Genetic and phenotypic variation among *Galaxias maculatus* populations reflects contrasting landscape effects between northern and southern Patagonia

CECILIA CARREA*, VICTOR E. CUSSAC* AND DANIEL E. RUZZANTE[†]

INIBIOMA (Universidad Nacional del Comahue-CONICET), Bariloche, Argentina
[†]*Department of Biology, Dalhousie University, Halifax, NS, Canada*

SUMMARY

1. Understanding the influence of landscape characteristics on genetic and phenotypic intraspecific variability can yield insights into how evolutionary processes work as well as provide essential information for the conservation of biodiversity.

2. Our aim was to compare the genetic structure and phenotypic variation among *Galaxias maculatus* populations inhabiting two Atlantic Ocean river basins in Patagonia in relation to historical and contemporary landscape characteristics associated with latitude.

3. Population genetic analysis (based on eight microsatellite loci, 505 individuals) indicates that genetic structure is more pronounced in the Río Negro basin (RN) at 39–41°S (10 localities) than in the southernmost Santa Cruz River basin (SCR) at 49–50°S (seven localities). Spatial autocorrelation analysis showed different effects of geographic distance in shaping population differentiation patterns in the two basins.

4. While in northern Patagonia, fragmented populations could have survived the severity of Quaternary climate cycles, at higher latitudes *G. maculatus* populations were probably extirpated by extensive ice sheets. Extant populations in the upper reaches of the southernmost basin probably originated from refugia close to the Atlantic Ocean.

5. Based on otolith microchemistry, we have documented facultative diadromy for the first time for the species in the SCR basin, while no evidence of migration to the sea was found in individuals from the RN basin. Vertebral number increased with latitude, and within the SCR basin, the higher counts are possibly associated with a migratory life style.

Keywords: facultative diadromy, *Galaxias maculatus*, genetic structure, intraspecific variation, microsatellite markers, vertebral count

Introduction

Observed patterns of population structure and connectivity are generally influenced by both historical and contemporary processes related to landscape characteristics (Costello *et al.*, 2003). Understanding such patterns and the processes affecting them is a focal issue in evolutionary biology and is fundamental for the design and implementation of scientifically informed initiatives for the conservation of biodiversity. The Patagonian landscape in South America has been affected by long-term geological processes related to the uplift of the Andes as well as by the climate cycles of the Quaternary (Rabassa, Coronato & Martinez, 2011). The combined impact of these processes on current biodiversity patterns is beginning to be understood (Ruzzante & Rabassa, 2011) for terrestrial (e.g. Mathiasen & Premoli, 2010; Pardiñas *et al.*, 2011; Sérsic *et al.*, 2011) as well as for freshwater organisms (e.g. Ruzzante *et al.*, 2008; Unmack *et al.*, 2009; Barber *et al.*, 2011; Zemlak *et al.*, 2011).

Freshwater fishes offer a good opportunity to study the influence of landscape on genetic diversity since they are intrinsically tied to the hydrological structure they inhabit (Gomez-Uchida, Knight & Ruzzante, 2009; Carrea *et al.*,

2012). The hydrological landscape in Patagonia was affected differentially across its latitudinal range by Quaternary glaciations. Thus, differential effects on genetic diversity patterns are to be expected for aquatic taxa. During the most extensive glaciations, such as the Great Patagonian Glaciation of the mid Quaternary (*c.* 1.1 Ma BP; Rabassa *et al.*, 2011), ice sheets reached the Atlantic Ocean at the southernmost tip of the continent and in Tierra del Fuego, eliminating habitat in headwater lakes and upper reaches of major river systems (Ruzzante *et al.*, 2011). In contrast, in northern Patagonia, glaciers remained confined to valleys and isolated mountain ice caps, which may have permitted the persistence of isolated populations (Mathiasen & Premoli, 2010; Sérsic *et al.*, 2011). Glacial refugia have been proposed for freshwater fish both in northern (i.e. north of *c.* 43°S) and southern Patagonia (Zattara & Premoli, 2004; Ruzzante *et al.*, 2006; Zemlak *et al.*, 2008).

In this study, we focus on the geographic structure and connectivity among populations of *Galaxias maculatus* (Jenyns, 1842), a fish species with a widespread distribution across the southern hemisphere. In South America, the species probably diversified in the northwestern region of Chilean Patagonia from where it extended its range into Argentina via marine dispersal through the southern tip of South America (Zemlak *et al.*, 2010). The Andes appear to have experienced limited large-scale direct trans-Andean dispersal as far as this species is concerned, as molecular evidence suggests headwater lakes located east of the Andes were most likely colonised from the sea through their Atlantic drainage basins (Zemlak *et al.*, 2010). Therefore, genetic diversity and distance to the Atlantic Ocean are expected to be negatively correlated along these basins due to the loss of diversity from repeated founder events (Petit *et al.*, 2003; Premoli, Mathiasen & Kitzberger, 2010).

Genetic structuring can be affected by life-history traits. *Galaxias maculatus* exhibits two life-history strategies (McDowall, 1970). Landlocked individuals complete their life cycle in freshwater lakes or rivers (resident populations). Diadromous individuals spawn in fresh water or estuaries, and their larvae migrate to the sea, where they stay for up to 6 months before juveniles return to fresh water (McDowall, 1997). Diadromy is considered the ancestral character for galaxiid fishes (McDowall, 1970, 1997; Waters, Dijkstra & Wallis, 2000; Waters & Wallis, 2001), and the repeated allocation of the migratory phase to fresh water (Chapman *et al.*, 2006; Barriga, Battini & Cussac, 2007) has probably facilitated their radiation throughout much of the southern hemisphere (Waters & Wallis, 2001; Burridge *et al.*, 2011). At the intraspecific

level, it has been shown that non-migratory galaxiid populations show higher genetic structuring than their diadromous counterparts (Allibone & Wallis, 1993; Waters *et al.*, 2000; Zattara & Premoli, 2004).

We examined the genetic variation of *G. maculatus* populations along two Atlantic drainage systems located at opposite ends of the Patagonian region: the Río Negro basin in northern Patagonia (39–41°S) and the Santa Cruz River basin (SCR) in southern (49–50°S) Patagonia. Environmental conditions associated with latitude in the SCR (e.g. low temperatures, lower food resources in fresh water compared to sea water) seem to have favoured the expression of the ancestral diadromous behaviour in the introduced salmonid, *Oncorhynchus mykiss* (Pascual *et al.*, 2001; Riva-Rossi *et al.*, 2007), while northern Patagonian populations of *O. mykiss* in the Río Negro basin are resident. A similar life-history pattern can be expected for *G. maculatus* with populations in southern Patagonia exhibiting diadromy and those in northern Patagonia being mostly resident (Cussac *et al.*, 2004; Zattara & Premoli, 2004).

