



Symposium Article

Contrasting Genetic Structure and Diversity of *Galaxias maculatus* (Jenyns, 1848) Along the Chilean Coast: Stock Identification for Fishery Management

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Abstract

Galaxias maculatus (Pisces: Galaxiidae) commonly known as “puye” has a disjunct distribution along the Southern Hemisphere including landlocked and migratory populations at latitudes over 30°S in South America, Australia, Tasmania, and New Zealand. Chilean artisanal fishery of *G. maculatus* has become less important as a resource due to multiple factors including overexploitation, pollution, introduction of predators, and competitors. At the same time, the current conservation status of the species in Chile is still uncertain. Here, we used mtDNA control region sequences (925 bp) to investigate main patterns of genetic diversity and structure in populations from 2 biogeographic areas along the Chilean coast. Extremely high levels of genetic diversity characterize the species, suggesting a low amount of influence of the last glacial cycle over its demography compared with other studies in freshwater and marine South American fishes. However, we recognized contrasting genetic patterns between the Intermediate Area (between 30°S and 42°S) and the Magellanic Province (between 42°S and 56°S). On the one hand, over a narrow geographical range (<200 km) each Intermediate Area estuarine population constitutes a different genetic unit. On the other hand, the Magellanic populations of the species exhibited low levels of differentiation in an area extending for more than 500 km. Such differences may be a consequence of different coastal configurations, oceanographic regimes, and Quaternary glacial histories. Finally, our results support the existence of different stock units for *G. maculatus* and this information should be integrated in future management strategies and aquaculture programs for this species.

Resumen

Galaxias maculatus (Pisces: Galaxiidae) comúnmente conocido como “puye” tiene una distribución disjunta a lo largo del Hemisferio Sur que incluye poblaciones residentes y migratorias a

latitudes sobre los 30°S en Sudamérica, Australia, Tasmania, y Nueva Zelanda. El recurso *G. maculatus* ha adquirido menor importancia para la pesquería artesanal chilena debido a múltiples factores incluyendo la sobreexplotación, la contaminación, la introducción de depredadores y la competencia. Al mismo tiempo, el estado de conservación de la especie en Chile es aun incierto. En este trabajo, utilizamos secuencias de la Región Control del mtDNA (925 pb) para investigar los patrones generales de diversidad y estructura genética incluyendo poblaciones ubicadas en dos áreas biogeográficas a lo largo de la costa Chilena. Niveles extremadamente altos de diversidad genética caracterizan a la especie lo que sugiere una baja influencia del último ciclo glacial sobre su demografía comparado con otros estudios realizados en peces sudamericanos marinos y de agua dulce. No obstante, se reconocen patrones de diversidad y estructura genética contrastantes entre el Área Intermedia y la Provincia Magallánica. Por un lado, a lo largo de un rango geográfico estrecho (> 200 kms) cada una de las poblaciones estuarinas del Área Intermedia constituye una unidad genética diferenciada. Por otro lado, las poblaciones magallánicas de la especie exhiben bajos niveles de diferenciación genética a lo largo de un área que se extiende por más de 500 kms. Tales diferencias pueden ser una consecuencia de diferencias en sus configuraciones costeras, regímenes oceanográficos, y sus historias glaciales Cuaternarias. Finalmente, nuestros resultados apoyan la existencia de distintas unidades de stock en *G. maculatus* y esta información debe ser integrada en futuras estrategias de manejo y en programas de acuicultura para la especie.

Subject areas: Population structure and phylogeography, Conservation genetics and biodiversity

Key words: Control region, Conservation, Galaxiidae, Genetic stock, mtDNA, Phylogeography

One of the main goals of most fisheries management and conservation plans is to supervise resources on a stock-specific basis to preserve the diversity and the evolutionary potential of the species as a whole (Dizon et al. 1992). Otherwise, stocks are of primary interest in fisheries management because the population replenishing effects of recruitment and reducing effects of mortality operates independently upon individual stocks. Indeed, to identify, discriminate and determine stock composition are integral elements of fishery management and constitutes the juncture between fishery biology and fish microevolution (Waldman 1999). The term “stock” includes many definitions and a widely accepted is consistent with concept of population: an intraspecific group of randomly mating individuals with temporal or spatial integrity (Dizon et al. 1992; Waldman 1999; 2005) which may exhibit differences in life history parameters compared with other stocks of the same species (Begg and Waldman 1999). Such differences in life history parameters are usually a consequence of particular strategies to which fish stocks have evolved, reflecting the underlying dynamics of populations showing different parameters such as abundances, growth, survival, physiology, behavioral, reproduction, maturity, and recruitment (Ihssen et al. 1981; Begg et al. 1999). Although classical fisheries approaches are focused on factors driving short-term demographic changes in stock units, the application of genetic principles to fisheries and management has increased interest in those factors sustaining the dynamics and resilience of exploited species (Cárdenas et al. 2009). In this context, adequate management strategies and culture programs require an understanding about the geographic partition of genetic diversity within a target resource (Shaklee and Bentzen 1998; Cárdenas et al. 2009).

