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Metapopulation dynamics of a diadromous galaxiid fish and potential effects of salmonid aquaculture

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SUMMARY

1. Direct ecological effects of biological invasions have been widely documented, but indirect genetic effects on native species are poorly known. In many cases, this is because of the lack of information on the genetic structure of species affected by invasions.
2. We used microsatellite DNA loci to estimate the genetic structure and gene flow patterns of *Galaxias maculatus*, a galaxiid fish endemic to the southern hemisphere, which is increasingly being threatened by salmonid invasions.
3. Analysis of nine diadromous populations of *G. maculatus* in Chilean Patagonia (an area heavily impacted by farming of non-native salmonids) indicates that dispersal is mostly a passive process, seemingly driven by wind and currents and resulting in high gene flow and weak population structuring.
4. Gene flow was asymmetrical, with three populations acting as sources and six populations acting as sinks. Sinks had lower habitat quality and had a greater incidence of adults than sources, which consisted mostly of juveniles.
5. Rivers invaded by salmonid escapees experienced significantly higher aquaculture pressure than rivers where salmonid escapees were apparently absent, but no effect on genetic diversity of *G. maculatus* could be detected.
6. We discuss whether salmonid aquaculture might affect the demography and connectivity of galaxiid metapopulations: indirectly through habitat alteration and directly through escapes of predatory fish.

Keywords: aquaculture, *Galaxias maculatus*, galaxiids, metapopulation, source–sink

Introduction

Biological invasions represent one of the most important threats to aquatic biodiversity, particularly for freshwater fish that are the second-most endangered vertebrates after amphibians (Cambray, 2003). Aquatic invaders can alter ecological conditions through predation, competition or introduction of new pathogens (Lodge, 1993), and these can have important evolutionary consequences for native species (Sakai *et al.*, 2001; Sax *et al.*, 2007).

Hybridisation and genetic introgression are the most commonly reported genetic consequences of invasions (Rhymer & Simberloff, 1996; Echelle & Echelle, 1997), and the ability of native fish to survive in the face of biological invasions may depend strongly on demography, genetic structure and population diversity.

Galaxiids tend to dominate the relatively impoverished fish fauna of the cool-temperate freshwater systems of the southern hemisphere (McDowall, 2006). However, they are increasingly endangered by the introduction of exotic salmonids (McDowall, 2006; Garcia de Leaniz, Gajardo & Consuegra, 2010), which in Chile have become widespread owing to the escape of an estimated 4 million salmon and trout from fish farms each year (Arismendi *et al.*, 2009). In Chilean Patagonia, exotic salmonids now represent 95% of the total biomass in many lakes and rivers (Soto *et al.*, 2006). The absence of large native predatory fish has resulted in low biotic resistance of native fish communities

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to salmonid invaders (Young *et al.*, 2009, 2010). However, there is little or no information on the conservation status of native galaxiids prior to salmonid introductions, and current knowledge is largely based on the abundance and distribution of species that are still poorly known (Habit *et al.*, 2010; Vanhaecke *et al.*, 2012).

Galaxias maculatus (Jenyns, 1842) is a widespread galaxiid that has suffered major local declines in abundance in response to the spread of non-native poeciliids (Habit *et al.*, 2010), as well as salmonids (McDowall, 2006). Most populations of *G. maculatus* are diadromous and semelparous, making them particularly sensitive to alterations in accessibility to spawning grounds and perturbations of connectivity between populations (McDowall, 1996, 1998, 2006). As the larvae of *G. maculatus* disperse out to sea soon after hatching in estuarine banks (McDowall, Robertson & Saito, 1975; McDowall, 2006), ocean currents can be expected to affect the gene flow (Knutsen *et al.*, 2007), either facilitating or hampering population connectivity depending on hydrography and spatial scale (Waters, Dijkstra & Wallis, 2000; Zemlak *et al.*, 2010). In addition, because diadromous fishes are affected by multiple disturbances across different spatial scales (McDowall, 1992), local differences in birth and mortality rates can give rise to source–sink dynamics, where negative population growth at sinks is compensated by immigration from sources, either by active or by passive dispersal (Pulliam, 1988).

Understanding the connectivity patterns of native fishes is essential for predicting the likely genetic impacts of salmonid invasions in Patagonia and other areas of the southern hemisphere, as the effects of invasive salmonids may extend beyond individual waterbodies (Pilliod *et al.*, 2010) and differ depending on propagule pressure and location of aquaculture facilities (Arismendi *et al.*, 2009; Consuegra *et al.*, 2011). We employed microsatellite markers to analyse the genetic structure and connectivity of nine diadromous populations of *G. maculatus* in the Gulf of Ancud, off Chiloé Island. Chiloé sustains the highest densities of open-net salmonid cages and salmonid escapees in Chile and possibly anywhere in the world (Muñoz, 2006; Soto *et al.*, 2006; Buschmann *et al.*, 2009). Current declines in native fish fauna have been causally linked to the spread of invasive salmonids because of predation and resource competition (McDowall, Allibone & Chadderton, 2001; Glova, 2003; Townsend, 2003; Macchi, Pascual & Vigliano, 2007; Arismendi *et al.*, 2009; Penaluna, Arismendi & Soto, 2009; Young *et al.*, 2010), and we have recently shown that many of these invaders originate from salmonid farms nearby (Consuegra *et al.*, 2011).