Movement between marine and freshwater environments has been documented in galaxiid fishes using otolith microchemistry (David *et al.*, 2004; Hicks *et al.*, 2005). Strontium (Sr) concentration in sea water is more than 10 times as high as in fresh water (Secor, Henderson–Arzapalo & Piccoli, 1995). Therefore, Sr levels in ototliths of fish exposed to sea water are substantially higher than in otoliths of those exposed only to fresh water. For example, in New Zealand, David *et al.* (2004) found fresh water rather than diadromous larval recruitment in coastal *Galaxias argenteus* (Gmelin, 1789) populations, a species considered to be diadromous. This finding highlights the need for further research in other diadromous galaxiid species in other regions. We used otolith microchemistry to ascertain whether *G. maculatus* individuals had experienced sea water at some point in their lives.

Diadromous galaxiid populations show a higher vertebral number than non-diadromous ones (McDowall, 2003a). Non-migratory *G. maculatus* populations from Australia and New Zealand show a reduced number of vertebrae, with 49 vertebrae at the extreme compared with around 62 in diadromous stocks (Ling *et al.*, 2001). Similarly, in South America, the lowest vertebral count recorded for landlocked populations is 50 (Carrea *et al.*, 2012). Furthermore, vertebral count is correlated with latitude for diadromous *G. maculatus* populations across its distribution range (McDowall, 2003a). For landlocked populations, however, this correlation differs among regions, perhaps reflecting specific relationships between latitude and local climate (McDowall, 2003a). For

example, for the six South American lake populations examined by McDowall (2003a), vertebral number and latitude were not positively correlated. However, this could be due to the small number of samples or to a masking effect of altitude in the Andean range (increasing northward). Diverse factors have been proposed to explain vertebral number variation in fishes in general, including temperature during ontogeny (Lindsey, 1988), fish size and mode of swimming (McDowall, 2003a,b), but the underlying mechanisms are not yet clear (McDowall, 2003a,b, 2007).

In this study, we compared genetic structure and connectivity patterns among *G. maculatus* populations inhabiting two Atlantic drainage systems at different latitudes in Patagonia. Such a comparison is complex because the two basins differ in hydrological characteristics, including the presence or absence of dams, and also the distance from the headwater lakes to the sea. We hypothesise that contemporary as well as historical landscape characteristics associated with latitude, and differences in life-history traits, will have affected the genetic structuring of *G. maculatus* populations in the studied systems. Specifically, we expected that the differential effect of past climatic events would result in a stronger population structure of the northernmost Río Negro basin than in the southernmost basin. Potential restrictions of gene flow resulting from the adoption of a resident life style, and the current hydrological structure in the Río Negro basin, could maintain higher isolation among populations. Finally, we predicted an increase in the incidence of diadromy (high Sr levels, high connectivity) and high vertebral counts at higher latitudes.

Methods

Sampling sites and study area

Galaxias maculatus individuals were collected by seine netting between January 2007 and March 2008 at 17 localities in two river drainages (Fig. 1): 10 sites at locations along Río Negro basin in northern Patagonia and seven sites along the SCR basin in southern Patagonia. Individuals were sacrificed using an overdose of benzocaine, and tissue samples were preserved in 96% ethanol. Details of each sampling site are shown in Table 1, including records of life history (for localities where precise information was available).

Both basins drain into the Atlantic Ocean. The Río Negro is formed by the confluence of Neuquén and Limay rivers, and its basin extends about 600 km from the headwater lakes in the west to the river mouth on the Atlantic Ocean in the east. The Santa Cruz basin extends about 250 km from the headwater lakes in the west to the Atlantic Ocean. Over the last 40 years, at least four dams have been built on the Limay River (indicated in Fig. 1); no dams have thus far been built on the SCR.

Genetic data collection

Genomic DNA was extracted from 505 tissue samples following the glass milk method described by Elphinstone *et al.* (2003). Approximately 5–10 mg of each tissue sample was first digested in 300 μL buffer (100 mM NaCl, 50 mM TrisHCl pH 8, 10 mM EDTA pH 8, 0.5 mM SDS) and 2 μL Proteinase K overnight at 55 °C and 200 rpm. The extractions were performed in 96-well filter plates using a
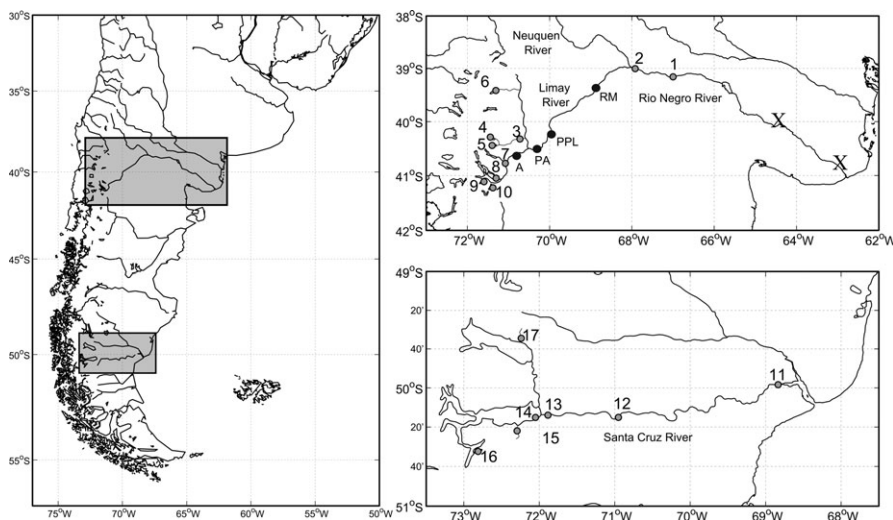

Fig. 1 Sampling sites along the Negro River basin (right top panel) and Santa Cruz River basin (right bottom panel). Location names and characteristics are given in Table 1. Xs indicate sampling sites where no *Galaxias maculatus* individuals were captured. Dams are indicated with black circles: RM, Ramos Mexía; PPL, Pichi Picún Leufú; PA, Piedra del Águila; A, Alicurá.