The Family Galaxiidae includes ~50 species of Gondwanan-distributed freshwater fishes at cool-temperate regions. Some species present a migratory life stage with a salt-tolerant larval phase with considerably greater dispersal capabilities than landlocked or resident ones (Zattara and Premoli 2005). Among them, *Galaxias maculatus* is one of the most widely distributed freshwater fish in the planet.

Resident and migratory populations are currently found in southern Australia, Tasmania, New Zealand, and surrounding islands, as well as in South America and the Falkland/Malvinas Islands (McDowall 1970). The distribution of the species has been explained by vicariance (Rosen 1978) and dispersal (McDowall 1978) hypotheses, but molecular evidence suggests that dispersal occurred from Australia towards other areas of the Southern Hemisphere following the West Wind Drift (Waters and Burrige 1999; Waters et al. 2000; Burrige et al. 2011).

Along the Pacific coast of South America, *G. maculatus* is found in freshwater and coastal areas along 2 main biogeographical marine areas: the Intermediate Area (between 30°S and 42°S) and the Magellanic Province (between 42°S and 56°S; Camus 2001). Major differences in terms of oceanographic regimes, geologic, and climatic processes explain a 42°S biogeographical break along the Chilean coast that constitutes a boundary for many marine (Camus 2001) and freshwater species (Dyer 2000). The species has a maximum size of 17 cm; the migratory juvenile stage is transparent or “crystalline” with a life span that can last for up to 7 months (McDowall et al. 1975; Mardones et al. 2008). Major differences in the reproductive behavior between migratory and resident populations of *G. maculatus* have been reported (Vega et al. 2013). For instance, the reproduction in migratory populations occurs during summer and autumn whereas resident ones have several spawning periods during the year (Peredo and Sobarzo 1993; Peredo and Sobarzo 1994). The species is commercially exploited in Chile, Argentina and New Zealand due to the great value of the crystalline phase (28–83 USD/kg) and is considered a luxury product (Mardones et al. 2008). Chilean artisanal fishery of *G. maculatus* is restricted to estuarine areas between the Tolten (39°S) and the Aysen (45°S) rivers and is mainly based on the capture of crystallines during spring. However, according to Vega et al. (2013), *G. maculatus* has become less important as a fishery resource due to overexploitation (Mardones et al. 2008), pollution (Pequeño 1987), introduction of predators (Macchi 2004; Habit et al. 2012), and competitors (Rowe et al. 2007). However, *G. maculatus* was included again in

the fishing Chilean statistics of SERNAPESCA in 1990, after being absent for decades (Vega et al. 2013). Currently, *G. maculatus* captures are low with ~1 ton/year (SERNAPESCA 2009, 2010) and are not enough to sustain local demand (Vega et al. 2013). In Chile, *G. maculatus* is considered as vulnerable (Habit et al. 2006), but Vila and Pardo (2006) regarded it as vulnerable along Central Chile and as out of danger in the Magellanic Province. No information concerning the conservation status of the species is available in the IUCN Red List of endangered species (<http://iucnredlist.org>). Finally, according to Vega et al. (2013), the collapse of the Chilean *G. maculatus*' fishery necessitated repopulation programs for the species through the development of massive farming technologies or "galaxiculture" (Bariles et al. 2003) following the experience in other countries (Mitchell 1989).

Genetic-based studies have provided important information in the delimitation of stock units for sustainable fisheries management (Carvalho and Hauser 1994; Thorpe et al. 2000; Cárdenas et al. 2009). Most marine and anadromous fish species that sustain harvest do so on more than one stock, and multiple stocks may also exist in distinct freshwater and marine bodies (Waldman 1999). In the particular case of *G. maculatus* in South America, no population-based studies have been performed to evaluate patterns of genetic structure in the species along its distribution in the Pacific coast of South America. In this context, stock structure information is required for efficient management of the species and aquaculture programs. However, phylogeographic analyses in the species along its distribution in the Chilean coast recognized high levels of genetic diversity and a minor influence of the glacial cycles on its demography (Zemlak et al. 2010; Zemlak et al. 2011). Female population sizes remained relatively constant until ~500

Ka when population sizes increased exponentially, reaching contemporary levels.

