Conserv Genet (2013) 14:1217–1231
DOI 10.1007/s10592-013-0510-y

RESEARCH ARTICLE

Spatio-temporal effects of stray hatchery-reared Atlantic salmon *Salmo salar* on population genetic structure within a 21 km-long Icelandic river system

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Received: 14 December 2012 / Accepted: 24 June 2013 / Published online: 25 July 2013

Abstract Although the tendency of Atlantic salmon *Salmo salar* to form differentiated populations among rivers and among tributaries within large river systems (>100 km-long) is well documented, much less is known about population structure within small river systems (<30 km-long). In the present study, we investigated the genetic effects of straying of hatchery-reared salmon on population structure and genetic composition within the Ellidaár river system, a small system (21 km total length) in SW Iceland. We analyzed spatial and temporal variation of wild and domesticated samples (farmed and ranched; $n = 931$) using seven microsatellite loci. Estimates of population differentiation [F_{ST}, genetic tree (D_A)] and Bayesian cluster analysis (STRUCTURE) revealed a significant population structure as well as relative long-term temporal stability of the genetic composition in the main river from 1948 to 2005. However, the genetic composition of the tributary populations was unstable and genetically homogenized in recent years. Wild-hatchery hybrids were detected during the influx of strays as well as few years after, suggesting that introgression has changed the genetic composition of the wild populations. More investigations are needed in Iceland and elsewhere on possible fine-scale population differentiation and factors leading to it. Fine-scale population differentiation as observed in the present study has implications for the resolution with which harvest and habitat management of salmon should be conducted. In addition, farming and ranching operations should be located to minimize potential negative effects of strays on wild fish.

Keywords Atlantic salmon · Population structure · River system · Introgression · Hatchery salmon · Temporal stability

Electronic supplementary material The online version of this article (doi:10.1007/s10592-013-0510-y) contains supplementary material, which is available to authorized users.

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Introduction

Atlantic salmon *Salmo salar* can be characterized as consisting of a large number of distinct, local populations or stocks (sensu Ricker 1972) resulting from their highly developed homing ability (Stabell 1984; Youngson et al. 1994), reproductive isolation, and specific adaptations to local differences in the environment (Taylor 1991; Garcia de Leaniz et al. 2007; Fraser et al. 2011). Genetic studies have identified not only distinct population structure among rivers (e.g. King et al. 2001) but also at a finer scale among tributaries within relatively large river systems (>100 km-long, Galvin et al. 1996; Primmer et al. 2006; Vähä et al. 2008).

In the past century, widespread declines and extirpations of these distinct, locally-adapted populations have occurred throughout most of the Atlantic salmon's natural range. Many factors, singly or in combination, have negatively affected salmon populations, including habitat destruction, construction of dams, overfishing, pollution, and changing ocean conditions (Parrish et al. 1998). In addition, large-scale aquaculture may threaten wild salmon populations through genetic and ecological interactions (Hindar et al. 1991; Naylor et al. 2005; Jonsson and Jonsson 2006). Escapees from sea cages and strays from ranching operations have entered salmon rivers and in some cases they have become a large proportion of the adult run (Gudjónsson 1991; Heggberget et al. 1993; Morris et al. 2008). Several genetic studies have shown that hatchery-reared escapees and wild salmon interbreed in nature (Crozier 1993; Clifford et al. 1998a, b) and that introgression has changed genetic composition of wild populations (Crozier 2000; Skaala et al. 2006; Bourret et al. 2011; Glover et al. 2012). In a hatchery, farmed and ranched salmonids undergo domestication selection which may result in a rapid change of fitness-related traits and reduced fitness in the wild (Araki et al. 2007), even after only one generation (Christie et al. 2012). Introgression from hatchery-reared salmon can therefore erode local adaptation, disrupt co-adapted gene complexes of wild populations and reduce their fitness (Utter 2001; Fleming et al. 2000; McGinnity et al. 2003). Furthermore, introgression may homogenize population structure (Araguas et al. 2004; Van Houdt et al. 2005; Williamson and May 2005), reducing genetic diversity and ultimately the ability of salmon as a species to adapt to a changing environment (McGinnity et al. 2009).

The use of temporal samples has become an increasingly common approach for studying human-induced impacts on genetic composition and population structure of wild fish populations (Nielsen and Hansen 2008). The genetic analysis of material collected over time from a putative salmon population using highly variable markers (e.g. microsatellite loci) can provide information relevant to management and conservation. Stocking impacts on genetic composition within populations and on population structure have been assessed for various salmonids (Guinand et al. 2003; Eldridge et al. 2009; Hansen et al. 2009), including Atlantic salmon (Martinez et al. 2001; Blanco et al. 2005; Finnegan and Stevens 2008). Similarly, introgression from reared escapees into wild salmon stocks has been inferred from temporal samples (Crozier 2000; Skaala et al. 2006; Bourret et al. 2011; Glover et al. 2012).

Studies of genetic material collected over time have also provided important information on population structure and its temporal stability and persistence in Atlantic salmon. Studies within and among river systems have provided evidence of metapopulation structure exhibiting temporal instability in unstable environments (Garant et al. 2000) and temporal stability in stable environments (Vähä et al. 2008). In various cases, populations may be characterized genetically in relation to different geographical hierarchical levels (Ensing et al. 2011), as well as riverine landscapes (Dillane et al. 2008) and life-history characteristics (Vähä et al. 2007).

Although such studies have provided information on population structure and its possible bases within larger rivers and river systems (>100 km-long), less information exists about spatial boundaries of populations (Verspoor et al. 2005) and population structure within very small river systems (<30 km-long). In Iceland, small river systems, many of which flow directly to the sea and have shown evidence of having distinct population characteristics, are found all over the island (Scarnecchia 1983; Gudjónsson 1990).

In the present study, we investigated temporal genetic stability and population structure of Atlantic salmon within the Ellidaár river system, a small system (21 km total length) in southwest (SW) Iceland. Furthermore, we assessed the genetic impact of hatchery-reared salmon, which strayed into the river system in large numbers in the 1980s and 1990s, on genetic composition and population structure of wild salmon in the river system.

Materials and methods

Study site and background

The Ellidaár river system, located in SW Iceland (64°07′N, 21°50′W) drains a catchment area of 286 km^2 (Rist 1990) before flowing into the Gulf of Faxaflói (Fig. 1). The mainstem Ellidaár flows through the capital city, Reykjavík, and is 6 km long, with an average annual flow (A_f) of 4.9 m^3/s. The river is the outflow of a lake (Ellidavatn; area 2 km^2), into which two tributaries enter, Hólmsá (11 km long, A_f of 2.3 m^3/s) and Sudurá (4 km long, A_f of 0.4 m^3/s; Birgisson et al. 1999). Two dams are located in the river system, both passable by salmon. The river system is spring fed (Rist 1956), resulting in relatively stable flow and thermal regimes throughout the year (Gudjónsson 1990). Salmon spawning and nursery habitat exist in the three rivers but most juvenile production occurs in Ellidaár, which is warmer than the tributaries [e.g., 1,335 degree-days (°C) in Ellidaár vs. 976 degrees-days in Hólmsá over the period Jun 1–Sept 24 1981] and benefits from nutrients emanating from the lake outflow (Gardarsson 1983).

