ICES Journal of Marine Science, 63: 1224–1233 (2006)
doi:10.1016/j.icesjms.2006.04.005

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Evidence of temporal genetic change in wild Atlantic salmon, *Salmo salar* L., populations affected by farm escapees

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Skaala, Ø., Wennevik, V., and Glover, K. A. 2006. Evidence of temporal genetic change in wild Atlantic salmon, *Salmo salar* L., populations affected by farm escapees. – ICES Journal of Marine Science, 63: 1224–1233.

A large number of farmed Atlantic salmon escape from sea cages and hatcheries annually. Selection programmes and domestication have changed the genetic composition of farmed salmon to improve their performance in the culture environment, which apparently occurs at the cost of their fitness in the natural environment. Therefore, gene flow from farmed salmon to wild salmon populations may have altered the genetic composition of wild salmon populations. To investigate the temporal genetic stability in seven wild Norwegian salmon populations, genetic profiles were produced from historical and contemporary scale samples. Historical and contemporary samples of salmon from the Namsen, Etne, Opo, Vosso, Granvin, Eio, and Hå Rivers were genotyped at the following eight microsatellite loci: *Ssa13.37*, *Ssa28*, *SsOSL85*, *Ssa197*, *Ssa20.19*, *SsaF43*, *Ssa202*, and *Ssa85*. A significant change in genetic profiles was observed over time in the Opo, Vosso, and Eio Rivers, but no changes in genetic profiles were observed in the Namsen, Etne, Granvin, and Hå Rivers. A small reduction in F_{ST} values and genetic distances among populations was observed in the contemporary samples compared with the historical samples, indicating a eduction in population differentiation over time.

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Keywords: Atlantic salmon, farmed escapees, genetic, temporal change.

Received 21 October 2005; accepted 12 April 2006.

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Introduction

Every year, farmed Atlantic salmon (*Salmo salar* L.) escape from sea cages and hatcheries in large numbers. In Norway alone, the average annual number of reported escaped farmed salmon in the period 1999–2003 was 333 000 fish (Anon., 2004), and in 2004 and 2005 it increased to 437 000 and 715 000 salmon, respectively (the Norwegian Directorate of Fisheries). According to the fisheries and aquaculture management authorities, however, the relationship between reported and unreported escapes is uncertain, but it is likely that the total number of escaped farmed salmon is higher than reported (Anon., 2004). At the national level, the average proportion of escaped farmed salmon in wild spawning populations varied between 11% and 35% from 1989 to 2003, with significant variation among years and among regions and rivers.

In some rivers, such as the Etne, located in the Hardangerfjord region, a major salmon farming region, the proportion of escaped farmed salmon in the spawning population has exceeded 50% in most years since the surveys began in 1989. In contrast, the proportion of farmed salmon has been very low in the Hå River, located in the area of Jæren, which has very little salmon farming activity. In rivers with large proportions of farmed salmon, the genetic composition of the wild populations may be altered by gene flow (e.g. Crozier, 1993, 1998; Clifford *et al.*, 1998a). Farmed salmon differ genetically from wild salmon both in genetic marker loci and in fitness-related traits. A number of studies conducted since the late 1980s have demonstrated reduced levels of genetic variability in farmed salmon, measured both at protein coding loci (Verspoor, 1988; Youngson *et al.*, 1991; Mjølnerød *et al.*, 1997; Skaala *et al.*, 2005) and at variable number of tandem repeats (VNTR) loci (Clifford *et al.*, 1998a, b; Norris *et al.*, 1999; Skaala *et al.*, 2004).

Selection programmes for farmed salmon have targeted a number of phenotypic traits such as growth rate, body size, late maturation, disease resistance, and flesh quality. It is not surprising, therefore, that several studies have

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also documented differences between farmed and wild salmon in phenotypic traits such as growth rate and behaviour (Einum and Fleming, 1997; Johnsson *et al.*, 2001; Fleming *et al.*, 2002). Furthermore, growth is faster, but survival is less, for farmed salmon in natural environments compared with that of wild salmon. Two recent experimental field studies found significantly reduced fitness and significantly lower productivity in farmed than in wild salmon. It was concluded that gene flow from farmed to wild salmon populations will reduce the productivity and fitness of wild salmon populations significantly (McGinnity *et al.*, 1997; Fleming *et al.*, 2000; McGinnity *et al.*, 2003). The occurrence of large proportions of escaped farmed salmon in wild salmon populations has therefore raised concerns about gene flow from farmed to wild salmon.

Until recently, genetic profiles of populations had to be developed from fresh tissue material. However, the development of molecular methods and polymerase chain reaction (PCR) techniques, in particular, has provided new opportunities for genetic studies based on archival material such as scale collections (Nielsen *et al.*, 1999). For most salmon populations, good genetic baseline data do not exist, but where archival scale samples are available, they can be reconstructed.

The aim of the present study was to investigate temporal genetic stability in Norwegian salmon populations subjected to high and low levels of immigration of farmed salmon by comparing genetic profiles developed from historical and contemporary scale samples.

