

**Research Article**

## **Genetic diversity in Chilean populations of rainbow trout, *Oncorhynchus mykiss***

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**ABSTRACT.** The rainbow trout *Oncorhynchus mykiss*, was first introduced in Chile between 1905 and 1920 and is currently widely distributed in Chile from Antofagasta (23°S) to Patagonia (55°S). The broad range of the geographic and climatic distributions of this species in Chile offers a unique opportunity to study the effect of naturalization of an introduced species on its genetic variability. It is of particular importance to observe the genetic variability of populations in the northern range of this species distribution, in a transition zone where a Mediterranean-type climate changes to an arid climate. The present study analyzed allozymic variability and distribution within and between populations of *O. mykiss* from the river basins of Elqui and Limarí rivers, and six culture strains, using starch-gel protein electrophoresis. Populations were found to be in Hardy-Weinberg equilibrium and the average values of  $H_e$  (0.045), polymorphism (13.9%) and allele per locus (1.19) are similar to rainbow trout in its native distributional range. About 77.8% of the genetic variability was within population, similar to the variability reported for wild populations in the northern hemisphere. However, a marked genetic differentiation between wild populations was also found. This is likely to be the consequence of initial founder effects followed by subsequent introgression of resident populations caused by reseeded with trout of different origins in both basins.

**Keywords:** *Oncorhynchus mykiss*, rainbow trout, Salmonidae, genetic variation, aquaculture, conservation.

## **Variabilidad genética en poblaciones chilenas de trucha arcoiris, *Oncorhynchus mykiss***

**RESUMEN.** La trucha arcoiris, *Oncorhynchus mykiss*, fue introducida en Chile entre 1905 y 1920, y actualmente está ampliamente distribuida entre Antofagasta (23°S) y la Patagonia (55°S). El amplio rango de distribución geográfica y climática de esta especie en Chile ofrece una oportunidad singular para estudiar los efectos de la naturalización de una especie introducida sobre su variabilidad genética. Es de particular importancia observar la variabilidad genética de las poblaciones de esta especie en el rango norte de distribución, una zona de transición de un clima tipo mediterráneo a una condición árida. El presente estudio analizó la variabilidad aloenzimática dentro y entre poblaciones de *O. mykiss* en las cuencas hidrográficas de los ríos Elqui y Limarí, así como seis cepas de truchas comerciales, usando electroforesis de proteínas en geles de almidón. Las poblaciones analizadas se encontraban en equilibrio de Hardy-Weinberg, con valores de heterocigosidad esperada (0,045), polimorfismo (13,9%) y alelos por locus (1,19) similares a los de la especie en su rango nativo de distribución. Aproximadamente el 77,8% de la variabilidad genética fue intrapoblacional, similar a lo informado para poblaciones silvestres de esta especie en el hemisferio norte. Esto probablemente es consecuencia de efectos fundadores iniciales seguidos por introgresiones subsecuentes de las poblaciones residentes debidas a resiembras con truchas de diferentes orígenes en ambas cuencas.

**Palabras clave:** *Oncorhynchus mykiss*, trucha arcoiris, Salmonidae, variabilidad genética, acuicultura, conservación.

## INTRODUCTION

The native distribution range of the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), extends along the North American Pacific coast and the rivers that drain into it, from Alaska to the north of Mexico (MacCrimmon, 1971), and was introduced to the rest of the world from this zone (MacCrimmon, 1971; Hershberger, 1992; Crawford & Muir, 2008). In Europe, the rainbow trout was introduced at the end of the 19<sup>th</sup> century from an original stock from the McCloud River in Baird, California (Hershberger, 1992). Study of these populations has led to greater understanding of the effects of introductions and later translocations on their genetic diversity, as well as their offspring in captivity. Studies of allozymes in introduced or domesticated populations have revealed genetic differences between strains, showing fixation of alleles in normally polymorphic loci in wild populations and reduction in levels of heterozygosity (Currens *et al.*, 1990). These changes are usually interpreted as reflecting the occurrence of the founder effect and genetic drift, both processes directly related with the effective population sizes (Quinn *et al.*, 1996; 1998; Winkler *et al.*, 1999). On the other hand, the historical isolation between different introduced stocks enables the observance of adaptive divergences between them (Moritz, 1999).