Historically, by Icelandic standards, Ellidaár is known for large salmon runs for such a small river. From 1935 to 2010, wild adults entering the river system averaged 2,960 per year, ranging from 694 individuals in 2001 to 7,184 in

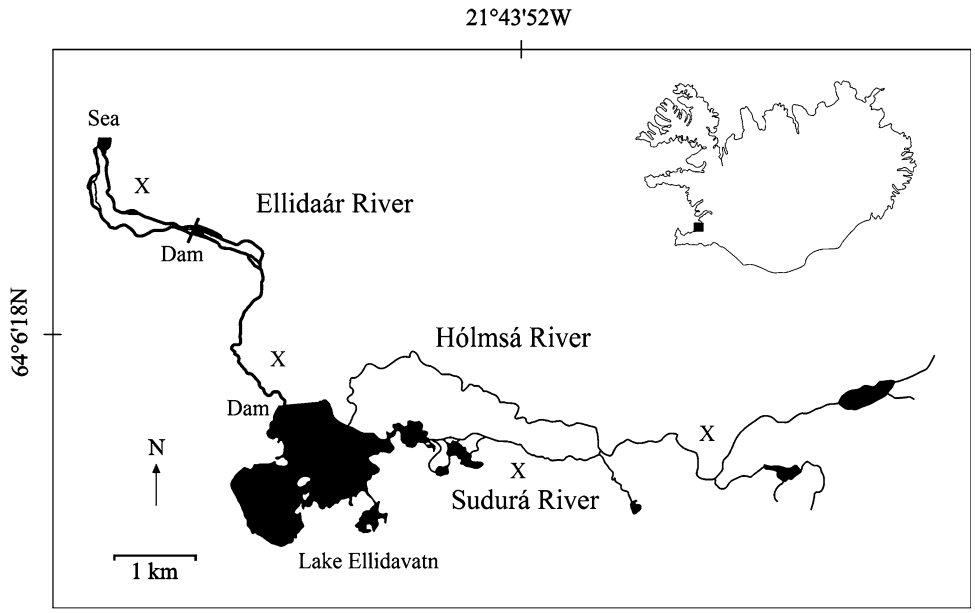

Fig. 1 Map of the Ellidaár river system showing approximate sampling locations of juvenile Atlantic salmon (*X*)

1975 (Fig. 2a; Antonsson et al. 1998; Antonsson and Árnason 2011). The number of adults entering the tributaries is unknown and no catch statistics are available as angling is prohibited in the tributaries. Harvest rate in Ellidaár has typically been 30–50 %, which is low compared to many Icelandic salmon rivers (Antonsson et al. 1998). In addition, salmon fishing at sea and coastal salmon fishing near Ellidaár stopped in 1932 and 1980, respectively (Antonsson et al. 1998). In the late 1990s and early 2000s, the population experienced the most serious decline recorded, manifested as a large drop in adult abundance and juvenile density (Fig. 2a, b). In the following years, juvenile density remained comparatively low in all rivers, especially in the tributaries, where density measured only 3 juveniles per 100 m^2 in Hólmsá in 2004 and 1 per 100 m^2 in Sudurá in 2007.

In the 1980s and 1990s, large-scale salmon ranching and farming (with sea cages) was carried out in SW Iceland (Gudjónsson and Scarnecchia 2009). Straying of ranched and escaped farmed salmon from these activities resulted in an influx of hatchery-reared salmon into many rivers, including Ellidaár (Fig. 2c; Gudjónsson 1991; Antonsson and Gudjónsson 2000). Straying of ranched fish began around 1984 and continued until 1999. Ranched fish constituted up to 40 % of the total catch of adults from the river during that period. Farmed strays entered the river system from 1987 to 1992 and constituted up to 24 % of the total catch of adults. Both types of strays were composed of a mixture of Icelandic populations. However, whereas a large fraction of the ranched strays likely originated from Ellidaár (Gudjónsson 1989), the composition of farmed is unknown (Sigurdur Gudjónsson, Institute of Freshwater Fisheries, Reykjavík, Personal Communication). These aquaculture activities later ceased due to financial failure. However, shortly after, sea cage rearing resumed in other distant parts of Iceland using a Norwegian salmon strain (Gudjónsson and Scarnecchia 2009).

Sample collection

Under our temporal and spatial approach, samples of wild salmon consisted of fish from Ellidaár and the two tributaries, Hólmsá and Sudurá. The samples from Ellidaár consisted of adults collected in 1948, 1962, 1989, 1991, 1992 and 2005 and juveniles collected in 1990 and 2002 (Fig. 1; Table 1). Samples from 1948 ($n = 33$) and 1962 ($n = 25$) were not genetically different ($F_{ST} = 0.000$, $P > 0.05$) and were pooled together to increase sample size. Likewise, samples from 1990 ($n = 38$) and 1991 ($n = 36$) were also pooled ($F_{ST} = 0.000$, $P > 0.05$). In Hólmsá and Sudurá, juveniles were sampled both in 1990–1991 and 2002 (Fig. 1).

In general, adult wild fish were caught by anglers. Each adult fish had previously been aged by examination of scale growth patterns (Institute of Freshwater Fisheries, Unpublished data). The juvenile samples consisted of mixed age groups, 1+ to 3+ in 1990–1991 (Daníelsdóttir et al. 1997) and 0+ to 3+ in 2002 (as estimated from their fork lengths), and sampled by electrofishing 100–400 m long river stretches in August (1990–1991) and October (2002). In the 1990–1991 sampling of juveniles from Ellidaár, Hólmsá and Sudurá, as well as adults from Ellidaár, muscle tissues were collected and immediately frozen at −75 °C (Daníelsdóttir et al. 1997). In 2002, fin clips were collected from juveniles and preserved in 95 % ethanol (non-lethal sampling).

