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Impact of climate change and human-mediated introgression on southern European Atlantic salmon populations

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Abstract

This study focuses on temporal changes in Atlantic salmon (*Salmo salar*) populations from the vulnerable periphery of the species range (northern Spain). Using microsatellite markers to assess population structuring and introgression of exogenous genes in four different temporal samples collected across 20 years, we have determined the relative weights of climate and stocking practices in shaping contemporary regional population genetic patterns. Climate, represented by the North Atlantic Oscillation Index, was identified as the main factor for determining the level of population genetic differentiation. Populations within the region have become homogenized through gene flow enhanced by straying of adult salmon from natal rivers and subsequent interchange of genes among rivers due to warmer temperatures. At the same time, and in line with documented changes in stock transfer strategies, evidence of genetic introgression from past stock transfers has decreased throughout the study period, becoming a secondary factor in erasing population structuring. The ability to disentangle the effects of climatic changes and anthropogenic factors (fisheries management practices) is essential for effective long-term conservation of this iconic species. We emphasize the importance of evaluating all factors which may be linked to stocking practices in vulnerable species, particularly those sensitive to climate change.

Keywords: anthropogenic-mediated migration, gene flow, NAOI, population structure, *Salmo salar*

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Introduction

Biological invasions constitute one of the main risks for wild ecosystems. As aquatic environments generally exhibit less physical barriers to migration than terrestrial landscapes, which are typically patchy, they are particularly prone to invasions (e.g. Sala *et al*., 2000). Exotic fish species are large-scale invaders of freshwater systems in all continents, from Europe to America (e.g. Gido *et al*., 2004; Jeschke & Strayer, 2005; Salmenkova, 2008), often following anthropogenic introductions. Salmonids are typical examples of deliberate human-mediated stock movements. For the purposes of fishing and aquaculture, they have been transferred to exotic regions such as South America and New Zealand, where they have adapted and in some cases become invaders (Pascual *et al*., 2002; Townsend, 2003; Valiente *et al*., 2007). Large-scale human-mediated stock movements have also occurred between regions within the natural geographic range of a species; for example, in the second half of the XX century farmed salmon and trout from central and north European countries have been transplanted to south European rivers, where native populations already existed (e.g. Moran *et al*., 2005; Ayllon *et al*., 2006). Farmed salmonids have the potential for impacting on population productivity, disrupting local adaptations and reducing the genetic diversity of wild salmon populations (e.g. Fleming *et al*., 2000; McGinnity *et al*., 2009). It seems prudent to treat farmed fish as exotic species and to recognize the potentially negative consequences for wild populations, particularly when the latter are of conservation concern (Weir & Grant, 2005). This is particularly the case for peripheral populations located at the edge of a species' range, for example, southern European Atlantic salmon populations (42–43°N), which are declining (Parrish *et al*., 1998) and are recognized as being severely endangered (Prouzet, 1990). Projections for the future suggest that further reductions in their population sizes (Boylan & Adams, 2006) are also likely.

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Climate change, however, has varied consequences for animal species: higher mortality in sheep (Hallett *et al*., 2004), variation in reproductive performance in marine birds (Croxall *et al*., 2002; Gjerdrum *et al*., 2003), changes in abundance, mortality and distribution of fishes (i.e. Finney *et al*., 2000; McFarlane *et al*., 2000, MacKenzie & Koster 2004). Among the species potentially sensitive to global warming (De Young *et al*., 2004), the migratory Atlantic salmon (*Salmo salar*, Salmonidae) provides an opportunity for a case study because of its broad distribution on both sides of the Atlantic Ocean and its sensitivity to environmental effects, particularly temperature (Mills, 1991). Recent stock declines have been attributed in part to global climate change, which affects both juveniles and adults in diverse ways, from growth to migratory behaviour (e.g. Friedland *et al*., 2003, 2009; Jonsson & Jonsson, 2004; Condron *et al*., 2005; Valiente *et al*., 2010). Another effect of climate change is the increase of sensitivity to invasions in aquatic ecosystems (e.g. Stachowicz *et al*., 2002; Rahel & Olden, 2008), yet the influence of climate change on introgression of genomes from farmed lineages into wild Atlantic salmon populations has scarcely been explored.

