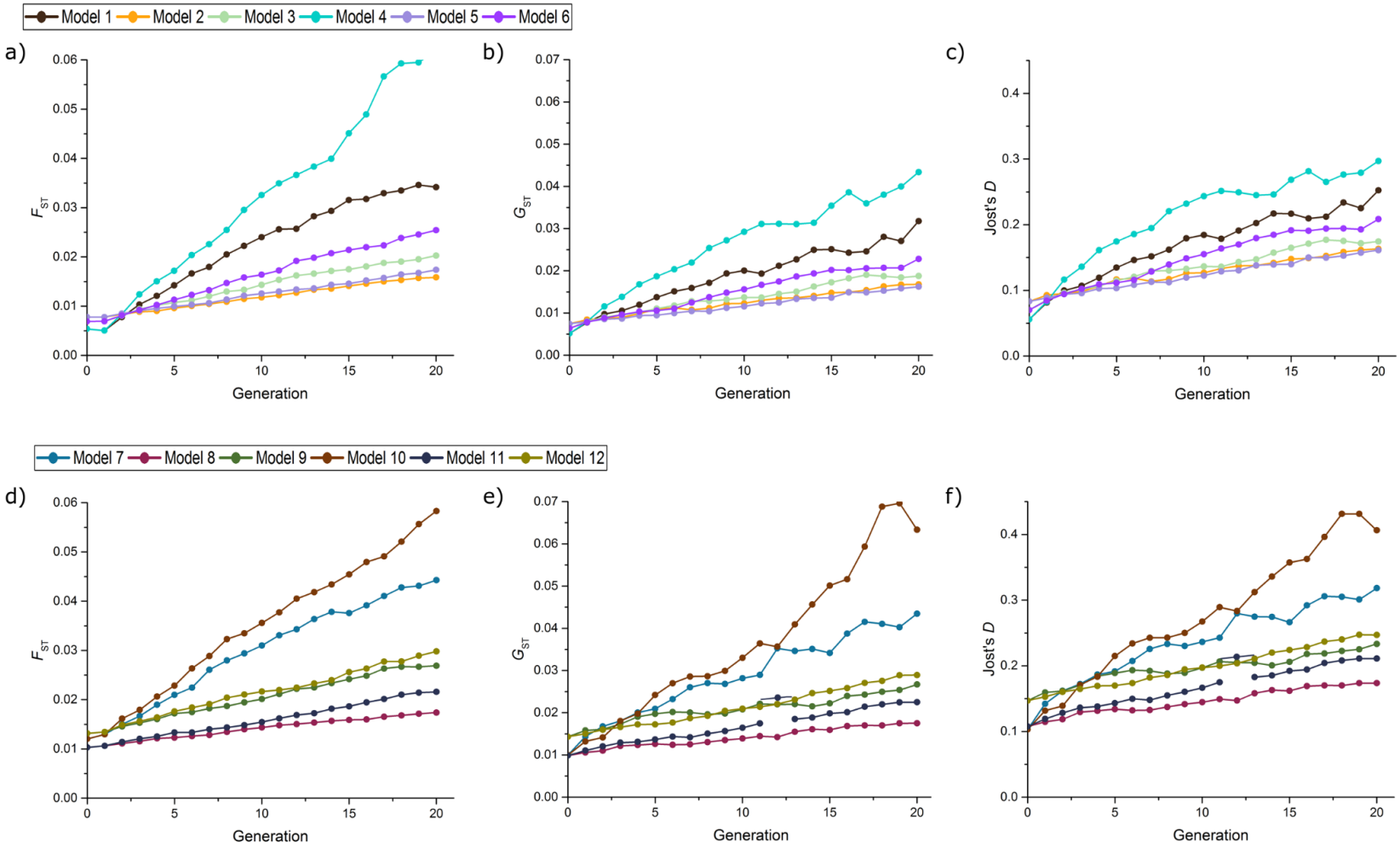

Figure 5.3. F_{ST} (Fig. 5.3a, 3d), G_{ST} (Fig. 5.3b, 3e), and Jost's D (Fig. 5.3c, f) for 12 dendritic metapopulation models simulated with asymmetric gene flow. Models correspond to those described in Figure 1. Generation 0 = the last generation before the change in the migration scheme. N_e downstream-increase (models with higher N_e in headwater lakes) showed the higher increase in the F_{ST}, G_{ST}, and Jost's D, especially in models with gene flow = 0.05 before the reduction of the upstream gene flow.