At the spatial scale of our study, two general dispersal scenarios were envisaged: (i) random dispersal of *G. maculatus* with no homing, which would result in panmixia and little or no genetic differentiation among rivers, and (ii) population structuring resulting from isolation by distance and genetic differentiation. We therefore expected different possible impacts of invasive salmonids, depending on the mode of dispersal of *G. maculatus*. In particular, we predicted that if *G. maculatus* populations were strongly structured and showed restricted dispersal, then (i) populations located closest to salmonid farms would have the lowest genetic diversity and effective population sizes as a consequence of direct impacts by salmonid escapees (predation and/or displacement) and (ii) gene flow would be restricted in those rivers most affected by salmonid farming. On the other hand, if *G. maculatus* displayed panmixia and extensive dispersal, any impact of fish farms as localised stressors would be much reduced, as populations would exchange migrants from affected and unaffected areas.

Methods

Study area and fish sampling

We employed single-pass electro-fishing (Smith-Root, Inc LR-24 Electrofisher, Vancouver, WA, USA) or fly fishing (one river) to sample *G. maculatus* in nine streams on the north-east of the Island of Chiloé and the Reloncaví inner sea during 2007–2009 (Fig. 1). We concentrated our sampling in the lower 30- to 350-m reaches of the study streams, as these will often represent the main invasion routes for aquaculture escapees (Young *et al.*, 2010; Consuegra *et al.*, 2011). All fish were identified to species, total length (mm) and weight (g) were recorded, and fin clips were collected for at least 28 *G. maculatus* per site and kept in 95% ethanol for subsequent analyses. *Galaxias maculatus* were classified as juveniles or adults according to pigmentation (translucent for juveniles, pigmented for adults) and the modes of a size frequency distribution of 1418 fish; these results confirmed the 50- to 55-mm-size cut-off for juvenile-to-adult transition found in other regions (McDowall, 2006). At each sampling site, we estimated the relative abundance of fines in the stream substratum and the percentage of substratum covered by submerged vegetation (Young *et al.*, 2010), as these have been shown to be important determinants of habitat quality and survival of *G. maculatus* and other galaxiids (Young *et al.*, 2010; Hickford & Schiel, 2011). Differences in silt and submerged vegetation among rivers were tested by Mann–Whitney tests, and fish abundance and temporal changes in size were compared using Fisher's exact tests and ANOVA, respectively. All statistical tests

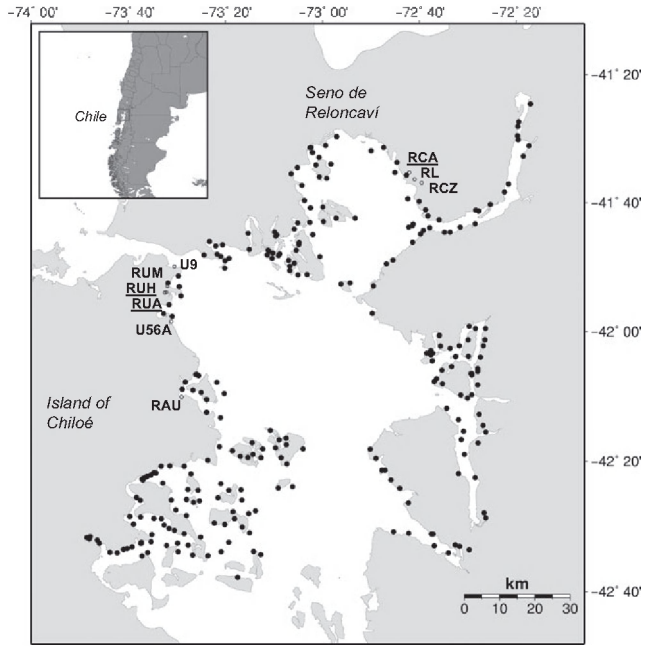

Fig. 1 Map of the Gulf of Ancud (Chiloé Interior Sea) showing the location of salmonid farms (dots) and *Galaxias maculatus* populations acting as genetic sources (river codes underlined, n = 3) and genetic sinks (river codes in normal typeface, n = 6) according to the direction and magnitude of gene flow.

were carried out in SYSTAT v.11 (Systat Software Inc., London, UK).