Table 1 Locations and characteristics of sample sites. Genetic diversity patterns by population include mean observed (H_o) and expected (H_e) heterozygosity with their standard error (SE) and Allelic richness (AR)

Sampling site	N	Drainage basin	Latitude/ Longitude	Habitat	Life history	Mean H_o/SE	Mean H_e/SE	AR	Private alleles
1-Villa Regina	34	Limay-Negro	39°16′/67°11′	Riverine	?	0.69/0.05	0.68/0.04	6.21	2
2-Isla Jordan	40	Limay-Negro	38°59′/67°58′	Riverine	?	0.71/0.06	0.67/0.06	6.12	1
3-Caleufu River	24	Limay-Negro	40°20′/70°45′	Riverine	Landlocked[*]	0.64/0.09	0.62/0.09	6.24	2
4-FiloHuaHum Lake	24	Limay-Negro	40°28′/71°15′	Lacustrine	?	0.59/0.07	0.54/0.08	5.62	0
5-Meliquina Lake	21	Limay-Negro	40°23′/71°17′	Lacustrine	Landlocked[†]	0.56/0.09	0.54/0.09	5.15	6
6-Quillén Lake	30	Limay-Negro	39°25′/71°17′	Lacustrine	Landlocked[‡]	0.58/0.07	0.57/0.08	5.17	3
7-Limay-Traful River	50	Limay-Negro	40°45′/71°05′	Lacustrine	?	0.57/0.09	0.52/0.08	4.91	0
8-Nahuel Huapi Lake	15	Limay-Negro	41°02′/71°10′	Riverine	Landlocked[†]	0.58/0.08	0.58/0.08	5.63	1
9-Morenito Lake	30	Limay-Negro	41°02′/71°32′	Lacustrine	Landlocked[§]	0.61/0.05	0.63/0.05	4.54	2
10-Gutiérrez Lake	23	Limay-Negro	41°17′/71°41′	Lacustrine	Landlocked[¶]	0.60/0.08	0.64/0.09	6.54	2
11-Piedra Buena	35	Santa Cruz	49°59′/68°54′	Riverine	Diadromous[**]	0.85/0.04	0.85/0.04	11.56	21
12-Condor Cliff	17	Santa Cruz	50°12′/70°54′	Riverine	?	0.69/0.04	0.74/0.05	7.46	0
13-Sta. Cruz River	42	Santa Cruz	50°16′/7153′	Riverine	?	0.72/0.03	0.75/0.04	7.33	2
14-Argentino Lake	38	Santa Cruz	50°12′/71°58′	Lacustrine	?	0.73/0.06	0.72/0.06	7.17	0
15-Calafate stream	20	Santa Cruz	50°33′/72°26′	Stream	?	0.71/0.05	0.74/0.04	6.40	3
16-Roca Lake	32	Santa Cruz	50°31′/72°47′	Lacustrine	Landlocked[††]	0.77/0.05	0.77/0.05	8.05	2
17-Río Cangrejo-L.Viedma	30	Santa Cruz	49°35′/72°15′	Stream	?	0.66/0.06	0.65/0.07	5.70	0

?, information not available.

[*]Barriga *et al.* (2007).

[†]McDowall (1972).

[‡]Ruzzante *et al.* (1998).

[§]Battini (1997).

[¶]Barriga *et al.* (2002).

[**]R. Hudson, pers. comm.

[††]M. A. Battini, pers. comm.

liquid handling robot (MultiprobeII; Perkin Elmer, Waltham, MA, USA).

Eight species-specific microsatellite markers (Carrea *et al.*, 2009) were amplified by 5-μL-volume polymerase chain reactions (PCR) in 384-well plates. PCR mixtures contained 10–50 ng DNA, 20 mM Tris–HCl, 10 mM (NH_4)$_2SO_4$, 10 mM KCl, 0.1% Triton X-100, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 U Tsg DNA polymerase (BioBasic D0081) and 0.1–0.2 μM of each primer. Microsatellite fragment lengths were visualised in polyacrylamide gels using a LICOR sequencer (Biosciences, Lincoln, NE, U.S.A.) and genotypes obtained using AUTOMATED MICRO-SATELLITE (version 3.3; LICOR). MICRO-CHECKER (Van Oosterhout *et al.*, 2004) was used to test for potential genotyping errors or technical artefacts. GENEPOP 4.0.10 (Raymond & Rousset, 1995) was used for tests of Hardy–Weinberg equilibrium as well as for tests of linkage disequilibrium between all pairs of loci.

Genetic diversity and population structure

Mean observed and expected heterozygosities and number of private alleles by locus and locality were estimated with GeneAlex (Peakall & Smouse, 2006). Allelic richness

was calculated using FSTAT 2.9.3 (Goudet, 2001) taking advantage of the fact that this program applies a rarefaction method (Petit, Mousadik & Pons, 1998). Expected Heterozygosities were regressed against geographic (waterway) distance to the sea using least squares in Matlab 7.0.1 (Mathworks, Natick, MA, USA).

Pairwise F_{ST} for all pairs of sampling sites was estimated with ARLEQUIN 3.1 (Excoffier, Laval & Schneider, 2005); the null hypothesis of no differences between populations was tested with 1000 permutations of the haplotypes between populations.

We estimated the number of genetic clusters using the Bayesian approach implemented in STRUCTURE (Pritchard, Stephens & Donnelly, 2000). Three rounds of analysis were performed hierarchically under an admixture model and correlated allele frequencies. Ten iterations for each K were run to apply the ΔK method (Evanno, Regnaut & Goudet, 2005) as implemented in STRUCTURE HARVESTER (Earl & von Holdt, 2012). A total of 350 000 Markov Chain Monte Carlo replicates were run, the first 150 000 of which were burn-in. Geographic data were obtained from ESRI World Data and Digital Chart of the World, using Arc View, to show the geographic distribution of the population genetic

structuring inferred with STRUCTURE. Finally, a hierarchical AMOVA was performed using ARLEQUIN 3.1 (Excoffier *et al.*, 2005) to estimate the percentage of genetic variance explained by the genetic clusters between and within basins.

Estimates of recent migration rates between inferred genetic groups were obtained with BIMr 1.0 (Faubet & Gaggiotti, 2008). Ten runs were performed, but only the results with the lowest Bayesian deviance are considered, as suggested by Faubet, Waples & Gaggiotti (2007). Each run consisted of 150 000 iterations, from which the first 50 000 were discarded. Sampling frequency was set once every 50 iterations.