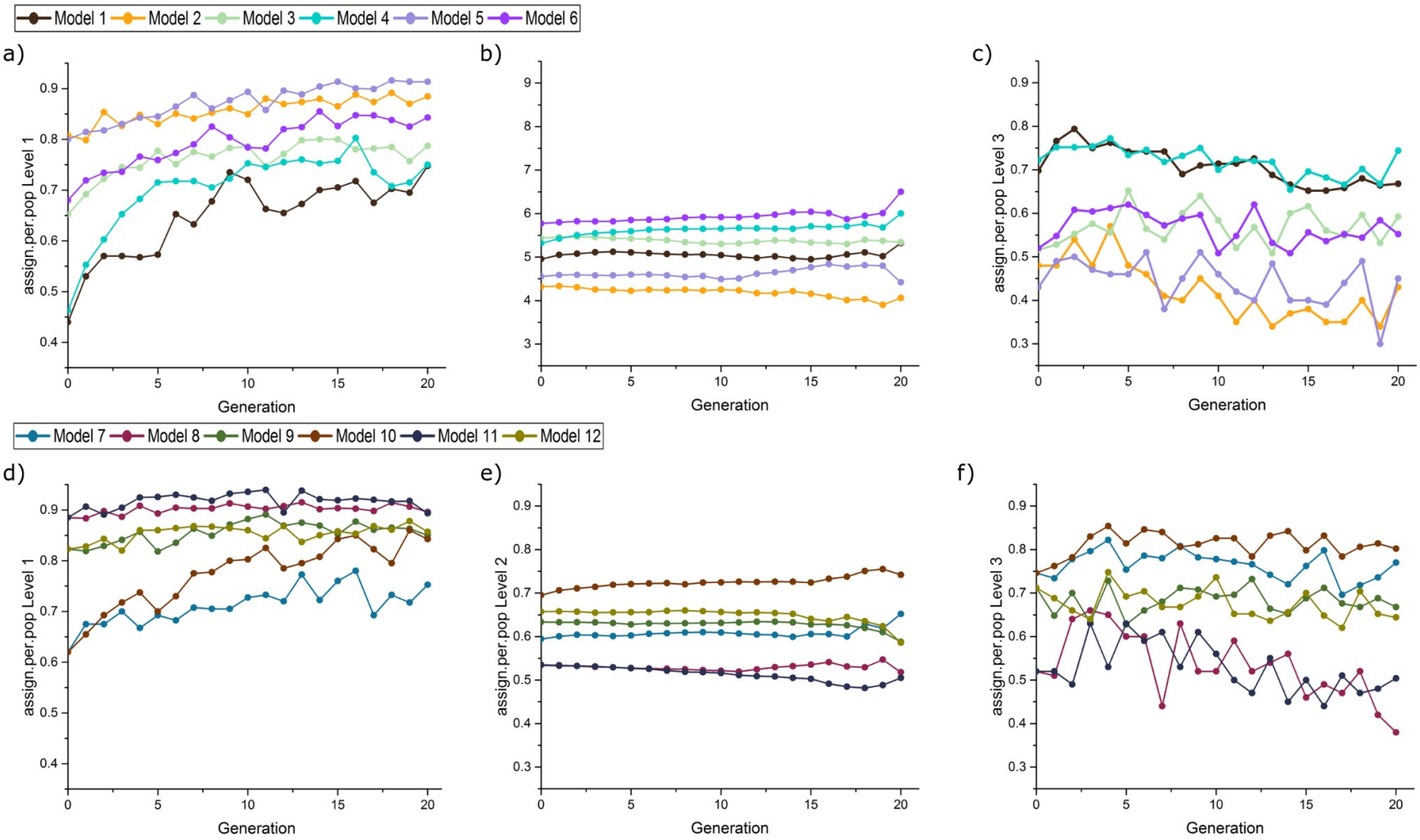
Model 1
Model 2
Model 3
Model 4
Model 5
Model 6
a)
b)
c)
assign.per.pop Level 1
assign.per.pop Level 3
Generation
Model 7
Model 8
Model 9
Model 10
Model 11
Model 12
d)
e)
f)
assign.per.pop Level 1
assign.per.pop Level 2
assign.per.pop Level 3
Generation

Figure 5.4. *assign.per.pop* the proportion of successful reassignment of individuals to their original population at level 1 (headwaters, Fig. 5.4a, 5.4d), at level 2 (Fig. 5.4b, 5.4e), and at level 3 (Fig. 5.4c, 5.4f) for 12 hierarchical dendritic models based on Figure 1. Figure 5 shows that the admixture is lower in populations at level 1(headwaters) and level 3 (terminal populations), and higher in populations at level 2 (nodal populations), mostly explained by the effect of local effective population sizes at level 1 and 3, and by population arrangement at level 2.

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Chapter 6 Discussion

6.1 A brief summary

In this thesis I tried to elucidate the effect of historical processes on contemporary populations and used a multitemporal perspective as a predictive tool for conservation of future populations. According to Lyell (1830) "the present is the key to the past", a phrase that tried to summarize how current contemporary geological processes could be used to understand past geological processes. Landscape changes through time affect species distribution and abundance, leaving traces in their DNA that can now be recovered to explain what happened to past populations. Our theoretical and empirical understanding of extant population dynamics allow us to comprehend how species might have responded to changes in landscape, providing us with information that can be used as feedback for the study of contemporary and future populations. Therefore, while the present is still key to understand past populations, reconstructions of the past becomes key to understand present as well as future populations.

During the last decades we have been able to focus on one of the key aspects of the theory of evolution, that is the variable "time", or the temporal dimension. We observe "time" mostly as a continuum axis where all other evolutionary variables enter into play. If we consider the various processes that affect evolution and act through time and partition them into processes that act over different time scales, we can analyze these processes using genetic markers. In this thesis I fragmented processes that take place over different time scales in- four different partitions that I studied separately: long-term historical processes (*i.e.*, Quaternary glaciations), short-term historical processes (i.e., introduction of dams during the last century), contemporary processes (*i.e.*, processes observed over the last few years or generations to the present), and future processes (*i.e.,* planned construction of dams). I used the freshwater fish *Galaxias platei* as a case study, a fish inhabiting Patagonian Andean lakes and rivers. *Galaxias platei* has survived the Quaternary glaciations *in situ* due to adaptations to survive under low temperatures and high turbidity (Milano et al. 2002). This species lives in connected and isolated lakes, with low to high

degrees of intervention in environments without and with the massive presence of salmonids that were introduced during the last century (Habit et al. 2010, 2012). The biological information available for the species and geological data available for the region makes it an ideal candidate for exploring the question of how landscape modifications during the past, present and future have in the past, and will in the future likely affect *G. platei* populations.

