



Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations

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Abstract

Farmed Atlantic salmon (*Salmo salar*) escape from net pens and enter rivers to spawn, potentially resulting in genetic introgression and reduced fitness of wild salmon. Here we quantify genetic introgression of farmed to wild salmon, using molecular genetic markers, in populations from 147 salmon rivers, representing three quarters of the total wild salmon spawning population in Norway. For 109 rivers with adult modern samples and sample sizes of 20 or more, the average level of farmed genetic introgression was 6.4% (median = 2.3%), with a range between 0.0 and 42.2%. Fifty-one of these rivers showed significant farmed genetic introgression when compared to historical reference samples. We observed a highly significant correlation between estimated farmed introgression and average proportion of escaped farmed salmon. We quantify levels of introgression as unweighted averages or weighted by population sizes, to compare geographical regions and to compare levels of introgression in rivers and fjords designated as locations deserving a high level of protection. We found a generally lower level of introgression in National Salmon Rivers and National Salmon Fjords subjected to formal protection by parliament. We conclude that farmed to wild genetic introgression is high in a large proportion of Norwegian salmon rivers, with the highest levels found in the most intensive areas of salmon farming. The extensive genetic introgression documented here poses a serious challenge to the management of farmed and wild Atlantic salmon in Norway and, in all likelihood, in other regions where farmed-salmon escape events occur with regularity

Key words: Atlantic salmon, genetic introgression, farmed salmon, aquaculture, genetics, SNPs

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36 **Introduction**

37 Farmed Atlantic salmon differ genetically from wild salmon because of a variety of causes.
38 Breeding programs of farmed Atlantic salmon were established in Norway in the early 1970s
39 based on salmon collected from several populations in Central and Western Norway
40 (Gjedrem *et al.*, 1991; Gjøen and Bentsen, 1997). The breeding program has successfully
41 changed the genetics of farmed Atlantic salmon to improve commercially important traits,
42 such as growth, utilization of feed, and filet quality (Thodesen *et al.*, 1999; Gjedrem and
43 Baranski, 2009; Solberg *et al.*, 2013). These genetic improvements have undoubtedly
44 contributed to the rapid expansion of the Atlantic salmon farming industry in Norway, with a
45 production close to 1.3 million tons in 2015.

46 Farmed Atlantic salmon also differ genetically from wild salmon because of selection to
47 captivity, and loss of genetic variation from a limited number of wild founders and
48 subsequent genetic drift (Hutchings and Fraser, 2008). Because of the reduced fitness
49 (Fleming *et al.*, 2000; McGinnity *et al.*, 2003; Skaala *et al.*, 2012; Reed *et al.*, 2015) and
50 lower genetic variation in farmed salmon (Mjølnerød *et al.*, 1997; Skaala *et al.*, 2004; Skaala
51 *et al.*, 2005; Karlsson *et al.*, 2010) compared to their wild conspecifics, there is a concern that
52 genetic introgression of escaped farmed salmon to wild salmon might reduce the viability of
53 wild Atlantic salmon. Reported numbers of escaped farmed salmon in Norway have ranged
54 from 39 000 to 920 000 since 1993, with an average of 380 000 (Norwegian Directorate of
55 Fisheries, <http://www.fiskeridir.no/English>). Inventories since 1989 have shown high
56 proportions of escaped farmed salmon in many Norwegian rivers, with large variations
57 between years (Fiske *et al.*, 2006) and rivers (Gausen and Moen, 1991; Diserud *et al.*, 2013).
58 A similar situation has been documented in eastern North America with a large number of

59 escaped farmed entering salmon rivers, in many rivers outnumbering the wild spawning
60 population and with extensive variation between rivers and years (Morris *et al.*, 2008).

61 Genetic introgression of escaped farmed salmon to wild salmon populations has been
62 modelled (Hindar *et al.*, 2006) based on relative fitness estimates (Fleming *et al.*, 2000;
63 McGinnity *et al.* 2003) and observed proportions of escaped farmed salmon (Fiske *et al.*,
64 2006). The spawning success of escaped farmed salmon (Fleming *et al.*, 1996; 1997) and
65 survival of their offspring (Fraser *et al.*, 2008; 2010; Skaala *et al.*, 2012; Sundt-Hansen *et al.*,
66 2015) depend on a variety of factors in wild populations, farmed escapes and the environment
67 in which they meet, and make it difficult to accurately predict farmed to wild genetic
68 introgression. The development of improved models with important and more precise
69 parameters requires quantification of the farmed to wild genetic introgression (Heino *et al.*,
70 2015).

71 Several molecular genetic markers for quantifying genetic introgression of farmed
72 escaped salmon in wild salmon populations have been identified (Karlsson *et al.*, 2011).
73 These markers were used to quantify genetic introgression in 20 Norwegian salmon
74 populations, based on observed temporal genetic changes and Approximate Bayesian
75 Computation (ABC) of the farmed-to-wild gene flow that is consistent with these changes
76 (Glover *et al.*, 2013). The ABC method is restricted, as it relies on the existence of historical
77 samples from each population to be analyzed. From the generic genetic differences observed
78 at the genetic markers identified by Karlsson *et al.* (2011), an alternative standardized method
79 was developed by Karlsson *et al.* (2014). This method does not rely on historical samples
80 from all populations, but uses the directional genetic change from farm-to-wild introgression,
81 and not genetic changes stemming from genetic drift and/or gene flow between wild
82 populations. In short, the method uses historical samples from many wild populations and

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83 samples from the Norwegian breeding kernels for farmed salmon, and estimates for each
84 individual of interest the proportion of membership to these two groups, using STRUCTURE
85 (Pritchard et al., 2000).

86 The objective of the present study was to obtain an extensive coverage of farmed to wild
87 Atlantic salmon genetic introgression using the new molecular genetic and analytical
88 methods. We analysed 21 562 Atlantic salmon hatched in the wild in 147 Norwegian rivers,
89 including 16 407 adults and 5 155 juveniles. Here, we first present a comprehensive
90 geographical coverage of status with respect to farmed to wild introgression. Second, we
91 compare estimates of introgression in samples based on juveniles with samples based on
92 returning adults from the same population. Third, we assess the relationship between long-
93 term proportions of escaped farmed salmon and genetic introgression. Finally, we assess to
94 what extent a major conservation policy decision in Norway, designating 52 rivers as National
95 Salmon Rivers and 29 fjords as National Salmon Fjords where important salmon populations
96 receive extra protection (e.g. Vøllestad *et al.*, 2014), has an effect on the levels of
97 introgression.

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99 **Material and Methods**

100 To quantify genetic introgression resulting from spawning of escaped farmed salmon in the
101 wild, we analysed only fish hatched in the wild. We excluded fish classified as escaped
102 farmed salmon, or with uncertain classification, based on their growth patterns in the scales
103 (Lund and Hansen, 1991; Fiske *et al.*, 2005). Samples of juvenile, pre-smolt salmon can
104 safely be regarded as hatched in the wild, because the escape of juvenile farmed salmon from
105 land-based facilities to rivers in this study is unlikely.