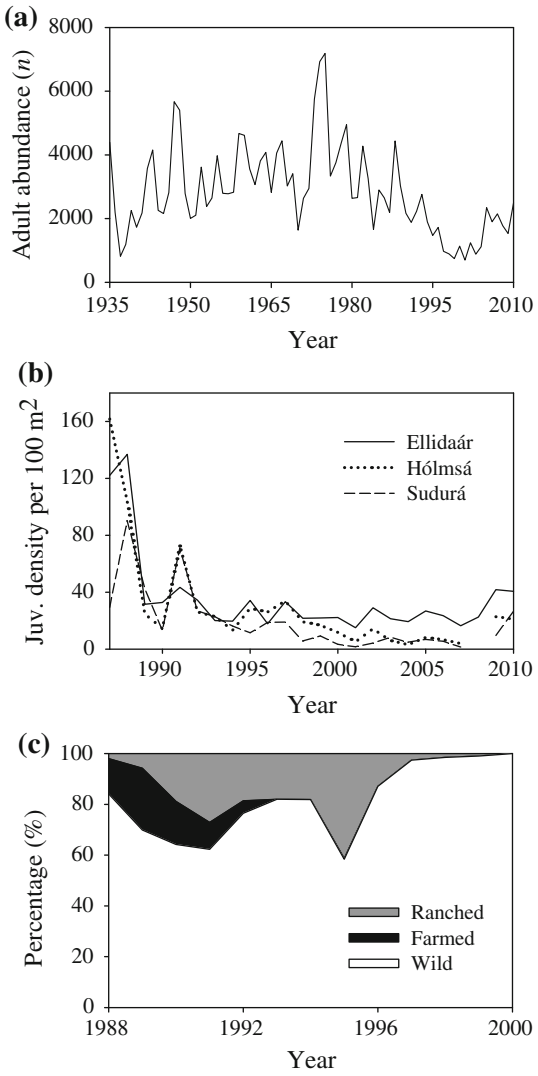

Fig. 2 **a** Estimates of the total wild (naturally spawned) adult Atlantic salmon abundance in the Ellidaár river system from 1935 to 2010 and **b** juvenile density, as measured by single-pass electric fishing (Árnason et al. 2005), in Ellidaár (*solid line*), Hólmsá (*dotted line*) and Sudurá (*broken line*) from 1988 to 2010. Juvenile density was not assessed in 2008 in Hólmsá and Sudurá due to bad weather conditions for sampling. **c** Proportions of Ranched (*gray area*), Farmed (*black area*) and Wild (*white area*) of the total catch of adult salmon in Ellidaár from 1988 to 2000

In general, genetic samples of hatchery-reared adults were also obtained from angler-caught fish. Samples of hatchery-reared fish consisted of the ranched strain (hereinafter called Ranched) and the farmed strain (hereinafter called Farmed). These strains had previously been distinguished from wild Ellidaár salmon based on different growth patterns of their scales (Gudjónsson 1991; Institute of Freshwater Fisheries, Reykjavik, Unpublished Data). However, growth patterns of scales can sometimes be similar between wild salmon and ranched (Gudjónsson 1991), and some ranched individuals might therefore have been misclassified as wild and included in the adult samples collected in Ellidaár in 1989–1992. To deal with this possibility, the Bayesian clustering method in STRUCTURE 2.3.3 (Pritchard et al. 2000; Falush et al. 2003) was used to identify and discard putative Ranched individuals from the wild adult samples, prior to full genetic analyses.

Molecular analyses

A total of 931 Atlantic salmon were genotyped for the present study (Table 1). As quality varied between tissue types and age of samples, different DNA extraction methods and polymerase chain reactions (PCR) were applied. We used Chelex-100 resin (Bio-Rad Laboratories) to extract DNA from muscle tissues and fin clips (10 mg of tissue) while the phenol/chloroform protocol of Taggart et al. (1992) was used to extract DNA from scales (four scales per individual). In addition, the historical DNA was purified and concentrated with Microcon YM-50 (Millipore) centrifugal filter tubes as described by Nielsen et al. (1999a).

Genetic variability was analyzed at seven microsatellite loci: Ssa85, Ssa197 and Ssa202 (O'Reilly et al. 1996), SSOSL25, SSOSL85 and SSOSL311 (Slettan et al. 1995) and Ssa404 (Cairney et al. 2000). Most loci were amplified in duplexes for DNA extracted from muscle and fin clips while each locus was amplified separately for DNA extracted from scales. PCR's were performed in 10 µL volumes, except 25 µL volumes were applied for PCR's with the historical DNA (1948 and 1962). PCR programs and protocols are listed in Table S1. In general, reactions contained 2.0 µL of extracted DNA, 250 µM of each dNTP, 1× reaction buffer (10 mM Tris–HCl, pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl and 0.1 % Triton X-100), 0.4 U polymerase (0.6 U for scale DNA), 1.5 mM $MgCl_2$ (2.0 mM for scale DNA, except for SSOSL311) and completed with distilled water. In addition, 0.5 µg/µl of bovine serum albumin (BSA) was added into the PCR solutions of the historical DNA to improve the amplification.

PCR products were visualized on an ABI PRISM 377 DNA sequencer (Applied Biosystems) and allele scoring was performed manually with the software GeneMapper ver. 3.0 (Applied Biosystems). Negative control samples were applied in all steps of the genotyping process to detect possible risks of cross- and aerosol contamination among the historical samples. Also, reproducibility was obtained by repeating the whole process from extraction of historical DNA to PCR. Positive control samples were also used to ensure that different PCR protocols did not affect the results.

Family sampling and genetic diversity

Prior to all statistical analyses, we assessed family sampling (Hansen et al. 1997) within each juvenile sample and

Table 1 Description of 12 Atlantic salmon samples caught in the Ellidaár river system

Code	Sampling	Origin	Sample size[a]	Life stage	F_{IS}	H_S	A_R[b]
Ell'48–62	Ellidaár 1948, 1962	W	58	a	0.04	0.74	6.4
Ell'89	Ellidaár 1989	W	91	a	0.01	0.73	6.8
Ell'90–91	Ellidaár 1990, 1991	W	73	j, a	0.00	0.75	6.6
Ell'92	Ellidaár 1992	W	87	a	−0.06	0.73	6.6
Ell'02	Ellidaár 2002	W	87	j	−0.03	0.74	6.5
Ell'05	Ellidaár 2005	W	89	a	0.01	0.73	6.7
Hol'90–91	Hólmsá 1990, 1991	W	70	j	0.04	0.73	6.6
Hol'02	Hólmsá 2002	W	79	j	0.02	0.69	5.9
Sud'90	Sudurá 1990	W	66	j	−0.02	0.73	6.6
Sud'02	Sudurá 2002	W	14	j	−0.04	0.64	4.4
Ranched	Ellidaár 1992	H-R	74	a	−0.01	0.76	7.8
Farmed	Ellidaár 1989	H-R	96	a	0.02	0.77	8.0

Sample code, river and year of sampling, origin [*W* wild, *H-R* hatchery-reared (i.e., ranched or farmed)], sample size, life stage (*a* adult, *j* juvenile), Weir and Cockerham's (1984) inbreeding coefficient (F_{IS}), expected heterozygosity (H_S) and allelic richness (A_R)

[a] Corrected sample sizes where full-sibling groups among juveniles and putative hatchery reared individuals from wild adult samples have been removed (see text for more detail)

[b] Calculations of A_R were based on a minimum sample size of 14 diploid individuals

removed full-sibling groups to reduce sampling bias. The analysis was performed using the maximum likelihood method implemented in Colony ver. 2.0.0.1 (Wang 2004), assuming polygamy for both sexes (Wang and Santure 2009). Apart from one randomly chosen individual, only full-siblings belonging to groups of four or more were deleted (see Lehtonen et al. 2009) as detection of false positive full-siblings among groups of less than four individuals might be important when using only seven microsatellite loci (Hansen and Jensen 2005).

Tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were calculated for each locus and sample using exact tests (GENEPOP 3.4; Raymond and Rousset 1995). Unbiased *P* values were estimated with Markov chain parameters set at 10,000 dememorizations, 1,000 batches and 10,000 iterations per batch. The sequential Bonferroni correction was used to minimize Type I error of multiple tests (Rice 1989).