Material and methods

Collection of samples

In all, 17 samples comprising 1066 individuals were genotyped from the salmon populations in the Vosso, Etne, Opo, Granvin, Eio, Namsen, and Hå Rivers (Figure 1, Table 1). The Namsen River supports one of the largest salmon

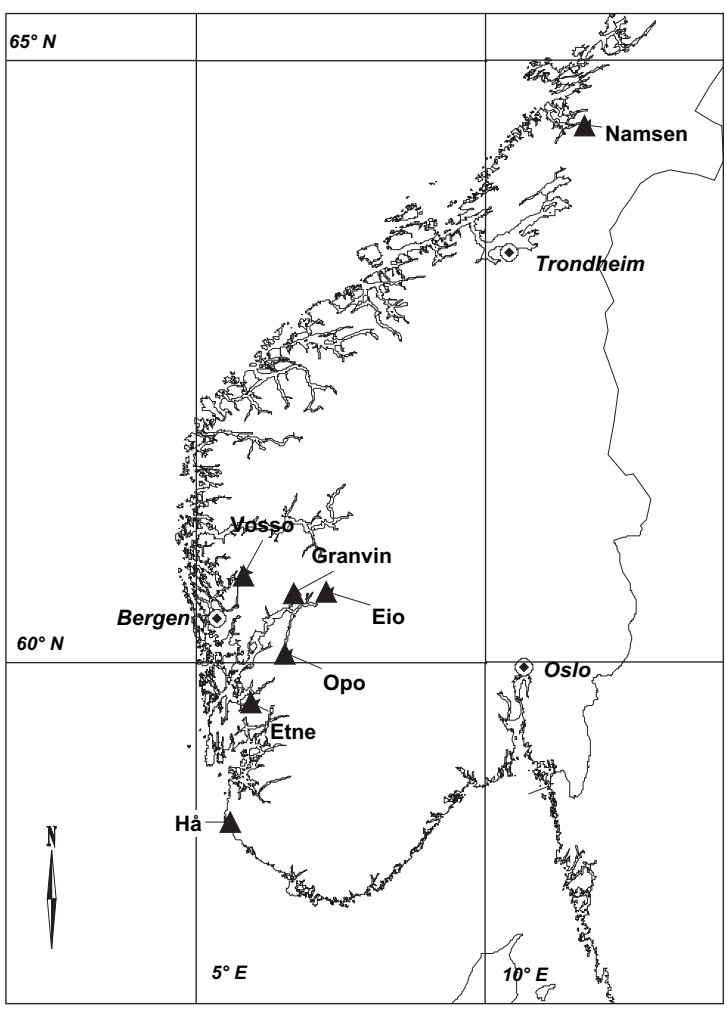

Figure 1. Map of southern Norway showing the locations of the seven populations of Atlantic salmon studied.

Table 1. Sample size, life stage of sampled fish, observed percentage of escaped farmed salmon in the rivers, and an indication of population status for the seven salmon populations studied. Information on population status was collected from Skurdal *et al.* (2001), official Norwegian statistics, and Mr Atle Kambestad, County Governor's Office for Hordaland.

Sample	Sample size	Life stage	Percentage of farmed salmon	Population status
Vosso 1980	50	Spawners		Historical river catches >3 000 kg
Vosso 1995–1997	48	Spawners	19–75	Reduced to <100 kg
Vosso 2003	84	Smolt		Salmon angling banned in 1991
Etne 1983	92	Spawners		Historical river catches >2 000 kg
Etne 1997–1998	81	Parr	31–79	Current catches about 2 000 kg
Opo 1971–1973	88	Spawners		Historical river catches >1 000 kg
Opo 2000	56	Parr	18–100	Reduced to <100 kg Salmon angling banned in 1998
Namsen 1977	92	Spawners		Historical river catches >25 000 kg
Namsen 1998	71	Parr	10–59	Current catches 22 000–40 000 kg
Namsen 2000	71	Spawners		
Granvin 1990–1996	28	Spawners		Historical river catches <500 kg
Granvin 2000–2001	67	Parr	>50	Reduced to ~50 kg Salmon angling banned in 2000
Hå 1986–1990	21	Parr		Historical river catches >3 000 kg
Hå 1996–1997	39	Parr	~5	Current catches 3 000–5 500 kg
Hå 1999–2000	91	Parr		
Eio 1987–1995	35	Spawners		Historical river catches >3 000 kg
Eio 2001	52	Parr	0–67	Reduced to <300 kg Salmon angling banned in 2000
Total	1 066			

populations in Norway. The Etne and Hå Rivers are also large, but are smaller than the Namsen. Large numbers of escaped farmed salmon have been reported in the Namsen River, while in the Etne, the proportion of farmed salmon has exceeded that of wild salmon for 15 years. There is little salmon farming in the Jæren area, and rivers there, such as the Hå, have very few escaped farmed salmon. Both the Opo and Granvin Rivers have small salmon populations and have experienced large proportions of farmed salmon over many years. Historically, the Vosso and the Eio Rivers supported large salmon populations, but both rivers have experienced severe population declines since about 1990.