In Atlantic salmon, it is commonly assumed that a strong homing instinct (returning to the natal river for reproduction; Lohmann *et al*., 2008) produces genetic isolation between populations inhabiting neighbouring rivers. Thus, the appropriate scale for biological conservation and management units tends to be at the level of an individual river, and large-scale genetic analysis across the species' distribution has demonstrated that this is generally true (King *et al*., 2001). Gene flow does occur between salmon populations, but is constrained by local adaptation at the regional scale and is influenced by both coastal distance and temperature regime (Dionne *et al*., 2008). Valiente *et al*. (2010) have recently demonstrated that warm climate conditions tend to increase straying and, consequently, gene flow among salmon in southern French rivers. It is also known that, as a consequence of the abundant deliberate stock transfers of the XX century, foreign genomes native to northern European countries have introgressed into southern European populations, contributing to the erosion of population structuring (Moran *et al*., 2005; Ayllon *et al*., 2006). Therefore, between-river genetic differences appear to have been diminished by the concomitant effects of two factors, human-mediated introgression of alien genomes and a climate-mediated increase in gene flow. Such a reduction of the spatial component of genetic diversity could destroy local adaptations, reducing population fitness (Utter, 2004). In the present circumstances of global climate warming, it is crucial to follow the evolution of those introgressed populations for preventing, if possible, future risks to vulnerable populations.

The case study presented here focuses on the largest Atlantic salmon populations in northern Spain, the natural southern edge of the species' distribution in Europe. Until 1992, local populations were supplemented with fish from a range of foreign stocks (originally from northern regions such as Ireland, Norway, Iceland and principally Scotland), which produced small but significant (up to 9%) introgression of foreign genomes (Ayllon *et al*., 2006). Populations are currently managed by limiting catches per angler and conducting supportive breeding (Machado-Schiaffino *et al*., 2007; Horreo *et al*., 2008) and we expect that foreign genomes introduced in the past are being diluted in those populations because farmed and nonnative individuals released in the wild generally exhibit less fitness than native ones, e.g. Garcia-Vazquez *et al*., 1991; McGinnity *et al*., 1997, 2009; Martinez *et al*., 2001; Finnegan & Stevens, 2008.

The question addressed in the current study is, is it possible to disentangle the relative weights of climate and fisheries management in shaping contemporary regional population genetic patterns? Using microsatellite loci as genetic markers we determined gene flow and the level of population structuring in the region of Asturias in northern Spain, and estimated the relative effects of human-mediated introgression and climate-driven migration on the population pattern encountered in 4 different years over the last three decades.

Materials and methods

Sample collection

In total, 924 tissue samples (adipose fin biopsy or scales) taken from returning adult Atlantic salmon captured by anglers were analyzed from five rivers in the region of Asturias in northern Spain (Eo, Esva, Narcea, Sella and Cares rivers; Fig. 1). The samples were collected during the angling and reproductive seasons (March–July and November–December, respectively) in the years 1988, 1996, 2002 and 2007, and were preserved in ethanol (fin) or dried (scales) until analysis. Approximately, 50 individuals were analyzed for each river per year, except for the river Narcea in 1988 (26 samples) and the river Eo in 1996 (21 samples). Samples from the river Sella were not available in 1988. The samples obtained each year covered all cohorts returning to the river that year (in these rivers lifespan is 4 years, adults being generally aged 1.1, 2.1, 1.2, or 2.2, where the first number represents the number of years in the river and the second the number of years at sea).

Climate index

The North Atlantic Oscillation Index (NAOI) measures fluctuations in sea-level air pressure between the Atlantic subpolar low-pressure zone around Iceland and the subtropic

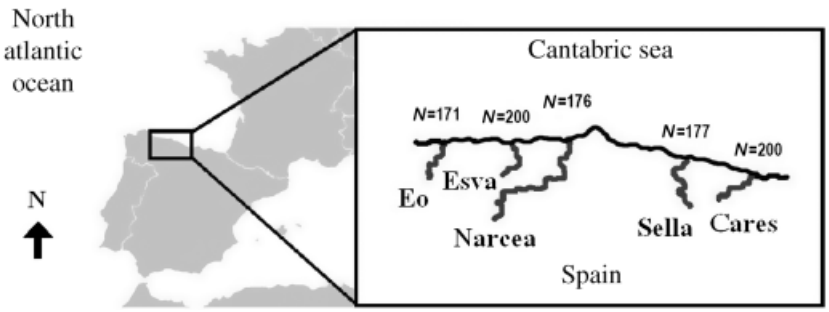

Fig. 1 Geographic location of the five rivers studied (Eo, Esva, Narcea, Sella and Cares) in northern Spain. N, total sample size for each river.

high-pressure zone around the Azores (Hurrell, 1995). We have chosen the normalized index Lisbon minus Stykkisholmur for the winter (December–March), obtained from http://www.cgd.ucar.edu/~ jhurrell. The use of the winter index is due to the fact that during this season the NAOI has the greatest effect on sea temperatures and hydrographic variability (Marshall *et al*., 2001; Ottersen & Stenseth, 2001; MacKenzie & Koster, 2004). Since the model species considered in this study is sensitive to climate oscillations in different life stages, and there are generation overlaps with breeders of different ages each year (e.g. Juanes *et al*., 2007), we have used the average NAOI of the 4 years of the species lifespan in this region as a proxy for the climatic conditions of each sampling period.