Allele numbers and observed heterozygosity (H_O) were calculated for each sample using GENETIX 4.05.2 (Belkhir et al. 2004). FSTAT 2.9.3.2 (Goudet 2001) was used to calculate Weir and Cockerham's (1984) inbreeding coefficient (F_{IS}), expected heterozygosity (H_S) and average allelic richness (A_R). Significant differences in H_S and A_R were assessed between each river, i.e. between pooled samples of Ellidaár, Hólmsá and Sudurá, and within rivers using 5,000 permutations. Similar comparisons of H_S and A_R were performed between wild and Ranched and Farmed, respectively.

Population structure and temporal stability among wild samples

Differences in allele frequencies among and within rivers were estimated with pairwise F_{ST} (Weir and Cockerham 1984) using Arlequin ver. 3.5.1.2 (Excoffier and Lischer 2010) and significance assessed with 10,000 permutations. Genetic relationships among the wild samples were also explored with a neighbour-joining (NJ) tree constructed from D_A distances (Nei et al. 1983) using the software POPULATIONS 1.2.32 (Langella 1999). Branch support was estimated with 10,000 bootstrap replications over loci and the resulting tree was visualized in TreeView 1.6.6 (Page 1996).

To assess the potential number of genetic clusters contained in our samples, a Bayesian cluster analysis was performed using STRUCTURE 2.3.3 (Pritchard et al. 2000; Falush et al. 2003). STRUCTURE jointly estimates admixture proportions (*q*) for each individual to *K* genetic clusters and the log-likelihood [Ln P(D)] of the data for a given *K*. The most likely number of clusters was inferred from log-likelihood values and the ad hoc ΔK statistics (Evanno et al. 2005), implemented in STRUCTURE HARVESTER v0.3 (Earl and vonHoldt 2012). As STRUCTURE tends to detect the uppermost level of population structure (Evanno et al. 2005), we adopted a hierarchical approach. The level of structure was therefore assessed within each detected cluster until no further structure was found or sample size was very small. In each run, the admixture model with correlated allele fre-

quencies was implemented and ALPHAPROPSD was set at 0.005, which increased stability of α. Furthermore, we used the LOCPRIOR setting, which considers sample information, e.g. information on location or time. This recently developed method (Hubisz et al. 2009) has been suggested to perform better than the traditional STRUCTURE methods when genetic structure is weak or when data sets have low information content. We first tested sample priors on river basis and then sample specific priors to search for additional structure within hierarchical groups. In all tests, a total of 10 independent runs consisting of a burn-in length of 10,000 and 40,000 MCMC were performed from $K = 1$–6. The software *distruct* (Rosenberg 2004) was used to visualize the results.

Analysis of introgression

We used two approaches to analyze hybridization and introgression of Farmed and Ranched salmon into the wild population(s). First, as introgression may homogenize allele frequencies between populations, we investigated temporal trends in genetic differentiation (F_{ST}) between each putative wild population (Ellidaár, Hólmsá and Sudurá) and each hatchery strain (Farmed and Ranched). For each pairwise comparison, standard error was estimated by jackknifing over loci using FSTAT. Second, introgression was inferred with hybrid analysis using STRUCTURE. We analyzed the Ellidaár and tributary samples separately in order to avoid bias arising from population structure within the system. The most likely value of K was assessed among the wild, Farmed and Ranched samples and individual q values of wild salmon explored given the most likely K. We applied the same parameter setting as above except that sample information was not included. In addition, the option of separating alpha for each population was applied, which aided in separating the wild and hatchery-reared individuals.

We used the threshold of $0.20 \leq q \leq 0.80$ to distinguish between hatchery-reared, hybrids and wild among the wild samples as the lowest q value observed among the historical individuals was 0.86. This threshold was also used to eliminate putatively wrongly identified Ranched individuals (see above in Sample collection) from the wild adult samples collected in Ellidaár in 1989–1992, which was done prior to any statistical analyses. However, as admixture proportions of the Ranched individuals were often similar to wild or intermediate, other misclassifications cannot be excluded.

Results

Family detection

A total of 47 full-siblings in nine groups were detected among the juvenile samples; one group ($n = 4$) in each of Hol'90–91 and Sud'90, four groups ($n = 12$) in Ell'02 and three groups ($n = 23$) in Hol'02. In all, 38 juveniles (9.7 % of the juvenile data) were therefore discarded from subsequent analyses.

Genetic diversity

Departures from HWE were detected in six of 84 single locus tests. These departures involved four loci, distributed among five wild samples; two due to heterozygote excess and four due to heterozygote deficiency but none remained significant after correction for multiple tests (initial $\alpha = 0.0071$ for seven comparisons). When probabilities were combined over loci, departures from HWE were observed in Ell'48–62, Ell'05 and in Hol'02, though none remained significant after correction for multiple tests (initial $\alpha = 0.0042$ for 12 comparisons).

Overall, linkage disequilibrium (LD) was detected between 26 of 252 locus pairs. Among the wild samples, the number of LD ranged from zero to four (most in the tributary samples Sud'90 and Hol'02), though significant LD was only observed in Sud'90 (Ssa202–SSOSL311, $P < 0.05$) and marginally significant in Hol'02 (Ssa85–Ssa404, $P = 0.0026$) after correction for multiple tests (initial $\alpha = 0.0024$ for 21 comparisons). Most LD was observed in the hatchery-reared samples, reflecting that they were mixtures of different populations. Two pairs of loci displayed significant LD in Ranched (Ssa85–Ssa404 and Ssa202–SSOSL25; $P < 0.01$, initial $\alpha = 0.0005$). In Farmed, five loci pairs displayed LD, of which only two were significant (SSOSL85–Ssa85 and Ssa197–Ssa404; $P < 0.05$).

In all, 106 alleles were observed, ranging from eight alleles at SSOSL25 to 27 at Ssa404. Gene diversity (H_S) and allelic richness (A_R) ranged from 0.64 to 0.77 and 4.4 to 8.0, respectively (Table 1). Within Ellidaár, genetic variability was relatively stable from 1948–1962 to 2005, where H_S ranged from 0.73 to 0.75 and A_R from 6.4 to 6.8. The level of genetic variability in the tributaries in 1990–1991 was similar to that observed in Ellidaár; it decreased in 2002, though not significantly. Both Ranched and Farmed were significantly more variable than the wild samples (Ranched, H_S, $P = 0.032$ and A_R, $P = 0.018$; Farmed, H_S, $P = 0.016$ and A_R, $P = 0.003$).

Population structure and temporal stability among wild samples

The pairwise F_{ST} estimates were highly significant among Ellidaár and its tributaries in 1990–1991 (Table 2). However, Hol'02 displayed somewhat lower F_{ST} levels than Hol'90–91 when compared to Ellidaár and not all comparisons were significant. Similarly, not all comparisons

between Sud'02 and Ellidaár were significant, though Sud'02 was based on small sample size. In 1990–1991, the tributary samples were highly significantly differentiated ($P < 0.0001$) but not in 2002. Temporal stability (F_{ST}) was observed within Ellidaár as 10 of 15 comparisons were not significantly different. The significant comparisons involved the same sample, Ell'89. Temporal stability was also observed within Hólmsá but not within Sudurá ($P < 0.0001$).