In Chile, the rainbow trout was originally introduced from Hamburg, Germany, between 1905 and 1910 (Hershberger, 1992; Soto *et al.*, 2001, 2006; Crawford & Muir, 2008). Reproduction of these stocks was initially done in Blanco River (Los Andes; 32°55'S, 70°16'W) and later in Cautín River (Lautaro; 38°31'S, 72°27'W). From these stocks, young fish were seeded in central and southern rivers of the country, where wild populations were established in various bodies of water (Gosluda, 1927; Mann, 1954; Campos, 1970). Several varieties of rainbow trout from different origins have been imported for commercial purposes and have been released into water bodies deliberately or accidentally (Uribe, 1988; Mardones & Vega, 1993). Among the commercial strains introduced in Chile, one was recorded as being from Sulfur Spring River (North Carolina, USA), referred to locally as the "American" strain, as well as the Donaldson (Donaldson & Olsen, 1957); Cofradex, a variety developed in Denmark; Steelhead from the McCloud River (California, USA) and Kamloops from Kooteney Lake (British Columbia, Canada) (Colihueque *et al.*, 2001). In Chile there is little information on the origin or lineage of populations spread in natural environments or their variability and genetic structure. Information is limited to cytogenetic studies of cultivated strains that indicate chromosomal

heterogeneity (Colihueque *et al.*, 2001), and allozyme variability analysis in three populations naturalized in the south of the country, showing levels of genetic diversity similar to trout in its native range of distribution (Gajardo *et al.*, 1998). This species, along with other salmonid species, is naturalized in rivers and streams throughout the country, establishing itself as an important species for sport fishing and farming (Gajardo *et al.*, 1998).

In Chile, the restocking and spreading of strains in wild environments and the policies followed to do this have not been accurately documented. Thus, the implementation of monitoring of genetic diversity of these wild and farmed populations is relevant given their influence on the fauna of local populations in the wild and the possibility of improving breeding (Gajardo *et al.*, 1998; Farrington *et al.*, 2004). In this context, the present study analyzed the genetic diversity of naturalized rainbow trout populations in two river basins in central-northern Chile, as well as six commercial strains introduced for cultivation.

## MATERIALS AND METHODS

### Sample collection

Elqui and Limarí rivers are two main river basins located in a semi-arid climate in northern Chile, characterized by scarce rainfall and large inputs of water from snowmelts. River flows fluctuate dramatically depending on season and heavy rainfalls associated with the periodic occurrence of the El Niño event (Vila *et al.*, 1999).

Sampling areas are shown in Fig. 1. Two populations were collected between September, 1994 and May, 1995 from La Laguna (30°02'S, 70°05'W) and Incaguas (29°30'S, 70°26'W) rivers, both belonging to the north-eastern branch of the Elqui River basin, and another sample from Claro River (30°02'S, 70°30'W), belonging to the south-eastern tributary of the Elqui River. Samples from Claro River were collected in late September, 1994 (one sample-RCL1) and in late May, 1995 (two samples-RCL2 and RCL3). Samples from the Limarí River basin were collected in May, 1996, one from Hurtado River (30°17'S, 70°42'W) and another from Grande River (30°55'S, 70°46'W). Fishes were collected using electric fishing. The Donaldson, Cofradex and Steelhead strains were provided by the Lautaro fish farm, Lautaro (38°31'S, 72°26'W); the American and Scottish strains were provided by the Blanco River farm (32°55'S, 70°16'W) and the Kamloops strain was provided by a fish farm in the Macul stream (Santiago; 33°30'S, 70°30'W). Specimens were frozen in the field using dry ice or liquid



**Figure 1.** Location of sampling sites for rainbow trout in the Elqui and Limarí River basins, Coquimbo Region, Chile.

nitrogen, and subsequently stored at  $-20^{\circ}\text{C}$  until analysis. For data analysis, samples from La Laguna and Incaguas rivers were grouped and treated as from the same geographical origin. The same procedure was followed with samples from Claro River taking into account the geographical proximity and the absence of obvious barriers to migration between the sampling places.

### Sample analysis

Muscle and liver tissue samples were obtained from each specimen. Each sample was homogenized in a grinding buffer (0.001M EDTA 2Na, 0.01M Tris). Electrophoresis was carried out on horizontal 10% starch gel following the general procedures described by Aebersold *et al.* (1987) and Hillis & Moritz (1990). The buffers and the 20 enzyme systems analyzed were; 1) Clayton & Tretiak (1972) buffer: Aspartate amino transferase (AAT; 2.6.1.1), Aconitate hydratase (ACO;

4.2.1.3), Malic enzyme (ME; 1.1.1.40), Glycerol-3-phosphate dehydrogenase (G3PDH; 1.1.1.8), Isocitrate dehydrogenase (IDH; 1.1.1.42), Malate dehydrogenase (MDH; 1.1.1.37), Glucose-6-phosphate dehydrogenase (6PGD; 1.1.1.49), Phosphoglucomutase (PGM; 5.4.2.2) and Superoxide dismutase (SOD; 1.15.1.1); and 2) Ridgway *et al.* (1970) buffer: Alcohol dehydrogenase (ADH; 1.1.1.1), Adenylate kinase (AK; 2.7.4.3), Creatine kinase (CK; 2.7.3.2), Diaphorase (DIA; 1.6.2.2), Esterase (EST; 3.1.1.\*), Glicerato dehidrogenasa (GLYDH; 1.1.1.29), Glucose-6-phosphate isomerase (GPI; 5.3.1.9), L-Iditol dehydrogenase (sIDDH; 1.1.1.14), Lactate dehydrogenase (LDH; 1.1.1.27), Transferrine (TF) and Xanthine dehydrogenase (XDH; 1.2.1.37).

