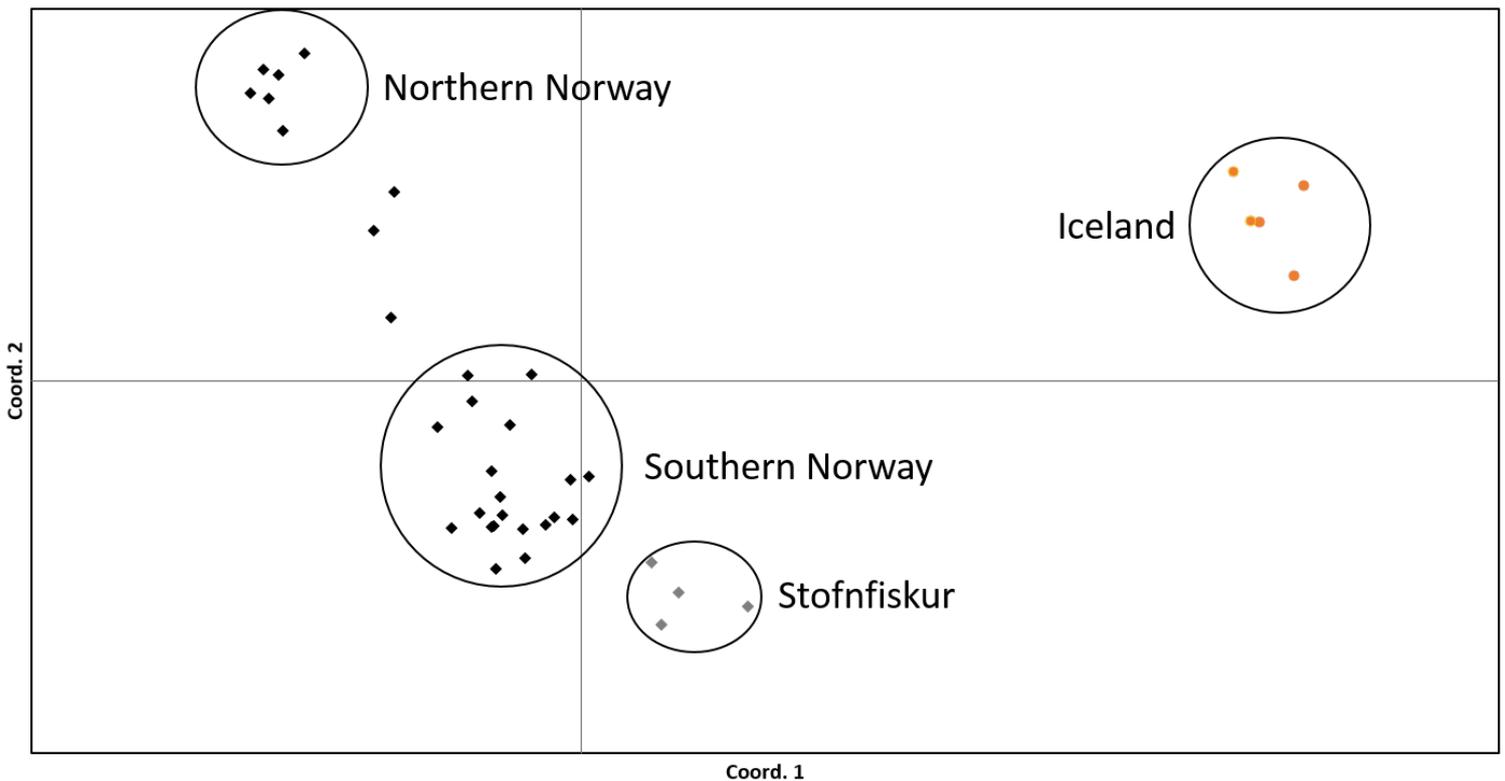


The genetic background of Stofnfiskur breeding lines of farmed Atlantic salmon

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Genetic clustering of Atlantic salmon populations from Norway, Iceland, and Stofnfiskur by Sten Karlsson

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Abstract

The genetic background of Stofnfiskur breeding lines of farmed Atlantic salmon - NINA Report 1401. 17 pp.

Stofnfiskur is a breeding company of Atlantic salmon located in Iceland, and delivers salmon eggs to the fish farming industry, including the Norwegian aquaculture industry. Escaped farmed salmon is considered one of the most important threats to wild salmon populations. Foreign genetic origin of the farmed salmon may possess an extra negative effect on wild salmon populations and is therefore discouraged in Norwegian legislation, and in NASCO guidelines. Documentation of the genetic origin is mandatory before approved to be used in Norwegian aquaculture. According to the information from Stofnfiskur, the Stofnfiskur breeding lines are of Norwegian origin and is a mixture of farmed salmon from the three major breeding companies in Norway: at that time Bolaks, Mowi and the National selective breeding program. The Norwegian Environment Agency has ordered a molecular genetic analysis to confirm the Norwegian origin. DNA extraction and genotyping at 96 Single Nucleotide Polymorphisms (SNPs) of fish from the Stofnfiskur breeding lines and of wild salmon from five Icelandic rivers was conducted at Matís, Icelandic Food and Biotech R&D, Iceland. We compared the genetic profiles of salmon from Stofnfiskur and the wild Icelandic salmon with the large database of Norwegian wild salmon at NINA. As a group, the Stofnfiskur salmon was genetically similar to the southern phylogenetic group in Norway and distinctively different from the Icelandic wild populations. At the individual level, 186 of 187 fish (99.5%) from Stofnfiskur assigned genetically to Norwegian rather than Icelandic wild populations, and one fish assigned with a high relative probability (98.4%) to Iceland populations. One out of 362 Icelandic wild salmon assigned to Norway rather than to Iceland, and two out of 2087 Norwegian salmon assigned to Iceland. Our analyses confirm that the salmon at Stofnfiskur is of Norwegian origin. Because Stofnfiskur is now in use in fish farms in Norway, we were also interested in to what extent we could trace this fish in events of escapes and interbreeding with wild Norwegian salmon. We used the same genetic markers and statistical analyses that are currently in use in Norway. The salmon from Stofnfiskur were not as genetically different from the Norwegian wild salmon as the current Norwegian breeding lines at the genetic markers developed to differentiate farmed from wild salmon. Unless a new set of markers are being developed including all farmed strains in Norway, genetic introgression of escaped salmon from the Stofnfiskur breeding line will lead to an underestimate of farmed genetic introgression, in events of escapes.