Stocking data

The juveniles released in the region for supplementing natural populations were obtained from two different sources: imported from commercial hatcheries in other countries (*foreign transfer*, FT), or from supportive breeding (the F1 generation produced from the artificial spawning of adults returning to the five rivers which are caught annually in the spawning season). In the second case, ova of one female are fertilized with sperm of one or two males. Embryos are reared in a hatchery and fed until the *parr* (juvenile) stage. Parr are released into the rivers of the region, most often in the river of origin of the breeders (*autochthonous*, A) or, less commonly, into another river within the region (*regional transfer*, RT).

Data concerning the release of parr were obtained from the Consejería de Medio Ambiente del Principado de Asturias, the administration in charge of managing the species in the region (jeronihr@princast.es). Reliable stocking reports with number of individuals released in each river were only available since 1981. Before that date, activity of FT had been reported only sporadically and therefore cannot be quantified.

DNA extraction and polymerase chain reaction methodology

Genomic DNA was extracted from tissue samples using Chelex resin (Estoup *et al*., 1996). Nine microsatellite loci were analyzed: SSsp2210, SSspG7 and SSsp1605 (Paterson *et al*., 2004), Ssa197 and Ssa202 (O'Reilly *et al*., 1996), SSOSL417, SSOSL85 and SSOSL311 (Slettan *et al*., 1995), and SS4 (Martinez *et al*., 1999).

PCR amplifications were performed on reaction mixtures containing about 50 ng of salmon DNA, 10 nM Tris.HCl pH 8.8, 2.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 0.35 mM of fluorescently labelled primers, 0.5 U of DNA Taq Polymerase (Promega, Fitchburg, WI, USA) and 250 μM of each dNTP in a final volume of 20 μL. Annealing temperatures were: 52° (SSOSL417), 55 °C (SSspG7, SSOSL85 and SSOSL311), 56 °C (Ssa202), 60 °C (SS4 and SSsp2210) and 61 °C (SSsp1605 and Ssa197). Possible contamination was checked by negative extraction and PCR controls. Size determination of the labelled PCR products was performed using an ABI 3100 automatic DNA Sequencer and the GENEMAPPER V.3.5 (Applied Biosystems, Foster City, CA, USA) software at the DNA Sequencing Unit of the University of Oviedo.

Estimates of genetic variability

Genotyping errors (null alleles and large allele dropout) were checked with the MICROCHECKER software (Van Oosterhout *et al*., 2004). The computer program GENETIX (Belkhir *et al*., 2004) was used for calculating sample variability and genotype frequencies. Conformity with Hardy–Weinberg equilibrium and linkage disequilibrium were calculated with GENEPOP (Raymond & Rousset, 1995). Microsatellite polymorphism in the region was quantified by allelic richness, gene diversity between samples (F_{ST}), observed (H_O) and expected (H_S) heterozygosity, relatedness (Rel) and corrected relatedness (Relc), and compared between sampling years using the program FSTAT version 2.9.3.2 (Goudet, 1995).

Population structure

Population structure across the region in each year of sampling was assessed using the program STRUCTURE 2.3.1 (Pritchard *et al*., 2000). This software estimates using a Bayesian method the minimum number of population units with a partial genetic identity in a dataset. The output from STRUCTURE provides Ln *P(D)* values for a given number of genetic units

in a dataset for each run. These values increase along K (the number of genetic units considered) until becoming stable. The number of genetic units is estimated in a conservative way, choosing the minimum number when Ln $P(D)$ becomes stable. The program organizes the dataset in groups of samples being a part of the same genetic unit. Parameter set: Length of Burn-in Period: 50 000, Number of MCMC Reps after Burn-in: 500 000, five runs for each K.

Estimates of migration rates

The estimates of the number of migrants between rivers (Nm) in each year were based on the private allele methodology of Barton & Slatkin (1986), using the program GENEPOP (Raymond & Rousset, 1995) with the following settings: 1000 dememorization steps, 100 batches, 1000 interactions per batch. Gene flow between populations was also estimated by Rho-values (Slatkin, 1995), which are based on differences in allele frequencies between populations, with the RSTCALC software (Goodman, 1997) using 1000 permutations and 1000 bootstraps.