Microsatellite analysis

DNA was extracted from dried scales; three or four scales were used for adult salmon and between 20 and 30 scales for parr. DNA was extracted from scales using the Qiagen DNeasy kit, following the procedure recommended by the manufacturer. Eight microsatellite loci were amplified: *Ssa28* (B. Høyheim, unpublished data), *Ssa13.37*, *SsaF43*, *Ssa20.19* (Sanchez *et al.*, 1996, 2000), *Ssa85*, *Ssa197*, *Ssa202* (O'Reilly *et al.*, 1996), and *SsOSL85* (Slettan *et al.*, 1995). PCRs were performed in 96-well plates with a total reaction volume of 12 µl on an Applied Biosystems GeneAmp 9700 thermal cycler. Each reaction consisted of 50 ng genomic DNA, 1.5 mM $MgCl_2$ (2.5 mM for *SsOSL85*, *Ssa171*, and *SsOSL438*), 0.2 µl of forward and reverse primers (Applied Biosystems, fluorescently labelled), 0.2 mM dNTPs, and 0.5 U Taq polymerase (Promega). The following programme was used for PCR: initial denaturation at 94 °C for 5 min, 30 (35 for *Ssa197* and *SsOSL85*) cycles of 94 °C for 50 s, 40 s at locus-specific annealing temperature, 50 s at 72 °C, followed by a final extension at 72 °C for 10 min. The annealing temperatures used for the different loci were as follows: 55 °C: *SsOSL85*, *Ssa85*, and *Ssa202*; 56 °C: *Ssa13.37*, *Ssa28*, and *Ssa197*; 60 °C: *Ssa20.19* and *SsaF43*. The PCR products were diluted to between 1:20 and 1:50 with deionized water and, to each well in Applied Biosystems Optical Well 96-well trays, 2 µl diluted product was added to 8 µl formamide and 0.1 µl Genescan™ Liz-500 size standard. Three to four different PCR products were combined and run on an Applied Biosystems ABI 3100 Genetic Analyzer. Alleles were scored using the program Genotyper Analysis Software version 3.7, with manual control of the automatically scored peaks.

Statistical analysis

Allele frequencies, F_{ST} values and exact tests of population difference between population pairs, tests for linkage disequilibrium, and deviations from Hardy–Weinberg equilibrium were calculated in the program Genepop 3.4 (Raymond and Rousset, 1995). Nei's (1978) genetic distance and

heterozygosities were computed in TFPGA (Miller, 1999). This program was also used to compute the dendrogram and the percentage of bootstraps supporting the nodes based on Nei's (1978) genetic distance. Allelic richness was calculated in FSTAT 2.9.3.2 (Goudet, 2001).

Assignment tests were conducted in the program Bayes (Pella and Masuda, 2001). Classical assignment tests (Paetkau *et al.*, 1995; Cornuet *et al.*, 1999) perform well when samples are sufficiently differentiated, although Bayesian estimation methods give better results than classical assignment methods in situations where genetic differentiation between samples is low (Koljonen *et al.*, 2005). For comparison, tests were also performed with GeneClass 2 (Piry *et al.*, 2004), using the Bayesian method of Rannala and Mountain (1997) implemented in the program.

The assignment power of the loci was evaluated with the program Whichloci (Banks *et al.*, 2003). This program uses successive assignment trials from one locus at a time, on a test data set generated by random sampling of the alleles according to their frequency in the populations in the given data set, to generate a ranking of the loci's efficiency for correct population assignment. In the present study, the population size parameter for the randomly generated populations was set to 500.

Assignment tests were performed, first, on the entire material, which included individuals from all 17 samples; second, only on the oldest samples from each river; third, only on the most recent samples from each river.

Results

Allelic variability and heterozygosity

In all, 1066 fish were screened at eight microsatellite loci (Table 2). Overall scoring was high (95%−97%) for all markers, with lowest values for *Ssa202* in the 2000 Opo River sample (86%) and *Ssa85* in the 2000 Namsen River sample (89%). For some samples more than 30 years old, reruns and more PCR cycles were necessary to achieve a satisfactory scoring percentage for some of the loci. For some individuals, accurate scoring could not be achieved at all eight loci. Where lower scores were obtained at markers with long alleles, no consistent trend could be seen in the total material.

Sample sizes varied from 21 to 92 individuals. The number of alleles at the different loci ranged from 2 (*Ssa13.37*) to 27 (*Ssa197*) across populations. The number of alleles observed for a given loci varied greatly among the samples. For example at the *Ssa197* locus, the number of alleles observed in a sample varied from 13 in the 1986−1990 Hå River sample to 24 in two of the Namsen River samples. This variation in number of alleles between samples is also reflected in the values for allelic richness across loci. The highest values for allelic richness were observed in the 1977 and 1998 Namsen River samples, while the lowest observed values were found in the 1971−1973 Opo River and the 1980 Vosso River samples. Observed heterozygosity across loci for the samples varied between 0.63 and 0.71.