The observed genetic pattern was largely supported by the NJ tree constructed from D_A distances (Fig. 3). The Ellidaár samples clustered together and separated from the tributaries with 59 % bootstrap support. The tributary samples, Hol'90 and Sud'90, separated with 46 % support but Hol'02 and Sud'02 branched from Sud'90 with 70 % support.

The Bayesian cluster analysis (STRUCTURE) suggested that the most likely number of genetic clusters was $K = 2$ (the ΔK signal at $K = 3$ was due to the large drop in log-likelihood values between runs for $K = 3$ and $K = 4$) among all the collected wild samples (Fig. S1a). Ellidaár samples were clearly distinct from those of Hólmsá and Sudurá, with strong assignment values (q) of individuals to the Ellidaár group (>0.95; Fig. 4). In contrast, individual assignment values (q) of the tributary salmon were not as clear, although high q's (>0.90) to the tributary group were frequent in the Sudurá samples.

In subsequent hierarchical runs, considering the tributaries only and when using river location as prior (LOCPRIOR), the most likely number of genetic clusters was $K = 1$ [average Ln P(D) from 10 runs; $K = 1$, −5251.7, $K = 2$, −5299.4, $K = 3$, −5297.1]. However, using unique sample priors for each sample, the most likely value of K was 3 (Fig. S1b). Each tributary clustered separately for the samples collected in 1990–1991 but tributary samples collected in 2002 clustered together (Fig. 4). A third hierarchical round of STRUCTURE, considering only tributaries samples from 1990 to 1991, separated the samples in a clearer pattern (Fig. 4). However, the analysis supported three clusters (Fig. S1c), where Hol'90–91 and Sud'90 clustered separately and few individuals in Hol'90–91 clustered together. No structure could be detected within the Ellidaár samples.

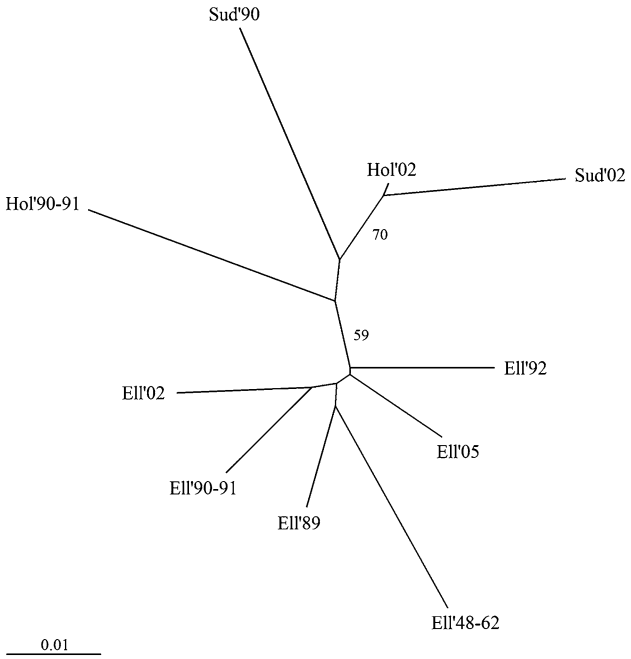

Fig. 3 Unrooted neighbor-joining tree for wild Atlantic salmon samples of the Ellidaár river system based on D_A genetic distances. Bootstrap support >50 % in 10,000 replicates is shown. Branch length of Sud'02 is reduced

Table 2 Pairwise estimates of genetic differentiation (F_{ST}) for wild Atlantic salmon samples from the Ellidaár river system and hatchery-reared samples (Ranched and Farmed)

	Wild samples									Hatchery-reared samples	
	Ell'89	Ell'90–91	Ell'92	Ell'02	Ell'05	Hol'90–91	Hol'02	Sud'90	Sud'02	Ranched	Farmed
Ell'48–62	0.008*	0.005	0.007*	0.006	0.007	0.020***	0.018***	0.017***	0.042***	0.005	0.032***
Ell'89		0.008**	0.005	0.008**	0.006*	0.012***	0.010**	0.014***	0.023*	0.012***	0.043***
Ell'90–91			0.002	0.003	0.002	0.013***	0.010**	0.012**	0.021	0.006	0.027***
Ell'92				0.003	0.000	0.011***	0.004	0.016***	0.021*	0.006*	0.025***
Ell'02					0.002	0.010***	0.010**	0.018***	0.025*	0.012***	0.033***
Ell'05						0.009**	0.003	0.013***	0.026*	0.006	0.026***
Hól'90–91							0.007	0.014***	0.028*	0.019***	0.040***
Hól'02								0.012**	0.018	0.014***	0.037***
Sud'90									0.035***	0.017***	0.046***
Sud'02										0.033***	0.067***
Ranched											0.014***

See sample details in Table 1

Significant values after Bonferroni correction are indicated with asterisk (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$)