The present study constitutes the first broad-scale population level study in *G. maculatus* along its distribution in the Chilean coast, including populations from the Intermediate Area and the Magellanic Province, 2 areas where the species has been historically exploited. We used nucleotide sequences of the mtDNA control region from *G. maculatus* samples to describe the geographic distribution of its genetic diversity, to infer the genetic population structure along the Chilean coast and to provide new information for stock identification and therefore fisheries and aquaculture management of this resource. Considering the broad distribution of the species, *G. maculatus* constitutes a suitable model to evaluate how historical biogeographical processes are related to current patterns of genetic diversity and structure along a latitudinal gradient. In this context, the Chilean coast constitutes an interesting area to evaluate the relative influence of habitat discontinuities, oceanography, biogeography, and historical events on the spatial distribution of genetic diversity in marine organisms.

Material and Methods

Sample Collection, DNA Extraction, PCR Amplification, and Sequencing

A total of 288 individuals were collected in 2007–2013 in estuarine areas at 4 localities of the Intermediate Area and at 5 localities in the Magellanic Province (Figure 1; Table 1). Whole specimens were fixed in ethanol (95%), and DNA was extracted using a salting-out method (Aljanabi and Martinez 1997). A partial fragment of the

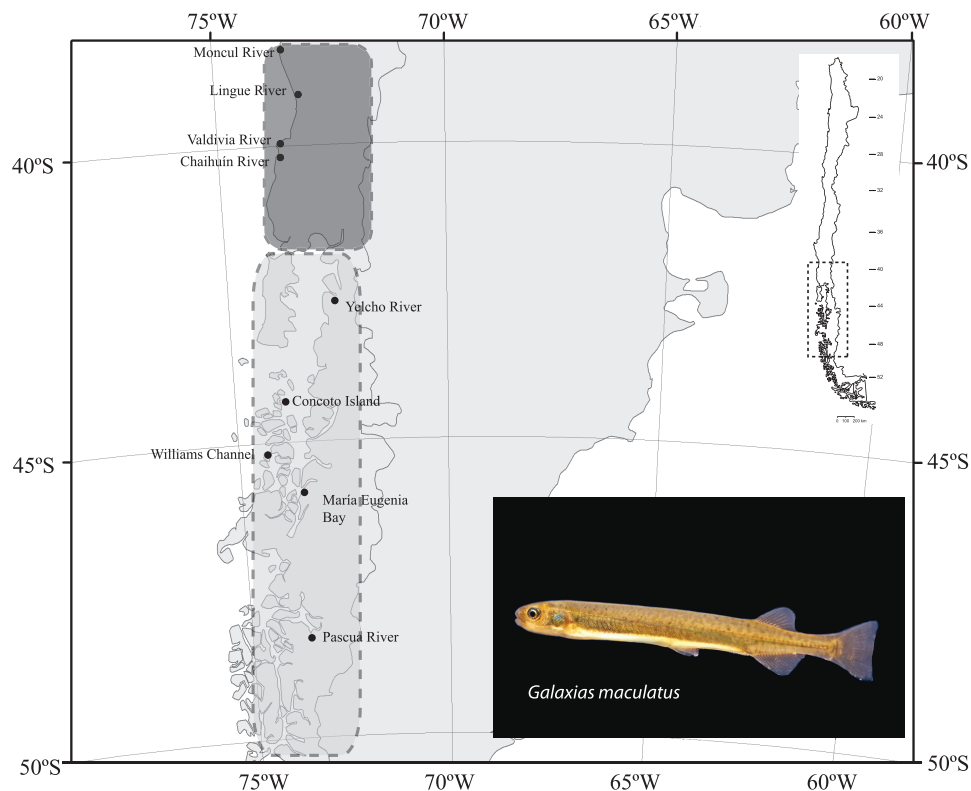


Figure 1. Sampling localities of *Galaxias maculatus* along 2 biogeographical provinces in the Chilean coast. Dark grey = Intermediate Area and Light grey = Magellanic Province.