6.2 Main conclusions

In Chapter 2, I contrasted two *G. platei* populations from two river basins, one from northern Patagonia (San Pedro) and the other from southern Patagonia (Serrano) to understand how population fragmentation and what changes in connectivity took due to long-term processes. Populations from the northern Patagonia river basin appear to have originated from two different refugial population located most likely on the eastern side of the Andes and the Pacific Coast, from where the colonization of extant lakes took place during the late Holocene. These populations changed from highly symmetrically connected populations to populations connected by asymmetrical gene flow. On the other hand, in the Southern Patagonian river basin (Serrano River) populations survived the Quaternary glaciations *in situ* in a great lake surrounded by the glaciers that gave rise to new lakes after the melting of the ice in eroded areas where glaciers used to exist. In these populations, connectivity was highly asymmetrical during the past and extremely low during the present. These results revealed the importance of analyzing river basins separately to recreate local historical patterns and overall revealed the importance of analyzing studies at different temporal scales. Additionally, in the northern Patagonian populations I found that the effect of dams built during the 60s had no great influence on contemporary patterns. Next, in Chapter 3, I studied another *G. platei* population, this time in Central Patagonia, where plans exist to construct dams along the river basin. Therefore, the focus of this chapter was to study the historical, contemporary and future processes affecting *G. platei* populations. Historical reconstructions of populations in this river basin showed that the formation of extant populations occurred very recently and from a single refugial population with low genetic diversity. Due to the low genetic diversity in this system, gene flow was critical for the survival of the populations. The study of future populations under

scenarios of changes in population size and reduction in connectivity restated the importance of gene flow for the survival of populations. Thus the construction of dams completely eliminating the exchange of individuals among populations would mean local extinctions while the construction of dams along with mitigation measures such as the construction of fishways allowing a 1% of exchange of individuals will produce similar genetic patterns to those obtained under the scenario without landscape modifications. These last two chapters brought my attention to the effect of short-term historical processes that could assist in explaining some patterns of diversity in the native fauna of Patagonia. Thus, in Chapter 4 I focused on short-term processes that could explain differences in genetic diversity observed among *G. platei* populations. The focus of Chapter 4 was on the effect of the introduction of salmonids on the genetic diversity of *G. platei* populations. Genetic diversity in *G. platei* populations in the presence of salmonids is relatively low. The difference in genetic diversity between *G. platei* populations existing without and with salmonids was similar to that observed among unaffected and overexploited commercial marine fish species (Pinsky & Palumbi 2014). Such comparisons likely reveal the impact of invasive on native species. Finally, in Chapter 5 I tried to answer an unresolved question from Chapter 1 and 2, namely whether the power to detect changes in genetic diversity after a drastic reduction in connectivity among populations (due e.g., to the construction of dams) is affected by the spatial arrangement of the populations and their asymmetric gene flow? With this in mind I simulated 12 scenarios trying to recreate conditions in nature. I found that changes in the genetic diversity after the reduction of gene flow were more likely to be detected in dendritic populations with relatively low effective population sizes in headwater environments. Changes in genetic diversity and divergence are less likely to be detected when the headwater populations exhibit relatively high effective population sizes compared to nodal or terminal populations.

Thus, by studying real and simulated populations over at different time scales I showed the importance of multitemporal studies and simulations for a better understanding of extant populations, and overall to the study of possible scenarios for species whose habitat will be intervened.

6.3 Implications of the results

By studying historical patterns we can distinguish the effect of processes acting over different time scales and use this information to predict how species are likely to respond to future changes in the landscape. In practical terms, we can use different molecular ecology tools to produce more robust conservation policies. By using this information we can improve predictive models under scenarios of habitat intervention, increasing the robustness of conservation policies. More specifically, some of the information obtained in this thesis could be used for future evaluations of habitat modification, as well as a guiding methodology for evaluation of construction of dams and design of fishways. The method used in Chapter 3 allows to evaluate what percentage of individuals of a population is necessary to obtain similar genetic patterns to the ones of populations without habitat modifications. With this information we can design fishways able to assure a minimum of individuals allowing to recreate natural conditions. The main conclusions of Chapter 5 could complement the use of the methodology from Chapter 3 to help with monitoring species after habitat intervention since the results reveal that we can expect to observe different amounts of change in genetic statistics after a variable number of generations depending on the population arrangements.

Results from Chapter 4 should be useful for the design of new conservation policies, especially in Patagonia, where salmonids are known to have a negative effect on native fauna (Arismendi et al. 2009; Habit et al. 2010, 2012). In the absence of an approximate number of individuals from sampled lakes I found that the population reduction of *G. platei* was close to a 99% in lakes with salmonids.

6.4 Study limitations

The research described in this thesis has some limitations that need to be emphasized. For instance, the analyses at different temporal scales were conducted separately, as is the case with the effect of Quaternary glaciations, population expansion during the Holocene and recent anthropogenic modifications. Nevertheless, the various processes help explain the genetic composition of contemporary populations to a varying degree. Here I showed that in general, long-term historical processes help explain contemporary genetic patterns of *G.*

platei populations in Patagonia. I also showed that in lakes with introduced salmonids genetic diversity is relatively low suggesting a negative effect of salmonids on the genetic diversity of our focal species, *G.platei.* The effect of salmonids on the genetic composition of native species is a process that has been identified for by the first time here for Patagonia. I should also mention that I did not include competition with other native species in this analysis (Chapter 4). It is very unusual to observe a negative interaction among native species from Patagonia, therefore I discarded the effect of other native species and focused on the effect of invasive species.