Table 2. Number of salmon per sample, number of alleles at each microsatellite locus, allelic richness, and observed heterozygosity (H_O) in the sampled populations.

		Locus								Allele summary		Allelic richness		
Sample	*n*	*Ssa202*	*Ssa197*	*Ssa20.19*	*SsaF43*	*Ssa13.37*	*Ssa28*	*SsOSL85*	*Ssa85*	Total	Mean	Total	Mean	H_O
Vosso 1980	50	7	17	3	6	2	3	9	13	60	7.5	49.7	6.2	0.68
Vosso 1995−1997	48	10	20	3	6	2	4	10	14	69	8.6	57.4	7.2	0.68
Vosso 2003	84	12	17	3	6	2	3	11	13	67	8.4	52.8	6.6	0.71
Etne 1983	92	11	22	4	7	2	4	10	16	76	9.5	58.0	7.3	0.68
Etne 1997−1998	81	12	21	3	7	2	4	12	17	78	9.8	58.5	7.3	0.69
Opo 1971−1973	88	8	19	5	5	2	4	11	12	66	8.3	49.8	6.2	0.63
Opo 2000	56	9	17	4	5	2	4	11	12	64	8.0	54.3	6.8	0.66
Namsen 1977	92	12	24	5	8	2	4	12	16	83	10.4	62.1	7.8	0.69
Namsen 1998	71	12	23	5	6	2	4	12	17	81	10.1	62.2	7.8	0.69
Namsen 2000	71	11	24	5	7	2	4	11	16	80	10.0	59.8	7.5	0.66
Granvin 1990−96	28	10	14	4	5	2	4	9	13	61	7.6	55.7	7.0	0.69
Granvin 2000−2001	67	10	17	5	7	2	5	11	11	68	8.5	53.5	6.7	0.68
Hå 1986−1990	21	8	13	4	7	2	3	9	12	58	7.3	57.6	7.2	0.65
Hå 1996−1997	39	9	16	5	7	2	4	13	13	69	8.6	60.7	7.6	0.70
Hå 1999−2000	91	12	19	4	7	2	3	15	17	79	9.9	59.3	7.4	0.66
Eio 1987−1995	35	9	19	5	5	2	4	10	13	67	8.4	58.9	7.4	0.64
Eio 2001	52	12	14	5	6	2	4	10	14	67	8.4	56.5	7.1	0.66
Total	1066	16	27	7	8	2	6	21	19	106	13.3	59.5	7.4	0.68

The lowest values were found in the 1971−1973 Opo River sample and the 1987−1995 Eio River samples, while the highest value was found in the 2003 Vosso River sample.

Tests of Hardy−Weinberg equilibrium and genotypic disequilibrium

Considering the different loci in different samples, the null hypothesis of Hardy−Weinberg equilibrium could only be rejected in four of 136 tests after sequential Bonferroni correction for multiple tests. These four deviations were in four different samples and at four different loci (*Ssa85*, *Ssa202*, *Ssa197*, and *SsOLS85*). Consequently, no consistent trend in deviations was observed.

Four of 28 tests of linkage disequilibrium between locus pairs across populations were significant after sequential Bonferroni correction for multiple tests. These were *Ssa202* and *Ssa197*, *Ssa197* and *SsOSL85*, *Ssa85* and *Ssa202*, and *Ssa197* and *Ssa85*. However, because there is no indication that these loci are genetically linked (Gilbey *et al.*, 2004), it is unlikely that this has affected the outcome of the analyses. Within samples, only the 2000 Opo River sample had more than one instance of significant linkage disequilibrium, with significant *p*-values found at the locus pairs *Ssa197* and *Ssa85*, *Ssa202* and *Ssa85*, *Ssa197* and *SsOSL85*, and *Ssa197* and *Ssa28*.

Temporal and spatial patterns of genetic differentiation

None of the temporal intrariver comparisons in the Namsen, Etne, Hå, and Granvin Rivers were significantly different. However, significant temporal changes were found in the Opo, Vosso, and Eio Rivers.

The closest sample pairs according to F_{ST} and D_A were from the 1983 and 1997−1998 Etne River samples and the 1996−1997 and 1999−2000 Hå River samples, with F_{ST} estimates of 0.0002 and 0.0003, respectively (Table 3). Furthermore, very small differences were found between the 1996−1997 Hå River sample and the two Etne River samples. The highest values were found in comparisons between the 1980 Vosso River sample and samples from the Hå, Eio, and Namsen Rivers, with values ranging from 0.038 to 0.057.

To investigate potential temporal changes in interpopulation variability, overall F_{ST} values were compared among the oldest and most recent samples from the Hå, Vosso, and Namsen Rivers, excluding the "middle" samples. There was a modest reduction in both estimates for population differentiation over time. D_A declined from 0.0389 in the oldest samples to 0.0343 in recent samples, while F_{ST} declined from 0.0183 in the oldest samples to 0.0152 in recent samples.