### Data analysis

Duplicate loci (*IDH-3,4\** and *MDH-3,4\**) were treated as two disomic loci with identical allele frequencies

because allele variation could not be assigned to a specific locus (Allendorf & Thorgaard, 1984), although this treatment tends to give conservative estimates of heterozygosity and genetic differentiation (Krueger *et al.*, 1989). Alleles for all enzymes were named according to their relative electrophoretic mobility, outlined by Krueger & May (1987).

Hardy-Weinberg equilibrium expectations for genotype frequencies at polymorphic loci were tested using a Chi-square test for goodness-of-fit, and the probability of the null hypothesis was estimated using a Monte Carlo simulation with 10,000 iterations. The genetic structure within and between populations was analyzed using the *F*-statistic (Wright, 1978; Nei, 1987). The *F*-statistic estimates were calculated following the method of Nei & Chesser (1983) and its statistical significance was tested using a Chi-square test for homogeneity of allele frequencies across samples, and the probability of the null hypothesis was estimated using a Monte Carlo simulation with 10,000 iterations. The sequential Bonferroni correction after Hochberg (1988) was applied to avoid the type I error resulting from multiple significance testing of the same null hypothesis (Rice, 1989). Estimates of genetic variability were calculated for each sample (Nei, 1987). The computer program Bottleneck v1.2.02 (Cornuet & Luikart, 1996) was used to detect whether there was a recent reduction in effective population size. In a bottleneck population, gene diversity will be higher than that expected at equilibrium. Gene diversity was estimated under three models: SMM, IAM and the two-phase model (TPM) of Di Rienzo *et al.* (1994) which may be closer to the true model of mutation for most loci. Recent bottleneck (heterozygosity excess) effects were tested using those three models with a Wilcoxon sign-rank test (Luikart *et al.*, 1998).

Nei's (1978) unbiased genetic distances (*D*) among population pairs were estimated. The resulting pairwise genetic distance matrices were used to build Neighbor-Joining (NJ) trees (Saitou & Nei, 1987). To assess the confidence of the obtained trees, 1,000 bootstrap replicates of each data matrix were generated (Felsenstein, 1985). A Neighbor-Joining analysis was done using the 10 allozymic loci shared between the present study and that reported by Gajardo *et al.* (1998) in southern Chile, Gregg *et al.* (2001) in England, and Farrington *et al.* (2004) in Australia, (*ADH-1\**, *G3PDH\**, *GPI-1,2\**, *GPI-3\**, *IDH3-4\**, *MDH -1,2\**, *MDH3-4\**, *ME-1\**, *ME-2\** and *SOD\**) (Table 1 for alleles comparison).

The samples were also ordered with a nonmetric multidimensional scaling (MDS) of the pairwise *F<sub>ST</sub>* matrix, and the final stress was calculated (Dunn & Everitt, 1982). The genetic data were analyzed using the

FSTAT v2.9.3 (Goudet, 2002), GeDis v1.8 (Peña *et al.*, 2009), GENEPOP v4.1.1 (Raymond & Rousset, 2000), Bottleneck v1.2.02 (Cornuet & Luikart, 1996) and Phylip v3.69c computer programs.

## RESULTS

Of the 20 enzyme systems analyzed, a total of 38 presumptive loci were resolved, 9 were found to be polymorphic in at least one of the populations in the study (*ACO\**, *DIA\**, *GLYDH\**, *G3PDH-3\**, *PGM-2\**, *SOD\**, *TF\**) and the duplicate loci *IDH-3,4\** and *MDH-3,4\** (Table 2).

Alleles per locus and allele frequencies at each population are showed in Table 2. Samples from the Limarí river basins did not show variation for the *ACO\**, *DIA\**, *GLYDH\**, *G3PDH\** and *TF\** locus, but in these populations an exclusive *PGM-2\*60* allele was observed with significant differences in frequency among them ( $P < 0.05$ ). Samples from the Elqui basin did not show variation for *ACO\** and *GLYDH\**. In the La Laguna sample an allele (*DIA\*95*) was only shared with the Cofradex strain. The Claro River sample was the only wild population that showed the allele (*G3PDH\*120*), which was also observed in the cultured Steelhead and Donaldson strains. The allele *IDH-3,4\*70* in the La Laguna sample was also observed only in cultured strains, except for Steelhead, but the allele *IDH-3,4\*90* was exclusive to the Cofradex strain. In both basins, Elqui and Limarí, *F*-values for the locus *IDH-3,4\** were significantly different from zero ( $P < 0.01$ ), and also *PGM-2\** in the Limarí basin populations and in the Kamloops strain (Table 2).