Isolation by distance

We estimated spatial autocorrelations within each basin using the procedure implemented in GENALEX (Peakall & Smouse, 2006). The analysis allows the detection of the distance categories at which the spatial autocorrelation is significant. Autocorrelation patterns are shown in correlograms, which plot the spatial correlation coefficient *r* versus increasing distance classes. Confidence intervals were obtained with 999 permutations. Distance classes were selected so as to minimise potential bias due to differences in sample sizes. Under restricted gene flow and with no selection acting, the expected pattern of isolation by distance is one of positive spatial autocorrelations at short distances, decreasing towards 0 and becoming negative as distance classes increase.

Phenotypic analysis

A technique known as energy dispersive spectroscopy was used to characterise otolith chemical composition. The largest of the three otoliths (sagittae) present in the inner ear of the fish was extracted from each individual under a stereomicroscope. This analysis was conducted using a Phillips 515 (Amsterdam, Netherlands) scanning electron microscope equipped with a spectroscope EDAX 990 available at the Balseiro Institute (Bariloche, Argentina). Four measurements were taken per individual: two on each half of the otolith, each lasting 500 s.

According to the observed frequencies, Sr was considered present when the signal was higher than 2% of the total weight, constituting a qualitative analysis of the presence/absence type. A total of 10 individuals were measured by sampling site, for each basin. Given that populations closer, or with easier access, to the sea are more likely to exhibit diadromy than populations further from the sea, we started measuring the samples from the

sites close to the sea and stopped measuring when two consecutive sites resulted in no individuals with Sr present in their otoliths. Thus, a total of 60 individuals were measured: 10 from each of Villa Regina and Isla Jordan on the Río Negro basin, and 10 from each of Piedra Buena, Condor Cliff, Head of the SCR and Argentino Lake on the SCR basin.

Vertebral count

X-rays were taken on a subsample of individuals corresponding to five localities in Río Negro basin: Villa Regina (*n* = 21), Isla Jordán (*n* = 25), Caleufu River (*n* = 21), Nahuel Huapi Lake (*n* = 97) and Gutierrez Lake (*n* = 17), and four localities on the SCR basin: Piedra Buena (*n* = 25), head of river Santa Cruz (*n* = 20), Argentino Lake (*n* = 12) and Roca Lake (*n* = 34). Vertebral counts were measured from the X-ray plates under a stereomicroscope. When resolution was low, the count was performed directly on dissected individuals under a stereomicroscope. Individuals from other localities could not be included due to poor preservation status.

Results

The null hypothesis of HWE could not be rejected for 120 of 136 exact tests (Table S1, see Appendix S1). However, no tests remained significant after a sequential Bonferroni correction (Rice, 1989). Genetic diversity as measured by mean expected heterozygosity by locality was negatively correlated with distance to the sea within the SCR basin ($P < 0.05$, $R^2 = 0.57$, Fig. 2). In addition, the locality closest to the sea (Piedra Buena) showed the highest number of private alleles (Fig. 2). Mean expected heterozygosity was also negatively correlated with distance to the sea in the Río Negro basin ($P < 0.05$, $R^2 = 0.43$, Fig. 2). Genetic diversity (heterozygosity, allelelic richness, number of private alleles) was higher in localities from the SCR basin than in the Rio Negro basin (Table 1).

Pairwise F_{ST} between localities within the Río Negro Basin ranged from 0.012 to 0.264, and all pairs were genetically differentiated ($P < 0.05$ for each pair, Table S2). In the case of SCR basin, pairwise F_{ST} ranged from 0.001 to 0.068 (Table S2) and only the two outermost populations, Piedra Buena (located in the mouth of the river) and Viedma Lake (one of the headwater populations), were significantly differentiated from each other and all other sites within this basin.

The first round of analysis with STRUCTURE, including the complete data set, inferred $K = 3$ genetic clusters based on ΔK calculations (Figure S1). Two of the inferred
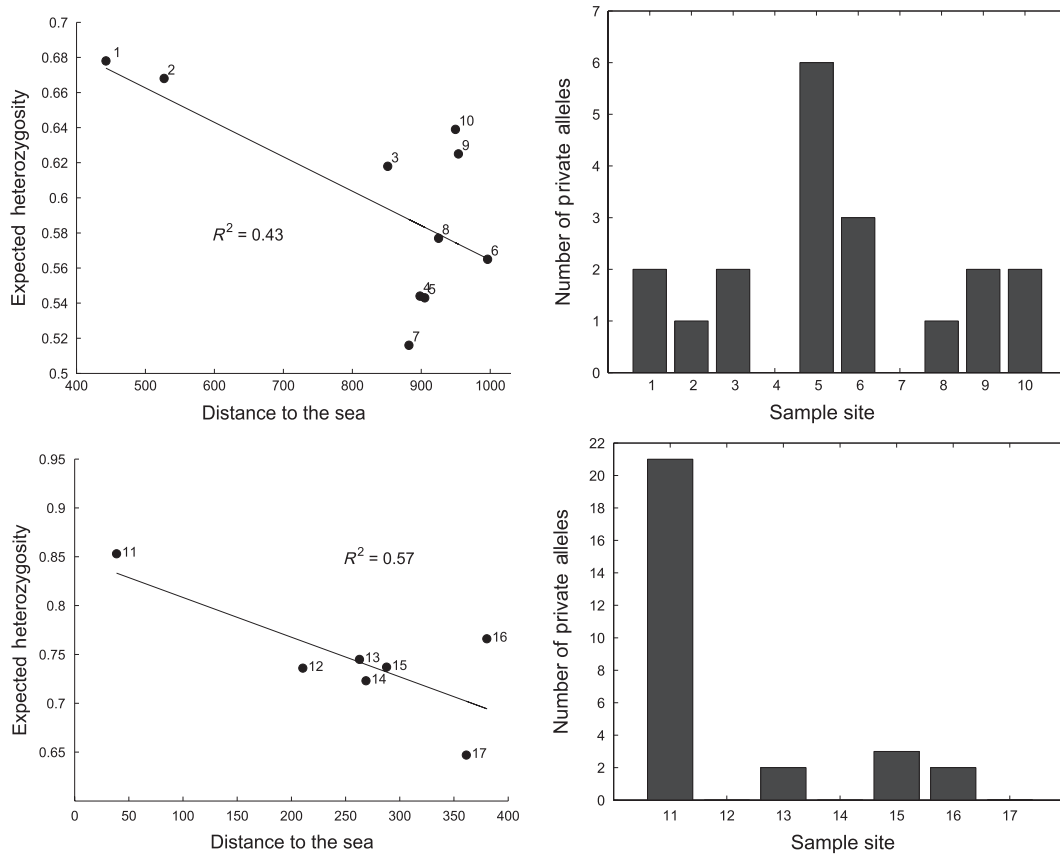
Fig. 2 Regressions of genetic diversity (as measured by expected heterozygosity) against distance from each sampling site to the sea for the Negro River basin (NR, top left) and the Santa Cruz River basin (SCR; bottom left). Private allele numbers per sampling site are shown for the NR basin (top right) and SCR basin (bottom right). Location names and characteristics are given in Table 1.

