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Research Article

Genetic population structure and evidence of genetic homogeneity in populations of the Argentinian silverside *Odontesthes bonariensis* (Teleostei: Atherinopsidae) inhabiting central and northwestern Argentina

Evelyn Valencia[1], David Veliz[1], Andrea Tombari[2] & Caren Vega-Retter[1]
[1]Departamento de Ciencias Ecológicas, Facultad de Ciencias
Instituto de Ecología y Biodiversidad (IEB), Núcleo Milenio de Ecología y Manejo
Sustentable de Islas Oceánicas (ESMOI), Universidad de Chile, Ñuñoa, Chile
[2]Universidad Nacional de Río Negro, Río Negro, Argentina
Corresponding author: Caren Vega-Retter (carenvega@ug.uchile.cl)

ABSTRACT. The study of species in their native geographic ranges is key to understanding how human activity has influenced spatial fragmentation or species homogenization. The Argentinian silverside *Odontesthes bonariensis*, of interest for aquiculture and sport fishing, is a relevant subject of study. The species has been introduced in a number of countries and re-introduced in some areas of Argentina with unknown effects. The objectives of this study were to determine the population structure, genetic diversity (GD) and effective population sizes (N_e) of *O. bonariensis* in Argentina. Six microsatellite loci were amplified in individuals collected from four water bodies affected by commercial and sport fishing: Cabra Corral Reservoir (CC), Chascomús Lake (CH), Chasicó Lake (LCH) and the Río de la Plata (RLP). Three genetic groups were detected: one in CC, one in RLP and the last inhabiting CH and LCH. Interestingly, CH and LCH are located 768 km apart, but showed no difference in allele frequencies; suggesting the introduction of individuals from CH into LCH. The largest allele richness, GD and N_e were found in RLP indicating that the largest population of *O. bonariensis* may be found in this area. Current N_e were lower than historical N_e in all areas, suggesting a change in the GD over time. This study provides information on the genetic structure and genetic diversity of *O. bonariensis* across its native distribution and over time, demonstrating the first evidence of a possible genetic homogenization in this species probably linked to human activities.

Keywords: genetic population structure, genetic homogenization, microsatellite, human impact.

INTRODUCTION

Anthropic intervention is one of the main threats to biodiversity, where environmental alterations and assisted species dispersion are the main factors producing large changes in the distribution of the biota (Olden *et al.*, 2004) and in their population genetic structures at different geographic scales (Bohonak, 1999; Sharma & Hughes, 2011; Husemann *et al.*, 2012). Although movement and dispersal patterns determine population structure (Short & Caterino, 2009), evidence indicates that humans may perturb these movements, especially by changing the natural connections of the species (Crook *et al.*, 2015). Ray *et al.* (2012) suggested that this intervention may alter the genetic signals of species by increasing or decreasing population connectivity. For example, perturbations in the landscape, such as dams, may limit species migration (Reid *et al.*, 2008; Beneteau *et al.*, 2009), while assisted dispersion due to intentional or accidental introductions may increase connectivity artificially in freshwater systems (Cegelski *et al.*, 2006; Husemann *et al.*, 2012; Crook *et al.*, 2015). These changes alter the natural dispersion of the species and natural population structure (Walter *et al.*, 2009; Husemann *et al.*, 2012). Thus, the study of population genetic structure is fundamental to understanding the impact of anthropic intervention on ecological connectivity in order to develop conservation strategies for freshwater species (Crook *et al.*, 2015).

Freshwater fish are expected to have high levels of genetic differentiation due to the natural fragmentation of the rivers and lakes (Ward, 2006). However, intentional introductions for fishing, aquaculture and to

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repopulate freshwater systems, in mitigation of population reductions, may homogenize populations (McBride *et al.*, 2015). Behnke (1992) demonstrated evidence of homogenization in *Oncorhynchus clarki*, while McBride *et al.* (2015) provided evidence of homogenization of *Alosa pseudoharengus*, showing that genetic isolation by distance was non-significant among stocked populations, but significant among natural populations. Overall, the anthropic effect on the structure of the natural populations of freshwater fish is evident. On the other hand, human activity, specially overfishing has strongly impacted the aquatic systems affecting population sizes and reproduction (Hoarau *et al.*, 2005). In this context, reduction in the population size produces a reduction of the genetic variability, an important component for species adaptation (Cruzan, 2001).