Other major changes in the landscape that were not considered here were the influence of volcanic eruptions and earthquakes. Eight major earthquakes (magnitude 8-9.5) have taken place in Patagonia during the last 500 years, changing the course of the rivers in North and South Patagonia. There are 36 active volcanoes in Chile, half of them are located in Patagonia and have erupted at least once during the last century. Patagonian lakes (e.g. those from the Valdivia River Basin) are located near active volcanoes which might have affected conditions of lakes such as turbidity and temperature during the past. Nevertheless, its effects on freshwater fish has not been evaluated here due to the lack of information about what lakes were more affected, at what magnitude and for how long.

6.5 Recommendations and future research

Future researchers should investigate possible bias on the use of the approaches used in this thesis, especially to reconstruct the past history of populations and the use of simulations to predict what would happen with future populations as consequence of habitat modifications. More studies are needed on the reliability of backward in time and forward in time analyses. Although several researchers have compared different software and approaches (Carvajal-Rodríguez 2010; Hoban et al. 2012) there is a need for the identification of possible problems with these software packages and methods. The methodologies used in these thesis, using multitemporal analyses, could be very relevant for the study of changes in the landscape as consequence of climate change. Especially by including backwards in time, contemporary and forward in time analyses. Thus we could include more genetic-based methodologies in conservation programs and evaluate the potential threats of future populations.

In a more philosophical perspective, there are two concepts of time that can be observed in this thesis and should be worked more properly in future studies. Ancient Greeks had two concepts for time: *chronos* and *kairos* (Smith 1969). *Chronos*, a quantitative character, can be defined as a measurable time and it is more related to questions of " how old" something is, as occurred with the estimations of divergence time of population and changes in the gene flow related to Chapters 2 and part of Chapter 3. The concept *kairos* it is a more abstract concept, a qualitative character. *Kairos* can be defined as a significant position in time an event occurs and is more related to simulated scenarios from Chapters 3, 4, and 5 where the central questions where related to "when" an event occurred and "at what time" will occur and what is the significance of that event. Ancient Greeks also had two terms for the concept of space: *choros* and *topos*, the first referring to the abstract space and the second to the concrete space. Time and space play a major role along this thesis and in the conservation of populations itself. Here I tried to respond to questions regarding the *chronotopos*, the time when some events took place in concrete places but also questions regarding the *kairochora*, the meaningful time in an abstract place in order to express its significance in the *kairotopos* or when significant events will take place in the real world (Rämö 1999). Most of conservation analyses consider one or two of the three concepts of space and time, usually disconnecting the *kairotopos*, the materialization of the ideas we obtain from the experience and our abstract thinking to predict and put in practice what we know. All the efforts in these thesis were focused in the understanding of the evolutionary variable time from the real world and the abstract world (simulations) to provide better tools for the conservation of future populations. Hopefully the results from this thesis could be used for the conservation of the populations.

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APPENDICES

APPENDIX 1 THE COMPLETE MITOCHONDRIAL GENOME OF THE FRESHWATER FISH *Galaxias platei*

This Appendix has been published as "Vera-Escalona I, Habit E, Ruzzante D.E. The complete mitochondrial genome of the freshwater fish *Galaxias platei*" in Mithocondrial DNA 28(2)176-177

Introduction

Galaxias platei is a freshwater fish widely distributed in lakes and rivers of the Chilean-Argentinean Patagonia, Tierra del Fuego archipelago and the Falkland/Malvinas Islands (Dyer et al. 2000). As a member of the Galaxiid family, its relationship with other species has been historically reviewed using various approaches (e.g. morphology and genetics) resulting in different classifications (Burridge *et al*. 2012). It has been suggested that this galaxiid fish diverged from its sister group during the Eocene-Oligocene (38-16 Mya), most likely due to the loss of suitable ocean currents or Antarctic habitats during the Antarctic cooling (Barker & Thomas, 2004), not discarding the loss of diadromous life as an alternative hypothesis to the divergence of its sister species (Burrdige *et al*. 2012).

Several studies suggest that during the Quaternary glaciations *G. platei* populations might have survived in glacial refugial far from the ice-covered areas, as well in in lakes surrounded by ice during the glacial cycles of the Quaternary (i.e. Ruzzante et al. 2008; Vera-Escalona *et al*. 2015; Zemlak *et al*. 2008, 2011). Survival in situ has been explained by the tolerance of *G. platei* individuals to a wide range of oxygen and temperature, as well to its low metabolic rate which might have helped it to subsist even in lakes with a frozen surface (Milano *et al*. 2002). Its distribution in the Tierra del Fuego archipelago and Falkland/Malvinas Islands and its lack of capability to migrate through the sea suggest the connectivity of this island with the mainland during the periods of lowest sea-level during the glacial cycles, while the interruption of the connectivity and the loss of the diadromy might triggered the differentiation of populations in Tierra del Fuego and the Falkland/Malvinas Islands after the increase in the sea level occurring during the Holocene (Lambeck *et al*. 2014).

During the Holocene, *G. platei* populations have been exposed to an increase in the suitable habitats due to the appearance of lakes and rivers, which has been modeled by volcanic and seismic activity in the Andean regions and the alteration of its habitat due to anthropogenic activity. One the most important anthropogenic changes in its habitat occurred due to the introduction of salmonids which might increase the vulnerability of its conservation status. *Galaxias platei* is rare to find it when in sympatry with invasive salmonids, while is very abundant in areas with lower presence of salmonids (Habit *et al*. 2012). The genetic consequences of the interaction with salmonids remains unknown for this species. Because of the multiple historical processes affecting the population of *G. platei* and the possible consequence of recent processes triggered during the Anthropocene, the availability of new markers and a deeper knowledge on the genetic patterns of these species could help to understand more about how changes in the landscape have affected the genetic patterns of this species. Here we present the complete mitochondrial genome of *G. platei* and compare it with the ones available for three other species of the same genus.