Microsatellite genotyping

Genomic DNA was isolated using the Wizard SV96 Genomic Purification kit (Promega, Southampton, UK). All samples were amplified for ten microsatellite loci in two multiplex reactions of five microsatellites each: (i) Gmac4, Gmac6, Gmac8, Gmac9, Gmac10 and (ii) Gmac1, Gmac2, Gmac3, Gmac5, Gmac7 (Carrea *et al.*, 2009) using the Qiagen Multiplex Kit (Qiagen, Crawley, West Sussex, UK). PCR products were resolved on an ABI3130xl sequencer (Applied Biosystems, Paisley, UK) and analysed using GENEMAPPER v 4.0 (Applied Biosystems). A minimum of 44 individuals were rescored for all loci in order to estimate the repeatability of scoring. All microsatellite loci were examined for potential errors in genotyping (null alleles, allele dropout and false alleles) using MICRO-CHECKER (Van Oosterhout *et al.*, 2004).

Genetic diversity and effective population size analyses

Linkage disequilibrium between microsatellite loci was computed using GENEPOP (Raymond & Rousset, 1995), and all markers were tested for neutrality using the Fdist2 method implemented in LOSITAN (Antao *et al.*, 2008) under the stepwise mutation model (SMM) (Valdes, Slatkin & Freimer, 1993). Tests for Hardy–Weinberg equilibrium were conducted using TFPGA v. 1.3 (Miller, 1997), and levels of significance were adjusted by sequential Bonferroni correction for multiple tests (Rice, 1989). We also used TFPGA to estimate the allele diversity, as well as observed and expected heterozygosities (H_o, H_e). Allelic richness (A_R) was calculated by FSTAT v.2.9.3.2 (Goudet, 1995), with a minimum sample size of five individuals per locus. Pairwise genetic distances F_{ST} and D_{est} (Jost, 2008) were calculated in FSTAT (10 000 permutations) and SMOGD (Crawford, 2010), respectively. Pairwise allelic differences between populations were calculated by GENEPOP using 10 000 iterations. Differences in heterozygosity, allelic richness and N_e between groups were estimated with a two-sample t-test, and the probability was estimated using 1000 bootstrapping iterations in SYSTAT 11.

We used two different methods to estimate the effective population size (N_e) of *G. maculatus* populations, as our data were based on a single temporal sample per population. Our first N_e estimate was based on the full-likelihood sibship model implemented in COLONY2, which assumes a polygamous breeding system without prior information (Wang, 2009). A second estimate of N_e was obtained with an approximate Bayesian approach implemented in ONESAMP (Tallmon *et al.*, 2008) with a prior of N_e max = 500. The two approaches (COLONY2 and ONESAMP) had been shown to produce the most reliable results in a comparison of different methods using similar sample sizes and number of markers as in our study (Beebee, 2009).

Number of migrants per generation (Nem) were estimated between all pairs of populations using microsatellites by the maximum-likelihood approach implemented in MIGRATE v.3.0.3 (Beerli & Felsenstein, 1999) with 20 short chains, two long chains and four chains under a heating scheme with temperatures 1.00, 1.20, 1.50, 3.00 (Hemmer-Hansen *et al.*, 2007). The results of two independent runs of the data set were congruent. Migration rates were also estimated between the regions (Chiloé Island and Seno Reloncaví), pooling the populations within each region in order to reduce the number of parameters estimated in the model.

Populations were tested for recent bottlenecks using BOTTLENECK v.1.2.02 (Piry, Luikart & Cornuet, 1999) and for recent expansion using the k test (within locus) (Reich & Goldstein, 1998) implemented in KGTESTS (Bilgil, 2007). Significances of the k test values were determined by the proportion of loci giving a positive k value using a

one-tailed binomial distribution (Reich, Feldman & Goldstein, 1999).

Population structuring

We used POWSIM (Ryman & Palm, 2006) to assess the statistical power of our tests for genetic homogeneity. Genetic structuring was examined using the hierarchical analysis of molecular variance (AMOVA) implemented in ARLEQUIN, using Seno Reloncaví versus Chiloé as regional groups and significance derived from 10 000 permutations. To examine the extent of isolation by distance (IBD) among study populations, we regressed genetic distance – measured as $F_{ST}/(1 - F_{ST})$ – on geographical distance using IBDWS v.3.16 and conducted 10 000 iterations to derive significance values.

We also estimated the most likely number of genetically homogeneous groups or genetic clusters (K) using TESS 2.3.1 (François, Ancelet & Guillot, 2006). We used the admixture model and ran 100 replicates for each K between 2 and 9. The maximum number of clusters (K max) was inferred from the Deviance Information Criterion (DIC) (Spiegelhalter *et al.*, 2002). Results from the 20 lowest DIC replicates were averaged and represented using DISTRUCT 1.1 (Rosenberg, 2004).