The Argentine silverside fish *Odontesthes bonariensis* (Valenciennes, 1835) is a species that has acquired much commercial and sport interest, activities through which its biology has been documented since 1959 (Boschi & Fuster de Plaza, 1959). Artificial reproduction has been carried out in hatcheries to repopulate depleted streams and lakes (Barros *et al.*, 2004). The attention paid to this species in aquaculture and sport fishing is due to the excellent quality of its flesh (Berasain *et al.*, 2010), easy cultivation using artificial fertilization, resistance to low water temperature, well-known tolerance to high concentrations of salts (Avigliano *et al.*, 2014) and general adaptability (López & García, 2002). These features observed in *O. bonariensis* and widespread repopulation efforts have allowed its establishment in most freshwater environments of Argentina (Barros *et al.*, 2004); concentrated particularly in the lakes in the pampas (Mancini & Grossman, 2001).

It is important to consider that the original distribution of *O. bonariensis* only included the Río de la Plata, Río Paraná and the waters of the Río Salado watershed. *O. bonariensis* fish hatcheries began in Chascomús Lake as early as 1904 and the first fry and ova were extracted from this area to be stocked elsewhere (Berasain *et al.*, 2010). The available records indicate that repopulation stocking began in the 1920s, primarily concentrated in the Buenos Aires Province to repopulate pampa lakes after an intense drought. The species was introduced into lakes, dams and ponds in other provinces in the 1940s, widening its distribution in central and southern Argentina (Tombari & Volpedo, 2008). Most introductions were successful, such as in the Cabra Corral Reservoir, where 86% of the fishing activity has been concentrated (Volante *et al.*, 1997). *O. bonariensis* is currently widely distributed in the continental waters of Peru, Bolivia, Brazil, Uruguay and Chile, as a result of human introduction and natural migration (López & García, 2002; Tombari & Volpedo, 2008).

In spite of the intense exploitation of *O. bonariensis*, many aspects of its biology and ecology, as well as population structure, are unknown (Avigliano & Volpedo, 2013). Information about the current state of its population structure would allow us to determine if its movement by humans may have influenced its population genetic structure (Wong *et al.*, 2004; Ward, 2006). Thus the goal of this study was to determine the population genetic structure of the Argentine silverside in four important areas used for recreational, commercial and repopulation purposes (Chascomús Lake, Chasicó Lake, Cabra Corral Reservoir and Río de la Plata). We hypothesized a complex genetic structure and high diversity in natural populations and depleted and homogenized diversity in repopulated areas.

MATERIALS AND METHODS

Collection, DNA extraction and microsatellite amplification

A total of 82 individuals of the silverside *O. bonariensis* were collected in: i) Cabra Corral Reservoir (CC) located in the Río Juramento, from the Salta Province with a surface area of 113.6 km^2 (Barros *et al.*, 2004); ii) Chasicó Lake (LCH) in the Buenos Aires Province, that has a size of 50.3 km^2 (Volpedo *et al.*, 2013); iii) Chascomús Lake (CH) with an area of 30 km^2 (Diovisalvi *et al.*, 2010) and Río de la Plata (RLP) with an area of about 3.1×10^6 km^2 (Acha *et al.*, 2008) (Fig. 1). The sample consisted of 18 specimens collected from CC, 22 from LCH, 19 from CH and 23 from RLP.

DNA was extracted by the salt method of Aljanabi & Martinez (1997). We used seven dinucleotide microsatellites, four (Odont02, Odont07, Odont09, Odont38) described for *Odontestes perugiae* (Beheregaray & Sunnucks, 2000) and three (Obo19TUF, Obo59TUF and Obo71TUF) described specifically for *O. bonariensis* (Koshimizu *et al.*, 2009). Microsatellites were amplified by PCR in a volume of 10 µL, including 1.3 µL 10x PCR buffer (Invitrogen), 0.5 µL $MgCl_2$ (50 mM) (Invitrogen), 0.5 µL of direct and inverse primers (50 ng μL^{-1}) (Applied Biosystems), 2.4 µL dNTPs (2.5 mM) (Invtrogen), 4.68 µL H_2O and 0.12 µL Taq polymerase (Invitrogen), to which 1.5 µL DNA (50 ng μL^{-1}) were added. The PCR touchdown method of Beheregaray & Sunnucks (2000) was used to amplify microsatellites Odont02, Odont07, Odont09 and Odont38, with the modifications of Muñoz *et al.* (2011). Loci Obo19TUF, Obo59TUF and Obo71TUF were amplified using the protocol of

Figure 1. Sampling sites of *Odontesthes bonariensis* in Argentina.