genetic clusters correspond to localities within the Río Negro basin: one downstream and the other upstream of the confluence of Traful and Limay rivers. The third group corresponds to all localities within the SCR basin. The second round of analysis with STRUCTURE was conducted separately on each genetic cluster. Figure 3 shows the geographic arrangement of the seven genetic groups (groups A–G) along both basins.

Three genetic groups were detected within the downstream genetic cluster in Río Negro; these corresponded to groups A (1-Villa Regina, 2-Isla Jordan and 3-Caleufu River), B (4-Filo Hua Hum and 5-Meliquina Lakes, which drain their waters to Limay River through Caleufu River) and C (6-Quillén Lake). The upstream Río Negro genetic cluster (group D) did not show substructuring in the second round of analysis of collections within the Rio Negro basin. Substructure was detected within the SCR basin where $K = 3$: these genetic clusters correspond to groups E (including 11-Piedra Buena in the river mouth), F (including 12-Condor Cliff, 13-upstream SCR, 14-Argentino Lake, 15-Calafate stream, and 16-Roca Lake) and G (17-Viedma Lake).

A hierarchical analysis of variance (AMOVA) showed that 20% of the genetic variability is explained by differences between basins (Table 2). When basins were analysed separately, the genetic variation explained by the genetic clustering inferred by STRUCTURE was 7% in the Río Negro and 4.8% in the SCR.

Contemporary migration patterns

Within the Río Negro basin, there was relatively high gene flow from groups A towards B: 20% of genes within group A were observed migrating towards Group B. To a lesser extent, the headwaters of Limay River (Groups D and C) also received migrants from the riverine localities included in group A (6 and 4% for groups D and C, respectively, Table 3a). Groups B, C and D had 100% of resident genes (see bold values of the diagonal in Table 3a). Migration rates from these groups into group A were zero, suggesting that fish in the headwater lakes of the Río Negro basin have adopted a landlocked or resident life history while riverine populations have maintained their migratory habits mainly in the upstream direction.
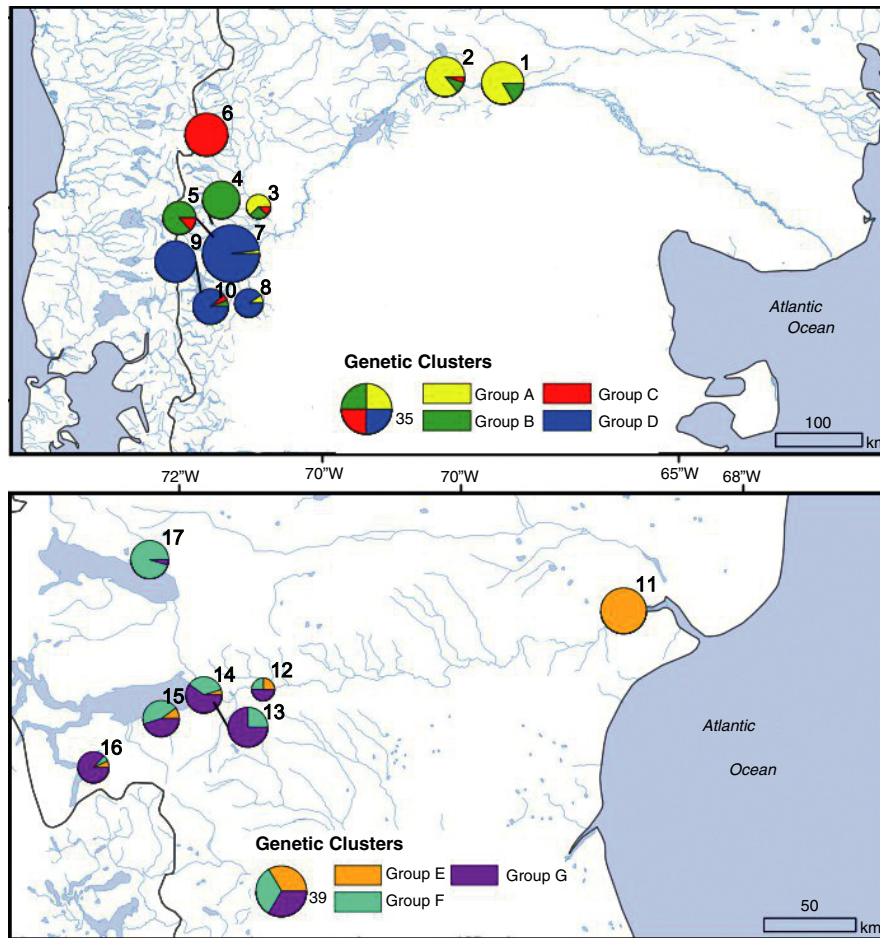
Fig. 3 Geographic distribution of genetic clusters (A–G) inferred by STRUCTURE. Pie charts represent the percentage of individuals assigned to each genetic cluster by a membership coefficient (*Q*) higher than 80%. Location names and characteristics are given in Table 1.

Within the SCR basin, all genetic groups had <100% of resident genes (Table 3b). However, migration from Piedra Buena (Group E) into the rest of the groups was very low. This was also true for Viedma Lake (Group F). Group G showed the highest proportion of migratory genes (50%). Again, the migration from this group occurred mainly upstream towards Viedma Lake (Group F).

Isolation by distance

Río Negro basin. The Río Negro system exhibited a spatial autocorrelation pattern that is more complex than expected if we assume isolation by distance (Fig. 4a). Correlation became negative at distances of about 100 km and then oscillated between negative and non-significant (Fig. 4a). Accordingly, genetic and geographic distances were not correlated at this broad scale (Mantel test, Rxy = 0.05, *P* ≥ 0.406). However, when we analysed the spatial structure within the genetic groups inferred with

STRUCTURE, an isolation by distance pattern could be observed within group A, which includes only riverine localities (Fig. 4b). For group D, the correlation became negative at a distance of about 20 km with a negative maximum at a distance of 30 km; no significant structure was detected at larger geographic distances (Fig. 4c). This group includes a riverine locality and three headwater Lakes (Nahuel Huapi, Morenito and Gutierrez). The observed pattern probably reflects higher dispersal ability among riverine *G. maculatus* aggregations (Group A) than among those inhabiting lakes (Group D).