Wild rainbow trout populations showed a similar range of variation in heterocigosity, polymorphism (P%) and number of alleles per locus than commercial strains (Table 2). In turn, wild populations from the Elqui basin showed higher genetic variability than populations from the Limarí River basin (Table 2). Only the sample from Claro River showed a significant departure from Hardy-Weinberg equilibrium in genotype frequencies ( $P < 0.05$ ), having an excess of observed homozygotes. This population was built by pooling three subsamples from the same river with different size distributions (Fig.4).

The *F<sub>ST</sub>* value of all populations was 0.222, ranging between 0.014 for locus *DIA\** to 0.361 for locus *TF\**, with all values being different from zero ( $P < 0.05$ ), except for *DIA\** ( $P > 0.05$ ). *F<sub>ST</sub>* between the wild populations was 0.155 and for between hatchery samples it was 0.213, both highly significant ( $P < 0.001$ ). Similarly, pair-wise comparisons between popu-

**Table 1.** Approximate size and size inferred from allele frequencies of loci in *O. mykiss* analyzed by different authors.

Loci	Alleles	Gajardo <i>et al.</i> (1998)	Gregg <i>et al.</i> (2001)	Farrington <i>et al.</i> (2004)
<i>ADH-1</i>	Monomorphic	Monomorphic	Monomorphic	Monomorphic
<i>G3PDH</i>	120	Monomorphic	Monomorphic	120
	100			100
<i>GPI-1,2*</i>	Monomorphic	Monomorphic	Monomorphic	Monomorphic
<i>GPI-3*</i>	Monomorphic	Monomorphic	Monomorphic	Monomorphic
<i>IDH-3,4*</i>	120	110	----	120
	100	100	100	100
	90	90	----	----
	70	----	70	80
	40	----	40	60
<i>MDH-1,2*</i>	Monomorphic	Monomorphic	Monomorphic	Monomorphic
<i>MDH-3,4*</i>	125	----	Monomorphic	----
	100	100		100
	----	95		80
	60	90		60
<i>ME-1*</i>	Monomorphic	105	110	Monomorphic
		100	100	
<i>ME-2*</i>	Monomorphic	100	Monomorphic	Monomorphic
		95		
<i>SOD-1*</i>	140	115	150	150
	100	100	100	100
	40	----	50	----

lations were highly significant ( $P < 0.001$ ), and only the comparisons between rivers within the Limarí basin, and between the Limarí basin and the Steelhead strain did not show differences ( $P > 0.05$ ; Table 3). The analysis to detect recent effective population size reductions from allele data frequencies showed a significant departure from the IAM model only for the Grande River samples ( $P < 0.05$ ).

The Neighbor-Joining tree (Fig. 2a) grouped rainbow trout populations from the Limarí basin in a cluster together with the Steelhead strain. Populations from the Elqui basin are enclosed in a broader cluster that includes the Donaldson and Cofradex commercial strains, the cluster of Limarí basin populations and the Steelhead strain. The next Neighbor-Joining tree and MDS, including data of populations from southern Chile, England and Australia (Fig. 2b, 3) showed that, with the exception of the American, Kamloop and Scottish commercial strains, the remaining populations are grouped in a large cluster that include all the rainbow trout populations studied at present in Chile, and also strains from England and Australia (75%). Within this group, samples from southern Chile and Australia (70%) are clearly distinct from the stocks of the Limarí basin and Steelhead (56%).

## DISCUSSION

The results show that rainbow trout populations in the basins of the Limarí and Elqui rivers in north-central Chile retain relatively high levels of genetic variability, demonstrating genetic differences among them and with southern populations. However, rainbow trout populations from Elqui basin had higher genetic variability than those from the Limarí basin, as shown heterozygosity levels. They also show genetic associations with different cultured strains.

Intrapopulation analysis showed a private allele in populations in the Limarí basin (*PGM-2\*60*), and rare alleles in the Elqui basin were not detected in Limarí, but in cultured strains they were. This, coupled with the absence of statistically detectable bottlenecks, indicates that populations have retained similar levels of variability compared to the original populations (Allendorf & Phelps, 1981; Hershberger, 1992; Krueger *et al.*, 1994).