Table 1. Standard diversity indices and neutrality tests in *G. maculatus* from the Chilean coast

Locality	Latitude	Longitude	<i>N</i>	<i>K</i>	<i>H</i>	<i>S</i>	Π	π	TAJIMA'S D	FU'S FS	M.D.
1 Moncul River	38°42' S	73°24' W	24	24	1.000	87	21.21	0.023	-0.726	-9.83***	M
2 Lingue River	39°26' S	73°09' W	24	20	0.982	81	23.68	0.027	-0.016	-2.18	M
3 Valdivia River	39°53' S	73°24' W	28	24	0.989	69	24.65	0.026	1.00	-3.79*	M
4 Chaihuín River	40°01' S	73°27' W	26	22	0.978	102	25.45	0.027	-0.66	-2.72*	M
Intermediate Area			102	86	0.988	134	32.80	0.036	0.174	-33.44***	M
5 Yelcho River	43°03' S	72°33' W	22	22	1.000	73	15.83	0.017	-1.18	-10.58***	M
6 Concoto Island	44°06' S	73°43' W	26	24	0.994	54	10.88	0.011	-1.09	-12.79***	M
7 Williams Channel	45°25' S	74°21' W	26	17	0.938	42	10.66	0.011	-0.36	-2.71	M
8 María Eugenia Bay	45°55' S	73°31' W	23	23	1.000	63	15.33	0.016	-0.63	-11.71***	M
9 Pascua River	48°15' S	73°18' W	42	40	0.998	75	13.28	0.014	-1.18	-28.45***	M
Magellanic Province			139	123	0.997	129	13.49	0.014	-1.74	-156.35***	M
Total			241	208	0.997	170	26.11	0.031	-0.83	-235.72***	M

Notes: M.D. = Distribution of pairwise differences between haplotypes (Mismatch Distribution); M = Multimodal. *n* = number of sampled individuals; *k* = number of haplotypes detected; *S* = polymorphic sites; *H* = haplotype diversity; Π = average number of nucleotide differences; π = nucleotide diversity. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

mtDNA control region was amplified using specific primers GAL-F 5'-TAA CTC TCA TTA ACT AAA G-3' and GAL-R 5'-TGA TAG TAA AGT CAG CAA GCC-3' designed from the complete mitochondrial genome of the species (ACN: AP004104; Salinas 2011). PCR amplifications were performed in a 25 µl volume containing 2.5 µl 10× Buffer (50mM KCl, 10mM Tris-HCl, pH 8.0), 1.0 µl 50mM MgCl₂, 200mM dNTPs, 0.5 µl of each primer (10 pg/µL), 1 U Taq (Invitrogen), 17.5 µL double-distilled water, and 5 ng of DNA. Thermal cycling parameters included an initial denaturation step at 94 °C for 5 min, followed by 35 cycles at 94 °C for 90 s, 60.7 °C for 90 s, and 72 °C for 90 s, and a final 10-min extension at 72 °C. Double-stranded amplicons were purified using QIAquick Gel Extraction Kit (QIAGEN) and sequenced in both directions with an Automatic Sequencer ABI3730 x 1 at Macrogen Inc. (Seoul, South Korea). All *G. maculatus* mtDNA control region sequences have been deposited in GenBank under Accession Numbers: KP298433–KP298673.

Genetic Diversity and Haplotype Genealogy in *G. maculatus*

Control region sequences were edited using Proseq v. 3.5 (Filatov 2009) and aligned with ClustalW (Larkin et al. 2007). We performed a DNA saturation analysis following Xia and Xie (2001) in Data Analysis and Molecular Biology and Evolution to evaluate how saturation of transitions is accumulated in relation to nucleotide divergence in the entire data set. Levels of genetic polymorphism were estimated using standard diversity indices including number of haplotypes (*k*), number of segregating sites (*S*), haplotype diversity (*H*), average number of pairwise differences (Π), and nucleotide diversity (π) for each locality and for the entire data set with DnaSP v.5.00.07 (Librado and Rozas 2009). We performed statistical neutrality tests (Tajima's D and Fu's *F_s*) for each locality and for the entire data set to estimate whether sequences deviate from expectations under a neutral model. Genealogical relationships in *G. maculatus* were constructed using Maximum Parsimony networks computed in Hapview (<http://www.cibiv.at>).

Genetic Structure in *G. maculatus*

We determined the levels of genetic differentiation between the analyzed localities using mean pairwise differences (Φ_{ST}) and haplotype frequencies (F_{ST}) in Arlequin v. 3.5 (Excoffier et al. 2005).

The statistical significance of genetic differences was estimated using permutation tests with 10 000 iterations of haplotype identities. We inferred the spatial genetic structure in the species by estimating the number and composition of groups that were most differentiated based on pairwise comparison results using analysis of molecular variance (AMOVA). Finally, we performed a test for isolation by distance using a Mantel test with 1000 permutations in Arlequin to determine the correlation between Slatkin's linearized population genetic differentiation (Slatkin 1995) and the linear geographic distance (km) between populations.