Methods

Whole genome DNA was extracted from muscle tissue of a *G. platei* individual from Lake Dickson in Torres del Paine National Park using a glass milk protocol. DNA quality was tested in a 1% agarose gel, and the concentration was measured using a PicoGreen protocol in a Perkin Elmer Fusion DNA Quantifier (Perkin Elmer, Waltham, Massachusetts). Next, we used 1 ng of genomic DNA, following a standard protocol of the Illumina Nextera DNA Sample Preparation Kit (Illumina Inc., San Diego, California). DNA sequencing was conducted using MiSeq Benchtop Sequencer (Illumina Inc., San Diego, California). Short reads obtained from the sequencer were analyzed with the software CLC Genomics Workbench 7 (CLC bio, Aarhus, Denmark) in order to obtain longer sequences. Sequences were assembled in Genius Pro 7.0 (Biomatters Ltd.), and compared with those in Genbank using the Megablast option for highly similar sequences. Three sequences of the whole mitochondrial genome of species of the genus *Galaxias* showed a high similarity with a long sequence of *G. platei*, corresponding to two New Zealand species *G. golluminoides* and *G. sp. southern* and the widely distributed *G. maculatus*. The four sequences were aligned and used to contrast the gene composition of the sequences of *Galaxias*. The

mitochondrial genome of *G. platei* was uploaded to MitoFish (http://mitofish.aori.u-tokyo.ac.jp/) to detect missing parts of the genome and also to obtain a graphical representation of the mitochondrial genome and information about the position and length of the genes. No presence of missing fragments was detected. The mitochondrial genome was uploaded and made available in Genebank (Accession number KT456550).

Finally, we constructed a Bayesian phylogenetic tree in MrBayes 3.2.5 (Ronquist *et al*. 2003) with the four species of *Galaxias* using *Retropinna retropinna*, another species of the suborder Galaxoidei, as outgroup. This outgroup was the closest species with a whole mitochondrial genome available. We ran an analysis with $2x10^6$ generations, sampling every $5x10^2$, discarding the 25% of the first samples, and using a model selected with PhyML online (Guindon *et al*. 2004). The resulting phylogenetic tree was observed and edited in FigTree v1.4.2. (http://tree.bio.ed.ac.uk/software/figtree/).

Results and Discussion

From the sequencing we obtained a total of 1,346,239 short fragments with an average read length of 35 to 151 pb. A total fragment of 16502 bp was obtained, corresponding to the whole mitochondrial genome of *G. platei* (Fig. 1; Table 1), comprising a 23.7% of Adenine, 25,2% of Thymine, 20.3% of Guanine, and 30.8% of Cytosine. We found 13 coding-regions, 22 tRNAs, 2 rRNAs and the D-Loop. Most mitochondrial genes were encoded in the high strand, except for the nad6 and eight tRNAs (tRNA-Gln, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser, tRNA-Glu, tRNA-Pro). The length of the 12S and 18S genes was 949 and 1671 bp respectively and the D-loop was 861 bp. The total length was similar to the three Galaxias sequences compared, varying from 3-7 bp less than the other sequences. When comparing the four *Galaxias* sequences we found differences in the length of four coding-regions (COII, ND3, ND5, and ND6), four tRNAs (tRNA-Trp, tRNA-Cys, tRNA-Lys and tRNA-Gly), and the two rRNAs (12S rRNA and 18S rRNA).

The best model selected with PhyML was a GTR+I+G model with ti/tv = 0.429 and gamma = 0.740. We found a high support for the New Zealand species (0.92) as well for the G. platei + *G. golluminoides* and *G. sp. southern* and *G. maculatus* as an outer group.

The results of our phylogenetic tree, while not comparable to earlier studies using a higher number of species, differ in the position of *G. platei* and *G. maculatus*. Burrdige *et al*. (2012) reconstructed the phylogenetic relationship of galaxiid fish using mitochondrial and

nuclear sequences (1154 bp from the cytochrome-b, 599 bp from the 16S, 1695 bp from the RAG-1, and 1083 from the S7) and morphological characters (181 characters), while here we used the whole mitochondrial genome only (16502 bp). The results of Burridge *et al*. (2012) show that *G. maculatus* is closely related to the New Zealand species *G. gollumoides* and *G. sp. southern*, while our results suggest a different arrangement, where *G. platei* is closer to the New Zealand species, and *G. maculatus* is the most distant species of the four compared.

The difference in both results could be taken cautiously because using the whole mitochondrial genome bring more information from a single organelle, but for the same reason it also increases the risk of recovering of not representing the true relationship among taxa in the phylogenetic tree (Edwards & Bensch, 2009; Toews & Brelsford 2012). Nevertheless, it has been shown that the phylogenetic signal of whole mitochondrial genome is comparable to the one nuclear and mitochondrial genes concatenated and in some cases yielding to better resolved trees (Powell *et al*. 2013), being a trustable tool to resolve difficult nodes (Botero-Castro *et al*. 2013). The difference in our results with the ones from Burridge *et al*. (2012) suggest the need to deepen into the phylogenetic relationship of galaxiids using the whole mitochondrial genome, and contrasting the results with the ones from nuclear genes only, as well by a combination of both sources of information. These result contribute to the discussion of the whole mitochondrial genome for group of species under complex historical processes like those related to the Gondwanan divergence and the Pleistocene glaciations.

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Zemlak TS, Walde SJ, Habit EM, Ruzzante DE (2011) Climate-induced changes to the ancestral population size of two Patagonian galaxiids: the influence of glacial cycling. Mol Ecol 20, 5280-5294.