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Sammendrag

The genetic background of Stofnfiskur breeding lines of farmed Atlantic salmon - NINA Report 1401. 17 pp.

Stofnfiskur er et islandsk avlsselskap for oppdrettslaks og leverer rogn til akvakulturindustrien. Stofnfiskur leverer også rogn til Norge. Rømt oppdrettslaks vurderes som en av de alvorligste truslene mot norsk villaks. Fremmed fylogenetisk opphav kan påføre en ekstra negativ effekt på villaksbestander og er derfor uønsket. Før godkjent i bruk i Norge må derfor opphavet kunne dokumenteres. I henhold til informasjon fra Stofnfiskur er deres laks av kun norsk opphav og består av en blanding av oppdrettslaks fra Bolaks, Mowi og det nasjonale avlsprogrammet (senere Salmobreed, Mowi og AquaGen). Miljødirektoratet har pålagt selskapet om å bekrefte dette ved molekylærgenetiske analyser. DNA-ekstraksjon og genotyping av 96 enkelt nukleotid polymorfismer (SNPer) for laks fra Stofnfiskur og for laks fra fem forskjellige islandske ville populasjoner ble gjort ved Matís, Icelandic Food and Biotech R&D, på Island. Vi sammenliknet den genetiske profilen til laksen fra Stofnfiskur og laksen fra de islandske populasjonene med en stor genetisk database over norsk villaks ved NINA. Som gruppe var laksen fra Stofnfiskur genetisk lik den sør-norske fylogenetiske gruppen og genetisk distinkt forskjellig fra de islandske ville populasjonene. På individnivå ble 186 av 187 fisk (99,5 %) fra Stofnfiskur tilordnet norske populasjoner istedenfor de islandske populasjonene og én ble tilordnet de islandske populasjonene med en høy relativ sannsynlighet (98,4 %). Blant 362 villaks fra Island ble én tilordnet norske populasjoner og to individer fra 2087 laks fra Norge ble tilordnet de islandske populasjonene. Våre analyser bekrefter at det genetiske opphavet til Stofnfiskur er norsk. Siden laksen fra Stofnfiskur allerede er i bruk i Norge, var vi interessert i å undersøke hvorvidt denne laksen kunne spores i tilfelle rømming og innkrysning med villaks. Vi brukte de genetiske markørene og statistiske analysene som brukes i dag for å skille mellom villaks og oppdrettslaks i Norge. Laksen fra Stofnfiskur viste ikke like store genetiske forskjeller til norsk villaks som oppdrettslaksen fra Salmobreed, Mowi og AquaGen. Ved å bruke de samme markørene som i dag vil derfor i tilfelle rømming, innkrysning med villaks fra nye avlslinjer som den fra Stofnfiskur, bli underestimert. Det vil derfor bli et behov for å utvikle nye genetiske markører som inkluderer alle nye avlslinjer.

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Foreword

According to the company, the Stofnfiskur Atlantic salmon breeding line is of Norwegian origin and was formed from a mixture of farmed salmon from Bolaks, Mowi, and the National breeding program; and the Norwegian breeding lines were all initiated from Norwegian wild salmon. Escaped farmed salmon is regarded as one of the most important threats to wild salmon populations in Norway because of interbreeding with wild salmon. Foreign wild origin of the farmed salmon may add an extra negative effect in interbreeding with wild salmon and is therefore unwanted. The Stofnfiskur company has informed Norwegian authorities that the farmed Atlantic salmon they produce are based on Norwegian farmed salmon strains. The Norwegian Environment Agency has ordered the company to conduct molecular genetic analyses to demonstrate that the Stofnfiskur strain has Norwegian origin. As farmed strains can deviate from their wild salmon origin for various reasons (selection, genetic drift, etc.) the critical test is whether the Stofnfiskur strain has descended from a Norwegian wild salmon origin, as opposed to an Icelandic wild salmon origin or a mix of Norwegian-Icelandic wild origin. NINA got the assignment to undertake these analyses. NINA has a large genetic database of Norwegian wild salmon populations, and in collaboration with Matís, samples from Stofnfiskur and samples from Icelandic wild salmon populations provided by The Marine and Freshwater Research in Iceland were genotyped and compared with Norwegian wild salmon.

We would like to thank Stofnfiskur for the assignment and for providing tissue samples from their breeding lines, and Salmobreed for coordinating the work between NINA, Matís, Marine and Freshwater Research in Iceland, Stofnfiskur, and the Norwegian Environment Agency.