We extracted total genomic DNA from scales of adult salmon and from fin-clips of juvenile salmon using DNEASY tissue kit (QIAGEN). Initially, we used the Sequenom SNP-genotyping platform for genotyping of 5897 individuals at 99 SNP loci, with PCR amplifications in 4 multiplexes. Primer extension reactions followed recommendations from Sequenom (www.sequenom.com) and fragments were separated and identified using Sequenom Mass ARRAY™ analyzer (Autoflex mass spectrometer). We conducted genotyping in real time depending on the presence or absence of a mass peak in expected mass range for each locus (Tang *et al.*, 1999) using the MassARRAY™ RT 3.4 software. We obtained reliable genotypes from 59 SNPs described as being collectively diagnostic in differentiating between wild and farm salmon (Karlsson *et al.*, 2011; Jensen *et al.*, 2013). For the remaining 15 293 individuals, we used the EP1™ 96.96 Dynamic array IFCs genotyping platform (Fluidigm, San Francisco, CA). Reliable genotypes were obtained for 48 of the same SNPs genotyped by the Sequenom platform (Karlsson *et al.*, 2011). The SNP genotypes from the Sequenom and the Fluidigm SNP-genotypes were merged for the 48 common SNP loci (Table S1).

As a reference for farmed salmon, we used genotypes from 503 individuals from the three leading breeding companies (Marine Harvest, Salmobreed and AquaGen) from the year classes 2004–2009 (MH), 2004–2007 (SB) and 1998–2001, 2008 (AG). Each yearclass represented one of four different breeding kernels from each breeding company. In 2005, AquaGen pooled the four breeding kernels into one big kernel, represented by the 2008 sample. To investigate historical genetic signatures of farmed salmon, we used 129 samples from 1982–1988 from the four AquaGen breeding kernels. As references for non-admixed wild salmon, we used historical samples of 2187 wild individuals from 39 populations, geographically distributed in rivers from southern to northern Norway. In agreement with previous studies (Bourret *et al.*, 2013; Jensen *et al.*, 2014), the Norwegian populations

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clustered into an Atlantic and a Barents-White Sea phylogenetic group, with the latter including populations from Finnmark County and the former including populations south of Finnmark (Figure 1). All founder populations for the farm strains are from the Atlantic Sea phylogenetic group, as judged from the genetic contributions from source populations in the third generation of the breeding program (Gjøen and Bentsen, 1997). Although the Atlantic and the Barents-White Sea phylogenetic groups are well separated, some populations in Troms County represent a transition between them. We analysed samples from 147 Norwegian rivers, including 5155 juvenile individuals and 16 407 adult individuals. From 109 of these populations, we had adult modern samples with sample sizes of more than 20. For the remaining 38 populations we had only juveniles, historical samples, sample sizes less than 20 (for detailed information see Table S2). Scale samples of adult salmon were obtained from sport fishing, and from catches of broodfish for stocking or during autumn monitoring, while juvenile samples were obtained by electrofishing.

Statistical analyses

We applied the method by Karlsson *et al.* (2014) to estimate the level of farmed to wild genetic introgression. This method uses the STRUCTURE program (Pritchard *et al.*, 2000) in a manner that avoids bias from the level of heterogeneity and different sample sizes as described by Kalinowski (2011) and standardizes the estimates of admixture when introgression occurs from several farmed populations. We generated an idealized wild and farmed population in Hardy-Weinberg proportions from a pool of reference individuals of wild and farmed salmon using the HybridLab program (Nielsen *et al.*, 2006). For the farmed salmon, we used all modern samples from the three breeding companies. For the wild salmon, we generated one population for the Atlantic phylogenetic group and one for the Barents-White Sea group. Samples used for creating these wild center points are indicated in Table

S2, column “REF Year”. These ideal populations ($n = 100$) represented center points for the three groups to which the probability of belonging was estimated (Figure 1). In an analysis of molecular variance (AMOVA), including the farmed reference samples and the historical wild reference samples from the Barents-White-Sea group, 18.01% of the variance was ascribed to variations between these groups ($P < 0.001$), and 4.08% to variance among populations within the groups ($P < 0.001$). In a comparison between farmed populations and the historical wild reference population from the Atlantic group, 7.19% of the variance was ascribed to variations between these groups ($P < 0.001$) and 3.48% to variance among populations within groups ($P < 0.001$). Single individuals were analysed with the farmed center point and the two wild center points representing the correct phylogenetic group for that individual, using an admixed model, 50 000 repetitions as burn in and 100 000 repetitions after burn in as implemented in STRUCTURE (Pritchard *et al.*, 2000). For each fish, the probability of belonging to the wild center point, hereafter $P(Wild)$, was recorded.

For statistical analyses of farmed introgression, we generated probability distributions of belonging to the wild center point for historical wild salmon (all samples in column “REF Year” in Table S2) and for modern farmed salmon. Four populations (rivers Skibotnelva [river ID 205.Z], Signaldalselva [204.Z], Målselv [196.Z], and Skipsfjordelva [202.11Z]) represented genetic transitions between the Atlantic and the Barents-White Sea phylogenetic groups and could not be analysed using the Atlantic or the Barents-White Sea center points. Instead, they were analysed by generating in silico populations from historical samples for each of these populations, except for Signaldalselva for which we did not have historical samples. Samples from the nearby River Skibotnelva were used as the analytical center point for the Signaldalselva population.

From the distribution of individual probabilities of belonging to the wild center point $P(Wild)$ for a given sample (population and year), we estimated genetic introgression from

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181 escaped farmed salmon into this year’s wild Atlantic salmon populations (Karlsson *et al.*,
182 2014). $P(Wild)$ was logit-transformed before the statistical inference (Warton and Hui, 2011).
183 For each contemporary sample with a historical reference from the same river, we tested
184 whether this population was introgressed with a two-sample test for comparing means,
185 assuming random sampling and equal variances for contemporary and reference samples.
186 Further, we assumed that all wild populations had the same variance, estimated as the
187 weighted average of the historical wild reference sample variances. Although the distributions
188 for logit-transformed $P(Wild)$ for wild reference samples are relatively symmetric (see
189 example in Figure 2), they depart too much from normality to perform standard tests for
190 homogeneity of variances. By resampling squared deviations from all wild references, we
191 found that 8.6% (3 of 35) of the wild population variances were significantly different from
192 the pooled variance with a 5% significance level and were close to what we expected under
193 the homogeneity assumption. Several of the wild reference populations have significantly
194 different averages, so when testing whether a population without historical reference is
195 introgressed we needed to consider this variance in wild population average values within a
196 phylogenetic group. For populations without a historical reference from the same river, the
197 contemporary average was therefore compared to the overall wild average for this
198 phylogenetic group with this additional variance component included in the sampling
199 distribution. For both tests, the null hypothesis states no genetic introgression; that is, mean
200 $P(Wild)$ from the contemporary population equals the mean $P(Wild)$ from the historical
201 reference population. The alternative hypothesis states that the contemporary mean $P(Wild)$ is
202 smaller than the historical mean.