The UPMGA dendrogram (Figure 2) shows that close clusters are formed by the three samples from the Namsen River, the two samples from the Etne River, the two oldest samples from the Vosso River, and the 1996−1997 and 1999−2000 Hå River samples. Other samples, e.g. the

Table 3. Pairwise genetic distances (Nei, 1978) in the upper diagonal and F_{ST} values in the lower diagonal, calculated from eight microsatellite loci. Non-significant differences between samples are indicated by * in the lower diagonal.

Pop	VO80	VO95−97	VO03	ET83	ET97−98	OP71−73	OP00	NA77	NA98	NA00	GR90−96	GR00−01	HA86−90	HA96−97	HA99−00	EI87−95	EI01
VO80		0.0107	0.0498	0.0483	0.0464	0.0569	0.0484	0.0903	0.0972	0.0825	0.0274	0.0382	0.1213	0.0491	0.0742	0.0959	0.0773
VO95−97	0.0061		0.0242	0.0215	0.0224	0.0335	0.0182	0.0465	0.0478	0.0345	0.0161	0.0375	0.0688	0.0186	0.0387	0.0418	0.0413
VO03	0.0247	0.0115		0.0185	0.0127	0.0312	0.0207	0.0214	0.0303	0.0172	0.0051	0.0350	0.0298	0.0046	0.0179	0.0364	0.0406
ET83	0.0233	0.0097	0.0083		0.0005	0.0284	0.0244	0.0270	0.0322	0.0201	0.0061	0.0238	0.0182	0.0013	0.0156	0.0174	0.0457
ET97−98	0.0227	0.0102	0.0059	0.0002*		0.0256	0.0166	0.0220	0.0198	0.0156	0.0046	0.0365	0.0222	0.0008	0.0050	0.0195	0.0422
OP71−73	0.0277	0.0154	0.0144	0.0130	0.0117		0.0388	0.0392	0.0518	0.0436	0.0254	0.0543	0.0530	0.0208	0.0368	0.0422	0.0430
OP00	0.0241	0.0088	0.0100	0.0113	0.0079	0.0180		0.0493	0.0478	0.0423	0.0262	0.0583	0.0416	0.0130	0.0270	0.0472	0.0658
NA77	0.0417	0.0208	0.0097	0.0116	0.0097	0.0178	0.0224		0.0022	0.0054	0.0065	0.0577	0.0323	0.0215	0.0234	0.0074	0.0178
NA98	0.0458	0.0221	0.0141	0.0144	0.0092	0.0237	0.0226	0.0012*		0.0117	0.0120	0.0701	0.0283	0.0155	0.0224	0.0103	0.0260
NA00	0.0382	0.0152	0.0077	0.0083	0.0068*	0.0194	0.0194	0.0023*	0.0054*		0.0169	0.0615	0.0256	0.0162	0.0255	0.0205	0.0225
GR90−96	0.0152	0.0080*	0.0029*	0.0031*	0.0023*	0.0114	0.0129	0.0032*	0.0061*	0.0077*		0.0176	0.0308	0.0075	0.0158	0.0075	0.0279
GR00−01	0.0190	0.0162	0.0153	0.0097	0.0153	0.0238	0.0255	0.0241	0.0299	0.0247	0.0082*		0.0593	0.0224	0.0425	0.0573	0.0703
HA86−90	0.0567	0.0308	0.0139	0.0077	0.0100*	0.0238	0.0198	0.0146	0.0134	0.0108*	0.0151	0.0249		0.0048	0.0162	0.0348	0.0877
HA96−97	0.0248	0.0086	0.0027	0.0003	0.0003*	0.0096	0.0069	0.0093	0.0074*	0.0066*	0.0043*	0.0089	0.0023*		−0.0013	0.0252	0.0445
HA99−00	0.0358	0.0183	0.0087	0.0075	0.0025	0.0172	0.0131	0.0110	0.0107	0.0119	0.0076*	0.0190	0.0075*	0.0003*		0.0172	0.0446
EI87−95	0.0465	0.0199	0.0176	0.0084	0.0094	0.0197	0.0226	0.0038*	0.0052*	0.0097*	0.0038*	0.0257	0.0166	0.0124	0.0084		0.0243
EI01	0.0366	0.0184	0.0181	0.0191	0.0182	0.0193	0.0295	0.0079	0.0121	0.0095	0.0130	0.0287	0.0378	0.0188	0.0205	0.0114	