Estimates of introgression of foreign genomes

Introgression of foreign genomes (mostly Scottish) had been estimated already for 1988 (Moran *et al*., 2005) and 1997 (Ayllon *et al*., 2006) samples. For reasons of internal consistency of the study we have applied the same methodology as used by Ayllon *et al*. (2006), based on the Monte-Carlo simulation implemented within the IMMANC program (Rannala & Mountain, 1997). Individuals of foreign origin (immigrants of first, second and consecutive generations) were identified based on their microsatellite genotypes (with 10 000 replications).

Principal component analysis (PCA)

A PCA was carried out using the PAST version 1.90 software (Hammer *et al*., 2001) to find hypothetical variables (components) which account for variance in multidimensional datasets of genetic characteristics (parameters of genetic variability, number of genetic units, estimated introgression) and environmental factors (NAOI) of the studied populations. Significant components of the PCA were identified, based on the Jolliffe cut-off value (Jolliffe, 1986), as those providing Eigenvalues >0.7. The program estimates the proportion of variance contributed by each component, and the sign (positive or negative) and relative contribution of each factor (in this case each population characteristics) within each component. The data were analyzed with default settings and the 'correlation matrix' option. A correlation matrix (linear r-values) was obtained with the same software before the PCA, to identify those factors or parameters which were significantly correlated ($P<0.05$). To avoid internal bias, one factor of each pair of significant correlates was eliminated from the PCA.

Results

Stocking history and foreign introgression

Two periods with markedly different stocking strategies can be identified (Table 1). The first period, from 1981 to 1992, was characterized by intense FT activity with some minor stocking of types A and RT. In the second period (1992–2007), both RT (at low level) and A stocking were carried out. FT represented between 13.1% and 59.7% (Sella and Eo rivers, respectively), RT between 0% (Narcea and Cares rivers) and 9.1% (Eo river) and A was 31.2–86.7% (Eo and Sella rivers, respectively) of the total number of individuals released in a river during the last three decades (1981–2008). In summary, three populations were stocked mainly with native salmon (Narcea, Sella, Cares, 76–87% of historical releases) and two populations were stocked with both foreign and native salmon (Eo, Esva), with regional transfers being scarce.

Variation at microsatellite loci

Variability was generally high in all samples (Table 2), with mean number of alleles per locus ranging from 9.5 to 12.1 in the Eo and Sella rivers, respectively. No evidence of scoring errors, allelic dropout or stuttering was detected with the MICROCHECKER software, except at

Table 1 Documented releases (in thousands of juveniles) of hatchery-reared Atlantic salmon into the main Asturian rivers from 1981 to 2008

	Enhanced river				
Origin	Eo	Esva	Narcea	Sella	Cares
Eo	188.8	–	–	–	–
Esva	–	466.6	–	–	–
Narcea	60	1.2	2975.2	7	–
Sella	–	–	–	4551.5	–
Cares	–	–	–	2.5	1731.4
Scotland	356	383	328.5	368.5	390.3
Iceland	–	25	73.1	63.5	110.7
Ireland	50	127.5	239	212.6	25
Norway	10	33	40	42.5	8.5
Total FT	416	568.5	640.6	687.1	534.5
	(59.7%)	(54.8%)	(17.7%)	(13.1%)	(23.6%)
Total RT	60.0	1.2	0	9.5	0
	(9.1%)	(0.1%)		(0.2%)	
Total A	188.8	466.6	2975.2		
	(31.2%)	(45.1%)	(82.3%)	4551.5	1731
				(86.7%)	(76.4%)

FT, foreign transfer (1981–1992); RT, regional transfer (1992–1996); A, autochthonous (1996–2008).

Table 2 Genetic variability at the microsatellite loci considered in the five rivers studied across the four sampling years (allele range/number of alleles per locus, Na)

Locus/sample	EO	ESVA	NARCEA	SELLA	CARES
SSsp2210	124–164/8.0	128–164/9.0	112–164/9.0	116–164/10.5	124–164/9.5
SSsp1605	218–250/6.0	218–246/6.0	218–250/7.0	214–250/7.5	218–250/6.0
Ssa202	236–268/8.5	236–272/9.5	236–272/8.0	236–268/8.0	236–272/9.5
SSOSL417	155–209/12.7	155–209/13.7	157–207/12.0	145–209/15.6	157–207/13.5
Ssa197	171–259/9.2	175–259/11.7	175–259/12.7	167–259/17.0	171–263/16.0
SSspG7	116–212/12.0	116–192/13.5	108–208/11.0	108–204/12.5	116–188/12.5
SSOSL85	180–230/10.0	180–228/13.2	170–228/11.5	124–228/12.3	164–228/13.2
SS4	176–232/9.7	176–238/11.2	176–254/9.5	176–254/10.3	176–254/11.5
SSOSL311	122–164/9.2	120–180/13.7	124–168/11.7	114–174/15.3	120–180/15.5
Mean Na (SD)	9.5 (2.0)	11.3 (2.7)	10.3 (1.9)	12.1 (3.4)	11.9 (3.2)