20.06.2017 Sten Karlsson

1 Introduction

Escaped farmed salmon has been identified as the largest threat to wild salmon populations in Norway (Forseth et al. 2017). Official annual reported numbers of escaped farmed salmon has ranged from 39 000 to 921 000 in the period 2001 to 2016 (<http://www.fiskeridir.no/English>). The true numbers of escapes are likely 2-4 times higher (Skilbrei et al. 2015). Inventories since 1989 have revealed large proportions of escaped farmed salmon in many Norwegian salmon rivers (Fiske et al. 2006, Anon 2017). The escaped farmed salmon interbreed with wild salmon (Fleming et al. 2000, Glover et al. 2013) and significant genetic introgression of farmed to wild salmon has been documented in almost half of 109 wild salmon populations investigated (Karlsson et al. 2016). Furthermore, in a recent study by Bolstad et al. (2017) it was shown that introgression of farmed salmon is leading to changes in important life history characters on a large geographical scale, and that the effect on the wild populations is dependent on the phylogenetic origin of the farmed salmon. Consequently, the negative genetic effect from introgression of farmed salmon on wild salmon populations cannot only be ascribed to the large genetic change from its wild origin from selective breeding but also to phylogenetic origin. Farmed salmon in regions with a phylogenetic origin different from the wild populations should therefore be avoided. This is also reflected in NASCO guidelines on responsible aquaculture.

Atlantic salmon eggs and fingerlings from Stofnfiskur are imported to Norway from Iceland by Salmobreed AS, and according to information from Stofnfiskur this fish is of Norwegian origin and was originally imported from the Norwegian farmed salmon lines from Bolaks, Mowi, and the National breeding program in the early nineteen eighties. In order to confirm the Norwegian origin, the Norwegian Environment Agency ordered the company to conduct molecular genetic analyses of the fish. The main question was whether the fish have pure Norwegian origin or if they have been mixed with salmon of Icelandic origin. Previous genetic analyses of wild Atlantic salmon throughout its distribution range show large genetic differences between salmon populations from Iceland and Norway (Verspoor et al. 2005, Bourret et al. 2013), and that they represent different phylogenetic groups. Determining the origin of the Stofnfiskur salmon should therefore be possible with high confidence.

The genetic analyses were conducted as a collaboration between Matís in Iceland and NINA in Norway. Matís received tissue samples from the Stofnfiskur breeding lines and from wild salmon in five rivers in Iceland provided by The Marine and Freshwater Research in Iceland, and conducted the DNA extraction and genotyping at 96 Single Nucleotide Polymorphisms. Together with the genotypes from Matís and a large genetic database of Norwegian wild salmon populations, NINA conducted the statistical analyses.

2 Material and Methods

The large database consisting of genotypic data at 96 SNP-loci for a large number of individuals from a large number of Norwegian salmon rivers was utilised for the analyses of the phylogenetic origin of the Stofnfiskur salmon. As a Norwegian reference, we utilised genotypic data from 29 rivers along the Norwegian coast (Figure 1). As an Icelandic reference, wild salmon from five Icelandic rivers (Figure 1) were genotyped for the same SNP-loci as included in the database at NINA. Finally, salmon from four year-classes from Stofnfiskur were analysed at the same SNP-loci. After quality control and excluding individuals with a genotyping success less than 80% of the loci, 2 636 individuals were included in the analyses; 2 087 from Norway, 412 from Iceland, and 187 from Stofnfiskur (Table 1).

Table 1. Samples of Atlantic salmon genotyped at 96 SNP loci included for the analyses of phylogenetic origin of the Stofnfiskur breeding lines. The Norwegian samples are ordered from south to north.

Rivers name	Sampling year	N
Norway		
Skienselva	1989	60
Håelva	1989-90	59
Suldal	1979-80	59
Etne	1967	40
Eidfj. Vassdraget	1988-89	54
Vosso	1990,-92,-95	50
Vikja	1985-87	122
Årøyelva	1981-82	78
Rauma	1986-94	75
Eira	1987-90	283
Driva	1977	35
Bævra	1986	63
Surna	1977-78	50
Børselva	1978	33
Salvassdraget	1989-90	59
Vefsna	1972,-79	94
Rana	1975-76	37
Alsvåg	1990-91	59
Åneselva	1990	60
Laukhelle	1989-90	185
Måselva	1978	71
Skipsfj.elva	1989-91	60
Skibotnelva	1976-80	47
Reisa	1989-90	59
Kvænangen	1991-92	58
Repparfj.elva	1989-90	60
Kongsfj.elva	1990-91	61
Vestre Jakobs elv	1989-91	56
Neiden	1989-90	60
Stofnfiskur		
Stofnfiskur 11-2	2014	45
Stofnfiskur 12-2	2015	43
Stofnfiskur 13-2	2015, -16	49
Stofnfiskur 15-2	2017	50
Iceland		
Viðidalsá	2014	82
Hofsá	2004	46
Ölfusá	2004	46
Haukadalsá	2016	94
Víkurá	2016	94
Total		2636