Table 1. Mitochondrial genome content and location of the Puye grande *Galaxias platei*. H = heavy strand, L = light strand

Name	Position		Strand	Length
	Start	Stop		
tRNA-Phe	1	68	H	68
12S rRNA	69	1017	H	949
tRNA-Val	1018	1089	H	72
18S rRNA	1090	2760	H	1671
tRNA-Leu	2761	2835	H	75
ND1	2836	3804	H	969
tRNA-Ile	3813	3884	H	72
tRNA-Gln	3884	3954	L	71
tRNA-Met	3954	4022	H	69
ND2	4023	5063	H	1041
tRNA-Trp	5073	5143	H	71
tRNA-Ala	5145	5213	L	69
tRNA-Asn	5215	5287	L	73
tRNA-Cys	5319	5383	L	65
tRNA-Tyr	5384	5454	L	71
COI	5456	6994	H	1539
tRNA-Ser	7007	7077	L	71
tRNA-Asp	7080	7152	H	73
COII	7166	7849	H	684
tRNA-Lys	7857	7930	H	74
APT8	7932	8096	H	165
ATP6	8075	8770	H	696
COIII	8773	9555	H	783
tRNA-Gly	9558	9629	H	72
ND3	9630	9977	H	348
tRNA-Arg	9979	10048	H	70
ND4L	10049	10342	H	294
ND4L	10339	11709	H	1371
tRNA-His	11720	11788	H	69
tRNA-Ser	11789	11856	H	68
tRNA-Leu	11858	11930	H	73
ND5	11931	13757	H	1827
ND6	13769	14290	L	522

tRNA-Glu	14288	14356	L	69
Cyt b	14360	15493	H	1134
tRNA-Thr	15501	15572	H	72
tRNA-Pro	15572	15641	L	70
D-loop	15641	16502	H	861

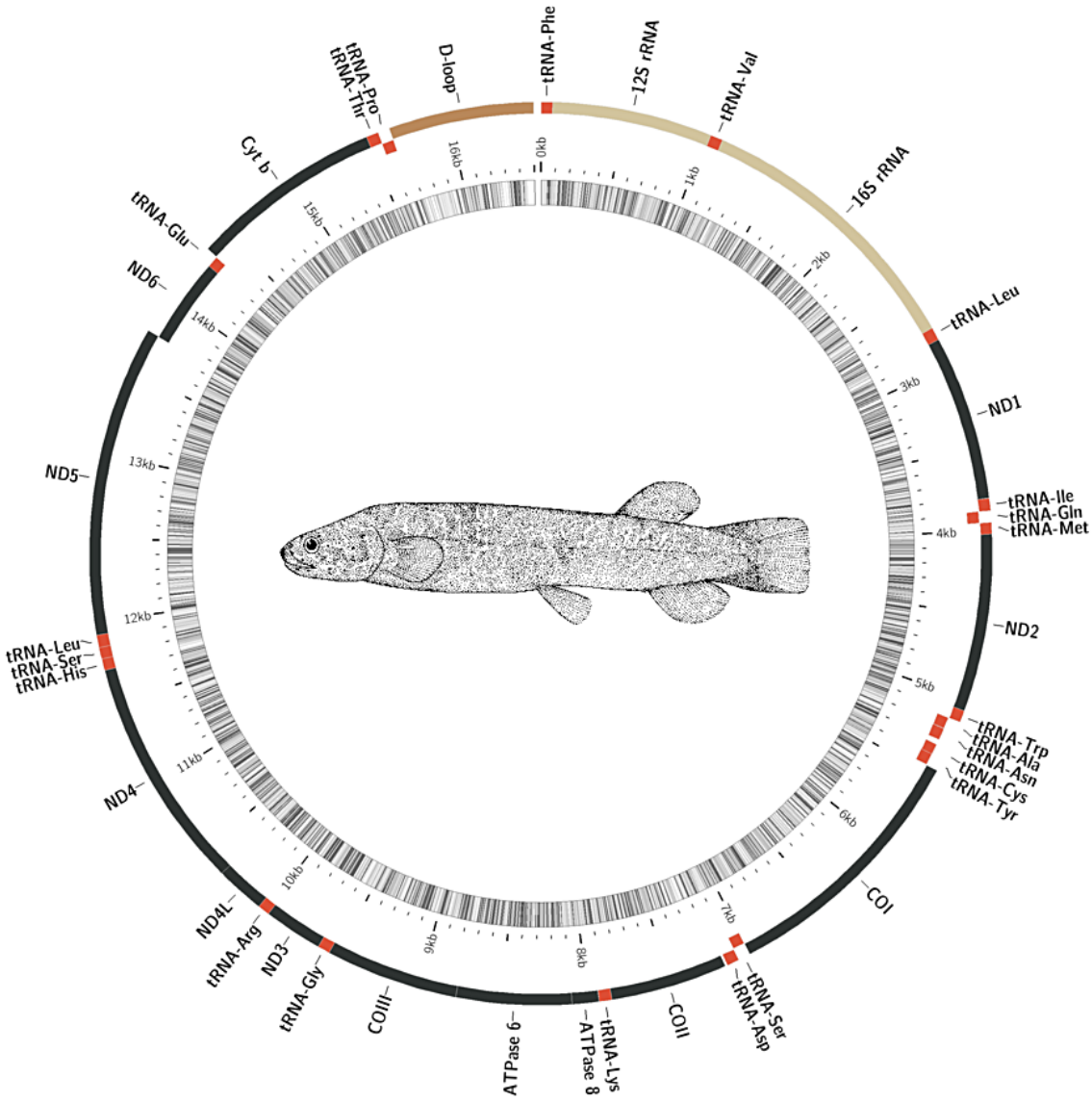

Figure 1. Representation of the whole mitochondrial genome of the Puye grande *Galaxias platei* obtained with MitoFish. The thirteen coding regions are in black, twenty two tRNAs are in red, two rRNAs are in light brown and the D-loop region is in brown.