Mean Na of the loci analyzed per population (SD in parentheses).

locus SS4 in years 2002 and 2007, which exhibited signs of null alleles and was eliminated from further analyses. Genotype frequencies at the remainder of microsatellite loci were mostly in Hardy–Weinberg equilibrium after sequential Bonferroni correction (about 80% of tests). When they were not in equilibrium, deviation was due to heterozygote deficit. No evidence of linkage disequilibrium (generated by physical linkage of pairs of loci) was observed within the dataset.

Population structure and gene flow over time

Population structure changed over the period considered, and was generally consistent with changes in stocking practices (Fig. 2). In the 1980s (FT-based management), the five rivers of the region exhibited population structure indicative of two genetic units. For $K = 2$, maximum $\ln P(D)$ values [mean $\ln P(D) = -3493.92$, SD = 3.13] were obtained which were consistent across runs. The River Eo was alone in one cluster and the other three, Esva, Narcea and Cares rivers (there were no data for the River Sella), constituted another unit, with some mixture in the river Esva (Fig. 2). It should be noted that FT was more intense for the River Eo than for the rest (Table 1). In the 1990s (immediately post-FT), the five rivers may be considered to be one single genetic unit; the highest $\ln P(D)$ values, consistent across runs, were found for $K = 1$ [mean $\ln P(D) = -4803.76$, SD = 0.28; Fig. 2]. By 2002, about two generations later (generation length being 2.6 years in the region, Consuegra *et al*., 2005), the regional population structure suggested by the microsatellite loci was consistent with two genetic units [mean $\ln P(D) = -7950.72$ for $K = 2$, SD = 0.65], which also corresponded well to geographic relationships between the clusters or rivers. One unit comprised the Eo and Esva rivers, located in the west of the region, with the other eastward unit comprising the Narcea, Sella and Cares rivers (Fig. 2). In 2007, after 5 additional years under only type-A stocking, at least three units were identified with the STRUCTURE software [mean $\ln P(D) = -7989.9$ for $K = 3$, SD = 1.51]. One unit contained the westernmost river, the Eo, the second included the mixed rivers Esva, Narcea and Cares, and the third unit corresponded to the River Sella (Fig. 2).

The number of migrants per generation (Nm) among the five rivers calculated using private alleles oscillated between 4.16 and 11.4 in 1988 and 2002, respectively (Table 3). When estimated using Rho-values, among-river migration in the region followed similar oscillations. Overall, these results indicate that since the 1980s gene flow increased steeply until 2002 and decreased in 2007.

Introgression of foreign genomes, climate indices and population genetic parameters

Some individuals caught in the region were identified as immigrants (from the first to the fourth generation) in the four temporal periods analyzed: 2.16%, 6.81%, 2.96% and 1.31% in the 1988, 1996, 2002 and 2007 samples, respectively (Table 3). The proportion of individuals with immigrant ancestors was at a maximum in 1996 and seemed to decrease later. The population genetic parameter F_{ST} and the number of genetic units K (Table 3), also experienced fluctuations over time, following a similar pattern (increasing from 1988 to 1996, and then decreasing). A significant increase of mean allelic richness was found from 1988 to 1996 (7.264 and 9.132, respectively, P-value 0.026) and continued at a high level in the third and fourth sample years. The climatic index NAOI experienced high oscillations over the lifespan of the 1996 sample, ranging from −3.78 to 3.96, with somewhat less but still high variations in