Propagule pressure from salmonid aquaculture

Coordinates of each sampling site were obtained using a GPS (GARMIN Colorado™ Series), while coordinates of each of the registered salmonid farms in the study area were obtained from official sources (Department of Aquaculture, SUBPESCA, September 2008), from the Chilean Aquaculture Farm Guide (4th Edition 2001, La Tene Maps, http://www.latenemaps.com) and from Google Earth (Fig. 1). Salmonid escapees cannot always be distinguished visually from naturalised salmonids in Chile (Schröder & Garcia de Leaniz, 2011; Consuegra *et al.*, 2011), as some fish may escape at a young age or may display plastic phenotypes. We used therefore an index of aquaculture propagule pressure to estimate the likely impact of salmonid escapees on *G. maculatus* populations. We assumed that rivers closer to salmonid farms were more likely to be impacted than those further away, but the form of this spatial relationship was unknown. Consequently, we calculated two indices of salmonid propagule pressure based on the cumulative weighted distance (d) from each sampling site to every salmonid farm within a 50-km radius using MATLAB (The MathWorks, Natick, MA, U.S.A.): one with a weighting that declined linearly with distance ($\Sigma 1/d$) and one with a weighting that declined with distance squared ($\Sigma 1/d^2$), thus relating aquaculture propagule pressure to an area of influence around each salmonid farm.

There are no data on the movements of salmonid escapees in Chile, but results from Norway indicate that salmonids can travel up to 25–40 km within a week after escaping from fish farms (Skilbrei & Wennevik, 2006); hence, our choice of a 50-km radius seems justified. The influence of salmonid fish farms on genetic diversity and population structuring of *G. maculatus* was then assessed by examining the strength of the nonparametric Spearman's rank correlation coefficient (rho) between genetic diversity, number of migrants per generation (H_o, Ar, N_e and Nem) and aquaculture propagule pressure, as calculated above.

Salmonid CPUE (catch per unit effort) was estimated as a measure of relative abundance, taking into account

Table 1 The study rivers and their sample sizes of *Galaxias maculatus* (N), salmonid catch per unit effort (fish/min) and estimated salmonid aquaculture propagule pressure based on linear ($\Sigma 1/d$) or random diffusion ($\Sigma 1/d^2$) distance models to each salmonid farm within a 50-km radius

River	Area	Code	Latitude	Longitude	N	Salmonid CPUE	Propagule pressure	
							$\Sigma(1/d)$	$\Sigma(1/d^2)$
Pte. Metri	Seno Reloncaví	RCA	−41.588	−72.701	30	0.96	4.67	1.09
R. Lenca	Seno Reloncaví	RL	−41.606	−72.683	30	0.85*	4.30	0.40
R. Chaica	Seno Reloncaví	RCZ	−41.616	−72.658	33	0.17†	4.19	0.29
R. Aucar	Chiloé Island	RAU	−42.167	−73.483	28	2.14	5.67	0.58
R. Unnamed 9	Chiloé Island	U9	−41.832	−73.508	35	0.00	3.17	0.31
Pte. Maquina	Chiloé Island	RUM	−41.883	−73.532	29	0.00	4.14	1.61
Pte. Huenopoicohue	Chiloé Island	RUA	−41.900	−73.542	50	0.00	3.40	0.42
Pte. Huelden	Chiloé Island	RUH	−41.899	−73.536	50	0.00	3.52	0.46
Pte. Metrenquen	Chiloé Island	U56A	−41.974	−73.518	50	0.00	3.93	0.68

*Fly fishing.
†Minimum estimate.

effective fishing effort (minutes). Salmonids were not detected in five rivers on the north-east coast of Chiloé Island (Table 1; Young *et al.*, 2010). H_o, Ar and N_e were compared between populations with and without salmonids using Mann–Whitney nonparametric tests implemented in SYSTAT v. 11.

Results

Genetic diversity

Repeatability of allele scoring was high for all loci (average 94.8%). Eight populations showed evidence of significant deviations from Hardy–Weinberg (HW) equilibrium at one to three loci after Bonferroni correction (Supplementary information, Table S1), in all cases because of the deficiency of heterozygotes. We found no evidence of allele dropouts or scoring errors owing to stutter peaks. However, results from MICROCHECKER indicated that one of the microsatellites showed evidence of null alleles in six of nine populations (Gmac 7). Evidence of null alleles was also found for Gmac 5, Gmac 6 and Gmac 2, albeit only in one population (Gmac 6, Van Oosterhout value = 0.21; Gmac 2, Van Oosterhout value = 0.20) and two populations (Gmac 5 Van Oosterhout values = 0.22–0.24). Evidence of selection was found for two microsatellites (Gmac 4 and Gmac 7), and these were excluded from further analyses. Analyses of genetic differentiation, genetic variation and migration estimates were carried with and without loci that presented HW deviations in some of the populations (Gmac 3, Gmac 5 and Gmac 6), but as the results did not change the direction or significance of the results, we included them.

Analysis of linkage disequilibrium was significant in only 6% of a total of 424 pairwise comparisons (between loci and populations, data not shown). Our simulations using POWSIM estimated that we had power of at least 0.99 to reject the null hypothesis of no differentiation with an $F_{ST} = 0.0025$ under different scenarios of N_e and number of generations of drift (t). Comparisons between populations from Seno Reloncaví and populations from Chiloé showed no significant difference in either allelic richness (range = 6.37–6.86, $P = 0.61$) or heterozygosity (range = 0.66–0.73, $P = 0.19$).