Santa Cruz River basin. Galaxias maculatus in the SCR basin exhibited an isolation by distance pattern. There was a positive correlation up to a distance of 25 km; the correlation then became non-significant between 25 and 151 km, and negative at distances >151 km (Fig. 4d). A Mantel test of correlation between genetic and geographic distances was also consistent with an isolation by distance

Table 2 Results of the analysis of molecular variance (AMOVA) showing variance percentage explained by individuals of different basins (a) and individuals from different genetic clusters within Rio Negro basin (b) and Santa Cruz basin (c)

Variance source	Variance percentage	F-statistic (P-value)*
(a) Between basins	19.93	F_{ST} = 0.26 (0.001)
Between localities within basins	6.74	F_{SC} = 0.08 (0.001)
Within localities	73.32	F_{CT} = 0.19 (0.001)
(b) Between genetic clusters within Río Negro basin	7.09	F_{ST} = 0.13 (0.001)
Between localities within genetic clusters	6.04	F_{SC} = 0.06 (0.001)
Within localities	86.85	F_{CT} = 0.07 (0.001)
(c) Between genetic clusters within Santa Cruz River basin	4.79	F_{ST} = 0.06 (0.001)
Between localities within genetic clusters	1.67	F_{SC} = 0.001 (0.001)
Within localities	93.5	F_{CT} = 0.04 (0.001)

*Nonparametric permutation tests for the fixation indexes (Excoffier, Smouse & Quattro, 1992) consisting of the permutation of individual genotypes between groups (F_{ST}), between localities within groups (F_{SC}) and within localities between groups (F_{CT}).

pattern (Rxy = 0.836, P < 0.01). When the spatial autocorrelation analysis was performed within the genetic Group G in the SCR (Fig. 4e), the autocorrelation was positive up to 6 km but negative at a distance of 93 km.

Phenotypic variation

No evidence of rearing in the sea was found in any individual from the Rio Negro Basin. In contrast, strontium (Sr), as an indication of rearing in sea water, was detected in the otoliths of five of 10 individuals examined in Piedra Buena, the sampling locality closest to the sea in the SCR basin. In Cóndor Cliff, 170 km upstream of Piedra Buena, Sr was detected in the otoliths of two of 10 individuals (Fig. 5). These results show that *G. maculatus* populations in these localities comprise a mixture of diadromous and resident individuals. No individuals with Sr levels of 2% or more were detected in the upstream samples from the Head of the SCR and Argentino Lake.

Vertebral counts varied between 51 and 56 in the Río Negro basin and between 54 and 61 in the SCR basin, representing a significant difference in vertebral counts between these two drainages (P < 0.001, nonparametric Mann–Whitney test). Within the Río Negro basin (N = 181), vertebral counts did not vary among sampling localities (Kruskal–Wallis, P = 0.06). *Galaxias maculatus* from the SCR basin (N = 91), on the other hand, differed in vertebral counts among localities (Kruskal–Wallis, P < 0.001). Individuals collected from Piedra Buena, the location closest to the sea, had more vertebrae than those collected from all other localities in this river (multiple pairwise comparisons, Dunn test, Piedra Buena: 59–61 versus 56–60 elsewhere). Individuals from lakes Argentino and Roca did not differ in their vertebral numbers from the riverine collections taken from the head of SCR (Table 4).

Discussion

The differences found in genetic structure and phenotypic traits among *G. maculatus* populations inhabiting the two Patagonian basins seem to respond to both landscape characteristics associated with latitude as well

Table 3 Mean migration rates and 95% highest posterior density intervals (HPDI) between genetic groups. (a) Río Negro basin (Groups A–D) and (b) Santa Cruz River basin (Group E–F)

(a) Río Negro basin: migration rates into: mean (95% HPDI)			
From	Group A	Group B	Group C	Group D
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Group A (*n* = 98)	**0.71 (0.47–0.9)**	0.19 (0.02–0.4)	0.04 (0.01–0.23)	0.06 (0.00–0.29)
Group B (*n* = 45)	0.00 (0.00–0.00)	**1.00 (0.99–1.00)**	0.00 (0.00–0.00)	0.00 (0.00–0.00)
Group C (*n* = 30)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	**1.00 (1–1)**	0.00 (0.00–0.00)
Group D (*n* = 118)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	**1.00 (1–1)**

(b) Santa Cruz River basin: migratory rates into: mean (95% HPDI)		
From	Group E	Group F	Group G
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Group E (*n* = 98)	**0.99 (0.79–1)**	0.00 (0.00–0.14)	0.00 (0.00–0.16)
Group F (*n* = 45)	0.00 (0.00–0.00)	**0.98 (0.69–1)**	0.01 (0.00–0.2)
Group G (*n* = 30)	0.05 (0.01–0.16)	0.46 (0.28–0.63)	**0.49 (0.29–0.69)**