Koshimizu *et al.* (2009). The fragments were genotyped by Macrogen Inc. (Seoul, Korea).

Genetic analyses, genetic diversity and population structure

The allelic matrix was generated with GeneMarker software (SoftGenetics Inc). Microchecker 2.2.3 (Van Oosterhout *et al.*, 2004) software was used to identify possible genotyping errors such as stuttering, dropout and the presence of null alleles. We estimated the mean number of alleles (NA) per locus, allele frequencies, and linkage disequilibrium for all pairs of loci, as well as observed (Ho) and expected (He) heterozygosity, and deviations from the Hardy-Weinberg Equilibrium (HWE) for each locus using Genetix 4.05 (Belkhir *et al.*, 2000) software. Fstat software (Goudet, 2002) was used to determine genetic diversity (GD) and allele richness (AR) for each locus and site.

Three approximations were used to analyze the population genetic structure. First, we used Structure 2.3.4 software (Pritchard *et al.*, 2000) to estimate the probable number of genetic groups present in the samples. This analysis uses a Bayesian method, which maximizes the H-W equilibrium to find the number of populations (k) that approaches the equilibrium most closely. Second, Flock 3.1 software (Duchesne & Turgeon, 2012) was used to determine the most probable number of populations (K). This analysis was run, as suggested by the authors, with an initial random partition, with 20 re-assignments per run, 50 runs and a log-likelihood minimum (LLOD) of 0.3. Third, Genetix 4.05 software (Belkhir *et al.*, 2000) was used to estimate the genetic distances between pairs of sites with Wright's F_{ST} (Weir & Cockerham, 1984). 10,000 individual permutations between sites were used to estimate the statistical significance of this index. Since there were multiple paired comparisons (6 in total), the Bonferroni correction was adjusted to $\alpha = 0.008$.

Effective population size and recent bottlenecks

Historical effective population size (N_e) was estimated for each of the populations from Theta ($\theta = 4\ N_e\ \mu$),

obtained with Migrate 3.6.8 software (Beerli & Felsnstein, 1999) using a mutation rate of 2×10^{-3}. The mutation rate used is an average value taken from *Lepomis marginatus* (Holbrook) (Mackiewicz *et al.*, 2002) and *Syngnathus typhle* (Linnaeus) (Jones *et al.*, 1999); both species are phylogenetically close to *O. bonariensis*. Contemporary N_e was estimated using a Bayesian approximation implemented in ONeSAMP 1.2 (Tallmon *et al.*, 2008). The Wilcoxson test implemented in R software (R Core Team, 2015) was used to test for differences between historical and current effective population sizes.

Finally, Bottleneck software (Cornuet & Luikart, 1996) was used to detect signals of a recent bottleneck in each of the *O. bonariensis* sampling sites studied. Bottleneck estimates the probability of recent reductions in N_e by comparing the expected heterozygosity in (HWE) equilibrium with the expected heterozygosity under mutation-drift equilibrium. Settings included the use the two-phase mutation model with 70% gradual mutation. The significance was evaluated with the Wilcoxson test in the same software.

RESULTS

Genetic diversity and population structure

Of the 7 microsatellites analyzed, the locus Obo19TUF consistently showed the presence of null alleles in all sample sites, thus this locus was eliminated from the analyses. The other six loci showed no consistent evidence of null alleles in all sampled sites. Nor were significant departures from the HWE or evidences for linkage disequilibrium were observed in these 6 loci. With a mean of 13 alleles per locus, the number of alleles varied from 9 (Odont38) to 26 (Odont38). Ho varied from 0.2941 to 0.8571 with a mean of 0.63, while He varied from 0.2851 to 0.9388 with a mean of 0.69 (Table 1). Two sites demonstrated global conformance with HWE (CC Fis = -0.02, $P = 0.59$; LCH Fis = 0.01, $P = 0.38$) and two sites showed significant departures to HWE (CH Fis = 0.21, $P < 0.021$; RLP Fis = 0.15, $P < 0.01$). RLP had the highest values of GD, AR and total NA, followed by CH and LCH, while CC had the lowest values. Population inhabiting RLP (NA = 65) had 2.32 times more alleles than the population of CC (NA = 28) (Table 2).