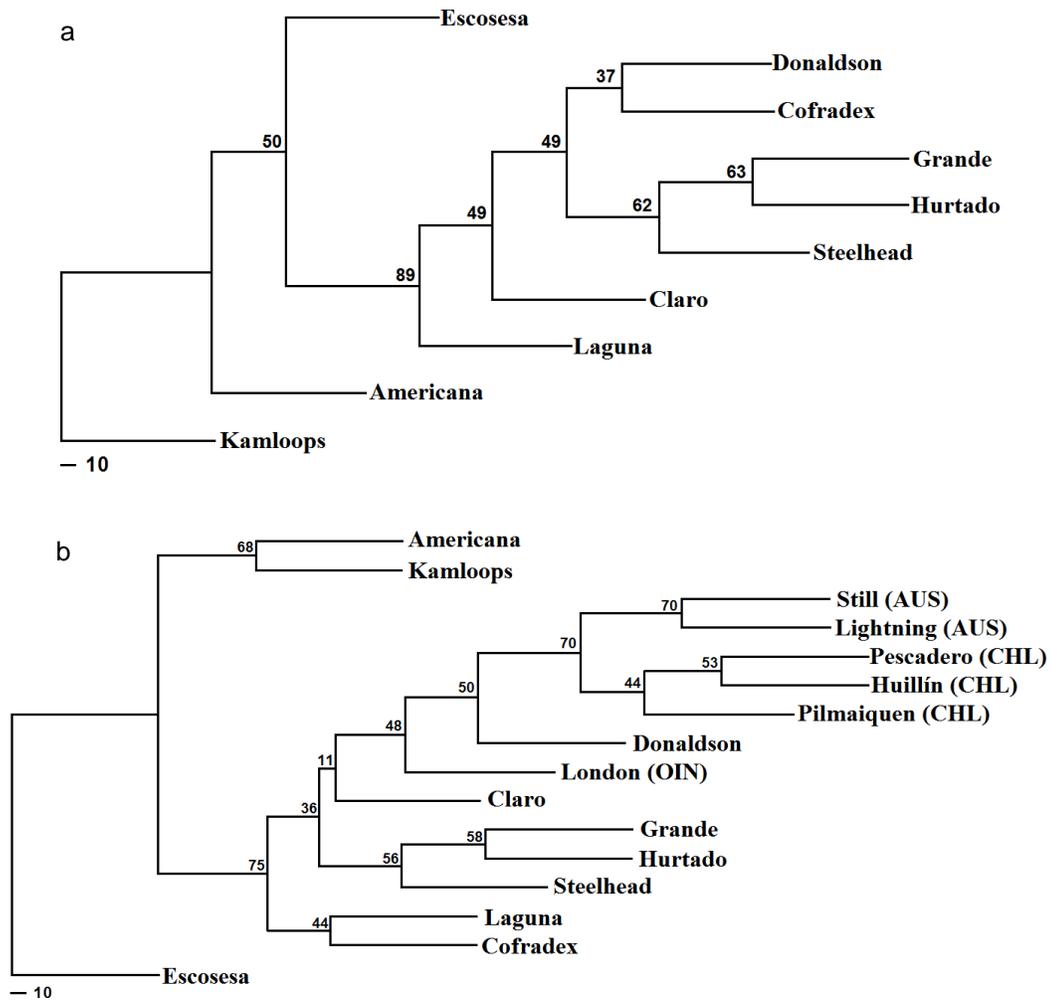
Only the population of Claro River showed a significant departure from Hardy-Weinberg equilibrium (excess homozygotes). This could be due to the "Wahlund effect" caused by mixing of different year-class individuals with different allele frequencies in the

**Table 2.** Allele frequencies of rainbow trout for 9 polymorphic loci in four naturalized populations from northern Chile and six farmed stock. Number of individuals (N), and Heterozygote deficiency (F) by locus in each population. The superscripts indicate those significant  $\chi^2$  test values after a Bonferroni's correction (<sup>a</sup>:  $P < 0.05$ , <sup>b</sup>:  $P < 0.01$ ).