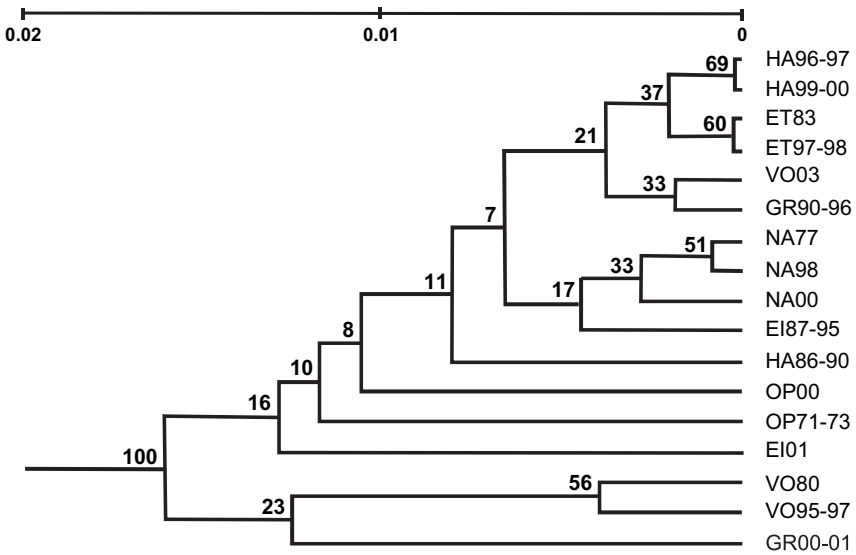

Figure 2. UPMGA dendrogram which shows one cluster for the Namsen River (NA), one for the Etne River (ET), and one for two samples from the Hå River (HA). The two oldest samples from the Vosso River (VO) also cluster together, while other samples are scattered around in the tree. Numbers at nodes show the proportion of similar replicates from 1000 permutations.

2003 Vosso River sample and the samples from the Opo, Granvin, and Eio Rivers, are scattered around the dendrogram and do not cluster with samples from the same river.

The exact tests of genetic differentiation, using loci combined, showed significant differences between most sample pairs. Of 136 pairwise tests, 110 resulted in significant *p*-values after sequential Bonferroni correction, while 26 tests gave non-significant *p*-values (Table 3).

Assignment

The overall assignment success, including all 17 samples, was 41% when using the Bayes program. The test conducted in GeneClass resulted in an overall assignment of 24%. The highest correct assignment was observed for parr from Granvin River, spawners from the 1971−1973 Opo River and 1980 Vosso River samples. For the smallest sample, 1990−1996 Granvin River, all individuals were assigned to other samples. As expected, where individuals were not assigned to the originating sample, they were most frequently assigned to one of the other samples from the same river. Separate assignment tests, including only the oldest and the most recent samples from each river, were also conducted (Figure 3). More accurate assignment was achieved by using these subsets of the samples, with

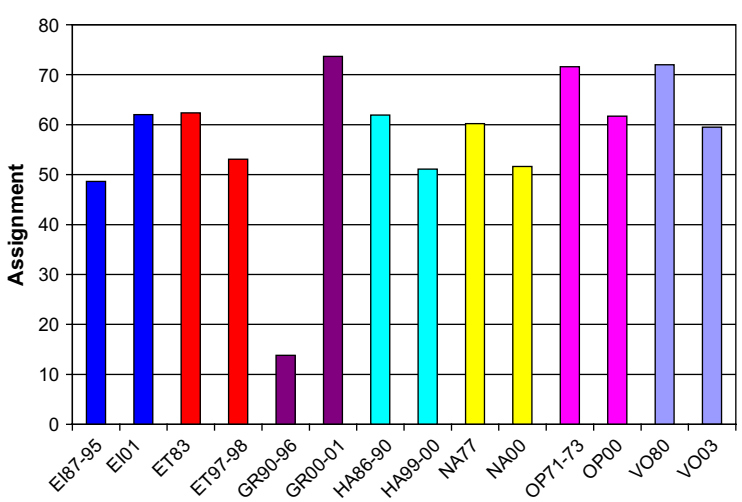

Figure 3. Percentage correct assignment in historical and in contemporary samples of Atlantic salmon (ET, Etne River; GR, Granvin River; HA, Hå River; OP, Opo River; VO, Vosso River; EI, Eio River).

60% and 58% correct assignment, respectively. The percentage of correct assignment in the oldest samples was higher for all rivers except the Eio and Granvin, which had very low values in these case. The reduction in assignment success between historical and contemporary samples for the other rivers was 10.2% on average, with the largest reduction (12.5%) observed in the Vosso River.

The analysis of the performance of the different loci conducted with Whichloci showed that the more variable loci contributed most to correct assignment. The two most variable loci, *Ssa197* and *Ssa85*, were also the most informative loci in the classical assignment test performed in Whichrun, while the di-allelic locus *Ssa13.37* was the least informative. The ranking and relative scores of the loci are given in Table 4.

Discussion

Significant genetic differences between the oldest and most recent samples were observed for three of the seven Atlantic salmon populations investigated (Opo, Vosso, and Eio). In the remaining four populations (Namsen, Etne, Hå, and Granvin) no significant differences were observed between the oldest and most recent samples. Except for the Hå River, which is located in an area with little salmon farming, the other rivers, particularly the Etne, Opo, and Eio, are located in regions with large concentrations of salmon farms. Farmed salmon have been recorded in large proportions in all the studied populations except for the Hå River. Consequently, changes observed in allelic frequencies between the oldest and the most recent samples in three of the populations may either have been caused by gene flow from immigrating farmed salmon or, alternatively, by genetic drift where the populations are small.