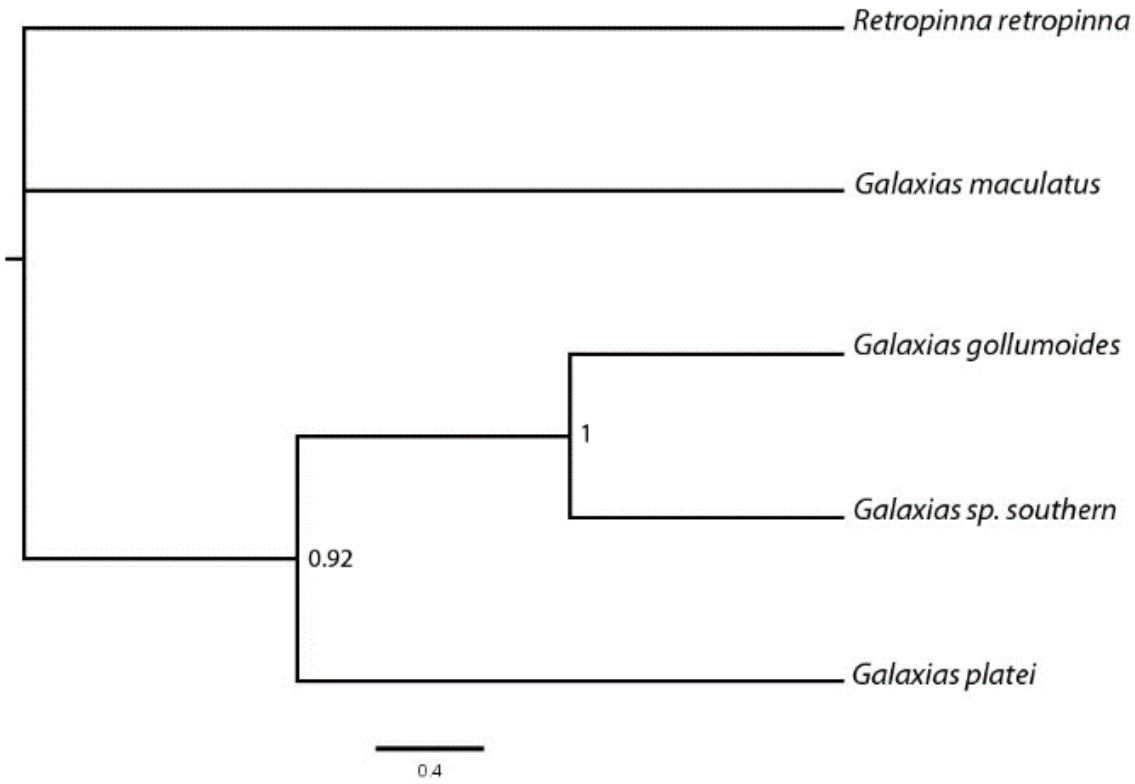

Figure 2. Phylogenetic tree of the whole mitochondrial genome of four galaxiid fish created in MrBayes. Bayesian probabilities are shown in the nodes.

Appendix 2 DEVELOPMENT AND CHARACTERIZATION OF 15 NOVEL MICROSATELLITE MARKERS FOR THE FRESHWATER FISH *Galaxias platei*

This Appendix has been published as "Vera-Escalona I, Habit E, Ruzzante D.E. Development and characterization of 15 novel microsatellite markers for the freshwater fish *Galaxias platei*" in Conservation genetics resources 6(4)899-901

Abstract

Galaxias platei is a fish species restricted to lakes and rivers from Patagonia. In this study, fifteen novel microsatellite loci were developed and characterized for 64 individuals of *G. platei* collected from a Patagonian lake. Eight loci were polymorphic and seven were monomorphic. The number of alleles in the polymorphic loci ranged from 3 to 6, and the expected and observed heterozygosities ranged from 0.08 to 0.68, and 0.08 to 0.62, respectively. These new markers are being used in an ongoing landscape genetics study on *G. platei*.

Galaxias platei is a freshwater fish restricted to lakes and rivers of Andean Patagonia in southern Chile and Argentina. The species appears to have survived the climate cycles of the Quaternary in-situ (Zemlak *et al*. 2008; 2011), likely due to its tolerance to large differences in temperature and low levels of oxygen, as well as to its low metabolic rate (Milano et al. 2002). *Galaxias platei are* rare and appear in low numbers when in sympatry with salmonids but the species can be found in abundance in high latitude and altitude lakes of the Andean Patagonian region not invaded by salmonids (Habit *et al*. 2012). An improved understanding of the patterns of connectivity among *G. platei* populations can assist in our understanding of the factors that affect the spatial distribution of genetic diversity in this freshwater species. Here we characterize 15 novel microsatellite markers in *G. platei* to increase the number of microsatellite loci over those already available (Arias *et al*. 2009). The loci were tested with a set of N=64 individuals from lake Azul, a Patagonian Lake located in the Torres del Paine National Park.