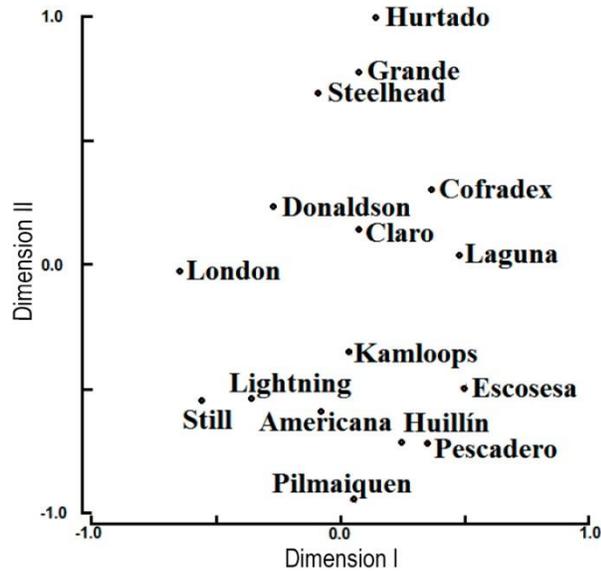
Locus	Allele	Populations											
		Elqui Basin			Limari Basin			Commercial strains					
		Laguna	Claro	Grande	Hurtado	Steelhead	Donaldson	Cofradex	Escocesa	Kamloops	Americana		
<i>ACO*</i>	N	22	58	30	30	20	20	20	56	89	102		
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.920	0.956	1.000		
	83	-	-	-	-	-	-	-	0.043	0.031	-		
	67	-	-	-	-	-	-	-	0.036	0.013	-		
<i>DIA*</i>	F	0.977	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000		
	95	0.023	-	-	-	-	0.025	-	-	-	-		
	F	-0.023	-	-	-	-	-0.026	-	-	-	-		
<i>GLYDH*</i>	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.889	0.871	1.000		
	78	-	-	-	-	-	-	-	0.111	0.129	-		
	F	-	-	-	-	-	-	-	-0.029	-0.140	-		
<i>G3PDH-3*</i>	120	-	0.008	-	-	0.025	0.225	-	-	-	-		
	100	1.000	0.992	1.000	1.000	0.975	0.775	1.000	1.000	1.000	1.000		
	F	-0.008	-0.008	-	-	-0.025	-0.290	-	-	-	-		
<i>IDH-3,4*</i>	120	0.023	0.043	0.167	0.033	0.250	0.100	-	-	0.026	-		
	100	0.523	0.448	-	-	-	0.150	0.200	0.818	0.647	0.599		
	90	-	-	-	-	-	-	0.075	-	-	-		
	70	0.023	-	-	-	-	0.200	0.150	0.100	0.064	0.156		
<i>MDH-3,4*</i>	40	0.431	0.509	0.833	0.967	0.750	0.550	0.575	0.082	0.263	0.245		
	F	-0.673 <sup>b</sup>	-0.757 <sup>b</sup>	0.532 <sup>b</sup>	1.000 <sup>b</sup>	-0.310	-0.256	-0.223	0.073	-0.074	-0.178		
	125	-	-	-	-	-	-	-	0.058	0.067	-		
	100	0.409	0.552	0.533	0.500	0.650	0.850	0.400	0.558	0.762	1.000		
<i>PGM-2*</i>	60	0.591	0.448	0.467	0.500	0.350	0.150	0.600	0.384	0.171	-		
	F	0.083	-0.106	-0.054	-0.049	-0.295	-0.151	0.191	0.005	-0.013	-		
	200	-	-	-	-	-	-	-	0.042	0.236	0.250		
	100	0.955	0.888	0.867	0.967	0.975	0.975	0.700	0.958	0.768	0.750		
<i>SOD*</i>	80	0.045	0.112	-	-	0.025	0.025	0.300	-	-	-		
	60	-	-	0.133	0.033	-	-	-	-	-	-		
	F	-0.034	-0.117	-0.137	-0.178	-0.026	-0.026	-0.407	-0.043	0.252 <sup>a</sup>	-0.072		
	140	0.386	0.009	0.121	0.150	0.150	0.200	0.375	0.170	0.104	0.338		
<i>TF*</i>	100	0.614	0.991	0.879	0.850	0.850	0.800	0.625	0.688	0.813	0.662		
	40	-	-	-	-	-	-	-	0.143	0.083	-		
	F	-0.055	-0.117	-0.120	0.361	-0.151	0.088	0.277	-0.120	-0.164	-0.160		
	100	0.675	0.632	1.000	1.000	1.000	0.500	0.650	1.000	1.000	1.000		
He	95	0.325	0.368	-	-	-	0.500	0.350	-	-	-		
	F	-0.254	0.333	-	-	-	-0.115	0.146	-	-	-		
	0.057	0.046	0.033	0.024	0.033	0.058	0.068	0.052	0.045	0.037			
Ho	0.065	0.056	0.031	0.020	0.039	0.066	0.067	0.040	0.037	0.041			
	1.21	1.18	1.11	1.11	1.13	1.21	1.21	1.30	1.30	1.11			
	15.79	15.79	10.53	10.53	15.16	15.79	15.79	15.79	15.79	7.90			

**Table 3.**  $F_{ST}$  values for 38 allozyme loci between population pairs of four naturalized populations of rainbow trout in northern Chile and six farmed stock. The superscripts indicate those significant  $\chi^2$  test values after a Bonferroni's correction ( $^b$ :  $P < 0.01$ ).

Populations	Laguna	Claro	Grande	Hurtado	Steelhead	Donaldson	Cofradex	Escosesa	Kamloops	Americana
Laguna	-----									
Claro	0.080 <sup>b</sup>	-----								
Grande	0.200 <sup>b</sup>	0.161 <sup>b</sup>	-----							
Hurtado	0.232 <sup>b</sup>	0.198 <sup>b</sup>	0.010	-----						
Steelhead	0.144 <sup>b</sup>	0.137	0.026	0.047	-----					
Donaldson	0.101 <sup>b</sup>	0.147 <sup>b</sup>	0.241 <sup>b</sup>	0.271 <sup>b</sup>	0.081 <sup>b</sup>	-----				
Cofradex	0.038	0.101 <sup>b</sup>	0.152 <sup>b</sup>	0.181 <sup>b</sup>	0.093 <sup>b</sup>	0.079 <sup>b</sup>	-----			
Escosesa	0.130 <sup>b</sup>	0.185 <sup>b</sup>	0.316 <sup>b</sup>	0.360 <sup>b</sup>	0.303 <sup>b</sup>	0.319 <sup>b</sup>	0.233 <sup>b</sup>	-----		
Kamloops	0.177 <sup>b</sup>	0.162 <sup>b</sup>	0.272 <sup>b</sup>	0.318 <sup>b</sup>	0.296 <sup>b</sup>	0.358 <sup>b</sup>	0.246 <sup>b</sup>	0.055 <sup>b</sup>	-----	
Americana	0.254 <sup>b</sup>	0.261 <sup>b</sup>	0.334 <sup>b</sup>	0.379 <sup>b</sup>	0.384 <sup>b</sup>	0.450 <sup>b</sup>	0.301 <sup>b</sup>	0.154 <sup>b</sup>	0.064 <sup>b</sup>	-----