Results

Control Region Sequence Characterization in *G. maculatus*

We included in the analyses a total of 241 individuals collected from 9 coastal localities along the Chilean coast encompassing the Intermediate Area and the Magellanic Province. Control region sequences included 925 nucleotide positions, and as expected analyzing a noncoding region, several insertion and deletions were recorded. Sequences were A–T rich (57.3%) compared with G–C content (42.7%). Levels of genetic diversity in the species were extremely high with 181 variable positions (19.56%), 140 of which were parsimony-informative. In spite of the high levels of genetic diversity found, Control region sequences were not saturated in the species.

Genetic Diversity and Haplotype Genealogy in *G. maculatus*

Levels of genetic diversity in the species were remarkably high for all the estimated indices in all the analyzed localities (Table 1), as well as for the entire data set. Haplotype diversity (*H*) ranged between 0.938 (Williams Channel) and 1.000 (María Eugenia Bay, Moncul River, and Yelcho River). Similarly, average number of nucleotide differences (Π) and nucleotide diversity (π) were also very high in most of the localities and ranged between 25.45/0.027 (Chaihuín River) and 10.66/0.011 (Williams Channel). Permutation test analyses (10 000 iterations) detected significantly higher (*P* = 0.003) values for Π and π in Intermediate Area populations compared with those from the Magellanic Province. At the same time, the number of polymorphic sites was significantly

greater ($P = 0.02$) in Intermediate Area populations than in Magellanic ones. Tajima's D and Fu's neutrality tests showed similar trends for each locality and for the entire Control Region data set in the species. On the one hand, Tajima's D neutrality test was negative but not statistically significant at each locality and for the entire data set (Table 1). On the other hand, Fu's FS was also negative and statistically significant in most of the localities and for the whole data set. Similarly, the distribution of pairwise differences recorded multimodal distribution at each locality and for the whole data set and did not fit to the sudden expansion model ($P < 0.007$; Supplementary Figure 1 online).

The maximum parsimony haplotype network constructed in *G. maculatus* recovered a total of 216 haplotypes with a very expanded genealogy (Figure 2). Of the total haplotypes detected in the species, 196 were unique and only 20 were present in 2 or 3 individuals. As previously recognized through mean standard diversity indices, Intermediate Area populations showed a more expanded genealogy than Magellanic ones.

Genetic Structure in *G. maculatus*

Considering the levels of genetic diversity and the very low frequency of shared haplotypes detected, population pairwise comparisons