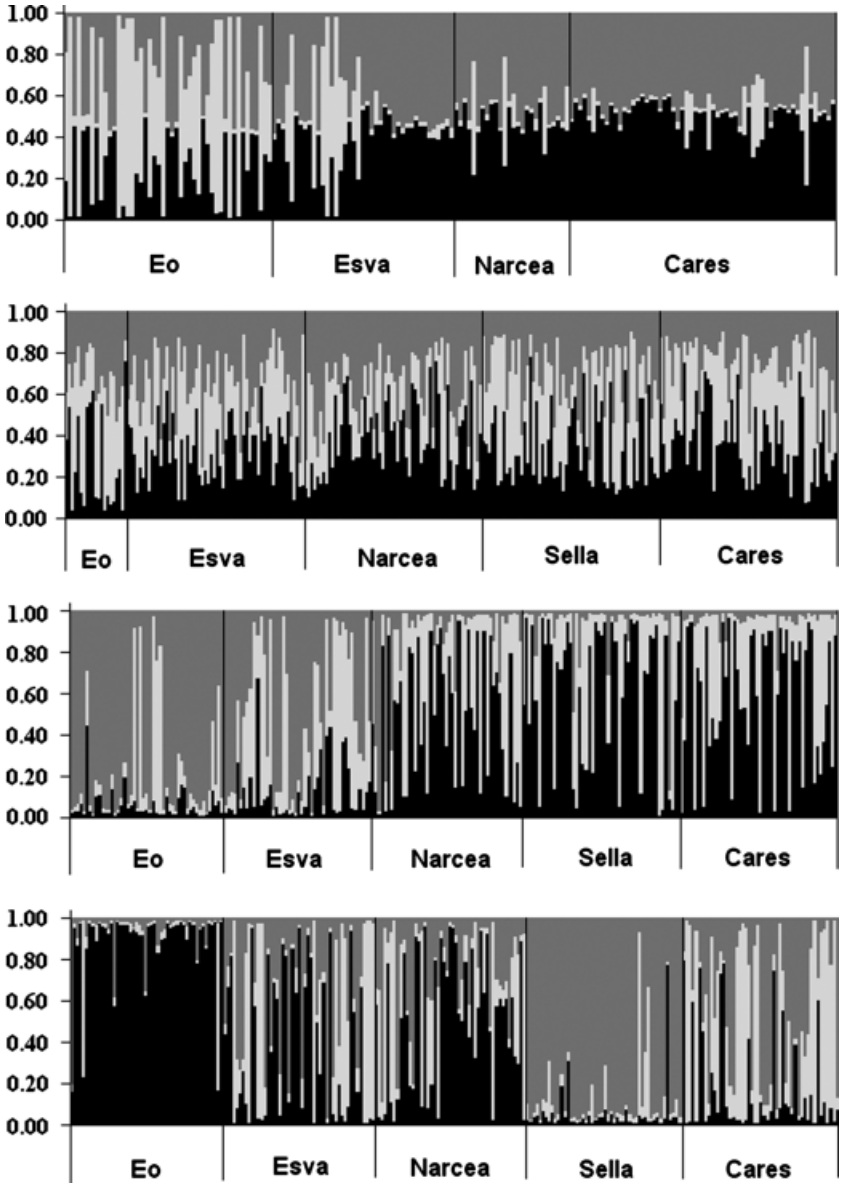

Fig. 2 Estimation of the membership fraction to each of three inferred clusters for North Spanish Atlantic salmon in (from top to bottom) 1988, 1996, 2002 and 2007, based on microsatellite loci. Each vertical bar represents one individual and vertical lines sticking out from the graph mark river limits. Each cluster is represented by one colour: dark grey, light grey, black. River Sella 1988 samples are missing.

those of the 2002 and 2007 samples. Maximum winter NAOI, however, was less pronounced and oscillations were lower for the lifespan of 1988 samples (Table 3). The 4-year average NAOI of 1996 was the highest and that of 1988 the lowest.

Although only four temporal points were studied, three significant correlations were found (Table 4). The two estimates of gene flow were also correlated to each other ($r = 0.983$, $P = 0.017$). The level of introgression was positively associated with the expected heterozygosity ($r = 0.982$, $P = 0.018$), as expected from introduction of new alleles in a region. The global climate index NAOI was negatively associated with F_{ST} ($r = -0{,}962$, $P = 0.037$), indicating that warm conditions decrease between-population diversity. F_{ST}, expected heterozygosity and Rho estimates were eliminated from the PCA in order to reduce biases due to internal correlations between population traits.