Whole genome DNA was extracted from muscle tissue using a glass milk protocol. The quality of the DNA was tested in 1% agarose gel and the DNA concentration was measured using a PicoGreen protocol using a Perkin Elmer Fusion DNA Quantifer (Perkin Elmer, Watham, Massachussetts). The individual with the highest quality was selected for sequencing using 1ng of genomic DNA, following a standard protocol of the Illumina Nextera DNA Sample Preparation Kit (Illumina Inc., San Diego, California). DNA sequencing was conducted using MiSeq Benchtop Sequencer (Illumina Inc., San Diego, California). The result from the sequencer containing unpaired short read fragments was analyzed with the software CLC Genomics Workbench 6 (CLC bio, Aarhus, Denmark) to obtain contigs with longer DNA fragments. These contigs were used to detect the presence of microsatellite loci with the software MSATCOMMANDER 0.8.2, identifying 259 pairs of primers with an m13 tag added. A total of 78 sequences with presence of dinucleotide and tetranucleotide repeats were found. All of them were tested using an m13-20 tag (5'-GTA AAA CGA CGG CCA GTG-3'). The microsatellites were amplified in a 5µl reaction consisting of 2.35µl of dd-H2O, 0.5µL of 10× reaction buffer (Bio Basic Inc., Markham, Ontario), 0.5µL of 2.0 mM of $MgSO_4$ (Bio Basic Inc., Markham, Ontario), 0.5µL of 200 µM dNTPs (Bio Basic Inc., Markham Ontario), 0.05µL µM of fluorescently labeled M13-20 tag 0.05µL 0.1 µM of un-tailed primer, 0.05µL of 0.01 µM of M13 tailed primer, 0.05 µL of 0.25 U TSG Polymerase (Biobasic Inc., Markham, Ontario), and 20-50 ng of genomic DNA. The PCR products were analyzed using Li-Cor 4200/4300 analyzers (Li-Cor Biosciences, Lincoln, Nebraska) and genotyped using SAGA software (Li-Cor Biosciences, Lincoln, Nebraska). The detection of potential genotypic errors, null alleles and stuttering was assessed in MICRO-CHECKER 2.2.3. Arlequin 3.5.1.2 was used to test Hardy Weinberg Equilibrium (HWE).

A total of 15 markers were successfully amplified, 8 were polymorphic and 7 monomorphic in the tested population (Table 1). The number of alleles per locus ranged between 2 and 6 and the observed and expected heterozygosities ranged from 0.08 to 0.68, and 0.08 to 0.62, respectively. MICRO-CHECKER showed no evidence for scoring error or technical artifacts and no evidence of genotyping linkage disequilibrium was found in any of the polymorphic markers. After applying the Bonferroni correction, none of the 8 polymorphic loci were found to deviate from Hardy-Weinberg Equilibrium

Table 1. Characterization of 15 microsatellite loci for *Galaxias platei*. Ta = annealing temperature, Na = Number of observed alleles, He = expected heterozygosity, Ho = observed heterozygosity, N = sample size.

Locus	Primer sequence (5'- 3')	Repeat motif	T_a	N_a	Size range	H_o	H_e	N	Genbank Accession Number
Gpla1	ACAAGGAGCTGCATTTGGC	(ACCAG)^2	58	3	127-142	0.48	0.54	64	KJ739527
	TGAGGCCGGCTAATTAAGTG								
Gpla2	AGCAGCTGTCCCAACGAAG	(AC)^3	59	6	249-261	0.64	0.62	64	KJ739528
	ACACTCACAGTCTGCCCTG								
Gpla3	GGTCATTCCTTAACATCTGAATGGG	(GT)^4	60	3	168-180	0.27	0.28	64	KJ739529
	AGGTGAGGTCCATTACGGC								
Gpla4	AGTACCATACAAAGGCCTGC	(GT)^2	61	4	270-278	0.08	0.08	64	KJ739530
	GGAACTGAAGCTGTTGCCC								
Gpla5	AGTGAAATGCACGCGGAAC	(AC)^3	59	4	172-178	0.37	0.20	64	KJ739531
	CACGGTAAGAACGCACTCTG								
Gpla6	TGTCCTTACCTCTGCTGAACC	(AG)^3	59	3	161-175	0.30	0.33	64	KJ739532
	CAGGTAACGTGCGATTTATTTGAC								
Gpla7	CTCTACACAGAGGGCCGTG	(AC)^8	59	5	258-278	0.11	0.12	64	KJ739533
	AGACAATGTGGGTATAATTGCGG								
Gpla8	TCTGGGTGGATTACCCTGC	(AT)^6	58	4	183-187	0.68	0.54	64	KJ739534
	GGTATGTGAGAGACCCTTGC								
Gpla9	CACCTGATTCCCACACTGC	(GT)^3	59	1	142	NA	NA	64	KJ739535
	ACAGGCTAGTCCAGAGCAC								
Gpla10	CTCCCTGTCTTATCCACATGC	(AC)^6	61	1	157	NA	NA	64	KJ739536
	TCTGCCTTTGCTGGAGGAC								
Gpla11	GACAGTGCACACCTAACCTG	(AG)^7	59	1	193	NA	NA	64	KJ739537
	GCTAGCACTACAGTCAGCC								
Gpla12	AGAGGGCTGCATCGAAAGC	(AC)^6	60	1	205	NA	NA	64	KJ739538
	TGCCTATGCAACTGCCTG								
Gpla13	TGGTGAGAGCGGGAAACTC	(AT)^4	60	1	151	NA	NA	64	KJ739539
	TCTAAGCGCTCCGAGGAAG								
Gpla14	ACGCCAGAGGCAGGTTG	(AC)^5	59	1	165	NA	NA	64	KJ739540
	CAGCCCTGATCAAGCCCTC								
Gpla15	TGACAGCCTCAAGCATCTG	(AC)^9	59	1	118	NA	NA	64	KJ739541
	GTCAGGGACCAAGCAGCC								

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