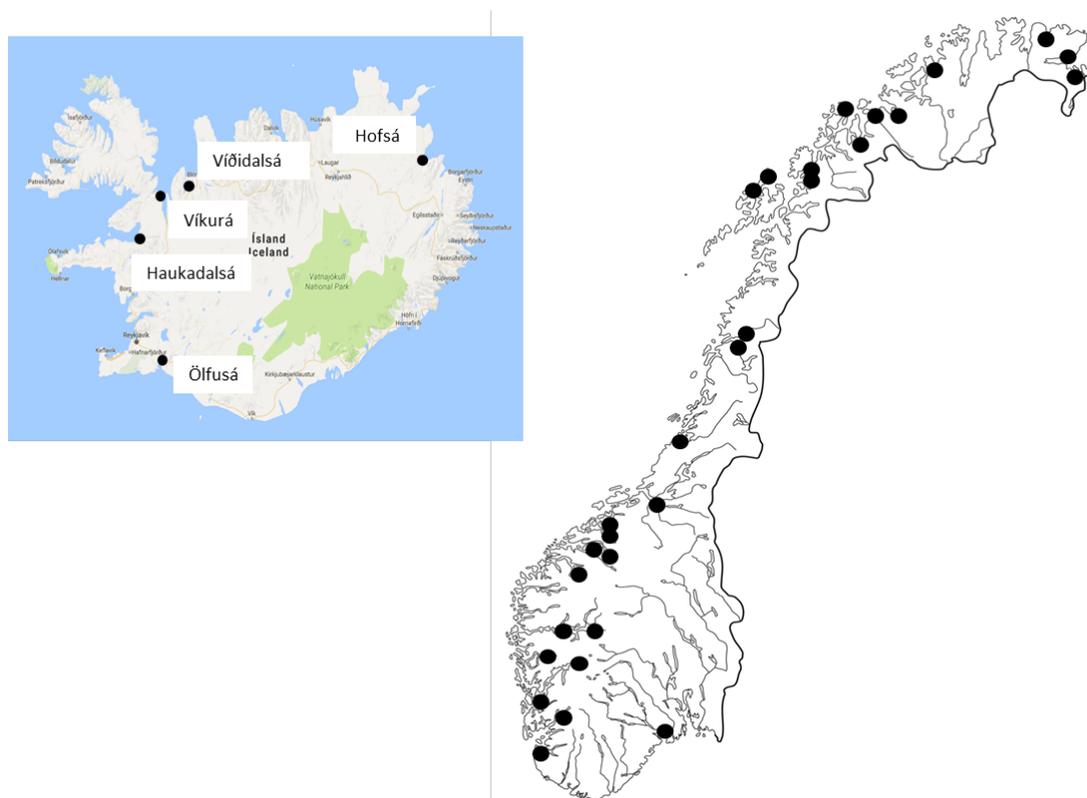


Figure 1. Geographical position of 29 salmon rivers in Norway and five salmon rivers in Iceland included as reference samples for the analyses of phylogenetic origin of the Stofnfiskur breeding lines.

2.1 DNA extraction and SNP genotyping

We extracted DNA with the DNEASY kit from Qiagen and the HotShot method. Ninety-six SNPs were genotyped on an EP1™ 96.96 Dynamic array IFCs (Fluidigm, San Francisco, CA.). The Norwegian samples were analysed at NINA, and the same assay design was applied, on the same type of SNP-genotyping platform at Mátis for the Icelandic and the Stofnfiskur samples. Although NINA and Mátis used the same assays and the same SNP-genotyping platform, NINA conducted the inspection of the genotype clusters for each SNP to prevent bias in the datasets from NINA and Mátis. Of the 96 SNPs, 81 were nuclear SNPs, previously identified as collectively showing generic genetic differences between Norwegian farmed and wild salmon (Karlsson et al. 2011). The remaining SNPs were located in the mitochondrial genome (Karlsson et al. 2010).

2.2 Statistical analyses for tracing the genetic origin of Stofnfiskur

In order to explore the genetic differences between the Icelandic and the Norwegian populations, we performed an Analysis of MOlecular VAriance (AMOVA) as implemented in Arlequin Ver. 3.5 (Excoffier et al. 2010), using the 81 nuclear SNPs. We estimated pairwise genetic distances (F_{ST}) between all Norwegian, Icelandic and Stofnfiskur populations, and visualised the genetic distances in a Principal Coordinate Analysis (PCoA) plot as implemented in Genalex 6.0 (Peakall & Smouse 2006). From the composite 15 mitochondrial SNPs, we identified and compared the different haplotypes and relative frequencies between the Norwegian, Icelandic and Stofnfiskur samples.

To explore the genetic origin of individual fish from Stofnfiskur, each fish was allowed to be genetically assigned to the Norwegian and Icelandic populations, using the direct assignment approach as implemented in GeneClass v. 2.0 (Piry et al. 2004). We also applied a self-assignment approach where all fish from Norway, Iceland, and Stofnfiskur were assigned to all possible populations, including the population from where each fish was sampled. The latter approach identify possible first-generation migrants, or outliers, in the reference samples from Norway and Iceland, in relation to the fish from Stofnfiskur.

2.3 Statistical analyses of Stofnfiskur in relation to Norwegian farmed salmon

Among the 81 nuclear SNPs, 48 have been used in a large-scale monitoring program for tracing farmed genetic introgression in Norwegian wild salmon populations (Karlsson et al. 2016). These SNPs are collectively diagnostic in differentiating between farmed and wild salmon regardless of the farmed strains from AquaGen, Salmobreed, and Mowi, and regardless of Norwegian wild population (Karlsson et al. 2011), but these SNPs have never been tested on other farmed strains. We applied the statistical approach for estimating level of farmed origin in individual fish developed by Karlsson et al. (2014) to test if the farmed salmon from Stofnfiskur also differentiate from wild Norwegian salmon using the 48 diagnostic SNPs. One fish at the time is analysed in STRUCTURE (Pritchard et al. 2000), together with one *in silico* generated wild population and one *in silico* generated farmed population, assuming two populations, applying the admixture model, and using 50 000 repetitions as burn-in and 100 000 repetitions after burn-in. The *in silico* populations were generated from a weighted pool of individual genotypes from historical samples of wild populations and from a pool of individuals from the different farmed strains followed by random mating using HybridLab (Nielsen et al. 2006). From STRUCTURE, we obtained the probability of belonging to the wild versus the farmed populations. By applying the same method for actual reference genotypes of wild and farmed salmon, we generated expected probability distributions for these probabilities to be compared with probability estimates obtained for the fish from Stofnfiskur.