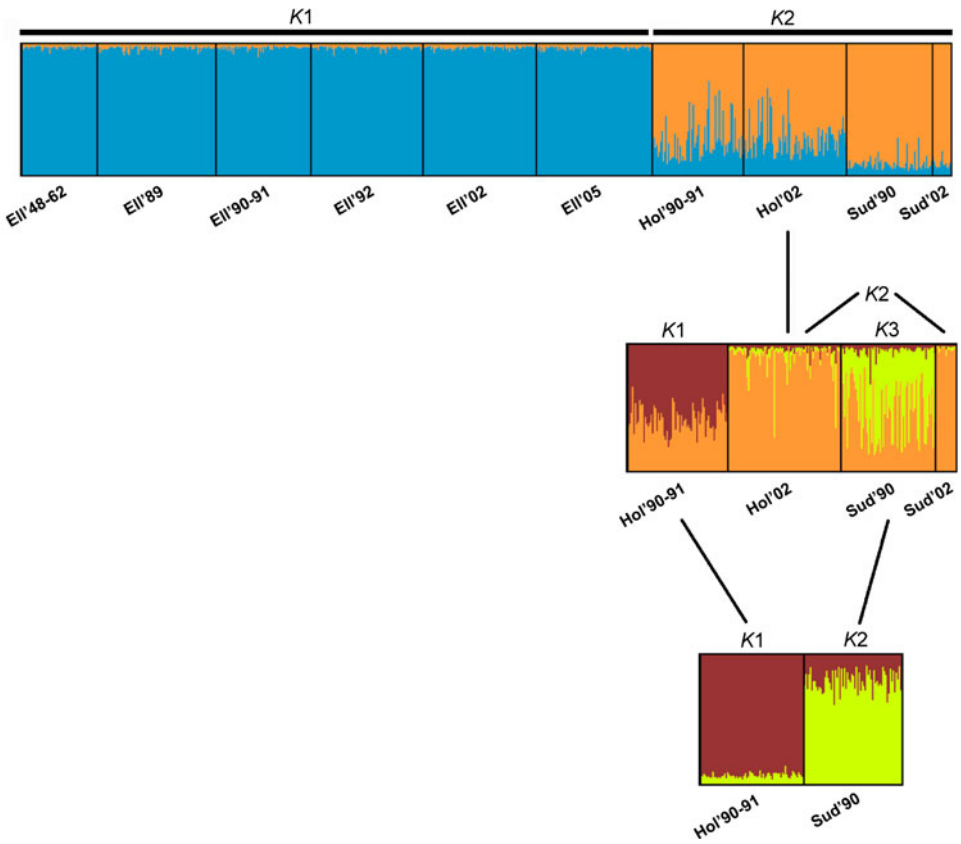

Fig. 4 Hierarchical Bayesian cluster analysis of wild Atlantic salmon samples using STRUCTURE. Each individual is represented by a *vertical bar* and admixture proportions are denoted with *different colors*. The first STRUCTURE graph presents the detected cluster (K) at the first hierarchical level, i.e. results including all rivers in 1948–1962 to 2005 at $K = 2$ (Ellidaár vs. tributaries), while the second shows the detected cluster at the second hierarchical level, i.e. results for the tributary samples in 1990–1991 and 2002 at $K = 3$ (Hol'90–91 vs. Sud'90 vs. Hol'02–Sud'02), and the last one displays analysis using only the tributary samples in 1990–1991 at $K = 2$. See sample code in Table 1. (Color figure online)

Analysis of introgression

Both Ranched and Farmed strains were generally highly significantly different ($P < 0.0001$) from the wild samples but the level of divergence was higher for Farmed (Table 2). Four of six comparisons between Ellidaár and Ranched were either weakly significant or not significant, supporting the partial Ellidaár origin of the Ranched strain. The level of divergence between Ellidaár and Farmed decreased somewhat after 1989, though not significantly (Fig. 5), and all comparisons were highly significant. F_{ST} between Farmed and Ell'48–61 and Ell'89 measured 0.032 and 0.043, respectively, while it averaged 0.026 for the more recent samples. The higher F_{ST}'s for Ell'89 than Ell'48–62 and the comparatively high divergence of Ell'02 ($F_{ST} = 0.033$) might indicate inflated F_{ST} values for those samples. The level of divergence between Hólmsá and both hatchery-reared strains decreased slightly from 1990–1991 to 2002 (Fig. 5).

The STRUCTURE analysis supported the presence of two genetic clusters ($K = 2$; Fig. S1d, e) among the hatchery-reared strains and wild samples, i.e. the Ellidaár and tributary samples vs. hatchery-reared strains, respectively. As the two hatchery strains did not separate into different clusters (Fig. 6a, b), we could not assess the importance of introgression specifically for each strain. In both tests, assignment proportions (q) of the hatchery-reared individuals ranged from strong assignment to the hatchery cluster to strong assignment to wild. Individuals

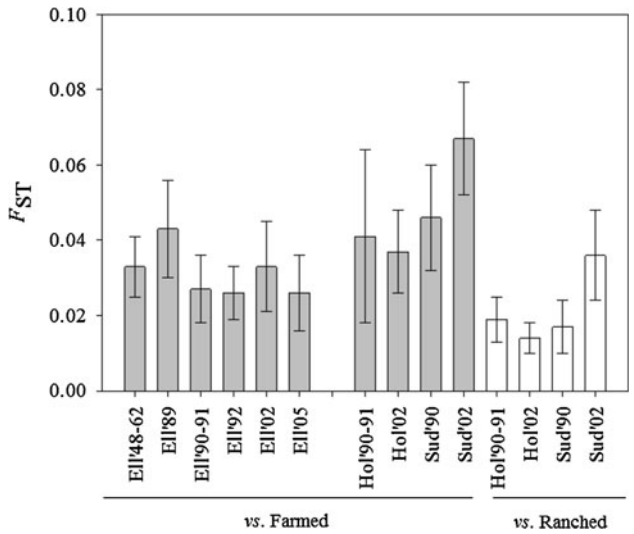

Fig. 5 Temporal trends in F_{ST} between wild Atlantic salmon and hatchery salmon; Ellidaár samples from 1948–1962 to 2005 vs. Farmed (*gray columns*) and tributary samples from 1990–1991 to 2002 vs. Farmed (*gray columns*) and Ranched (*white columns*), respectively. Standard errors of pairwise F_{ST} estimates were calculated by jackknifing over loci. See sample code in Table 1

of Farmed displayed more often strong assignment to the hatchery cluster than did individuals of Ranched.

Individual q values from the STRUCTURE analysis are shown for each wild sample in Fig. 7. In Ellidaár, identified hybrids were most frequent in Ell'05 ($n = 12$, 12.3 %), which was sampled approximately one generation after the influx of stray hatchery fish (see Figs. 6a, 7). However, fewest hybrids ($n = 2$, 2.3 %) were observed in the juvenile sample Ell'02, which should have represented fully or partially the same cohorts as the adult sample Ell'05. During the influx of strays, five (5.5 %), nine (12.3 %) and seven (8.0 %) individuals were assigned to the hybrid category in Ell'89, Ell'90–91 and Ell'92, respectively. In Ell'90–91, which was composed of both juveniles ($n = 38$) and adults ($n = 35$), three of the nine putative hybrids were juveniles or 7.9 % of the juvenile data. In the tributaries, 11 (15.5 %) and 12 (15.2 %) hybrids were detected in the Hólmsá samples Hol'90–91 and Hol'02, respectively, and in Sudurá, 13 hybrids were identified in Sud'90 (19.7 %) but none in Sud'02 (Figs. 6b, 7). Two Individuals in Hol'90–91 and two in Ell'02 were assigned to the hatchery category (i.e., $q \leq 0.20$).

Discussion

In this study we demonstrated population structure of Atlantic salmon within a small river system. The most distinguishable pattern of divergence was observed between the river Ellidaár and its tributaries. The divergence was established by highly significant levels of differentiation (Table 2), a genetic tree and Bayesian cluster analysis (Figs. 3, 4). Population differentiation was also detected between the tributaries in 1990–1991 (Table 2; Figs. 3, 4). These results are in agreement with previous studies, showing structures at the level of rivers and tributaries. Although the Ellidaár system may be the smallest river systems investigated to date, spatial heterogeneity has previously been observed over short distances, e.g. between samples separated by 11 km (Varzuga River system in Russia; see Primmer et al. 2006). The level of differentiation (F_{ST}) among Ellidaár and the tributaries in 1990–1991, i.e. prior to most of hatchery-reared salmon, was relatively high (1.2–1.4 %) compared to other studies. For example, in few recent microsatellite studies, differentiation among samples separated by less than 20 km did generally not exceed 1 % (Primmer et al. 2006; Dillane et al. 2008; Ensing et al. 2011). Furthermore, in Primmer et al. (2006) and Dillane et al. (2008), spatial autocorrelation analysis on individual genotypes suggested assortative mating above 34 and 29 km patch sizes, respectively, which is larger than the total length of the Ellidaár river system. The difference between studies may reflect smaller effective population sizes of the tributary populations and higher levels of drift, though environmental characteristics are likely important.