The values of Ln(k) obtained from Structure software showed a maximum likelihood value at k = 3. This analysis separated three clusters: one containing individuals from CC, the second containing individuals from RLP; and the third composed with individuals from LCH and CH (Fig. 2). The Flock assignment analysis did not determine an exact value of K, but indicated that there are two or more populations among the localities analyzed. The F_{ST} showed the same result as Structure software; all pairs of comparisons were significant ($F_{ST} > 0.033$, $P < 0.008$) except for LCH and CH ($F_{ST} = 0.013$, $P = 0.11$) (Table 3). Taken together, the three methods pointed to the presence of three genetic groups: CC, RLP and a third genetic group that includes the individuals of LCH and CH.

Effective population size and recent bottlenecks

Historical and contemporary N_e were small for each sampling site; where RLP was the only site with a value above 100. The smallest historical N_e was observed in CC, while the smallest current N_e was estimated for CH. The statistical analysis showed that current N_e were smaller than the historical measurements (Wilcoxon, $P = 0.0078$; historical mean = 52.77 ± 53.17 SD; current mean = 38.90 ± 50.23 SD). In the Figure 3 we observe the change in N_e over time for each sampling site. However, the low N_e values may not be related to recent bottlenecks, since the probabilities returned by Bottleneck were not statistically significant (CC, $P = 0.422$; CH, $P = 0.781$; LCH, $P = 0.078$; RLP, $P = 0.719$).

DISCUSSION

Population genetic structure

This study shows the existence of genetic structure in the Argentinian silverside *O. bonariensis*, detecting three differentiated genetic groups in part of its native range, one in CC, one in RLP and the last composed of CH and LCH. The observed genetic structure indicates that there is low connectivity between the bodies of water that Argentinian silverside inhabits, with a possible effect of population homogenization due to human activities in CH and LCH. The evidence from our study, showing a lower GD, AR and NA in LCH compared with CH, suggested a movement of individuals from CH to LCH. There are two possible explanations for this movement and homogenization. First, the silverside might have migrated to LCH by its only tributary, the Arroyo Chasicó, in a large-scale flood event (Tsuzuki *et al.*, 2000; Kopprio *et al.*, 2010; Avigliano *et al.*, 2012). The pampa is exposed to cycles of drought and floods, and floods tend to homogenize the lagoons (Quirós *et al.*, 2002). Although this is theoretically possible, the two lagoons are endorheic, closed and separated by approximately 768 km, therefore it is highly unlikely that they would be connected by floods. The second, and more likely, explanation is that similarities are the result of the release of fry and ova obtained from the former Vivero de Piscicultura del Ministerio de Agricultura y Ganade-

Table 1. Summary of the genetic data obtained for *Odontesthes bonariensis* in four sampling sites: Cabra Corral Reservoir (CC), Chascomús Lake (CH), Chasicó Lake (LCH) and the Río de la Plata (RLP). n: sample size, NA: number of alleles, He: expected heterozygosity, Ho: observed heterozygosity, Fis: consanguinity index and respective *P*-values obtained with 10000 permutations. **P* < 0.05.