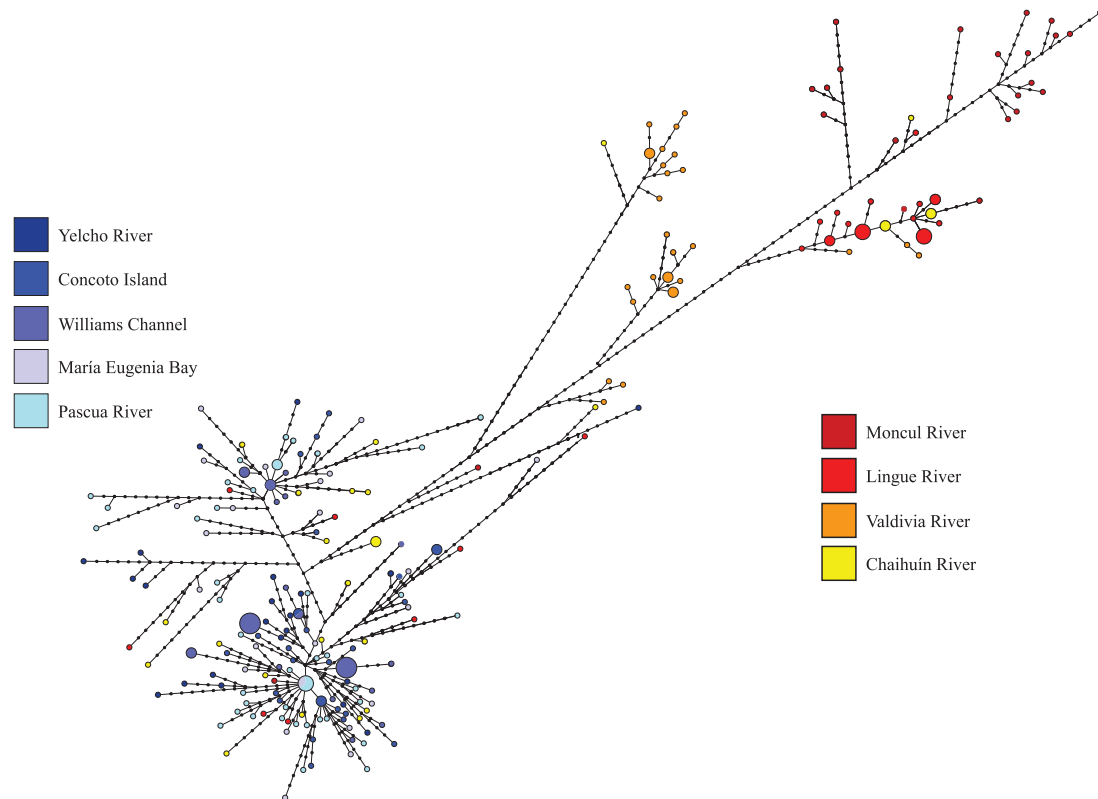


Figure 2. Maximum Parsimony haplotype network including mtDNA Control region sequences from 241 individuals of *Galaxias maculatus*. Each haplotype is represented by a colored circle that indicates the locality where samples were collected. The size of each haplotype represents its frequency in the whole Control region data set.

Table 2. Pairwise values of differentiation (Φ_{ST} ; below the diagonal) and their significances (after Bonferroni correction using 50 000 iterations; above the diagonal) between *G. maculatus* populations from the Chilean coast

Locality	1	2	3	4	5	6	7	8	9
1	—	***	***	***	***	***	***	***	***
2	0.337	—	***	***	***	***	***	***	***
3	0.489	0.323	—	***	***	***	***	***	***
4	0.437	0.183	0.286	—	***	***	***	***	***
5	0.612	0.425	0.461	0.113	—	ns	ns	ns	ns
6	0.657	0.470	0.500	0.132	0.059	—	ns	ns	ns
7	0.653	0.465	0.495	0.115	0.080	0.036	—	ns	ns
8	0.609	0.411	0.442	0.068	0.079	0.059	0.044	—	ns
9	0.649	0.467	0.500	0.116	0.069	0.031	0.034	0.008	—

Notes: Intermediate area: 1) Moncul River; 2) Lingue River; 3) Valdivia River; 4) Chaihuin River. Magellanic Province: 5) Yelcho River; 6) Concoto Island; 7) Williams Channel; 8) María Eugenia Bay; 9) Pascua River. ns = non-significant.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

were only estimated using Φ_{ST} (Table 2). Pairwise Φ_{ST} comparisons showed a greater degree of genetic differentiation between the Intermediate Area and Magellanic populations (Table 2), than among populations within each biogeographic area. Similarly, we detected contrasting patterns of genetic differentiation within each of the biogeographic areas here included. On the one hand, pairwise Φ_{ST} comparisons between Intermediate Area populations were highly significant and each one of them represented a different population unit (Moncul, Lingue, Valdivia, and Chaihuín). On the other hand, with the exception of 1 pairwise comparison (Yelcho River – Williams Channel), no significant genetic structure was detected among Magellanic populations. Such pattern of genetic differentiation was supported by AMOVA analyses that detected the same 5 groups with a maximal difference accounting for 45.68% of the total variation, and only 1.26% was due to within-group variation among localities (Supplementary Table 1). Again, the 5 AMOVA groups include: 1) Moncul River; 2) Lingue River; 3) Valdivia River; 4) Chaihuín River; and 5) Magellanic province populations from Yelcho River (43°S) to Pascua River (48°S). A strong ($r = 0.48$) but marginally significant ($P = 0.08$) correlation between genetic and geographic distances was observed among Intermediate Area populations (Figure 3). Magellanic populations exhibited a very low ($r = 0.045$) and not significant ($P = 0.41$) correlation between geographic and genetic distances (Figure 3).

Discussion

Stock identification represents an integral component of modern fisheries assessments, and therefore for effective fisheries and endangered species management (Begg et al. 1999). The analysis of a species' population structure is of primary importance in developing an optimal strategy for efficient management programs (Coyle 1997). A decision-making management process must consider the intraspecific variation of a resource species and hence requires information