203 The distributions for individual $P(Wild)$ values for the samples are illustrated for the River
204 Eira (104.Z) in Figure 2. Notable is the distinctiveness of the distribution for the farm
205 references (red line). The distribution for the contemporary sample (pooled sample for the

years 2012–2015; dashed black line) has a mean value significantly smaller than both the historical distribution for the River Eira population (solid black line) and the distribution for the whole Atlantic phylogenetic group (solid blue line).

For many samples, the observed change in mean value may not be significant, even if the populations show signs of genetic introgression. As in Figure 2, the contemporary distribution can indicate that a proportion of the population is introgressed by having a heavy left tail while the majority of the population is still mostly wild-like. Genetic introgression into a subpopulation can be tested by, for example, inspecting the lower 5-percentile of the distributions. Expected tail properties will be sensitive to distribution assumptions, so we opted for a randomization test approach. If the 5-percentile of a contemporary sample of a given size is much lower than expected from a sample of the same size from the historical distribution, it indicates that this sample has a too large proportion of individuals that genetically are admixed with farmed salmon. This effect was evaluated by simulating $n = 10\,000$ samples of the same size as the contemporary sample from the historical reference for the whole phylogenetic group, and registering the 5 percentiles of each simulated sample. The proportion of simulated 5-percentiles that was lower than the 5-percentile of the historical reference is the P -value of the test.

Juveniles of farmed and admixed origin show lower survival to adulthood than juveniles of pure wild origin (Fleming *et al.*, 2000; McGinnity *et al.*, 2003). We expected therefore to find a higher level of introgression in juveniles than in adults in the same cohorts. To explore this in our data, we compared juvenile samples with adult samples from the same river, using a quasi-cohort comparison. Specifically, we compared farmed introgression between juvenile and adult samples in 26 rivers, where sampling of juveniles occurred 3–5 years earlier than sampling of adults. Even though this is not a formal cohort analysis, at least some of the same year classes are likely represented in both the juvenile and adult samples.

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231 Regional averages of introgression were constructed as unweighted averages and as
232 averages weighted by spawning population size in each river studied (Forseth *et al.*, 2013).
233 We defined regions as counties from the northernmost, Finnmark County, to the southernmost
234 in western Norway, Rogaland County, whereas the counties from southernmost Norway to the
235 south-eastern border with Sweden, were treated as one region (Fiske *et al.*, 2006) denoted
236 Southeast.

237 A major conservation policy for wild Atlantic salmon in Norway, National Salmon Rivers
238 and National Salmon Fjords, was established by the Norwegian Parliament in 2003
239 (completed 2007) to increase the level of protection of Atlantic salmon, including protection
240 from fish farming. By the final decision in 2007, 52 rivers were designated as National
241 Salmon Rivers (of which we studied 48, cf. Vøllestad *et al.*, 2014) and 29 coastal areas were
242 designated National Salmon Fjords (all are represented by our samples). We calculated
243 unweighted and weighted averages for these groups of rivers in the same manner as for
244 counties.

245 To study associations between group levels of introgression and average proportions of
246 escaped farmed salmon, we used the method developed by Fiske *et al.* (2006) and Diserud *et*
247 *al.* (2010) to calculate an ‘annual incidence’ of escaped farmed salmon, by averaging
248 proportions of escaped farmed salmon in anglers’ catches in summer and in organized surveys
249 in autumn and by calculating a weighted average by river catches. At the individual river
250 level, Diserud *et al.* (2012, 2013) developed a long-term ‘average annual incidence’ for the
251 years 1989–2012 for all rivers that were represented by four or more years in the time series.

252
253 **Results**

Based on adult modern samples from 109 salmon rivers with a sample size of 20 or more, we observed significant genetic introgression from escaped farmed salmon in 51 wild salmon populations (47%) and an estimated level of introgression $>10\%$ in 27 populations, between 4–10% in 19 populations and $<4\%$ in 63 populations (Table S2, Figure 3). When all samples were considered, significant genetic introgression was observed in 77 of 147 rivers (Table S2).

Comparisons in 26 rivers of juvenile samples with adult samples taken 3–5 years later, presumably representing the same cohorts, showed an average reduction of 2.5 percentage points between estimates of introgression in juvenile and adult life stages. Variation between rivers was high ranging from a 13% increase to a 17% reduction in farmed introgression from juvenile to adult samples.

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266 **Geographical distribution of farmed to wild genetic introgression**

In the following, the presentation of level of introgression is based on pooled adult samples from recent sampling years in 109 rivers with a sample size of at least 20 individuals. National Salmon Rivers with special protection against anthropogenic impacts, including salmon farming, had on average lower levels of farmed genetic introgression (unweighted average, 4.5%) than salmon rivers without protection (unweighted average, 7.8%). The protecting effect of National Salmon Fjords appeared to be smaller as salmon rivers in and outside these fjords had similar (average, 6.4%) levels of farmed genetic introgression (Table 1). When considering population size (weighted averages), rivers within the National Salmon Fjords had however a lower level of introgression (1.8%) than other rivers (3.5%).

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Genetic introgression has occurred in all regions of Norway, and the highest genetic introgression is found in the most intensive salmon farming regions (Figure 3). Unweighted averages of genetic introgression were largest in Troms County (14.5%) and Hordaland County (13.9%) and smallest in Nord-Trøndelag County (0%) and Rogaland County (1.8%) (Table 2). However, four regional averages were based on less than 10 rivers, Troms and Nord-Trøndelag being two of them. We also found significant introgression in samples excluded because of sample sizes less than 20, including adult samples (Byaelva [128.Z] and Salvassdraget [140.Z]) from Nord-Trøndelag (Table S2). Hence, no region in Norway is without farmed introgression.

Weighted averages by wild population size in the sampled rivers within each county were largely determined by status of the largest rivers and illustrate the geographical distribution of farmed introgression relative to the number of genes of farmed origin (proportion of farmed genomes). Hordaland County had the largest proportion of genomes with farmed origin (11.1%), and Nord-Trøndelag County the smallest (0%). In the two phylogenetic groups of Norway, we found more introgression in the Atlantic group (unweighted average = 6.9%, weighted average = 2.6%) than in the Barents-White Sea group (unweighted average = 2.6%, weighted average = 1.0%). Nationally, unweighted and weighted estimated proportions of farmed genomes were 6.4%, and 2.1%, respectively (Table 2).

Genetic introgression relative to farmed escapees

We observed a highly significant relationship between accumulated genetic introgression and average annual proportion of escaped farmed salmon, explaining 24% of the variance in introgression between rivers (Figur 4). The relationship was stronger at the region level, with proportion of escaped farmed salmon explaining 56% of the variance when weighted by

population size (open diamonds in Figure 4). For populations in the Atlantic Sea phylogenetic group, the relationship was highly significant (red solid diamonds and dashed red line in Figure 4; $P < 0.01$, $R^2 = 0.19$, gradient = 0.3), while for populations in the Barents-White Sea phylogenetic group the relationship was weak and not significant (blue solid diamonds and dashed line, Figure 4; $P > 0.05$, $R^2 = 0.05$, gradient = 0.05).