The fine-scale population structure observed in the present study may in part be a result of the physical characteristics of the river system. The spring fed nature of the Ellidaár system (Rist 1956) and the presence of Lake Ellidavatn both act to stabilize the water flow. Lakes also increase habitat patchiness (Hillbricht-Ilkowska 1999). Both environmental stability and habitat patchiness have been shown to limit gene flow among salmonid populations

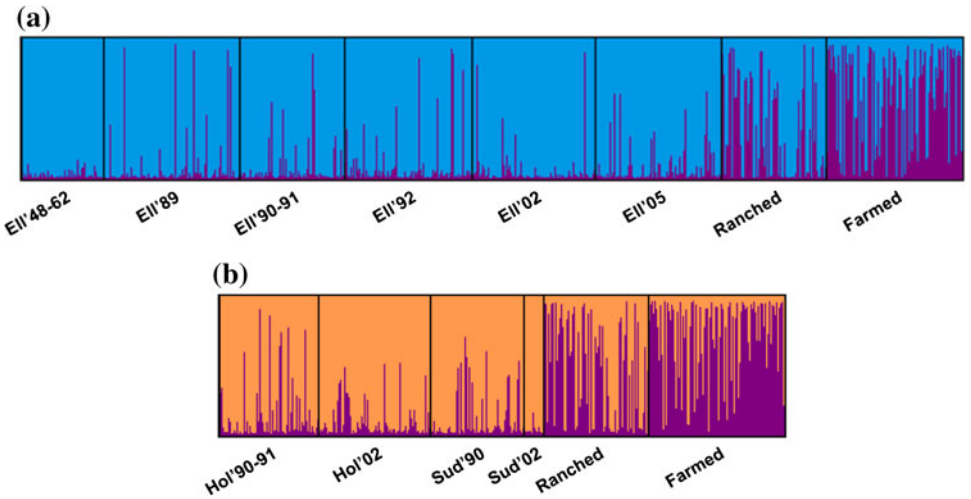

Fig. 6 Bayesian cluster analysis of wild Atlantic salmon vs. Ranched and Farmed using STRUCTURE. Each individual is represented by a *vertical bar* and admixture proportions are denoted with *different colors*. **a** Ellidaár samples from 1948–1962 to 2005 and Ranched and Farmed at $K = 2$, **b** tributary samples from 1990–1991 to 2002 and Ranched and Farmed at $K = 2$. Note that few individuals in Ell'89, Ell'90–91 and Ell'92 with strong assignment to the hatchery cluster were removed from the study (see text for more detail). See sample code in Table 1. (Color figure online)

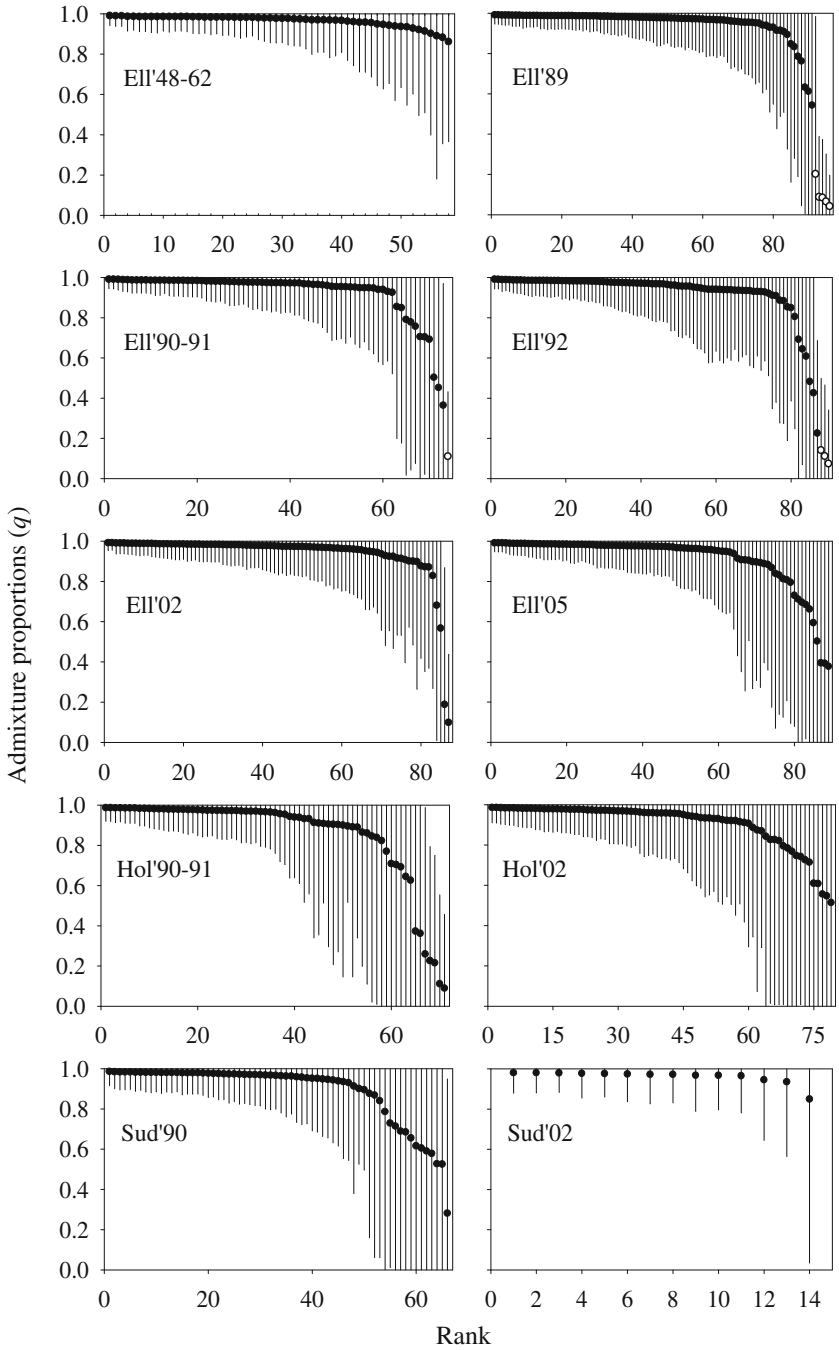

Fig. 7 Admixture proportions (q) and their 90 % probability limits for each individual in each sample of wild Atlantic salmon from 1948–1962 to 2005. q values close to 1 denote wild individuals and close to 0 denote hatchery fish. Intermediate values of q ($0.20 \leq q \leq 0.80$) may indicate hybrids of wild and hatchery salmon. q values have been ranked from the *left* to *right* in a decreasing order. *Non-filled circles* in Ell'89, Ell'90–91 and Ell'92 denote adults that were possibly wrongly identified as wild salmon by scale characteristics. These were eliminated from the samples prior to other statistical analysis. See sample code in Table 1

(Neville et al. 2006; Olsen et al. 2011). In the large Moy River system, Ireland, lakes were identified as the main barriers to gene flow among salmon populations (Dillane et al. 2008). Although the exact effects of lakes on salmon population structure have not been investigated, they may improve homing by creating differences in water chemistry (Young and Woody 2007). In particular, amino acids have been shown to be important olfactory cues (Yamamoto et al. 2010) and their concentration is higher below lakes during the spring bloom when parr-smolt transformation and imprinting occurs (Dittman et al. 1996). Lake Ellidavatn and its location within this small river system may therefore be important for the development of the observed population structure.