3 Results

3.1 Genetic differences between salmon populations from Norway, Iceland, and Stofnfiskur

Based on estimates of pairwise genetic distances (F_{ST}) from genetic variation at the 81 nuclear SNPs, the Stofnfiskur samples clearly clustered to the southern Norway phylogenetic group and not to the Icelandic populations (Figure 2).

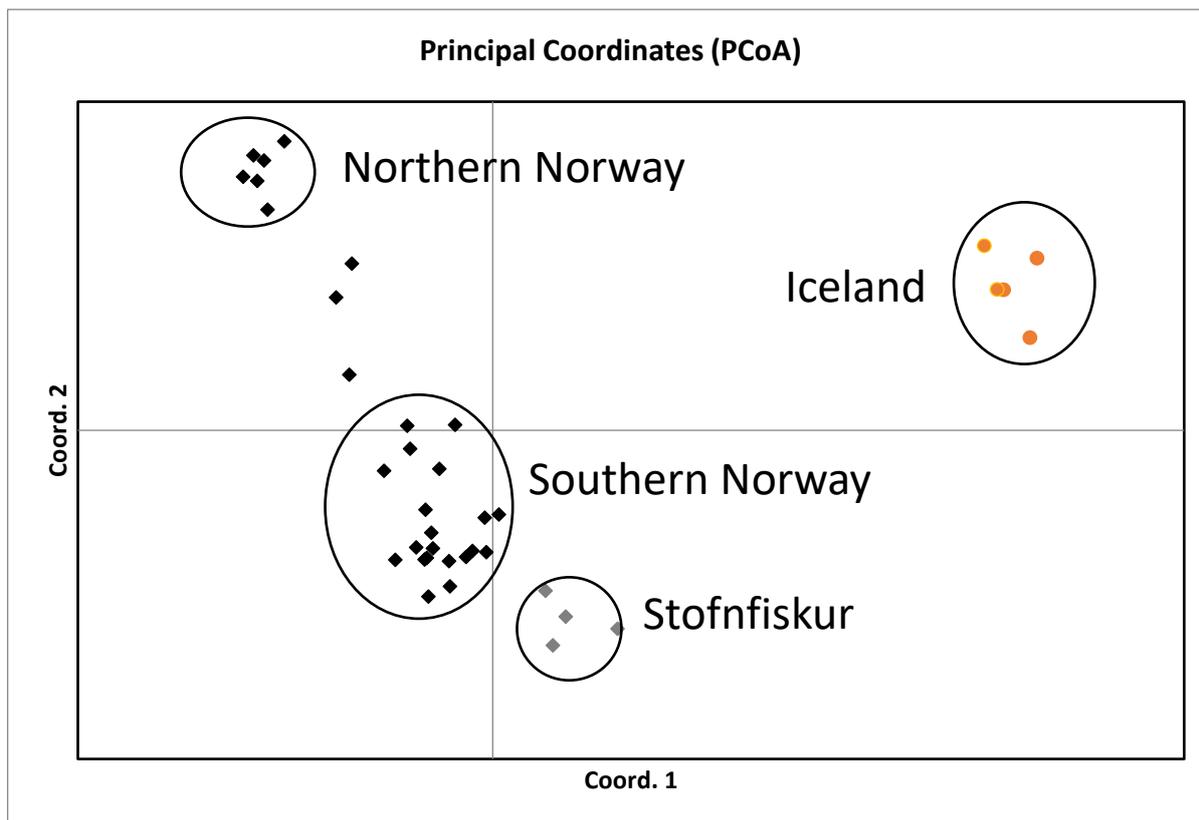


Figure 2. Principal Coordinate Analysis (PCoA) plot based on pairwise estimates of F_{ST} from genetic variation in 81 nuclear SNPs in Norwegian (black diamonds), Icelandic (orange filled circles), and Stofnfiskur (grey diamonds) populations. The first and second axis explained 28.2 and 23.1% of the variation, respectively.

From genetic variation in mitochondrial SNPs, we identified haplotypes. In agreement with the nuclear SNPs, the fish from Stofnfiskur had mitochondrial haplotypes more similar to Norwegian salmon than Icelandic salmon (Figure 3). Although the haplotype frequencies in Stofnfiskur are largely different from the pool of Norwegian populations, many haplotypes in Stofnfiskur were not observed in the samples from Iceland but were rather frequent in Norway. Furthermore, one haplotype (N) was common (8%) in the samples from Iceland and was only found in two of 2 014 individuals (0.1%) in the Norwegian samples, and not at all in the Stofnfiskur samples.