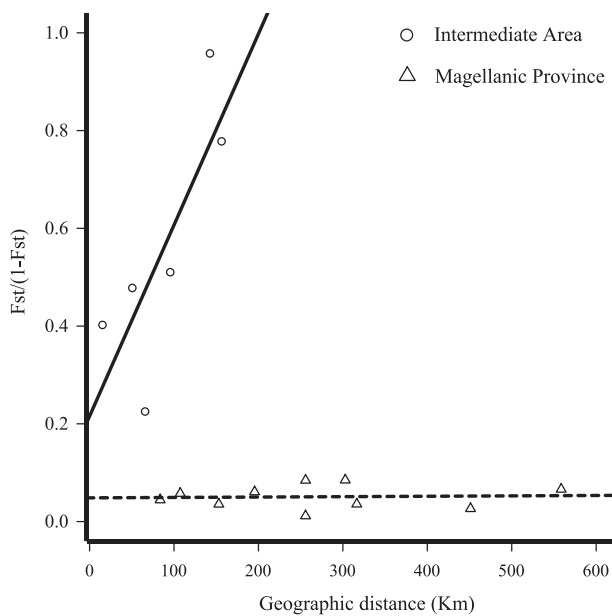


Figure 3. Relationship between linearized genetic differentiation (Φ_{ST}) and geographic distances (K_m) along the Intermediate Area and the Magellanic Province. Circles and triangles represent pairwise values between Intermediate Area and Magellanic Province populations, respectively.

about biological differences between discrete local groups to understand the genetic and ecological processes that influence its structure (Begg et al. 1999). Advances in molecular biology, biochemistry, image analysis, and tagging technologies have prompted revolutionary changes in many stock identification approaches. Therefore, stock identification has developed as an interdisciplinary field and has become a requisite component of fishery science and management programs worldwide. Studies on stock identification and management in Chilean resources have been mainly focused on parasite composition (Oliva and González 2004; Chavez et al. 2007; Oliva et al. 2008; Oliva 2013), otolith morphology (Leguá et al. 2013), and genetics (González et al. 1996; Galleguillos et al. 2000; Cárdenas et al. 2009; Canales-Aguirre et al. 2010).

This study provides new information about main patterns of genetic diversity and structure in *G. maculatus* along its distribution in coastal ecosystems of 2 biogeographic provinces in the Chilean coast. Our results corroborate previous phylogeographic studies in the species in South America (Zemlak et al. 2010, 2011) and New Zealand (Waters et al. 2000) showing the high degree of genetic diversity that characterizes the species. Indeed, *G. maculatus* maintains extremely high levels of genetic diversity along the biogeographic areas here analyzed, as well as in each of the localities. Out of 241 analyzed individuals, we recognized 216 different haplotypes; only 20 of these were found in more than 1 individual. Similarly, Zemlak et al. (2010) recorded in Patagonia a total of 273 different haplotypes, out of 299 analyzed individuals while in New Zealand populations of the species (Waters et al. 2000) identified 139 haplotypes in 144 individuals and only 5 haplotypes were present in more than 1 specimen. In spite of the general pattern of genetic diversity in the species, we found remarkable differences in standard diversity indices between the Intermediate Area and the Magellanic Province. In contrast to our expectations, Intermediate Area populations of *G. maculatus* are much more diverse than Magellanic ones, and therefore landscape heterogeneity does not seem to play a key role in limiting the degree of genetic diversity of the species.

Marked changes in genetic diversity and structure in *G. maculatus* between the Intermediate Area and the Magellanic Province coincide with the 42°S biogeographic break described along the Chilean Coast (Camus 2001; Escribano 2003). This break is considered as a major biogeographic discontinuity resulting from the divergence of the West Wind Drift into 2 main oceanic currents (Camus 2001). The Intermediate Area is washed by the flow of the Humboldt Current System, the northward branch of the West Wind Drift (Thiel et al. 2007). In contrast, the Cape Horn Current, the southward branch of the West Wind drift flows around Cape Horn and is responsible for the main oceanographic regime in the Magellanic Province. Moreover, the Intermediate Zone area represents an almost straight coastline while the Magellanic Province is characterized by irregular coastal environments with islands, inlets, coves, closed bays, peninsulas, and fjords (Strub et al. 1998; Camus 2001). Main differences in terms of genetic diversity and structure between Intermediate Area populations and Magellanic ones may also have been influenced by their contrasting Quaternary glacial histories (McCulloch et al. 2000; Hulton et al. 2002). The Magellanic Province was almost completely covered by ice during Quaternary glacial maxima and therefore shallow coastal and freshwater ecosystems should have been severely hampered. In contrast, glacial ice advances and retreats along the Intermediate Area were restricted to higher areas along the western slopes of the Andes and thus coastal areas were not severely affected (Gillespie and Molnar 1995; Harrison 2004). Nevertheless, as previously proposed by Zemlak et al. (2010, 2011) extreme levels

of genetic diversity recorded in *G. maculatus* along its distribution in the Chilean coast points toward a low amount of influence of the Last Glacial Cycle over its demography. Such result contrasts with main patterns of genetic diversity and structure in other South American aquatic taxa showing severe demographic impact associated with glacial advances and retreats, including marine invertebrates (Sánchez et al. 2011; González-Wevar et al. 2012), kelps (Fraser et al. 2010), freshwater (Ruzzante et al. 2006, 2008; Zemlak et al. 2008; Unmack et al. 2009, 2012), and marine fishes (Ceballos et al. 2011).