Temporal trends

We had samples from different periods (decades) in 27 populations, allowing us to examine temporal trends in the level of genetic introgression. Twelve of the populations showed an increase in genetic introgression, seven a decrease and six showing no introgression over time. In three populations for which we had more than two samples in time, there were increases followed by decreases in genetic introgression. Populations with downward trends had initial levels of genetic introgression between 1.8% and 6.1%, and in a more recent sample levels of introgression were between 0.0% and 3.8% (median = 0.2%). River Kinso (050.1Z) showed a decrease from a high of 24.7% in the 2000s to 12.7% in the 2010's. However, the trend in River Kinso is uncertain because there was only one sampling year representing the 2010s period and only 15 fish were analysed. A sample of juveniles from 2011 showed 29.4% introgression. A majority of the populations with an upward trend in genetic introgression had initial levels of genetic introgression between 0.0% and 7.5%, but showed large increases in genetic introgression with temporal differences in genetic introgression ranging from 1.5% to 23.7% (median = 11.2%).

Detecting early genetic introgression

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We tested to what extent our set of SNP markers and the standardized method for detecting introgression (Karlsson *et al.*, 2014) worked for characterizing earlier generations of farmed salmon than those used for selecting SNPs differentiating between farmed (breeding kernel year classes 1998–2009) and historical wild salmon (Karlsson *et al.*, 2011). A comparison of distributions of *P(Wild)* between historical (1982–1988) and contemporary AquaGen samples indicated that the historical farmed populations appeared to be more wild than modern samples of farmed salmon (Figure 5).

Discussion

We quantified genetic introgression of farmed Atlantic salmon into 21 562 wild salmon from 147 populations. Levels of introgression above 10% can now be found in any part of Norway in juveniles, as well as in adult salmon that have completed a life cycle in the wild. As expected, the level of introgression in wild populations is significantly associated with the average proportion of escaped farmed salmon in the river over the last 25 years.

Our method allows quantification of introgression from the individual level to populations, regions and the national level, and in rivers with and without a historical baseline. We found the highest levels of introgression in the counties of Norway where escaped farmed salmon have been present in highest proportions.

In Hordaland County, western Norway, one of the two cradles of fish farming, several populations show high levels of introgression, with the rivers Opo (048.Z), Granvin (052.1Z; juveniles) and Dale (061.Z) showing recent levels of introgression above 40% and three other rivers (Vosso (062.Z), Kinso (050.1Z), and Etne (041.Z)) showing introgression above 10%. Affected rivers are found both along the coast and within the major (Hardangerfjord) and minor fjords in the county. Highly affected rivers in this county were also found in a study of 20 Norwegian rivers by Glover *et al.* (2013).

Other rivers with high levels of introgression are found in Troms County in northern Norway, Sogn og Fjordane County and Møre og Romsdal County in western Norway. Rivers with low levels of introgression are most common in south-eastern Norway, Rogaland County in the southwest and Finnmark County in the northeast. The river holding Norway's largest Atlantic salmon population, River Tana (234.Z) on the border with Finland, has a low level of introgression (0 in our Table S2). The spawning population (or rather, populations, see Vähä *et al.*, 2008) of the Tana is so large (> 40 000 fish), compared the other salmon rivers (average = 1 900, range: 100-18 000), that it strongly affects regional weighted averages in Finnmark, the Barents-White Sea phylogenetic group and even the national average.

Current levels of introgression are likely underestimated

The farmed references in the present study are representative for introgression that occurred between the 1990s and present. They cover a large part of this period (year classes hatched 1998 to 2009) and may be representative of more years, as a previous study showed no significant change in allele frequencies at microsatellites in two breeding kernels sampled one generation (Karlsson *et al.*, 2010).

Intrusion of farmed Atlantic salmon on the spawning grounds of wild salmon was detected on a large scale from 1986 onwards (Gausen and Moen 1991). Introgression during this early time period is, however, likely underestimated, because we found a weaker genetic contrast between historical wild salmon and farmed salmon samples from the 1982–1988 than in the farmed salmon samples from 1998–2009 used as farmed references. Our statistical method has been tested against simulated data sets and has been shown to give precise estimates of introgression at the population level (Karlsson *et al.*, 2014). Precaution is therefore warranted in our evaluation of the status of populations with no or only weak levels of farmed introgression detected in the present study.

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Introgression varies by farmed intrusion and phylogeographic origin

Geographical variation in levels of introgression may have several explanations, the most immediate being that the proportion of escaped farmed salmon in spawning populations also varies. On both local (river) and regional (county) levels, we found a significant, positive correlation between average annual proportions of escaped farmed salmon 1989–2012 (Diserud *et al.*, 2013) and introgression in recent samples.

The most impacted rivers, with respect to long-term average proportions of escaped farmed salmon, are found in the counties of Hordaland (Opo [048.Z], Kinso [050.1Z], Eio [050.Z] and Frugardselva [044.3Z]) being highest among those studied genetically, all with average proportions 1989–2012 of escaped farmed salmon above 50% according to Diserud *et al.*, 2013) and Troms (River Salangselva [191.Z] with 65%, Diserud *et al.*, 2013).

Our samples of wild Atlantic salmon are represented by two phylogenetic groups, the Atlantic group and the Barents-White Sea group (Bourret *et al.*, 2013). Even though wild populations from both phylogenetic groups were represented among the source populations (Gjedrem *et al.*, 1991), only the Atlantic group was represented in the third generation of farmed salmon in the breeding programme (cf. GjØen and Bentsen 1997). Interestingly, we found a significant association between proportions of escaped farmed salmon and introgression for the Atlantic group and not for the Barents-White Sea group, and a steeper gradient in the Atlantic group (Figure 4). This might reflect differences in genomic architecture between the two phylogenetic groups and a higher barrier to introgression in the Barents-White Sea group. The barrier is not absolute, as we found significant introgression in several of the Barents-White Sea populations, even in numerically strong populations like River Alta (212.Z) and Vestre Jakobselv (240.Z). The number of samples from the Barents-

White Sea group is however limited, and a conclusion about barriers to introgression in relation to phylogenetic origin must await further study.

Additional explanations of variation in introgression

A large proportion of the variance in the level of introgression could not be explained by proportions of escaped farmed salmon (Figure 4). This is not unexpected, as one of the main conclusions from a review of genetic effects following releases was the wide variety of outcomes, ranging from no detectable effect to complete introgression or displacement of the native population (Hindar *et al.*, 1991). Experimental studies of farmed and wild salmon, however, point to some general findings about causes of variation.