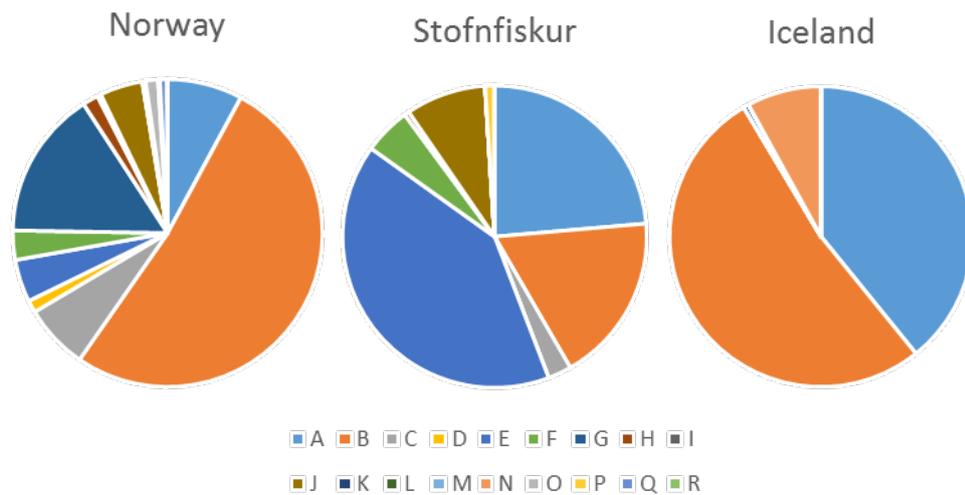


Figure 3. Relative frequencies of haplotypes (A - R) identified in salmon from Norway, Iceland, and Stofnfiskur, from genetic variation in 15 mitochondrial SNPs.

From an AMOVA including Norwegian samples as one group and Icelandic samples in a second group, 10.6% of the total genetic variation was ascribed to genetic differences between these two groups, 3.9% to variation between populations within groups, and 85.5% to variation between individuals within populations. The large genetic difference between Norwegian and Icelandic salmon has been reported in previous studies (Verspoor et al. 2005, Bourret et al. 2013) and enables us to identify genetic origin of individual fish.

3.2 Genetic assignment of individual fish

All but one fish from Stofnfiskur were genetically assigned to Norwegian populations rather than Icelandic populations (Figure 4), using the 81 nuclear SNPs.

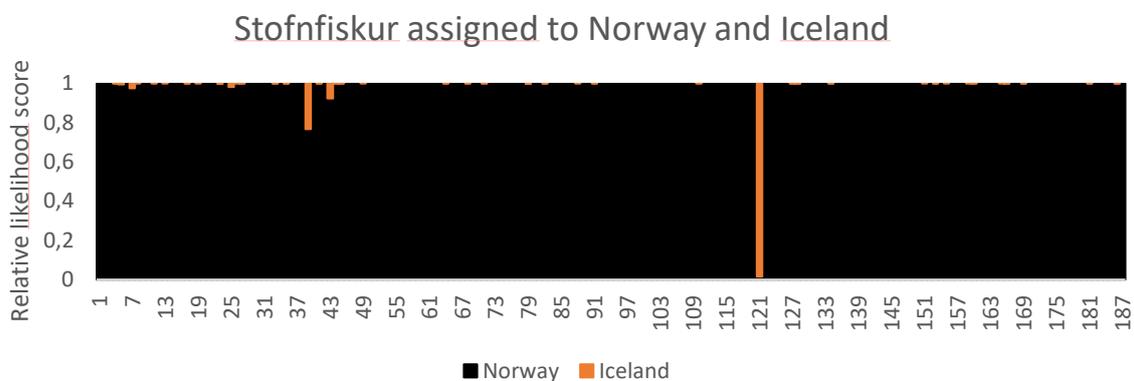


Figure 4. Direct genetic assignment of 187 salmon from Stofnfiskur to reference populations from Norway and Iceland. Relative likelihood scores are summed across all Norwegian (black bars) and all Icelandic (orange bars) populations, respectively.

The Stofnfiskur samples were also assigned to the different Icelandic populations, with the option of estimating the absolute probability of belonging to the five Icelandic wild populations. No fish had higher probability than 0.08 of belonging to any of the five Icelandic reference populations. The individual with a relatively higher probability of belonging to the Icelandic rather than the Norwegian populations (when forced to be assigned to the Norwegian and Icelandic wild salmon populations) had an absolute probability of no higher than 0.03 of belonging to any of the Icelandic populations. Further, we conducted a self-assignment approach whereby all fish from Norway, Iceland, and Stofnfiskur were allowed to assign to all populations, including the ones from where they were sampled (Figur 5).