Except for the Granvin, all seven rivers had spawning populations with several hundred spawners or more, but adult numbers in the Vosso, Opo, and Eio declined sharply around 1990. The temporal samples show stable levels of allelic variability and observed heterozygosity, and no evidence of reduced variability that could cause temporal instability through genetic drift. Furthermore, in the Opo River, four instances of linkage disequilibrium were detected. Departures from gametic equilibrium can be caused by natural selection, physical linkage, genetic drift, and population subdivision (Waples and Smouse, 1990). The test proposed by Waples and Smouse (1990) was developed for detecting mixtures of populations, but gametic disequilibria may also be detected in F1 progeny from such a mixture (Crozier, 1993). Therefore, the most likely explanation for the observed changes in the Opo, Vosso, and Eio Rivers is gene flow from the many farmed salmon in these rivers. The observed instances of gametic disequilibria found in the parr sample in the Opo River in 2000 support this conclusion.

Table 4. Ranking of loci in assignment with relative score (%).

Rank	Locus	% Relative score
1	*Ssa197*	35.8189
2	*Ssa85*	20.3728
3	*Ssa20.19*	13.3156
4	*SsOSL85*	13.3156
5	*Ssa202*	10.5193
6	*SsaF43*	6.1252
7	*Ssa28*	0.5326
8	*Ssa13.37*	0.0000

The eight microsatellite loci screened in the present study detected 106 alleles. This is somewhat lower than the 261 alleles detected by 12 microsatellite loci in a previous study on Norwegian salmon (Skaala *et al.*, 2004). It is also slightly lower than the number observed in European salmon populations (King *et al.*, 2001; Sälsä *et al.*, 2005; Tonteri *et al.*, 2005). However, the panel of microsatellites in the present study includes several low and moderately variable markers. The largest number of alleles was found in the Namsen River, where more than 80 alleles were detected. The lowest values for allelic richness were observed in the oldest samples from the Vosso and Opo Rivers; the latter also had the lowest observed heterozygosity. These observations most likely reflect the Namsen River's status as having one of the largest salmon populations in Norway, while the Opo River has a small salmon population, located at the head of the 160-km-long Hardangerfjord and a considerable distance from major salmon populations along the Norwegian coast.

For the populations in the Vosso, Opo, Granvin, and Eio Rivers, the stability observed in genetic diversity was unexpected because these populations are currently relatively small and presumably susceptible to genetic changes from the large number of escaped farmed salmon that enter the rivers. Although the salmon populations in the Granvin and Opo Rivers are naturally small, the populations in the Vosso and Eio Rivers were previously much larger and have declined markedly during the past 15 years. Therefore, a lower level of genetic diversity was expected in some of the populations. It is possible that the reduction in population size in the Vosso and Eio Rivers has not persisted long enough to cause a genetic bottleneck. Alternatively, immigration of farmed salmon may have counteracted a decline in genetic diversity. In the Opo, Eio, and Granvin Rivers, the proportion of farmed salmon in the spawning populations has been larger than that of wild salmon for several years. Therefore, the absence of a reduction in genetic diversity, and even an increase in observed heterozygosity in temporal samples from the Opo and Eio Rivers, most likely reflects gene flow from farmed salmon that have been recorded in these rivers in large proportions.

Analysis of the pairwise F_{ST} values for within-river samples indicates that the smallest values were observed in the Etne, Namsen, and Hå Rivers, all of which support large

salmon populations. None of the temporal comparisons in these rivers was significant, strongly suggesting that there has been no genetic impact from farmed salmon in these populations up to the most recent year classes analysed. The situation appears different in the Eio, Vosso, and Opo Rivers, where F_{ST} values are more than ten times greater than the values observed in the Etne, Namsen, and Hå. In the Vosso River, the F_{ST} value between the 1980 and 1995−1997 samples is low and more comparable with the values observed within the rivers with temporal stability. The F_{ST} value between the 1980 sample and the 2003 sample in the Vosso River is four times higher than that between the 1980 and the 1995−1996 samples, reflecting a larger change in year classes spawned after the 1995−1996 salmon were spawned. This could be a consequence of either increased genetic drift or, alternatively, gene flow from immigrating escaped farmed salmon. As there is an increase in allelic richness and observed heterozygosity over time in the Vosso River, the most likely explanation is gene flow from farmed salmon.

The genetic distances in Figure 2 further illustrate the temporal stability in some of the populations. The two samples from the Etne River cluster very closely, as do the three samples from the Namsen River. In the Hå River, two of the samples cluster very closely, but the oldest sample is located in a separate branch of the dendrogram. The shortest genetic distance was between the two most recent samples from the Hå River, which was not unexpected owing to the short time interval between the two samples. Nevertheless, the results demonstrate a genetic stability over year classes that probably would have been altered in the presence of gene flow from farmed salmon. The location of the oldest sample from the Hå River probably reflects the small sample size.