A concordance between phylogeographic and biogeographic patterns has been recognized in different areas of the planet, suggesting that the forces determining species distributions are also shaping the spatial patterns of population genetic structure (Waters and Roy 2004; Taylor and Hellberg 2006). Therefore, local adaptation and distinctive characteristics of the analyzed biogeographic areas in terms of coastal configurations, Quaternary glacial histories, and oceanographic regimes may be responsible for the general pattern of genetic structure recorded in *G. maculatus*.

Together with the clear differentiation between the Intermediate Area and the Magellanic Province populations, *G. maculatus* also exhibited marked differences within each of these major biogeographic areas in terms of the spatial distribution of the genetic diversity. On the one hand, the 4 populations in the Intermediate Area were highly differentiated and most of the genetic variance was partitioned among river drainages. This pattern of genetic structure suggests a limited amount of gene flow between river basin populations over a narrow geographic range (<200 km). These results are quite unpredicted considering the high dispersal potential described for migratory populations of this species with a marine juvenile stage that involves probably 6–7 months (McDowall et al. 1975). Although it is often assumed that recruitment is independent of local reproduction (Caley et al. 1996), there is an increasing amount of indirect evidence showing that physical and behavioral mechanisms facilitate larval retention near source populations (Chen et al. 1997; Cowen 2002; Thorold 2002; Warner and Cowen 2002). Mechanisms associated with this process in estuarine areas include vertical migrations (Sulkin 1984) and reversible tidal currents (Chen et al. 1997); these may be invoked to explain the marked genetic structure recorded in the species in the Intermediate Area. However, these processes have not been studied in estuarine populations of *G. maculatus* and according to McDowall et al. (1975) there may be little restriction in the movement of larval stages of *G. maculatus*, because specimens have been collected up to 700 km offshore. Alternatively, philopatric behavior could also generate small-scale genetic structure within the Intermediate Area, despite the high dispersal potential of the species (Waters and Burridge 1999). In contrast, populations from the Magellanic Province showed absence of or low genetic differentiation in an area that extends for more than 500 km. In spite of the geographic and oceanographic complexity within the Magellanic Province, patterns of genetic differentiation among *G. maculatus* populations are much more lower than those detected in the Intermediate Area. The Austral fjords region (42°–56°S) of Chile constitutes a protected area of fjords and channels with waters of Subantarctic origins, as well as those generated by the melting of resident glaciers. This input of melting fresh water in this province generates estuarine conditions along near-shore areas, as well as in the inlets (Escribano et al. 2003). Therefore, and according to our findings, the Magellanic province may represent a continuous ecosystem for *G. maculatus*. Otherwise, low levels of differentiation detected among Magellanic province populations could also reflect

a mixing of populations through a rapid process of recolonization of formerly glaciated areas from northern Patagonia marine refuge populations (Zemlak et al. 2010).

This study provides new information about main patterns of genetic diversity and structure of *G. maculatus* along its distribution in the Chilean coast in 2 biogeographic marine provinces with contrasting coastal configurations, oceanographic regimes, and glacial histories. Coastal populations along the Intermediate Area represent discrete genetic units while Magellanic Province populations are less differentiated suggesting the existence of a single genetic unit. Future exploitation management and aquaculture programs should integrate this information and implement differential plans for each of these main areas. For instance, each of the river basins in the Intermediate Area should be treated as different genetic stocks, while Magellanic Province populations could be considered as a single one. Nevertheless, it is important to consider the limitations of the genetic stock identification approach, because failure to reject the panmixia hypothesis does not prove the existence of a single stock (Shaklee and Currens 2003). Alternatively, contrasting patterns of genetic structure between major biogeographic areas here analyzed could also be a consequence of their different Quaternary glacial histories. As recently proposed by Haupt et al. (2013), evolutionary processes as range expansion and bottlenecks, and potential demographic histories may be also advocated to the current pattern of structure recorded in *G. maculatus* along its distribution in the Chilean coast. Hence, future studies in the species should include the use of fast-evolving genetic markers such as microsatellites in order to corroborate these findings and to help us to unravel the role of contemporary gene flow among Patagonian populations.

Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>.

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