Estimates of effective population size, bottlenecks and signatures of expansion

Estimates of contemporary effective population size (N_e) of *G. maculatus* were similar between methods. Effective population sizes computed by ONeSAMP were in general low for all populations, ranging from 16 to 296 (Table 2). Estimates of effective population size by the sibship method implemented in COLONY2 also indicated low to moderate effective population sizes (range 62–138, data not shown).

Table 2 Effective population size (N_e) and 95% confidence intervals estimated with ONeSAMP (Bayesian method). See Table 1 for explanation of population codes

		ONeSAMP (N_e max = 500)		
Population	N	N_e	0.05	0.95
RCA	30	16	14	18
RL	30	17	13	22
RCZ	33	289	192	465
RAU	28	294	170	620
U9	35	54	41	71
RUM	29	16	14	19
RUA	50	296	225	403
RUH	50	20	18	21
U56A	50	53	45	64

We did not find a significant evidence of recent bottlenecks in any of the populations (results not shown). We found evidence of recent expansion in five populations using the k test (RCZ, RUA, RUH and U56, $P = 0.03$; U9 $P = 0.003$).

Population structuring

Exact tests of genetic differentiation between juveniles and adults from the same population were not significantly different ($P = 1.000$) and were therefore pooled for analyses. After Bonferroni correction, 11 of 36 pairwise comparisons (31%) revealed significant differences in allelic frequencies among populations (data not shown). Overall population differentiation estimated by microsatellites was low ($F_{ST} = 0.0023$) but significantly different from random expectations ($P = 0.0001$). Pairwise F_{ST} comparisons between all populations ranged between 0.0002 and 0.007 (Table 3). D_{est} values were also low and generally showed the same pattern of population differentiation as F_{ST} (Spearman's rank correlation coefficient, $\rho = 0.7257$, $n = 36$, $P < 0.0001$; Table 3). We found a significant pattern of increasing genetic differentiation with geographical distance (Mantel test: $z = 5.3157$, $r = 0.458$, $P = 0.005$) and a significant positive correlation between the number of immigrants and river latitude ($r = 0.793$, $P = 0.011$), but genetic diversity was not correlated with latitude (He, $P = 0.139$; Ar, $P = 0.807$).

AMOVA analyses of populations grouped geographically (Seno Reloncaví versus Chiloé) indicated that 0.15% of variation was attributable to differences among geographical groups ($P = 0.003$), 0.28% of variation was

Table 3 Pairwise estimates of genetic distances (below diagonal F_{ST}, above diagonal D_{est}) between nine populations of *Galaxias maculatus* in Chilean Patagonia, calculated using FSTAT and SMOGD based on eight microsatellite loci. Value in boldface is significant at $P < 0.001$

	RCA	RL	RCZ	RAU	U9	RUM	RUA	RUH	U56A
RCA	–	−0.0007	0.0035	0.0138	0.0355	0.0335	0.0479	0.0271	0.0370
RL	0.0000	–	0.0017	0.0459	0.0523	0.0000	0.0099	0.0317	0.0163
RCZ	0.0019	0.0000	–	0.0899	0.0491	0.0287	0.0454	0.0304	0.0504
RAU	0.0000	0.0014	0.0080	–	0.0259	0.0043	0.0454	0.0457	0.0083
U9	0.0041	0.0025	0.0048	0.0007	–	0.0106	0.0299	0.0079	0.0027
RUM	0.0036	0.0026	0.0007	0.0008	0.0005	–	0.0026	0.0070	0.0117
RUA	0.0044	0.0011	**0.0067**	0.0025	0.0004	0.0000	–	0.0075	0.0456
RUH	0.0037	0.0016	0.0039	0.0050	0.0001	0.0000	0.0001	–	0.0385
U56A	0.0041	0.0036	0.0040	0.0000	0.0015	0.0037	0.0045	0.0054	–

attributable to differences among populations between groups ($P = 0.017$) and 99% of genetic variation was attributable to differences among individuals within groups. Results from TESS indicated that there was no population structuring. Analyses with $K = 2$ resulted in admixture values different from 50/50 in 98% of cases, with q values ranging between 0.01 and 0.91 represented in variable proportions within each sampling group.

Gene flow and metapopulation dynamics

The number of immigrants per generation estimated using microsatellites ranged from 0.4 to 23.4 between populations, while the number of emigrants ranged from 0.5 to 41.3 (Table 4). The resulting net migration patterns were therefore asymmetric, with six populations acting as sinks and three populations acting as sources. Taken together, populations in the Island of Chiloé donated more fish than they received, while populations in mainland Chile (Seno Reloncaví) received more fish than they donated. These results were fully congruent with the estimates of migration when populations from each geographical area were pooled [average migration from Seno Reloncaví to Chiloé = 19 (range 30–75) and average migration from Chiloé to Seno Reloncaví = 56 (range 0–40)].