The 1980 and 1995−1997 Vosso River samples cluster together, while the 2003 sample is located in a different part of the dendrogram. The F_{ST} and D_A values indicate that a greater change has taken place later than the 1995 sample would indicate. The result agrees with statistics showing that only since 1992 has a significant proportion (46%) of farmed salmon occurred in the Vosso River. This means that our sample of spawners collected in 1995−1997, originating in spawning around 1990, would be less affected by gene flow than subsequent year classes. Therefore, genetic data and cluster location of our smolt sample from 2003, originating in spawning around 2000, agree with annual observations of the proportion of farmed salmon in the Vosso River.

Temporal stability in the Etne River, also illustrated in the dendrogram (Figure 2), is particularly interesting and unexpected because the proportion of farmed salmon in this river has been large (74%) since monitoring began in 1989. This also agrees with the hypothesis that a large number of wild spawners, as in the Etne River, reduces the spawning success of farmed salmon and, consequently, their impact on the wild population. Stable levels of genetic variability were also found in a study of temporal samples of Danish salmon populations (Nielsen *et al.*, 1999). The scattered location of the individual samples from the Eio and Opo Rivers in the dendrogram further illustrates the temporal instability in these populations.

The genetic differentiation among the seven populations studied here is lower than reported in other studies (King *et al.*, 2001; Skaala *et al.*, 2004; Wennevik *et al.*, 2004; Koljonen *et al.*, 2005; Sälsä *et al.*, 2005; Tonteri *et al.*, 2005). This can be explained by the panel of microsatellites employed and the smaller geographical area covered in the present study. However, F_{ST} values observed in the seven Norwegian populations are comparable with those found by Nielsen *et al.* (1999) for Danish Atlantic salmon.

When only the oldest or the most recent samples were included in the assignment, the precision of identifying individuals to population of origin varied from approximately 50% to 70%. As some of the present markers, such as *Ssa29* and *Ssa13.37*, which are characterized by a small number of alleles, contributed little to the power of assignment, it is expected that precision would be increased significantly by including highly variable microsatellite markers. King *et al.* (2001) realized 100% correct assignment to continent of origin, while identification to individual population varied from 62% to 99%. High precision (97%−99%) was also obtained by Koljonen *et al.* (2005) in a study using eight microsatellite markers for Atlantic salmon from the Baltic Sea. In the present study, the greatest precision was achieved for the oldest samples from the Opo and Vosso Rivers and the most recent sample from the Granvin River. There was a reduction in the precision of assignment when the oldest and the most recent samples were analysed separately, indicating a reduction in genetic differentiation among the salmon populations over time. In the Granvin and Eio Rivers, there was an increase in assignment precision over time. The oldest samples from both of these populations consist of spawners collected over several years, which may be more affected by immigrants and, therefore, they are less population-specific than the more recent parr samples. For the Granvin River, the small sample size may also have reduced assignment precision. When all the samples were included, precision was reduced, which is not unexpected because several less-differentiated samples were included.

In general, immigration will reduce the interpopulation variance in allelic frequencies and reduce population differentiation. It has been postulated, therefore (Hindar *et al.*, 1991; Mork, 1991), that farmed salmon, in the numbers experienced in Norway, will gradually reduce the population differentiation of wild Atlantic salmon. Interestingly, when F_{ST} and D_A values for old and new samples were compared, there was a moderate reduction in population differentiation over time in both estimates. The most likely explanation for the observed reduction, therefore, is increased immigration of farmed salmon. In a similar study

on the genetic effects of compensatory hatchery releases in the Baltic Sea, Vasemägi *et al.* (2005) also detected a reduction in F_{ST} over time.

In summary, the genetic profiles developed in the present study strongly suggest reasonably unaltered gene pools in the Namsen, Etne, Hå, and Granvin Rivers up to the year classes analysed. In the Vosso River, there appears to have been a change in the gene pool in or shortly before 2000. In the Eio and Opo Rivers, significant temporal genetic changes in the gene pools were observed. As there was no indication of reduced genetic diversity in these populations, it is likely that the observed changes in the gene pools are the result of gene flow from farmed salmon, which have been reported to be present in these rivers in large numbers. As was observed in the estimators of genetic distance, the slightly lower correct assignment of contemporary samples may be an indicator of diminishing genetic differentiation between populations.

Acknowledgements

Samples were generously provided by Reidar Borgstrøm, Anton Rikstad, Erlend Waatevik, Rådgivende Biologer, Bjørn Barlaup, and Kjetil Hindar. The laboratory work conducted by Anne Grete Sørvik is gratefully acknowledged. Michele Masuda is acknowledged for assisting with running the Bayes program. The project was funded by the Ministry of Fisheries and Coastal Affairs. KAG was funded by project no. 153250/120 "Atlantic salmon genes influencing resistance to salmon lice (*Lepeophtheirus salmonis*)" from the Norwegian Research Council.

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