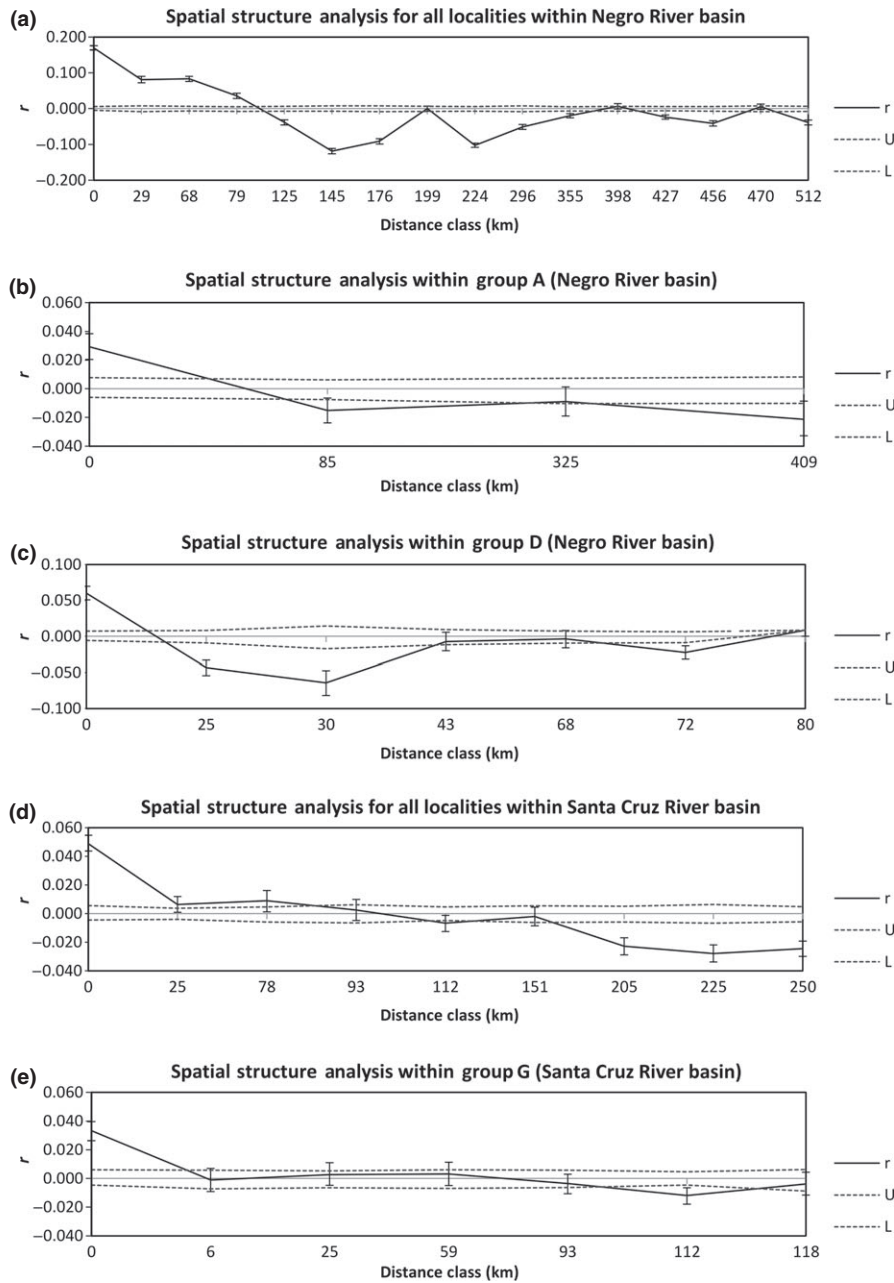Values are reported to two significant decimal places.

Fig. 4 Correlograms showing the genetic spatial structuring of *Galaxias maculatus* populations along the two basins. (a) Individuals within Negro River basin, (b) individuals within Group A, (c) individuals within Group D (Group B or C were not analysed separately because they include 2 and 1 localities, respectively), (d) individuals within Santa Cruz River basin and (e) individuals within Group G.

as to variation in the migratory behaviour of the species. Genetic structure was higher in the Río Negro basin (RN) than in the SCR basin, most likely reflecting differential effects of Quaternary climatic cycles in the latitudinal range studied, and the adoption of a resident life history at low latitudes. Evidence of life-history variation at the intra-population level was found in SCR, representing the first record of facultative diadromy for the species in Patagonia. Vertebral number varied with latitude and life history as well.

Long local persistence close to the Atlantic Ocean is suggested by a high number of private alleles in Piedra Buena population in the SCR basin. Lowland refugia have been proposed for both plants and terrestrial vertebrates along the Atlantic coastline of Patagonia at high latitudes (Fraser *et al.*, 2012), from about 51° to 53° (Sérsic *et al.*, 2011) and at 54° (Premoli *et al.*, 2010). For *G. maculatus*, headwater Andean populations at these latitudes were probably completely extirpated in the mid Pleistocene when glaciers covered vast areas of Patagonia, and
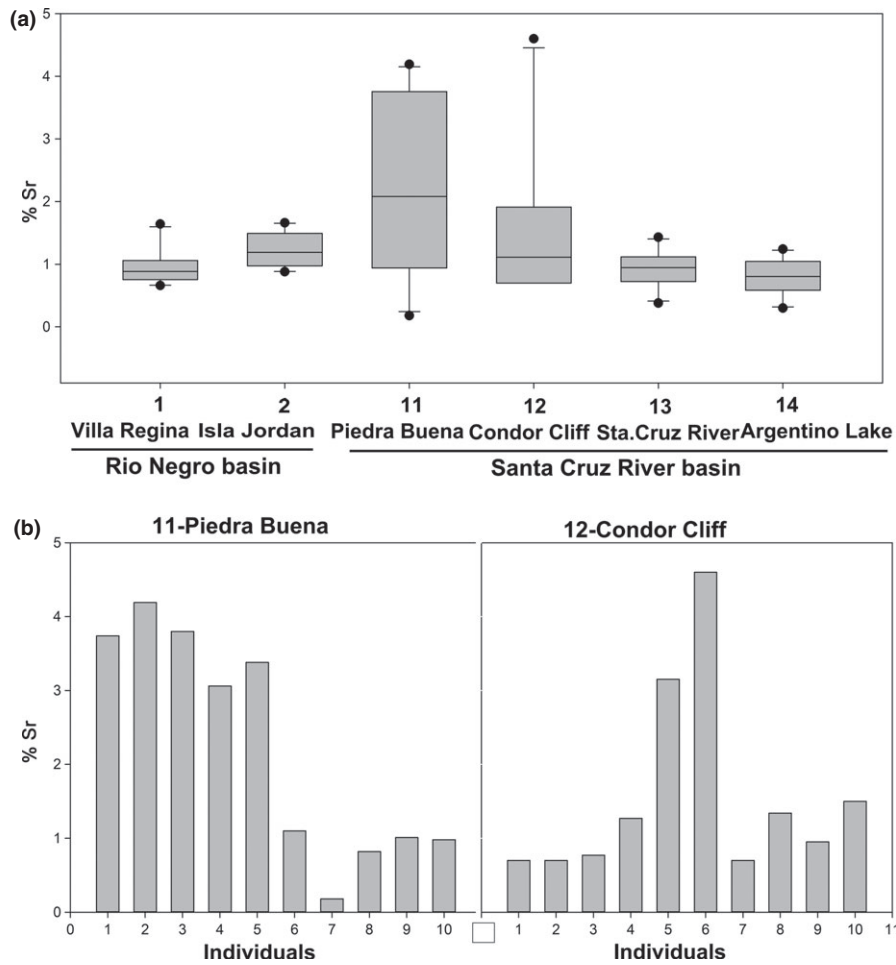
Fig. 5 Strontium (% by weight) in otoliths of fish by locality. (a) Box plots showing individuals from Piedra Buena and Condor Cliff with the highest %Sr. Histograms show total weight %Sr per individual in (b) Piedra Buena and (c) Cóndor Cliff.