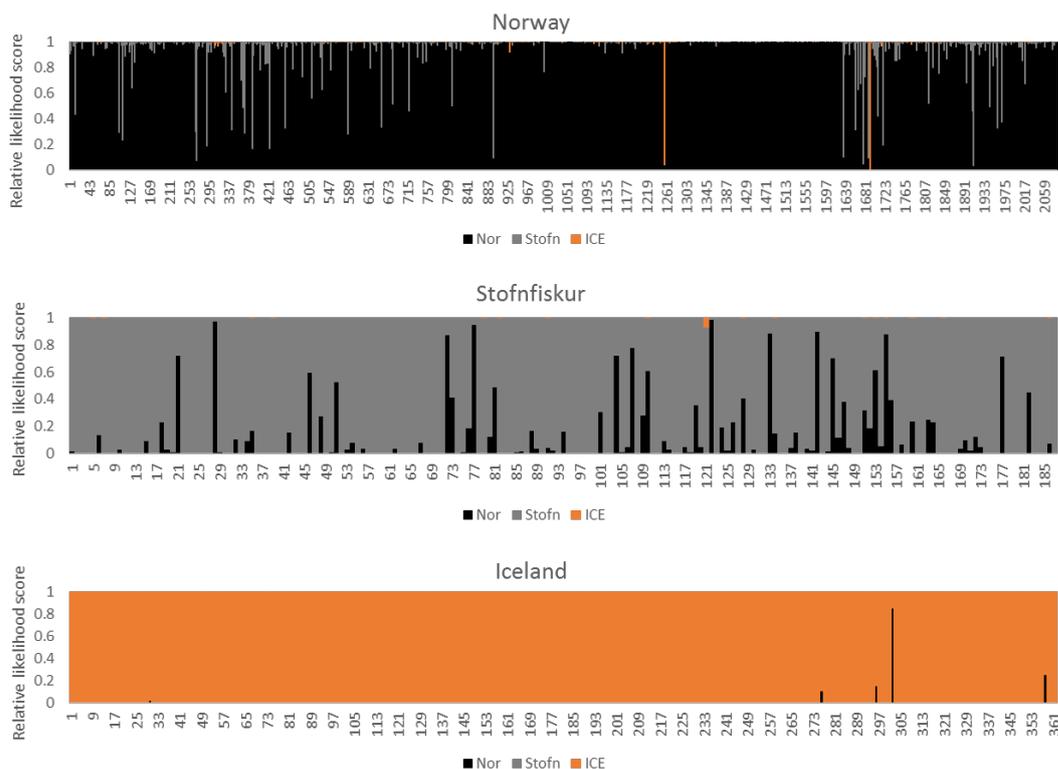


Figure 5. Self-assignment of salmon from Norway, Stofnfiskur, and Iceland, using 81 nuclear SNPs. The fish were assigned to all reference populations, including the populations from where they were sampled. Relative likelihood scores are summed across all Norwegian (black bars), all Icelandic (orange bars), and all Stofnfiskur (grey bars) populations.

From Figure 5, it is apparent that the fish from Stofnfiskur assigned with a higher probability to Norwegian populations, or to the year-classes from Stofnfiskur from where they were sampled, than to Icelandic populations, including the fish with a higher relative probability of belonging to Iceland than Norway. Interestingly, two fish from Norway assigned with a higher probability to Iceland and one fish from Iceland with a higher probability to Norway. These results indicate that these fish might represent first generation migrants, or, possibly, outliers with odd multi-locus genotypes.

3.3 Genetic identification of Stofnfiskur as farmed salmon

From pairwise F_{ST} estimates, the Stofnfiskur year-classes clustered between the Norwegian farmed populations and the southern Norway wild populations (Figur 6), using 48 nuclear SNPs routinely used for quantifying genetic introgression of escaped farmed salmon in Norwegian wild salmon populations (Karlsson et al. 2016).

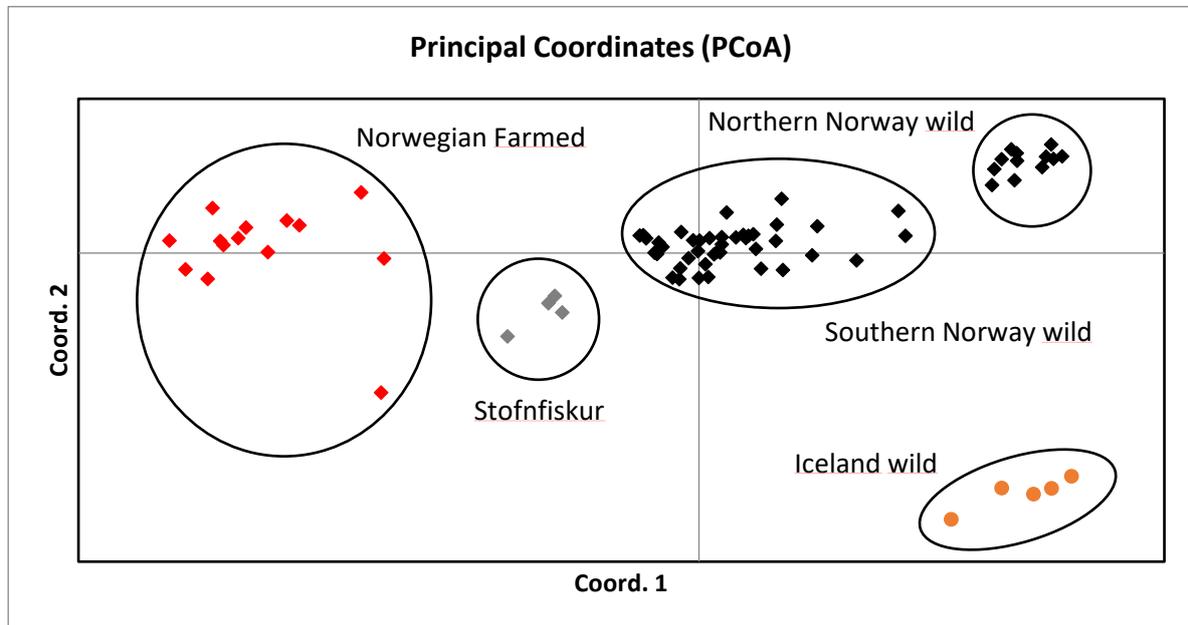
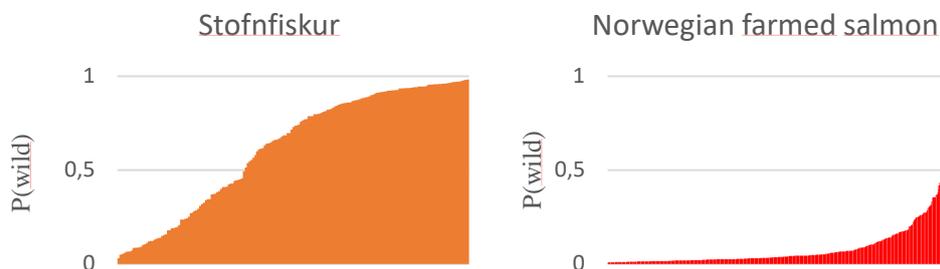