It has been shown experimentally that farmed salmon escaping early from captivity have higher reproductive success in competition with wild salmon than later escaping farmed salmon, i.e. comparing hatchery-released smolts with farmed adults (Fleming *et al.*, 1996; 1997). So far, this has not been accounted for in analyses of how escaped farmed salmon leads to introgression, but will be possible in the future as scale reading advances to include the likely size at which farmed salmon escape.

The density of wild Atlantic salmon on the spawning ground may also be important. The breeding behavior of Atlantic salmon involves female-to-female competition for access to high-quality spawning sites to excavate the nests, and male-to-male competition for access to females (Fleming and Einum, 2011). Lura (1995) suggested that the spawning success of escaped farmed females was density dependent because the contributions of eyed eggs, relative to their proportion among the spawners, were lower in rivers and years with high densities of spawners. Likely explanations may be that farmed females are outcompeted from

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the most favourable nest sites at high densities (Lura, 1995), and there may be a larger proportion of unspawned eggs in farmed than in wild salmon at high densities (Jonsson *et al.*, 1990; Fleming *et al.*, 1996; 2000). For males, Fleming *et al.* (1997) showed density-dependent spawning success in an experimental study of hatchery-reared vs. wild River Imsa males. In contrast, late-escaping farmed males showed poor reproductive success regardless of density in the same spawning arenas (Fleming *et al.*, 1996). We do not yet know whether there are differences in reproductive success among the various selected strains of farmed salmon, but we know that farmed fish vary in their genetic relationships with wild salmon (Karlsson *et al.*, 2011, 2010, 2014).

Lower average introgression is found in National Salmon Rivers and to a lesser extent in rivers within a National Salmon Fjord. This indicates that national salmon fjords and rivers provide increased protection from farmed introgression. One common factor among these populations is that emphasis was put on the numerically strongest populations when rivers were chosen for designation as National Salmon Rivers. Population size in itself may be a protective measure from introgression (Heino *et al.*, 2015), which is also supported by the difference between unweighted and weighted averages found here (Table 2). Another type of protection is the increased distance between aquaculture operations and wild salmon rivers, which makes it less likely for a salmon river in a National Salmon Fjords to receive escaped farmed salmon, than outside of such a fjord, other things being equal (Fiske *et al.*, 2013).

However, we do not see low introgression levels in all salmon populations with this protective regime. For example, River Daleelva (061.Z), River Vosso (062.Z), River Vikja (070.Z), River Årøyelva (077.Z), River Jølstra (084.Z), River Olden (088.1Z), River Røssåga (155.Z) and River Beiarelva (161.Z) are National Salmon Rivers or are situated in a National Salmon Fjord and have > 10% farmed genetic introgression. One explanation for the variation

in the protecting effect of National salmon rivers and fjords might be the size of the protected region, exemplified by the large Trondheimsfjord. The entire Trondheimsfjord is a National Salmon Fjord in a highly intensive farming region, where a high level of introgression was found in a coastal population (River Teksdalselva [134.Z]), but consistently lower levels were found in rivers inside the major Trondheimsfjord. In the Hardangerfjord system, on the other hand, only a small part (less than 5 % of the fjord area) is designated as a National Salmon Fjord, Etnefjorden. Most rivers in the Hardangerfjord show high levels of introgression, as does River Etne (041.Z). Another explanation for high levels of introgression in salmon populations within National Salmon Fjords is that some of these rivers have occasionally had low levels of wild spawners, because of the parasite *Gyrodactylus salaris* (Vikja, Røssåga, Beiarelva; Johnsen and Jensen, 1991), or of other anthropogenic factors. A likely mechanism is easier access to spawning opportunities when wild population size is low (Sægrov *et al.*, 1997).

An explanation for the variable effect of National Salmon Rivers and Fjords not yet highlighted is the possibility that an introgressed population may impact neighbouring populations through straying of wild offspring of cultured fish (Felsenstein, 1997). An important question in this regard is a potentially weaker homing of offspring from escaped farmed salmon compared to the locally adapted wild salmon, because of different genetic (Jonsson *et al.* 2003) or epigenetic origins (Christie *et al.*, 2016). In experiments with wild and farmed Atlantic salmon, hatchery-produced smolts of farmed origin showed a higher straying rate than hatchery-produced Imsa salmon released into the Imsa (Jonsson *et al.*, 2003).

Differential survival of introgressed individuals

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Levels of introgression were similar between juvenile and adult salmon samples in our study. Experimental studies generally show a lower lifetime survival of farmed offspring than wild offspring, with hybrid groups being intermediate (McGinnity *et al.*, 1997, 2003; Fleming *et al.*, 2000; Skaala *et al.*, 2012). It is therefore expected that within the same cohort, a general reduction in mean $P(Wild)$ should be observed across life stages from alevin, to parr, to smolt, to returning adults. In our material, we could not make a formal cohort analysis, but some populations could be compared between juvenile and adult samples that likely showed some year-class overlap. The average reduction was estimated at 2.5 percentage points, with a large variation between populations, including some where the level of introgression was higher among adults than among juveniles. Observational studies that control for year class (cohort) are needed before the effect of viability selection on introgression can be quantified more precisely.

What do the levels of introgression found in this study mean?

This question may be discussed at several different levels: genetics, fitness and viability, ecology and life-history, management, and conservation. With respect to genetics, three concerns are important: loss of genetic variation within populations, loss of genetic variation between populations and loss of fitness (Waples *et al.*, 2012). Farmed Atlantic salmon have in general lower genetic variation than wild Atlantic salmon (Mjølnerød *et al.*, 1997; Skaala *et al.*, 2004; Skaala *et al.*, 2005; Karlsson *et al.*, 2010), and the long-term prediction from escapes is that lower genetic diversity will eventually lead to a drop in diversity in recipient wild populations (Tufto and Hindar, 2003), even though in the short-term, genetic variation may increase from interbreeding with farmed salmon. Loss of genetic variation between populations as a result of introgression from farmed Atlantic salmon has been demonstrated

both theoretically (Mork, 1991) and empirically (Skaala *et al.*, 2006; Glover *et al.*, 2012; 2013). Loss of fitness has been demonstrated in controlled rivers in Ireland (McGinnity *et al.*, 1997; 2003) and Norway (Fleming *et al.*, 2000; Skaala *et al.*, 2012) and in large-scale experiments in Canada (Fraser *et al.*, 2010). The loss of viability is also indicated by these same studies, as well as in meta-analysis of the population dynamics of salmon populations near or far from aquaculture operations (Ford and Myers, 2008; Vøllestad *et al.*, 2009). For the latter studies, however, several mechanisms in addition to introgression may be at work, such as increased mortality caused by parasites associated with fish farming activities (e.g. Krkosek *et al.*, 2012).

Ecological change in introgressed individuals was evident from experiments in controlled, natural rivers showing changes in growth rate, condition factor (length-weight relationship) and age at smoltification and maturation (Fleming *et al.*, 2000; McGinnity *et al.*, 2003). This was also true in a large-scale observational study that tested whether *P(Wild)* had an impact on ecological key traits (Geir Bolstad, NINA, *et al.* in prep.). This change in ecological traits also likely has a negative effect on fitness (Tufto, 2001; Huisman and Tufto, 2012; Baskett *et al.*, 2013).