	Sampling site								
	CC	CH	LCH	RLP		CC	CH	LCH	RLP
Odont07					**Odont02**				
n	18	19	22	23	n	18	19	22	23
NA	4	5	4	10	NA	7	11	8	24
He	0.607	0.683	0.631	0.816	He	0.75	0.868	0.807	0.939
Ho	0.6	0.692	0.722	0.79	Ho	0.786	0.583	0.81	0.857
Fis	0.046	0.027	-0.116	0.059	Fis	-0.011	0.366	0.022	0.111
P	0.514	0.562	0.346	0.359	*P*	0.619	0.002*	0.505	0.040*
Odont38					**Obo59TUF**				
n	18	19	22	23	n	18	19	22	23
NA	4	4	4	5	NA	6	9	8	7
He	0.445	0.517	0.537	0.285	He	0.802	0.808	0.816	0.829
Ho	0.588	0.294	0.474	0.318	Ho	0.727	0.529	0.8	0.529
Fis	-0.296	0.456	0.145	-0.093	Fis	0.14	0.371	0.046	0.387
P	0.148	0.0131*	0.307	0.571	*P*	0.252	0.002*	0.419	0.001*
Odont09					**Obo71TUF**				
n	18	19	22	23	n	18	19	22	23
NA	3	6	6	10	NA	4	7	6	9
He	0.469	0.649	0.626	0.846	He	0.541	0.684	0.725	0.804
Ho	0.5	0.588	0.591	0.857	Ho	0.546	0.533	0.611	0.714
Fis	-0.023	0.123	0.079	0.011	Fis	0.04	0.253	0.185	0.148
P	0.649	0.275	0.38	0.56	*P*	0.558	0.066	0.131	0.164

Table 2. Mean values for genetic diversity (GD), allelic richness (AR) and the total number of alleles (NA) per site.

Site	GD	AR	NA
CC	0.627	4.429	28
CH	0.733	6.388	42
LCH	0.710	5.113	36
RLP	0.777	8.552	65

ría de la Nación and from the Estación Hidrobiológica del Ministerio de Asuntos Agrarios, Buenos Aires, Province both located in Chascomús. Records indicate that a process of repopulation, concentrated in the Buenos Aires Province, was undertaken after a period of intense drought (Berasain *et al.*, 2010). According to these authors, the current distribution of *O. bonariensis* is a consequence of these stocking measures, since the species was taken to different countries from the Chascomús hatchery.

Olden *et al.* (2004) suggested that anthropic alterations and assisted dispersion have produced genetic homogenization and reduced the spatial component of genetic variability in species or among their populations. Our results suggest a homogenization process in populations of the Argentinian silverside, as has occurred in other fish (Rahel, 2000), birds (Lockwood *et al.*, 2000), mammals and reptiles (Wilson, 1997), among others, causing impacts at ecological and evolutionary levels. The negative impacts of stocking are not restricted to species homogenization; for example, the introduction of the Argentinian silverside significantly affected the structure and function of Laguna El Estado, producing a cascade effect on other components (Grosman & Sanzano, 2003). Moreover, Conte-Grand *et al.* (2015) showed that the introductions have allowed the introgression of *O. bonariensis* genome into several populations of *O. hatcheri*. Thus it is important to take precautions when species dispersion is assisted by humans, especially when the species in question has invasive characteristics such as being a good colonizer (Grosman & Sanzano, 2003) and possessing broad environmental tolerance (Marchetti *et al.*, 2004).

Genetic variation and effective population size

The silverside population in RLP presents the greatest GD, AR and NA of the water bodies studied. Given that genetic variation relates to the effective size of the populations, it also showed the highest values of histo-

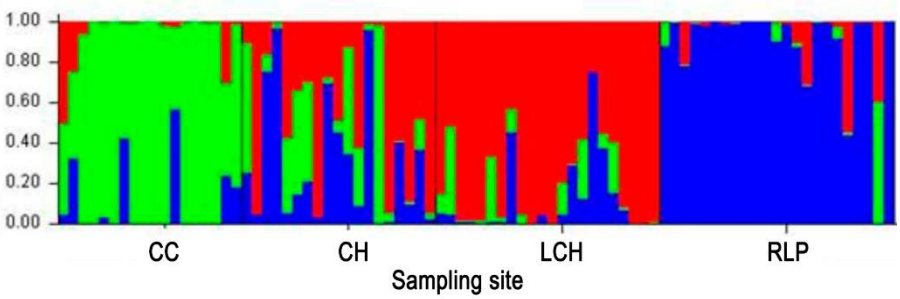

Figure 2. Results of the Structure software based on the four sampling sites and the resulting k = 3 clusters. CC: Cabra Corral Reservoir, LCH: Chasicó Lake, CH: Chascomús Lake, RLP: Río de la Plata. Each bar represents an individual.

Table 3. Pairwise F_{ST} values. *$P \leq 0.008$.