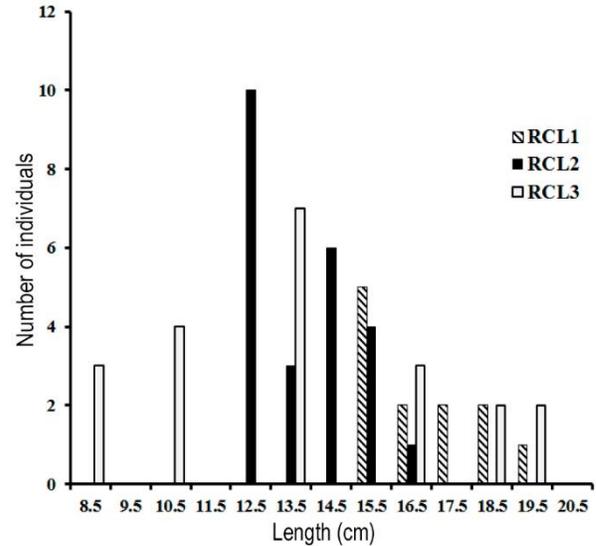
**Figure 2.** Neighbor Joining based on Nei's distances. a) Samples from Elqui basin, Limarí basin and strain culture, b) The samples from this study and samples from AUS (Australia wild; Farrington *et al.*, 2004), CHL (Southern Chile; Gajardo *et al.*, 1998) and OIN (Ohio-Indiana; Gregg *et al.*, 2001).



**Figure 3.** Multidimensional scaling (MDS) from the matrix  $F_{ST}$  by Reynolds *et al.* (1983) of the population from Elqui and Limarí basins, culture strain and samples from Gajardo *et al.* (1998) (Huillin, Pescadero and Pilmaiquen), Farrington *et al.* (2004) (Lightning and Still) and Gregg *et al.* (2001) (London).

same sample, as suggested by the differences in size distribution between subsamples from the river (Fig. 4). This type of phenomenon has been previously reported as being associated with the movement of older groups across the gradient of the inflowing waters, creating cohorts or spatial-temporal segregation between juveniles and adults (Biette *et al.*, 1981; Morán *et al.*, 1995). By contrast, southern feral populations analyzed by Gajardo *et al.* (1998) showed four out of the 10 loci with excess heterozygotes, three of them in the Huillin population. Taking into account the presence of rare alleles in Pilmaiquén trouts, they propose the existence of reproductive isolation between both populations.

Another way to assess the genetic diversity within populations is through heterozygosity, polymorphism and mean number of alleles. In our study, average values for wild and hatchery populations were  $H_e = 0.040$  and  $0.049$ ,  $P = 13\%$  and  $14\%$  and  $A_n = 1.15$  and  $1.21$ , respectively, without significant differences among them ( $P < 0.05$ ). Wild rainbow trout populations from southern Chile showed values of  $P = 21.8\%$  and  $A_n = 1.26$  (Gajardo *et al.*, 1998). Their average  $H_e$  estimations ( $0.07$ ) were higher than those observed in the present study. This is a consequence of differences in allele frequency at polymorphic loci. Northern Chile populations showed higher frequency of rare alleles, which inflate polymorphism and the number of alleles per locus, but not heterozygosity to the same extent



**Figure 4.** Size (total length) frequency distribution of rainbow trout sampled in the Claro River.

(Currens *et al.*, 1990). In Australia, wild rainbow trout introduced over 100 years ago and hatchery strains showed values of  $H_e$  between  $0.020$  and  $0.042$  and  $P$  from  $16\%$  to  $22\%$ , respectively, without significant differences between hatchery and wild naturalized populations (Farrington *et al.*, 2004). These results are very close to our findings in northern Chilean populations of rainbow trout.