The observed temporal stability in the genetic composition of the Ellidaár population from 1948 to 2005 (Table 2; Figs. 3, 4) was similar to that reported in other areas over several decades (e.g. Nielsen et al. 1999b). On the other hand, it contrasted with the observed instability of the tributary populations, i.e. Hólmsá and Sudurá (Figs. 3, 4). In the

latter, our results indicated homogenization of the populations in 2002 (Figs. 3, 4) and the pairwise F_{ST} estimates generally supported reduced divergence between the Ellidaár samples and Hólmsá in 2002 (Table 2). This suggests that factors other than genetic drift may have been important. Two non-mutually exclusive possible explanations can be identified: natural gene flow among the wild populations and introgression of hatchery-reared salmon. The first possible explanation, natural gene flow, might involve a source-sink system (e.g. Dias 1996), where demographic surplus from the high quality habitat in Ellidaár (source) immigrated to the lower quality habitat of the tributaries (sink). This scenario is supported by the observed differences in habitat quality within the system (e.g. viewed by higher juvenile density and growth rate in Ellidaár; Antonsson and Árnason 2011). Also, source-sink metapopulation systems may characterize some Atlantic salmon population complexes, composed of large and small populations (Hindar et al. 2004). However, source-sink dynamics may not explain the observed genetic pattern as the reduction in genetic divergence occurred after a period of population decline (Fig. 2a, b). Alternatively, it has been suggested that low density of adult spawners may increase straying due to difficulties in finding mates (Hindar 1992). Although this possibility cannot be ruled out in the tributaries, it was unlikely in Ellidaár given the relatively large population size.

A second, more likely explanation for the observed genetic pattern is that it resulted from introgression of hatchery-reared salmon. Although we did not find significant evidence of homogenization between the wild and hatchery populations (Fig. 5), we found evidence of hybridization between wild and hatchery-reared salmon (Figs. 6, 7). This evidence was observed not only during the influx of strays but also few years after, in both Ellidaár and Hólmsá. Although hybrids of wild and hatchery-reared salmon are expected to have lower survival (McGinnity et al. 2003), our results indicated that some survived and reproduced in this small river system. For example, eight individuals in Ell'05, which were identified as hybrids and could be aged, were spawned in 2000 or later and the last detected occurrence of hatchery-reared fish in the system was in 1999. We acknowledge that the level of divergence between wild and the hatchery-reared strains was low for hybrid analysis (e.g. Marie et al. 2011). However, strong assignment of the historical Ellidaár sample collected prior to the influx of stray hatchery-reared salmon, lends support to introgression. Also, the level of differentiation was influenced by many hatchery individuals with intermediate q values or strong assignment to wild (Fig. 6). Importantly, the impact of those individuals could not be assessed, but they comprised a large proportion of the Ranched salmon and overall a large proportion of the hatchery-reared fish entering the system (Fig. 2c). Indeed, more introgression was expected from Ranched than Farmed salmon, not only because Ranched entered the river system in larger numbers and for a longer period (Fig. 2c), but also because it is expected to have higher reproductive success (Fleming et al. 1996). Such potential differences likely reflect different physical conditions of the two types due to different rearing techniques.

Conservation implications

Results of this study have important implications for conservation and management practices. Population structure of Atlantic salmon can exist within very small river systems, which is consistent with stock concept ideas of decades ago based on life history and other stock differentiation techniques in other areas (Ricker 1972) and in Iceland (Scarnecchia 1983). In general, the appropriate levels for stock-specific management and conservation of Atlantic salmon have been rivers and tributaries within large river systems. Our results suggested that these recommendations may in some cases be extended to tributaries of small river systems such as the Ellidaár river system. In this context, mixed-population fisheries within river systems should be regulated and managed with regard to the existing degree of the fine-scale differentiation of populations. Similarly, if hatchery supplementation is considered necessary, broodstocks should also respect the degree of fine-scale differentiation, i.e., should be collected on spawning grounds during the spawning season for each tributary to be stocked. For effective management and conservation, a critical question is therefore whether the fine-scale population structure observed in this study is common in Iceland and elsewhere, so that conservation and management decisions can be made appropriately. Also, more work is needed to evaluate the effects of river type (Rist 1956), habitat stability, patchiness, and other physical characteristics on the development of fine-scale population structure of Atlantic salmon. Comparative spatio-temporal studies on small river systems, incorporating landscape features (e.g. presence of lakes), would be highly useful to address these issues. In addition, to ensure the maintenance of genetic diversity of metapopulations (Palstra et al. 2007) and to evaluate the existence of local adaptation in particular situations (Vähä et al. 2008), the importance of such small-scale structure and of small populations in general needs to be addressed.

Our results suggested that the introgression from hatchery-reared salmon in the main river and its tributaries has homogenized the population structure and significantly altered genetic composition of the tributary populations. These results are consistent with what Glover et al. (2012) reported for Atlantic salmon on a national scale in Norway. Studies have shown that introgression from hatchery-reared salmon may result in loss of local adaptation (Bourret et al.

2011) and reductions in fitness of wild salmon populations (Fleming et al. 2000; McGinnity et al. 2003; Fraser et al. 2010). It is therefore tempting to relate the observed overall decline in salmon numbers in the Ellidaár system to introgression from hatchery salmon. Although unfavourable conditions in the sea are likely one of the principal factors in the decline (as has occurred elsewhere in Europe; Friedland et al. 2009), the steepness of the decline in the Ellidaár river system raises the possibility that the large-scale straying of hatchery-reared salmon may partly be responsible for the decline through genetic effects, ecological interactions, or both (Jonsson and Jonsson 2006). Indeed, in Scotland, where similar population trends have been observed, the decline was faster in salmon populations exposed to salmon aquaculture (Ford and Myers 2008). Overall, given the expected negative effects of hatchery-reared salmon on wild populations through genetic and ecological interactions (Jonsson and Jonsson 2006), every effort should be made to prevent hatchery-reared salmon from escaping into the wild. However, as past experience showed that accidental escape of farmed salmon and straying of ranched salmon cannot be prevented, such activities should be avoided in areas with existing populations of wild salmon (Jonsson et al. 2003; Gudjónsson and Scarnecchia 2009).

Acknowledgments We are grateful to Robin S. Waples for valuable comments and discussion during the process of this study. We are also grateful to the editor and two anonymous reviewers for their many critical comments that considerably improved the manuscript. We thank Sigrídur V. Jónsdóttir and our co-workers at the Institute of Freshwater Fisheries and Marine Research Institute for their assistance and support. This study was supported by Orkuveita Reykjavíkur (Reykjavik Power Company) and the Committee of Agriculture Genetic Resources in Iceland.

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