Source and sink populations of *G. maculatus* differed greatly in average body size and in the juvenile-to-adult ratio. During March–April (just before the onset of the spawning season of the species in this part of Chile, Mardones, Vega & Encina, 2008), sink populations had an average size of 70.3 mm (SE = 0.62) and were composed mostly of adults (96%). In contrast, source populations had an average size of 55.9 mm (SE = 0.47) and were composed mostly of juveniles (55%, Fig. 2). Thus, the relative abundance of juveniles and adults differed significantly between sinks and sources (Fisher's exact test $P < 0.001$). Body size was significantly greater in sink than in source populations ($F_{1,560} = 213.74$, $P < 0.001$) and

Table 4 Number of migrants per generation among the nine study populations of *Galaxias maculatus* estimated using MIGRATE. Sources and sinks were estimated according to the net exchange of migrants for each population. Taken together, Chiloé populations acted as a net source (average emigrants–immigrants = 2.35) and populations in mainland Chile (Seno Reloncaví) acted as a net sink (average emigrants–immigrants = −4.7)

	Seno Reloncaví			Chiloé						
Donor	RCA	RL	RCZ	RAU	U9	RUM	RUA	RUH	U56A	Total
Recipient										
RCA	*	1.3	1.6	3.3	3.5	5.8	19.7	25.2	12.1	72.50
RL	0.74	*	0.78	4.72	4.32	19.35	17.41	13.57	25.90	86.79
RCZ	0.92	1.08	*	8.91	13.31	5.42	24.32	41.33	5.31	100.60
RAU	13.43	8.10	1.07	*	3.74	1.60	13.13	7.42	2.26	50.75
U9	11.99	15.13	12.03	3.09	*	1.10	4.31	2.19	5.91	55.75
RUM	15.63	5.00	18.95	0.89	2.56	*	1.22	1.03	1.86	47.14
RUA	9.41	11.89	11.09	8.34	1.14	0.94	*	0.52	2.57	45.90
RUH	15.69	16.75	23.35	8.26	3.01	0.60	0.45	*	2.60	70.71
U56A	12.22	16.68	20.97	4.71	3.31	1.98	2.59	3.99	*	66.45
Total	80.03	75.93	89.84	42.22	34.89	36.79	83.13	95.25	58.51	
Type	Source	Sink	Sink	Sink	Sink	Sink	Source	Source	Sink	

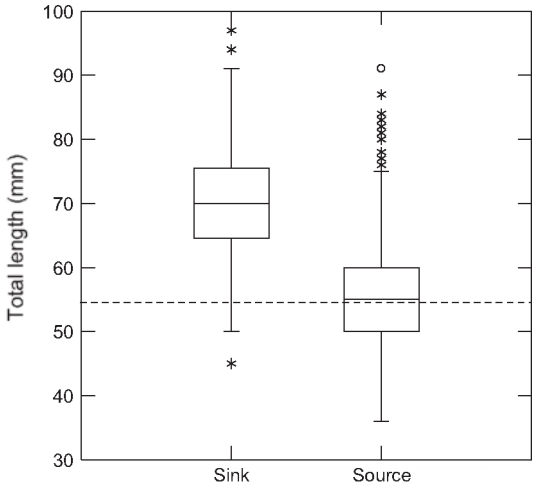

Fig. 2 Differences in body size (total length, mm) of source (n = 3) and sink (n = 6) populations of *Galaxias maculatus* in the study region. Dotted line indicates 55-mm size threshold for juvenile-to-adult transition.

increased significantly over time in sources, while it decreased significantly over time in sinks (month × type interaction $F_{1,560}$ = 61.06, $P < 0.001$), consistent with an emigration of young-of-the-year from sources to sinks.

Silt was much more abundant in the substratum of rivers that acted as genetic sinks (mean = 46.8%, SE = 15.33) than in the substratum of rivers that acted as genetic sources (mean = 1.7%, SE = 1.67; Mann–Whitney P = 0.035), while the extent of submerged vegetation did not differ significantly between sites (source = 16.7%, SE = 7.27; sink = 12.5%, SE = 5.74%; Mann–Whitney P = 0.500).

Aquaculture propagule pressure

Data on salmonid production of individual farms are confidential and not readily available, but mean farm area (a surrogate for production) was found to be 5052 m^2 (SE = 630.6) and not different among sites ($F_{2,15}$ = 0.368, P = 0.686). We used the weighted distance to salmonid fish farms as a proxy for aquaculture pressure, since all study rivers were potentially accessible to salmonid escapees. In total, we found 378 registered salmonid farms in the Chiloé Interior Sea. Salmonids were captured in relatively high abundances in four of the study rivers (Table 1). These included 117- to 480-mm rainbow trout (*Oncorhynchyus mykiss* Walbaum, four rivers) and 41- to 126-mm Atlantic salmon (*Salmo salar* Linnaeus; two rivers), which had phenotypes consistent with escapes from aquaculture facilities, as well as 72- to 280-mm resident (naturalised) brown trout (*Salmo trutta* Linnaeus; three rivers). The three salmonid species therefore included individuals well above the minimum 100-mm size threshold for piscivory in this part of Chile (Arismendi *et al.*, 2009) and therefore represented a predation threat for *G. maculatus*, particularly in the case of anadromous rainbow trout escapees, which were present in all four rivers.