Average expected heterozygosity values ( $H_e$ ) described in the literature in cultured strains of rainbow trout range from  $0.012$  and  $0.096$ , which is not an extremely wide range if we consider the large number of studied strains (Thompson, 1985; Paaver, 1988; Ferguson *et al.*, 1993; Gregg *et al.*, 2001; Farrington *et al.*, 2004). The  $H_e$  observed in almost all commercial strains in the present study were close to the upper limit of variability described for the species, consistent with the previous reports for all forms of *O. mykiss* (Ryman, 1983; Krueger & May, 1987; Berg & Gall, 1988; Hershberger, 1992). However, in the present study  $H_e$  in the Donaldson strain was almost double the value reported for the same strain in Russia ( $H_e = 0.023$ , Paaver, 1988) and close to values observed in Steelhead ( $H_e = 0.040$ , Paaver, 1988). Paaver (1988) did not detect variability at the *PGM-2\** locus in Steelhead nor in Donaldson strains, but in the present study both strains (20 individual in each sample) show an undescribed low frequency allele (80), a phenomenon that suggests that in Chile these strains could receive genes from other commercial lines. On the other hand, the retention of high levels of enzyme heterozygosity in rainbow trout culture strains has been associated with mixture of origins during its founding and subsequent

propagation or balanced selection followed by a population bottleneck and artificial selection (Busack *et al.*, 1979; Thompson, 1985; Ferguson *et al.*, 1993).

The relative genetic diversity estimated among all samples was 22%. This is the largest genetic diversity reported in studies in rainbow trout populations. Relative genetic diversity between wild populations was much lower (15%). However, it is larger than that observed between wild populations from eastern North America (3.6-5.5%; Krueger & May, 1987; Krueger *et al.*, 1994), western North America (1.8%-13.2%; Allendorf & Phelps, 1981; Berg & Gall, 1988; Reisenbichler & Phelps, 1989), between interior and coastal regions of Scandinavia and northern North America (15%; Gyllensten, 1985), or between wild stocks from southern Chile lakes (5.2%; Gajardo *et al.*, 1998). Limarí and Elqui basins are well separated by their geographic and hydrographic characteristics, and both are affected by strong seasonal and inter-annual flow variations. Water flow variations over 6,000% and 10,000% have been registered within one year in Elqui and Limarí basins, respectively, during the El Niño event (Dirección General de Aguas, Chile). Altered environmental conditions that accompany demographic expansion could shift fitness and survival patterns within and among populations creating a variety of effects on the gene pool (Nielsen, 1999). So, changes in effective population size (Ardren & Kapuscinski, 2003), gene flow among surviving groups and possible reintroductions must be considered to understand the population genetic structure within the basins (Avise, 1994; Allendorf & Waples, 1996). This could explain the high levels of genetic differentiation among populations accompanied with the retention of high levels of within-population genetic variation in the rainbow trout populations from northern Chile.

The cultivated strains of this species, as wild populations, normally retain high levels of within-population genetic variation (Milner *et al.*, 1979; Thompson, 1985; Paaver, 1988; Winkler, 1994; Cárcamo, 1999). In our study, the average genetic differentiation ( $F_{ST}$ ) among the six strains reaches 21%, close to the 23% obtained by Gregg *et al.* (2001) from the analysis of 12 strains, and higher than those described by Thompson (1985) from 5 strains (10%).

Allozyme analysis of rainbow trout populations from the different river basins in Chile showed genetic differences that could be caused by the source of trout used to first seed some river basins, but also by subsequent reseeding of the rivers with rainbow trout of different origins. As an example, Neighbor-Joining analyses with 38 and 8 loci, as well as the multidimensional scaling analysis strongly suggest that the rainbow trout populations of Limarí basin could

originate from the Steelhead strain with unsuccessful or non-subsequent reseeding of the rivers with trout of different origins. On the other hand, the Elqui basin populations like to be closely related with Donaldson (Washington, USA; Donaldson & Olson, 1957), Cofradex, (Denmark; Mardones & Vega, 1993), and London strains (UK; Gregg *et al.*, 2001). The latter strain was developed in London, but was obtained by interbreeding rainbow trout from Bowden Ohio, West Virginia, the Shepherd of the Hills Hatchery in Missouri and the Manchester Hatchery in Iowa (Gregg *et al.*, 2001). This situation involves two different scenarios, one in which there is a mixture in the origin of the introduction and subsequent naturalization or, a second option, where an initial introduction was followed by subsequent successful reintroductions of trout from different origins. Given the high levels of genetic variability, similar to its native range, we think the second option is the most feasible. By contrast, brown trout (*Salmo trutta*), another salmonid species introduced at the beginnings of the 20<sup>th</sup> century, shows lower genetic divergence for allozyme markers (9.5-12.64%) among Chilean populations (Faúndez *et al.*, 1997; Colihueque *et al.*, 2003), suggesting a more homogeneous origin.

The above discussed result shows that rainbow trout stocks from different river basins in Chile have substantive within and between genetic variations at allozyme loci. Rivers along Chile exhibit strong environmental variation in a north to south climatic cline (Miloslavich *et al.*, 2011). After 100 years of the presence of this introduced species, and assuming both selective and non-selective processes affecting the rainbow trout populations in the wild, it is possible that feral populations of rainbow trout in Chile can be a promising source of gene variability that can be exploited for aquaculture and/or conservation purposes. More detailed studies are necessary to map the amount and distribution of these resources in Chile.

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