Table 4 Vertebral counts for individuals in the Río Negro basin (group A) and the Santa Cruz River basin (group B and C)

Sampling site	N	51	52	53	54	55	56	57	58	59	60	61	Median	25%	75%	Dunn test
		Number of individuals with the following vertebral number														
Villa Regina	21		8	5	3	3	2						52	52	54.2	A
Isla Jordan	25	1	12	7	5								53	52	53	A
Caleufu	21	2	6	9	4								53	52	53	A
N Huapi Lake	97		20	53	23	1							53	53	53.25	A
Gutiérrez Lake	17	3	6	4	3	1							52	52	53.25	A
Piedra Buena	25								12	9	4		60	59	60	B
Naciente (SCRiver)	20							2	7	6	5		59	58	59.5	C
Argentino Lake	12				5				3	2	2		59	58	59	C
Roca Lake	34			1			5		10	15	3		57	56	58	C

evidence of a severe bottleneck has been reported for the species (Zemlak *et al.*, 2011). Our results suggest that *G. maculatus* may have survived in coastal refugia. The observed decline of genetic diversity as a function of distance to the sea within SCR can be explained by successive founder events during post-glacial colonisa-tion, as predicted by Petit *et al.* (2003). Furthermore, these results are in agreement with colonisation via Atlantic drainage systems as proposed by Zemlak *et al.* (2010).

The comparatively high divergence between *G. maculatus* populations in the RN basin probably reflects a more stable geomorphology during the Quaternary climatic

cycles allowing the local persistence of genetically diverse populations in multiple ice-free locations. Additionally, the adoption of a resident non-migratory lacustrine life history has probably contributed to the isolation among populations. Similarly, Wallis *et al.* (2001) showed that galaxiid populations inhabiting the geologically recent Canterbury Plains in New Zealand's South Island are genetically more homogeneous than in an adjacent geologically more stable area. The relatively shallow divergences found in the alluvial plains were in part attributed to gene flow among different catchments facilitated by unstable braided rivers. It could be argued that the divergence differences among localities between RN and SCR occur simply because RN is a larger basin and thus offers more scope for the detection of genetic structure. However, the spatial correlation coefficients and pairwise F_{ST} values indicate a higher genetic structuring among localities in RN separated by similar distances to those in SCR.

We did not detect genetic structure associated with the present day dams within the RN basin (i.e. locations downstream and upstream of the dams, namely Ramos Mexía, Pichi Picun Leufú and Piedra del Águila, did not differ from each other). However, these dams were built within the last 40 years, a time that might be insufficient for genetic drift to lead to differentiation (i.e. population structure) among these populations. On the other hand, sampling sites upstream and downstream of the Alicurá dam were genetically differentiated. The area where the Alicurá dam is located is characterised by steep riverbanks forming a canyon, which possibly reduced gene flow prior to the building of the dam. Similarly, the presence of gorges is known to inhibit gene flow in non-migratory *Galaxias* species in the Clutha river system of New Zealand (Burridge, Craw & Waters, 2007).

The estimates of recent migration rates between the inferred genetic clusters suggest that migration occurs predominantly in the upstream direction. Pollard (1971) and Chapman *et al.* (2006) observed upstream migration of landlocked *G. maculatus* populations in Australia. Accordingly, Barriga *et al.* (2007) documented upstream migration of metamorphic larvae of *G. maculatus* from Alicurá reservoir into Caleufu River. Our results show migration rates of 20% from the genetic group that includes Caleufu River individuals towards the headwater lakes of this river. Upstream migration has also been observed in Piedra Buena in the SCR basin (R. Hudson, pers. comm., Piscicultura Harengus S.A., Santa Cruz, Argentina). We obtained very low or zero estimates of downstream migration, suggesting that upstream migration is performed by a large number of larvae, whereas downstream migration is performed by a small number of individuals, most likely adults.

The presence of Sr in the otoliths of some individuals close to the mouth of SCR indicates that individuals with different life histories can be found in sympatry (e.g. no genetic structuring within individuals from Piedra Buena was detected suggesting that similar genotypes have different phenotypes). Facultative diadromy of *O. mykiss* populations in SCR has been previously reported (Pascual *et al.*, 2001; Riva-Rossi *et al.*, 2007), which could imply that the ecological conditions of this basin can result in the expression of the same life-history strategy in different taxa. The high genetic diversity observed among Piedra Buena individuals suggests that this is a large population, and the possibility of genetic exchange with other diadromous populations should not be discarded, for example populations in Tierra del Fuego (Boy, Morriconi & Calvo, 2007; Boy *et al.*, 2009) and along the Chilean coast (Zemlak *et al.*, 2010). Waters *et al.* (2000) showed high intraspecific genetic diversity in diadromous populations of the species, indicating life in the sea allows a higher gene flow between populations compared with resident populations. Future studies should examine the connectivity between Atlantic coastal populations in Patagonia.

The difference in vertebral number found between basins follows the latitudinal pattern described by McDowall (2003a) for diadromous populations (i.e. an increase in vertebral counts with latitude). Resident populations sampled in this study followed the same pattern. All individuals in SCR had vertebral numbers corresponding to the range observed by McDowall (2003a) for diadromous populations of *G. maculatus* (except for one individual in Roca Lake), even though we found no evidence of diadromy upstream of Condor Cliff. However, individuals from Piedra Buena had higher counts than remaining individuals at a similar latitude, pointing to a relationship between vertebral number and the presence/absence of diadromy. Disentangling the causes of such complex patterns of variability is not simple and will require future experimental efforts.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Hierarchical analysis implemented with (a) STRUCTURE and (b) STRUCTURE HARVESTER.

Figure S2. Results from STRUCTURE HARVESTER, showing K = 3 for the first round of analysis including 17 sampling localities along Río Negro and Santa Cruz river basins.

Table S1. Allelic frequencies per loci and per locality along Río Negro and Santa Cruz River.

Table S2. Paiwise F_{ST} between localities in (a) Río Negro basin and (b) Santa Cruz River basin.

Appendix S1. Paiwise FST between localities in Río Negro basin.

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