Rivers invaded by salmonids experienced significantly higher aquaculture propagule pressure than rivers where salmonids were absent (Mann–Whitney test, P = 0.015; Table 1) according to a linear distance model ($\Sigma 1/d$), but had similar values when the square distance model ($\Sigma 1/d^2$) was used (P = 0.624; Table 1). These results indicate that our approximation of salmonid propagule pressure under the linear distance model (d) is a good predictor of the presence of salmonid escapees in streams inhabited by *G. maculatus* in the region. We found no significant effect of propagule pressure on any of the measurements of genetic diversity (Ar: $\rho = -0.005$, P = 0.989; H_o: $\rho = -0.341$, P = 0.368; N_e: $\rho = -0.199$, P = 0.608). In addition, there were no differences in genetic diversity or number of migrants between rivers with and without salmonids (Ar: Mann–Whitney U = 15.000, P = 0.221; H_o: U = 12.000, P = 0.624; N_e: U = 10.500, P = 0.902; M: U = 7.000, P = 0.462). Salmonids were absent in four of the five populations of *G. maculatus*, which gave a signal of expansion, and were present in three of the four non-expanding populations, but the results are not significant (Fisher's exact test, one-tailed P = 0.103).

Discussion

Our results indicate that despite the existence of high levels of gene flow, we can reject the hypothesis of panmixia in nine populations of *G. maculatus* in Chilean Patagonia. Instead, we found low but significant spatial genetic structuring and isolation by distance at a regional scale, more consistent with a metapopulation model and with sea currents probably playing a role in shaping the intensity and direction of gene flow. Thus, the observed latitudinal cline in the number of migrants suggests that larval dispersal is predominantly northwards, reflecting the prevailing wind regimes and tidal currents in the area (Cáceres, Valle-Levinson & Atkinson, 2003; Bustos, Landaeta & Balbotin, 2008). High gene flow can help prevent the loss of genetic diversity among diadromous *G. maculatus* in the short term (Ruokonen *et al.*, 2010). However, given the importance that dispersal has for the evolutionary history of this species (Waters *et al.*, 2000), a disruption of connectivity could have long-term fitness

consequences. Population connectivity can be critical for the persistence of sinks, which rely on an adequate immigration from source populations (Taylor *et al.*, 1993), and may contribute disproportionally to the overall effective size of metapopulations among migratory fish (Kuparinen *et al.*, 2010).

The high levels of gene flow detected among diadromous populations of *G. maculatus* in our study could have been overestimated by the relatively small number of microsatellite loci used, but simulations indicated that the markers employed had enough statistical power to detect population structuring at the observed level of F_{ST} differentiation. Also, recalculation of F_{ST} estimates without the three loci that showed high heterozygote deficiency yielded the same results and did not affect the magnitude and direction of gene flow estimates (data not shown). These results are also consistent with previous estimates for this species that have suggested moderate to high levels of gene flow between rivers, and some structuring among populations (Barker & Lambert, 1988; Waters *et al.*, 2000). Our F_{ST} values were also broadly similar to those estimated by others using isozymes and mtDNA for diadromous populations of *G. maculatus* sampled at similar geographical scales (F_{ST} = 0.02–0.14), whereas higher values have been reported for landlocked populations (F_{ST} = 0.188; Zattara & Premoli, 2005). The relatively high genetic structuring observed among non-migratory populations of *G. maculatus* contrasts with the high levels of gene flow observed among diadromous populations and is in agreement with levels of phenotypic variation for this species across the southern hemisphere (McDowall, 2002). It is possible that further differentiation of diadromous *G. maculatus* populations might be uncovered with markers under selection (such as Gmac 4 with a global F_{ST} = 0.05 in our study), as long as selection pressures remain temporally stable (Andre *et al.*, 2010), because loci under selection can display high levels of differentiation in marine species, even when neutral markers suggest very low variability (White, Stamford & Hoelzel, 2010).

In species with marine larval dispersal, such as *G. maculatus*, source–sink dynamics depend on both demography and connectivity (Figueira, 2009), but uncovering metapopulation structure is challenging because marking and recapture of small larvae is difficult. Instead, we used molecular markers to infer the degree of connectivity among populations and to distinguish likely sinks from sources. Gene flow among *G. maculatus* populations was asymmetrical, with three populations acting as genetic sources and six populations acting as genetic sinks. Sinks had lower habitat quality (as inferred from silt deposition) and were composed mostly of adult fish, while sources had less silt and were dominated by juveniles. We have shown previously that connectivity estimates of migratory fish based on gene flow tend to produce results congruent with dispersal estimates based on physical marking (Consuegra *et al.*, 2005; Consuegra & Garcia de Leániz, 2007), and we are therefore confident that our molecular identification of migration patterns in diadromous *G. maculatus* is sound. Thus, although our results must be interpreted with caution, given that not all existing populations in the region could be sampled, they provide a general approximation to the dispersal of *G. maculatus* in the area.