The PCA identified two significant components (Table 5), accounting together for more than 95% of the variance. The main contributor for explaining the dataset variance within Component 1 (59% variance)

Table 3 Population structuring, climate indices and estimates of foreign introgression in Asturian Atlantic salmon

Year	NAOI	Migration (Rho)	Migration (Private)	F_{ST}	AR	H_s	H_o	K	Introgression
1988	−0.04 [−0.75/0.72]	2.12	4.16	0.053	7.264	0.782	0.669	2	2.16
1996	1.47 [−3.78/3.96]	10.9	8.87	0.017	9.132	0.834	0.742	1	6.81
2002	0.84 [−1.89/2.8]	12.7	11.34	0.027	10.346	0.784	0.731	2	2.96
2007	0.44 [−1.09/2.8]	9.02	8.41	0.033	10.073	0.778	0.746	3	1.31

NAOI, North Atlantic Oscillation Index (average value for the 4-year lifespan of the samples, range in brackets); Migration, gene flow estimated by Rho-values (Rho) and Private Allele methodology (Private); F_{ST} value, mean pairwise F_{ST} values between populations; AR, allelic richness; mean heterozygosity expected (H_s) and observed (H_o); K, number of genetic units estimated using the program STRUCTURE; Introgression, regional introgression of non-native individuals as mean introgression of the rivers analysed.

Table 4 Correlation between genetic and climate parameters (r-values for linear correlations and P-values, below and above the diagonal, respectively)

	NAOI	Migration (Rho)	Migration (Private)	F_{ST}	AR	H_s	H_o	K	Introgression
NAOI	0	0.206	0.319	**0.037**	0.508	0.162	0.279	0.342	0.137
Migration (Rho)	0.793	0	**0.017**	0.094	0.098	0.668	0.124	0.834	0.607
Migration (Private)	0.680	**0.982**	0	0.187	0.073	0.828	0.188	0.937	0.748
FST	**−0.962**	−0.905	−0.812	0	0.298	0.319	0.122	0.569	0.304
AR	0.491	0.902	0.926	−0.701	0	0.955	0.117	0.724	0.984
H_s	0.837	0.332	0.171	−0.680	−0.044	0	0.662	0.135	**0.017**
H_o	0.721	0.875	0.81	−0.877	0.882	0.337	0	0.954	0.695
K	−0.657	−0.165	−0.063	0.430	0.275	−0.864	0.045	0	0.075
Introgression	0.862	0.392	0.252	−0.695	−0.015	**0.982**	0.305	−0.924	0

Significant values (>0.05) in bold.

Table 5 Principal component analysis of the dataset

	Component 1	Component 2	Component 3
% variance	59.336 (3.559)	36.737 (2.204)	3.933 (0.236)
NAOI	**0.5002**	−0.2172	0.1488
Gene flow	**0.4628**	0.2535	−0.6373
Allele richness	0.398	0.4416	−0.1624
Heterozygosity	0.4622	0.2587	0.6242
Structure	−0.213	**0.6067**	0.3398
Introgression	0.3437	**−0.5085**	0.2006

Percent of variance contributed by each component (Eigenvalue in parentheses) and by each factor within each component. Jolliffe's cut-off Eigenvalue: 0.7.

was NAOI, followed by migration (Table 5). Within Component 2, which accounted for almost 37% of the variance, the parameter measuring the level of population structuring (K, the number of genetic units) and the level of introgression (in this case negative) were the most important quantitatively. Migration and heterozygosity (H_o) were almost coincident and indistinguishable in the scatter diagram (Fig. 3). Introgression and NAOI were both in the same direction and in the opposite direction of population structuring in the region (Fig. 3, negative r-values in Table 4); the NAOI projection was longer, indicating greater contribution to the dataset variance. Except for the number of genetic units (regional population structuring), the parameters: migration estimate, allelic richness and heterozygosity were projected as positive on the X-axis, as were introgression and NAOI, suggesting concomitant effects of the two latter factors with increased gene flow and within-population genetic diversity in the region.

Discussion

The results obtained in this study are consistent with high levels of migration among rivers in Spanish salmon. The rule of One Migrant Per Generation (OMPG, which is the maximum interchange allowed for considering two populations as independent; Mills & Allendorf 1996) was easily surpassed during the period 1996–2007 (Table 3). Despite a marked homing instinct (Stabell, 1984), and significant population structuring in the region (Fig. 2), it seems clear that there is a significant level of interchange of breeders between rivers. This is reflected in lower F_{ST} values (Table 3) than those

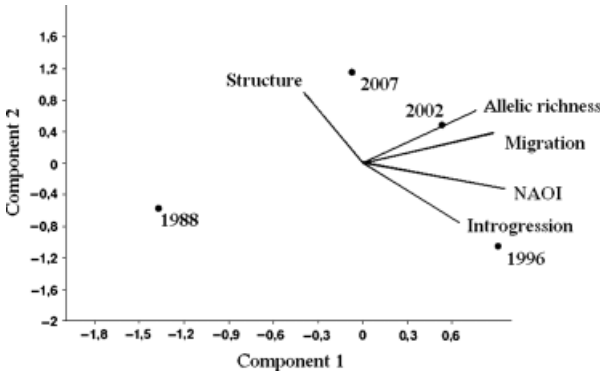

Fig. 3 Scatter diagram showing the principal components of the dataset. The length of the segments representing each factor is proportional to its relative weight in the principal component analysis (PCA).

reported by other authors at regional level for other latitudes (e.g. King *et al*., 2001). Genetic units comprised generally more than one river (Fig. 2), and mixture of different genetic units in the same river (migration) and mixed membership of some individuals was also observed.