Implications for management and conservation

In a management and conservation context, a pertinent question is how much introgression can be allowed (Ryman *et al.*, 1995). While there is no simple answer to this question, it is clear that near-zero limits need to be set in order not to compromise the genetic integrity of wild populations. Ryman *et al.* (1995) suggested that a defensible strategy, based on population genetic considerations, could be to allow gene flow at a rate that matched equilibrium levels of gene flow between semi-isolated populations, as quantified by Wright's

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513 fixation index, F_{ST} . This would allow only a small number of reproductively capable escaped
514 farmed salmon spawning in wild populations every generation.

515 In considerations of the Endangered Species Act listing of populations of westslope
516 cutthroat trout (*Oncorhynchus clarki lewisi*) in danger of hybridization with rainbow trout (*O.*
517 *mykiss*) and Yellowstone cutthroat trout (*O. c. bouvieri*), Allendorf *et al.* (2004) suggested
518 that listing only nonhybridized populations was the only alternative that could be defended
519 from the perspective of possessing local adaptations important for long-term persistence of
520 this sub-species. An alternative criterion, allowing 10% introgression from the other taxa, was
521 discarded because it could lead to hybridized populations acting as a source for further
522 introgression. These considerations deal with sub-species and species differences, and may be
523 too conservative for our Atlantic salmon study that deals with farmed and wild population
524 differentiation and introgression. The developmental and evolutionary forces acting on
525 farmed Atlantic salmon are so unlike those in the wild that two distinct biologies are being
526 created within the Atlantic salmon species (Gross, 1998; see also Roberge *et al.*, 2006 and
527 Christie *et al.*, 2016). Gross (1998) even suggested that farmed and wild Atlantic Salmon be
528 recognized as different “species”, and that farmed salmon be treated as “exotic” when they
529 escaped to the wild, as a measure to prevent further impact from aquaculture. The calculation
530 of $P(Wild)$ at the level of individuals has an immediate use in practical management and
531 conservation. In many rivers, hydropower companies have to compensate for the reduction in
532 natural productivity of a river by releasing hatchery-produced fish. In other rivers, releases of
533 offspring from local brood stock is practiced on a voluntary basis. Regardless of purpose, a
534 genetic test compulsory for all brood stock being used was introduced in 2014 by the
535 Norwegian Environment Agency to limit the likelihood of spreading farmed salmon
536 genotypes through stock enhancement. In autumn 2014, the calculation of individual $P(Wild)$
537 led to 14% of potential broodstock in Norway being discarded for genetic reasons, and in

2015, 18% of potential brood stock was discarded (Karlsson *et al.*, 2015, 2016). In the highly impacted Hardangerfjord rivers (average introgression 13.2%), calculations of $P(Wild)$ during autumn 2015 showed that only 83 of 141 fish (escaped farmed salmon excluded) qualified as wild-origin brood stock to create a live gene bank for the most impacted populations.

The probability distribution of $P(Wild)$ may help characterize the stage reached in an accumulation of farmed introgression. In some populations, the probability distribution for being wild shows distinct modes with fish at several stages of introgression, including “pure wild”, “hybrid” and “farmed” (Tufto, 2000). At later stages of introgression, with a wide range of admixed groups in the population, we expect a smoother distribution of individual $P(Wild)$ values without distinct modes. At this time, the proportion of individuals with pure wild origin is low, and management has to be cautious to preserve all ecotypes (e.g. late-spawning fish; upper river spawners) in the remaining historically wild populations (Hansen *et al.*, 2006).

To protect the genetic integrity of wild Atlantic salmon populations, only low levels of introgression from escaped farmed salmon can be allowed into wild populations. We found significant introgression in half of the populations studied, and levels of introgression above 10% in nearly one quarter of the populations. The rivers we studied represent three quarters of the entire Norwegian wild salmon spawning population. Further introgression is likely, unless substantial reduction of escaped farmed salmon in the wild, or sterilization of farmed salmon, can be achieved.

Acknowledgements

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For Review Only

Table 1. Farmed genetic introgression for Norwegian Atlantic salmon rivers with and without the protection status of being National Salmon Rivers, and for salmon rivers in and not in fjords with the a protection status of being National Salmon Fjords. Farm introgression values are given as averages and medians, both unweighted and weighted with estimated population size

Group	N Ind	N pop	Farm introgression - unweighted average / median	Farm introgression – weighted average / m edian
National Rivers	4347	47	0.045 / 0.016	0.016 / 0.000
Not National Rivers	4741	62	0.078 / 0.028	0.048 / 0.014
National Fjords	5337	59	0.064 / 0.018	0.018 / 0.000
Not National Fjords	3751	50	0.064 / 0.026	0.035 / 0.025

Table 2. Farmed genetic introgression in Norwegian geographical regions. Regions are set as counties (Figure 3), except for region Southeast which includes the south and the southeastern counties (Østfold, Akershus, Buskerud, Vestfold, Telemark, Aust-Agder and Vest-Agder).. Regional farm introgression values are given as averages and medians, both unweighted and weighted with estimated population size.

Region	N Ind	N pop	Farm introgression, u nweighted	Farm introgression, weighted
Southeast	899	11	0.038 / 0.000	0.015 / 0.000
Rogaland	1070	9	0.018 / 0.008	0.007 / 0.000
Hordaland	922	10	0.139 / 0.108	0.114 / 0.108
Sogn og Fjordane	1992	21	0.068 / 0.042	0.064 / 0.000
Møre og Romsdal	1946	16	0.062 / 0.044	0.039 / 0.014
Sør-Trøndelag	365	6	0.047 / 0.020	0.013 / 0.012
Nord-Trøndelag	162	4	0.000 / 0.000	0.000 / 0.000
Nordland	556	12	0.079 / 0.028	0.077 / 0.078
Troms	324	7	0.145 / 0.083	0.067 / 0.083
Finnmark	852	13	0.026 / 0.021	0.010 / 0.000
National	9088	109	0.064 / 0.023	0.021 / 0.000

Figure captions

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Figure 1. PCoA plot of pairwise F_{ST} estimates between historical samples from 39 Atlantic salmon populations and 13 farmed strain populations (diamonds), clustered into one farmed group (Farmed), one wild Atlantic salmon group from Finnmark (Barents-White Sea), and one wild Atlantic salmon group from South of Finnmark (Atlantic). Grey diamonds are populations (River Skibotnelva, River Målselva, and River Skipsfjordelva) outside the clusters and genetic introgression is analysed by using the local historical samples. Open circles are in silico generated populations from a pool of the historical samples within each cluster.