Sites	CC	CH	LCH	RLP
CC		0.122*	0.141*	0.138*
CH			0.013	0.033*
LCH				0.076*
RLP				

rical and current N_e. This fact is probably related with the location of RLP at the core of the original distribution of *O. bonariensis* and with the low impact of the repopulation programs, which focused mainly on lagoons in the Rio de la Plata watershed.

CC, LCH and CH had N_e <50, which is similar to the estimates of Dawnay *et al.* (2011) for the freshwater fish *Thymallus thymallus*, in which low values of N_e were found in 19 of the 27 populations studied. Theoretically, values of N_e between 50 and 500 are necessary to minimize the effects of endogamy and maintain genetic variation in the short and long term, respectively (Van Dyke, 2008; Rieman & Allendorf, 2001). Thus, populations with low Ne may not have capacity to respond to environmental changes due to inbreeding depression and/or accumulation of deleterious alleles (Frankham, 1995; Higgins & Lynch, 2001; Turner *et al.*, 2002). According to Cruzan (2001), the level of genetic variation is an indicator of the general vitality of a species and its potential for evolutionary response to environmental changes. Considering this indicator, the population in RLP is an important reservoir of genetic diversity for the species that should be preserved.

Our results showed lower contemporary than historical N_e in all populations, although no recent bottlenecks were detected. Both commercial and sport fishing activities have been active for decades in the bodies of water studied, concentrated in Chascomus Lake, which had the smallest effective population size.

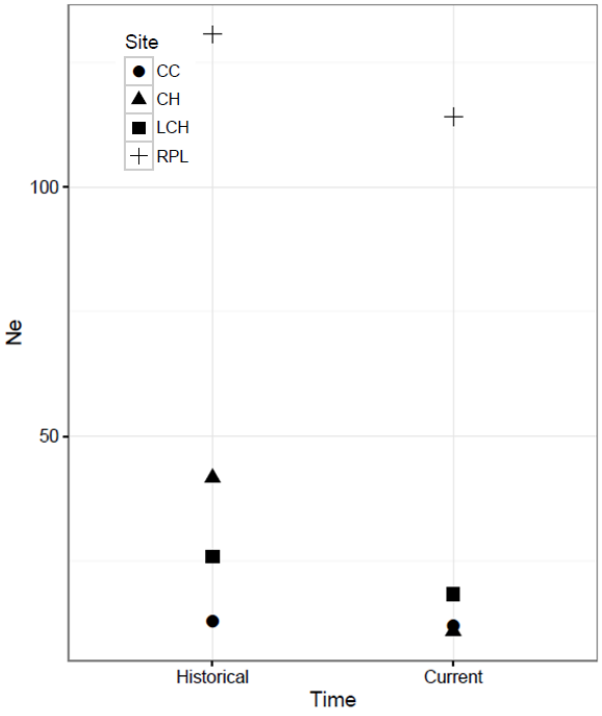

Figure 3. Plot of the historical and current effective population size for each *O. bonariensis* sampling site in Argentina. CC: Cabra Corral Reservoir, LCH: Chasicó Lake, CH: Chascomús Lake, RLP: Río de la Plata.

This may indicate that although fishing has not generated a drastic reduction in population size per se, it may have indirect effects, such as perturbation of the ecosystems, which may alter behavior and reproduction. This effect has been demonstrated in other species (Rowe & Hutchings, 2003) increasing a long-range risk of extinction (Turner *et al.*, 2002).

Due to low effective population sizes, the populations present in CC and LCH-CH likely have reduced capacity to respond to future environmental changes.

Take into account the information on the population genetic structure and genetic diversity is essential to the adequate management of fishing stocks. Understanding the genetic structure in the native range of the Argentine silverside is fundamental to recognizing the effect of human actions on its ecological connectivity and the possible consequences (*e.g.*, homogenization and therefore the reduction of the spatial component of genetic variation). It is also important to note that migration of this species has historically been restricted by natural habitat fragmentation (endorheic lakes), but have a great capacity to colonize, likely taking advantage of large flood events to disperse. Given the nature of the species and the current context, humans are an important vector for dispersal, but which should operate with informed control. Finally, this study provides a starting point for future studies on the adaptation of *O. bonariensis* to the different freshwater systems and its populations monitoring.

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