The existence of a significant interaction between month of capture and body size (with fish getting larger over time in sources, but smaller in sinks) reveals the interplay of different demographic processes in sinks and sources and is consistent with a net emigration of juveniles from sources in Chiloé Island into sinks in mainland Chile (Seno Reloncaví). This, along with results of individual assignment, adds support to the hypothesis that larval dispersal of *G. maculatus* in this area is in a northwardly direction and that this gives rise to weak structuring at the regional scale. Genetic differentiation of *G. maculatus* inhabiting these Patagonian rivers therefore seems to be explained largely by passive larval dispersal in relation to wind and currents, while demographic data would seem to support metapopulation inferences based on genetic connectivity alone. In this sense, our results are indicative of better growth of *G. maculatus* in sources, as well as of emigration of juveniles into sinks, although more data are needed to draw firm conclusions. Identification of sinks and sources is essential for efficient conservation of populations interconnected by high levels of gene flow (Cooper & Mangel, 1999), and further research would benefit from information on per capita reproductive rates of different subpopulations, as well as from knowledge on temporal stability of genetic and demographic parameters.

Predation by invasive species can result in the fragmentation of fish prey populations, particularly for migratory fish that move at different spatial scales during ontogeny (Labbe & Fausch, 2000). Predation and habitat disturbance have recently been shown to alter source–sink dynamics of native galaxiids in New Zealand, creating new demographic sinks that can make galaxiid populations more vulnerable to extinction (Woodford & McIntosh, 2010; Hickford & Schiel, 2011). An inverse association has been found previously between salmonid abundance and abundance of native fishes (including *G. maculatus*) in Patagonia (Soto, Jara & Moreno, 2001; Soto *et al.*, 2006; Arismendi *et al.*, 2009) and New Zealand

(Townsend, 1996). As salmonid escapees cannot always be identified phenotypically from naturalised (established) salmonids (Schröder & Garcia de Leaniz, 2011; Consuegra *et al.*, 2011), we used the weighted distance to salmonid fish farms as a proxy for salmonid propagule pressure. Salmonid propagule pressure was found recently to be a good predictor of relative abundance of non-native salmonids in a Patagonian lake (Arismendi *et al.*, 2009), and it was also a good indicator of the presence of salmonid escapees in the study streams (Consuegra *et al.*, 2011). However, although all the *G. maculatus* populations studied tended to have relatively small effective population size (N_e), we failed to detect any reductions in genetic diversity, effective population size or number of migrants that could be attributed to variation in salmonid propagule pressure or to the presence of salmonids in the study rivers.

There may be several reasons for this. First, it is possible that the impact of salmonid escapees may have been too recent (*c.* 30 years) or too weak to cause genetic changes upon native galaxiid species, particularly since the detection of temporal genetic shifts is hampered by the lack of baseline data before salmonids were introduced in the area. But it is also possible that extensive gene flow among *G. maculatus* may have helped buffer the potential genetic impacts of non-native salmonids because, as our study suggests, *G. maculatus* can exchange migrants from affected and unaffected rivers. Indeed, our results highlight the importance of marine dispersal for *G. maculatus*, not only at a continental scale, but also at a regional scale. The pattern of larval dispersal inferred from variation at microsatellite makers suggests that dispersal is mostly a passive process, possibly driven by tidal and wind currents, and that this results not only in high gene flow among populations, but also in weak but significant genetic structuring.

In conclusion, our study appears to represent the first analysis of the genetic structure of a native fish impacted by invasive salmonids escaping from aquaculture facilities. Yet, despite the widespread presence of predatory salmonids, we did not find any evidence to suggest that non-native salmonids might have caused reductions in genetic diversity, effective population size or number of migrants in *G. maculatus*. Nevertheless, disruption of larval dispersal of *G. maculatus*, for example as a consequence of salmonid predation or habitat changes although increased silt deposition from uneaten food and fish waste from aquaculture facilities nearby (Buschmann *et al.*, 2006, 2009), could result in the isolation of local populations, threatening the long-term persistence of sink–source metapopulations. Thus, further research examining the relationships between aquaculture propagule pressure, salmonid abundance and genetic diversity of native galaxiids is needed to elucidate the potential long-term genetic effects of salmonid invasions.

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Supporting Information

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

Table S1. Measurements of genetic diversity and results of the exact tests for Hardy-Weinberg equilibrium tests for Galaxias maculatus populations estimated using 8 microsatellites.

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