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Figure 2. Distribution of logit-transformed probabilities of being of wild origin $P(Wild)$, for farmed reference (red line; mean value indicated by the red diamond), wild references for the whole Atlantic phylogenetic group (blue line and diamond), wild reference for River Eira (black line and diamond) and contemporary sample for River Eira (dashed black line and open black diamond; $n = 786$ for years 2012, 2013, 2014, 2015 pooled).

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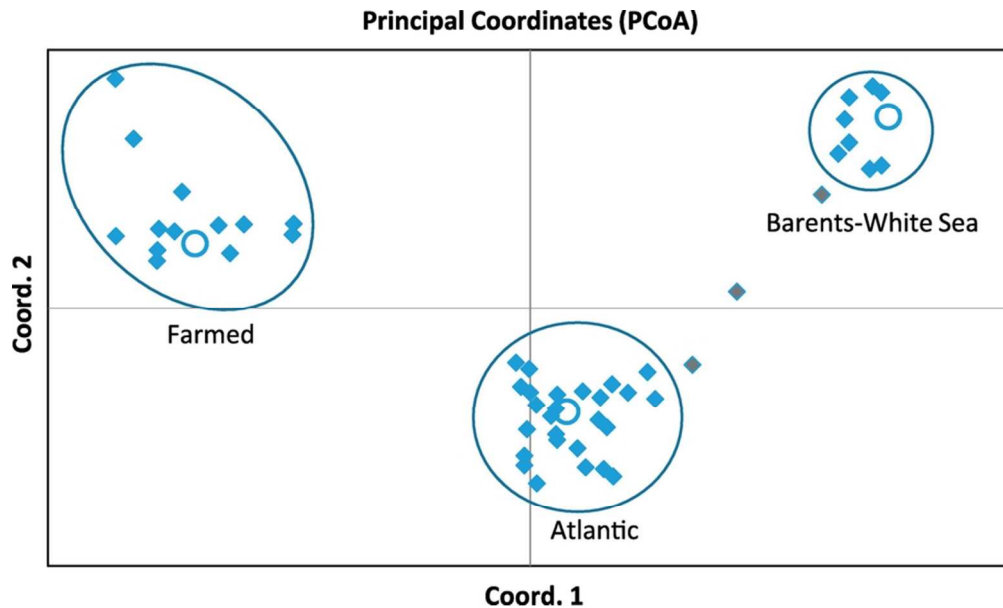
Figure 3. Map of Norway showing estimated farmed genetic introgression in 109 Norwegian salmon rivers from contemporary adult samples. Codes used for counties: FI = Finnmark, TR = Troms, NO = Nordland, NT = Nord-Trøndelag, ST = Sør-Trøndelag, MR = Møre og Romsdal, SF = Sogn og Fjordane, HO = Hordaland, RO = Rogaland, and SOUTHEAST is the southeasternmost counties pooled into one region.

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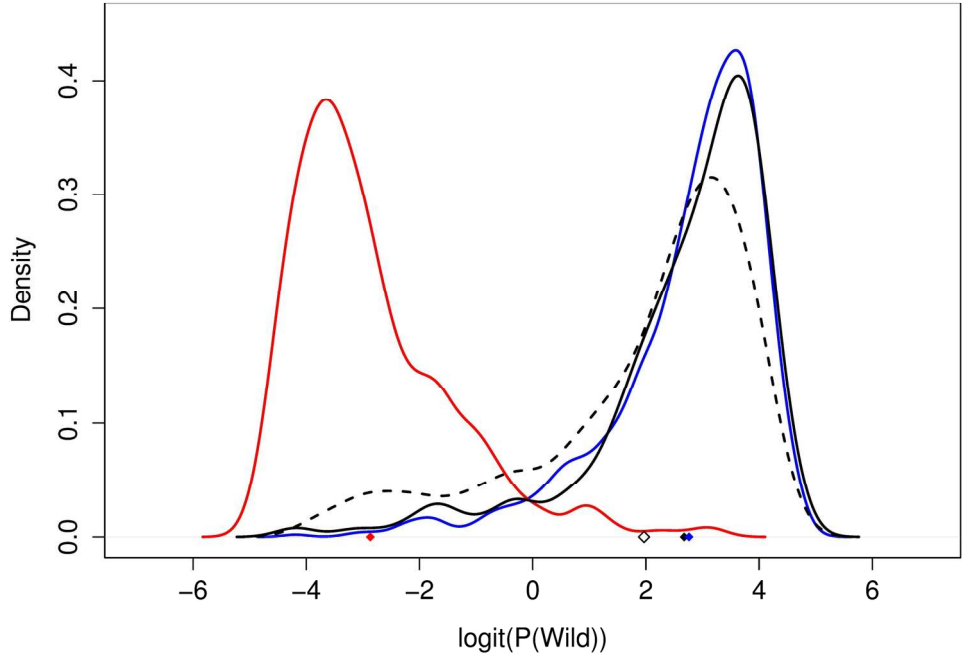
Figure 4. Relationship between mean annual proportions of escaped farmed salmon between 1989 and 2012 and estimated proportion of farmed genetic introgression from molecular genetic markers for 77 salmon populations (solid diamonds and dashed lines), and averaged for populations within geographical regions (open diamonds). Observations from the Atlantic Sea phylogenetic group are shown in red, from the Barents-White Sea phylogenetic group shown in blue, and two populations from the transition area are shown in grey.

Figure 5. Distribution of STRUCTURE-generated probabilities of being of wild origin $P(wild)$ for modern (red line) and historical (orange line) samples from the AquaGen farmed strains.