At least part of the high levels of gene flow observed in salmon populations in northern Spain may be attributed to anthropogenic causes. Stocking with the same foreign stock released in all the rivers at the scale described in Table 1 appears to be the likely origin of at least some of the high levels of migration detected in our data, and this represents human-mediated migration. At the beginning of the studied period (1988) gene flow was higher than OMPG, and increased noticeably during the FT years, accompanied by a significant increase of allelic richness in 1996, which could be attributed to introgression of some foreign genomes into local populations (Ayllon *et al*., 2006). In the next period, the consequences of previous foreign stocking and stock transfers were observed, with parr transferred between rivers returning as adults 2 or 3 years later. In addition, the levels of straying, i.e. the return of adults back to nonnatal rivers, usually neighbouring rivers, to breed (Jonsson *et al*., 2003), are generally higher with hatchery-reared fish (Jonsson *et al*., 2003; Jonsson & Jonsson, 2006), which may also have contributed to the high levels of migration observed in our data. The level of introgression decreased dramatically after 1996 (Table 3), which may indicate dilution of foreign genes in the wild gene pool consistent with decreased fitness of alleles originating from farmed individuals (e.g. McGinnity *et al*., 1997, 2009; Martinez *et al*., 2001).

However, introgression of foreign genomes was not the sole or even the main reason for oscillations in gene flow and, consequently, population structuring. Climate seems to be quantitatively more important, at least from the results observed in the PCA (Table 5) and the significant negative correlation between NAOI and F_{ST} (Table 4). Other climate indices such as the Atlantic Multidecadal Oscillation have also been found to be closely related with other Atlantic salmon population characteristics, such as recruitment (Friedland *et al*., 2009). In the present case, we have considered average NAOI for the entire lifespan of the adults sampled in the region, as, without many additional studies, it is not possible to determine the exact moment or life stage when the population structuring was eroded. Long datasets of well-monitored populations (survival at hatching, juvenile growth, smolt age, age at maturity, fertility and many other traits) could serve for this purpose, and would facilitate investigation of the association of population data with various climate indices (NAOI, Atlantic Multidecadal Oscillation, others). Between-river migration of adults, or straying and entering a nonnatal river when returning from the sea, are possibilities; research by Valiente *et al*. (2010) for another region at the same latitude demonstrated that warm conditions enhanced straying, contributing to increased gene flow. Valiente *et al*. (2010) also reported indirect signals of increased contribution of freshwater-maturing males (juvenile-like or precocious parr) under warmer climate conditions; such a finding suggests that an increased contribution to spawning by mature male parr of foreign origin, which are known to be responsible for at least some genome introgression at this latitude (Moran *et al*., 1994), may also play a role in eroding population structuring. Thus, synergistic effects related to a warmer climate may act to accelerate the homogenizing effect of human-mediated introgression, even after the cessation of introductions of foreign stocks.

Critically, this study changes our perception of the current status of Atlantic salmon populations at the southern-most limit of their natural distribution, emphasizing the importance of considering anthropogenic management as an artificial source of gene flow and population homogenization added to (and likely enhanced by) climate effects. Human intervention (stocking) is known to have reduced population structuring in other species, for example, partridge (Chen *et al*., 2006), chukar (Barbanera *et al*., 2007) and many others (Laikre *et al*., 2006). In Atlantic salmon, hatchery releases may threaten genetic integrity of even geographically distant wild salmon populations (Vasemägi *et al*., 2005). The escape or deliberate introduction of captive bred animals into the wild can substantially depress natural recruitment and more specifically can disrupt the capacity of natural populations to adapt to

higher winter water temperatures associated with climate variability (McGinnity *et al.*, 2009). In addition, our results suggest that in the present conditions of accelerated climate warming the risk is even higher, because warm conditions will likely enhance mobility of hatchery individuals among rivers in a region. When a species is sensitive to climate, as are Atlantic salmon (Friedland *et al.*, 2003; Jonsson & Jonsson, 2004; Condron *et al.*, 2005), conservation programmes should be analyzed on a case-by-case basis and the population model assessed for each.

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