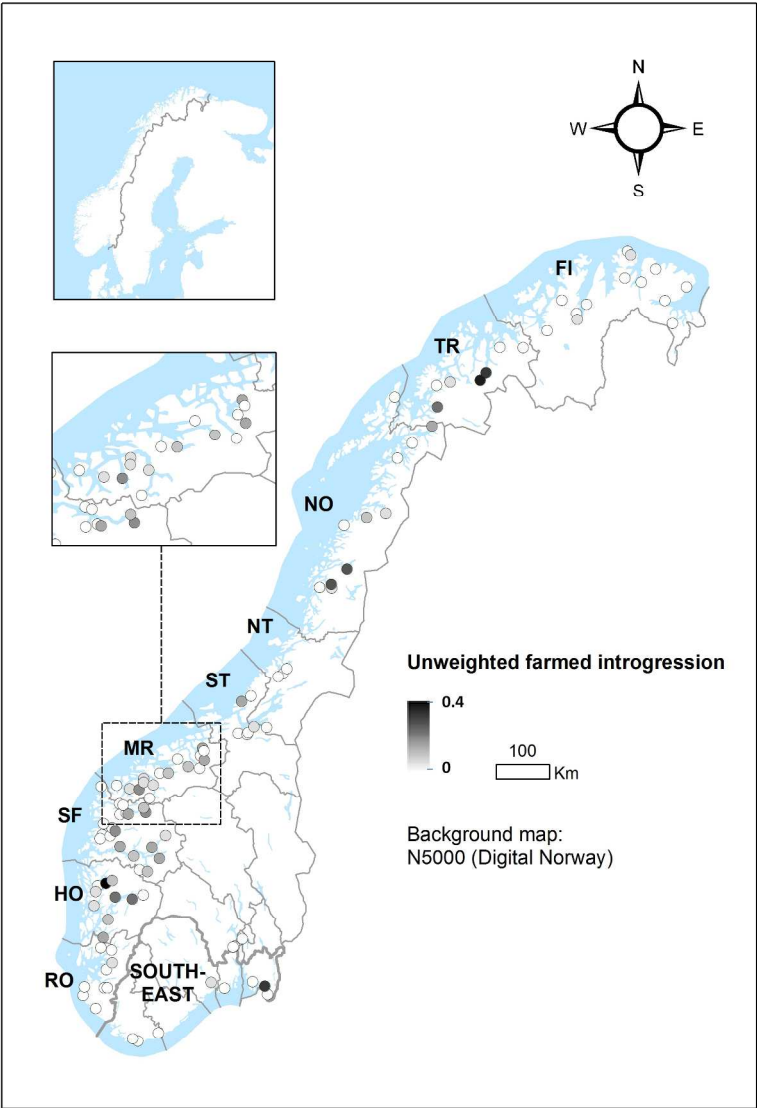


PCoA plot of pairwise F_{ST} estimates between historical samples from 39 Atlantic salmon populations and 13 farmed strains populations (diamonds), clustered into one farmed group (Farmed), one wild Atlantic salmon group from Finnmark (Barents-White Sea), and one wild Atlantic salmon group from South of Finnmark (Atlantic). Grey diamonds are populations (River Skibotnelva, River Målselva, and River Skipsfjordelva) outside the clusters and genetic introgression is analysed by using the local historical samples. Open circles are in silico generated populations from a pool of the historical samples within each cluster.

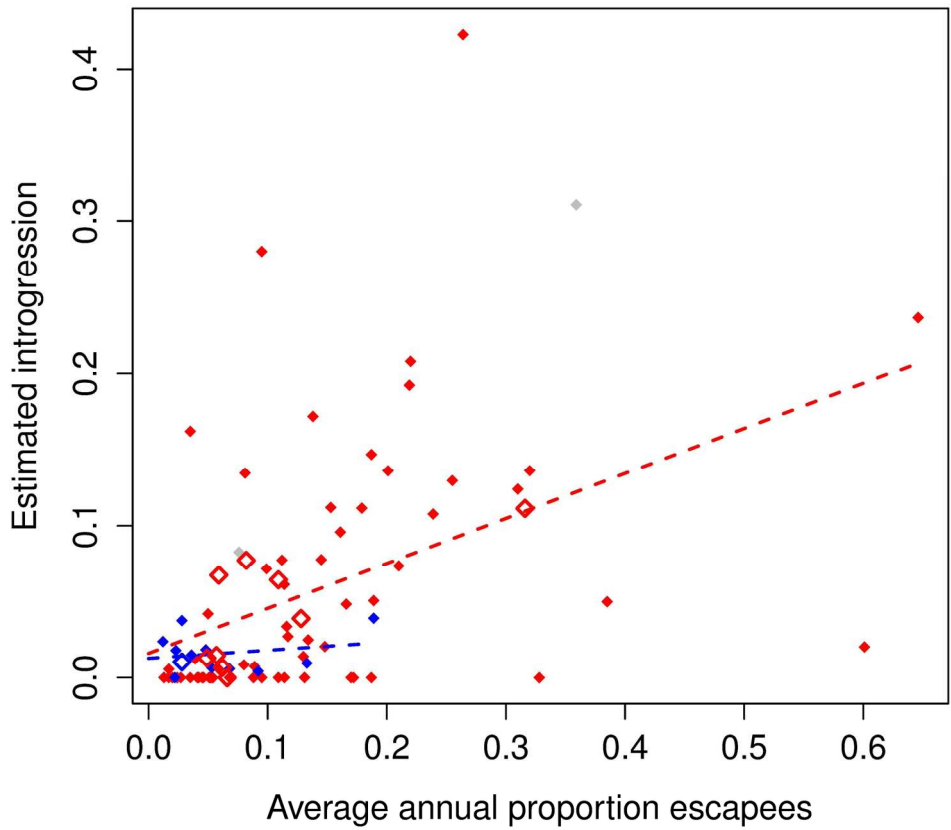
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Distribution of probabilities of being of wild origin ($P(\text{Wild})$), logit-transformed for Farm reference (red line; mean value indicated by the red diamond), wild references for the whole Atlantic phylogenetic group (blue line and diamond), wild reference for River Eira (black line and diamond) and contemporary sample for River Eira (dashed black line and open black diamond; $n=786$ for years 2012, 2013, 2014, 2015 pooled).
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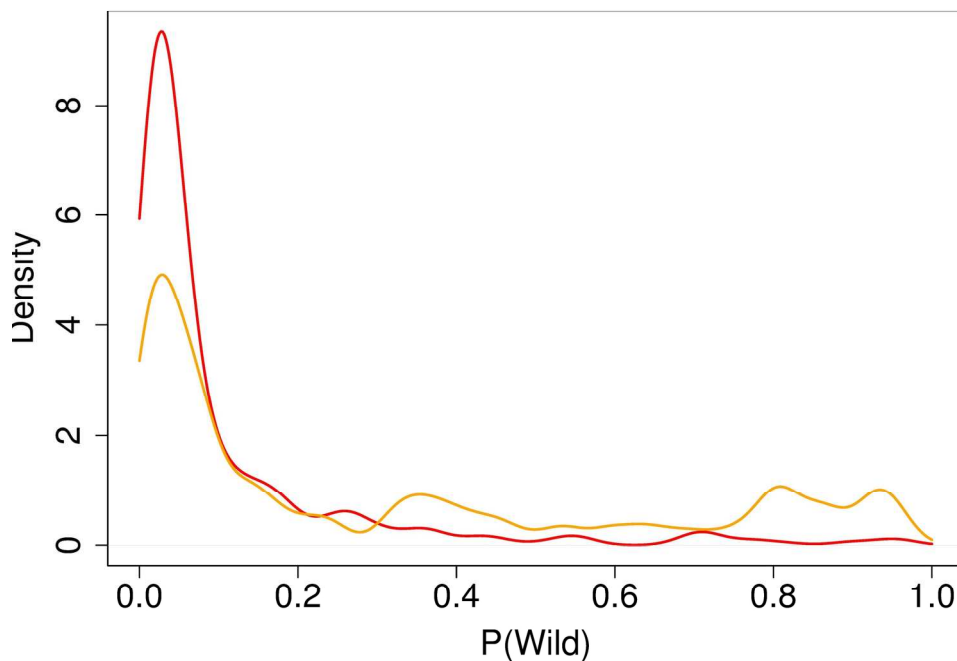


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Distribution of STRUCTURE-generated probabilities of being of wild origin ($P(\text{wild})$) for modern (red line) and historical (orange line) samples from the AquaGen farmed strains.
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