Figure 6. Principal Coordinate Analysis (PCoA) plot based on pairwise estimates of F_{ST} from genetic variation in 48 nuclear SNPs used for quantifying farmed to wild salmon genetic introgression in Norwegian wild populations (black diamonds), Norwegian farmed populations (red diamonds), Icelandic wild populations (orange filled circles), and Stofnfiskur (grey diamonds) populations. The first and second axis explained 34.6 and 13.0% of the variation, respectively.

Figure 6 indicates that the farmed salmon from Stofnfiskur does not differentiate from Norwegian wild salmon populations as well as the Norwegian farmed population at the loci specifically identified for the latter comparison. In potential events of escapees interbreeding with wild salmon, offspring from the farmed salmon from Stofnfiskur will therefore be difficult to trace and genetic introgression will be underestimated. The problem of estimating genetic introgression from Stofnfiskur is evident from comparing estimates of probability of belonging to wild salmon ($P(\text{wild})$) for farmed salmon from Stofnfiskur with Norwegian farmed strains (Figur 7).



Figur 7. Estimates of probabilities of belonging to wild salmon ($P(\text{wild})$) for farmed salmon from Stofnfiskur and from Norwegian farmed strains, using 48 SNPs identified as diagnostic in differentiating between Norwegian farmed salmon and wild salmon.

4 Discussion and conclusions

From molecular genetic analyses, we have demonstrated that the farmed salmon from Stofnfiskur with high probability have only Norwegian and not Icelandic origin. The Norwegian origin of Stofnfiskur was evident from the analyses of the samples from Stofnfiskur as a group, and from the genetic assignment of single individuals. An important assumption for the analyses is that the samples analysed from Stofnfiskur are representative for the entire strain, meaning that we would have reached the same conclusion with other samples from the strain. Another assumption is that the samples from the Icelandic wild populations are of pure wild Icelandic origin and not individuals of farmed escape ancestry from Stofnfiskur. In our analyses, we did not observe any indications of genetic introgression from Stofnfiskur in the wild salmon from Iceland. Furthermore, in case of farmed genetic introgression in the Icelandic wild samples we would expect the fish from Stofnfiskur to be more similar, and not more different to the Icelandic wild salmon. We have only compared the fish from Stofnfiskur with wild salmon from Norway and Iceland, and not with other wild populations from elsewhere in its distribution range, so we cannot conclude about other potential origins. Nevertheless, Stofnfiskur did not deviate much from the Norwegian references and it clustered closely to the southern Norwegian phylogenetic cluster, as expected from information given about their origin. One individual from Stofnfiskur were assigned to Iceland with a higher likelihood than to Norway, but we also found individuals in the reference samples from Norway and Iceland that did not assign to their country of origin. These individuals may represent migrants, or simply be individuals with rare multi-locus genotypes. We cannot conclude on one or the other explanation at this stage, but given the large geographical and genetic distance between Norwegian and Icelandic salmon populations, we would expect migration between Norway and Iceland to be very rare.

A set of markers for generic genetic differentiation between Norwegian farmed and wild salmon was identified from a comparison of Norwegian wild and farmed populations genotyped at 4514 SNPs (Karlsson et al. 2011). This set of genetic markers have successfully been used for quantifying genetic introgression from escaped farmed salmon in Norwegian wild populations (Glover et al. 2013, Karlsson et al. 2016). Because the Stofnfiskur strain has been established from salmon from the farmed strains Bolaks, Mowi, and the National breeding program, they have the same origin as the Salmobreed, Mowi, and AquaGen, included in the work by Karlsson et al. (2011), one would expect that the Stofnfiskur strain would also show large genetic differences to the wild Norwegian populations. To some extent, Stofnfiskur is different from the wild populations at these SNP-loci and in a direction similar to the Norwegian farmed strains, but with a shorter genetic distance to the Norwegian wild populations. A possible reason for the shorter genetic distance to the Norwegian wild populations is that Stofnfiskur is a mixture of farmed salmon from the Norwegian farmed strains and that this has cancelled out some of the differences between the wild and the farmed strains in the early nineteen eighties when they were included in Stofnfiskur. Another possible reason for the smaller genetic differences between Norwegian wild populations and Stofnfiskur compared to the genetic distances between Norwegian wild populations and Norwegian farmed strains is a weaker genetic drift in Stofnfiskur, compared to the Norwegian strains and therefore a slower genetic change since the Stofnfiskur strain was founded in the early nineteen eighties. This is supported by the observation that historical samples of Norwegian farmed salmon from the 1980's also show considerable less genetic differences to the wild populations compared to contemporary farmed salmon (Karlsson et al. 2016). Nevertheless, if farmed salmon from Stofnfiskur escape and interbreed with Norwegian wild salmon, farmed genetic introgression in Norwegian wild populations will be severely underestimated. The observations in this study demonstrate that introduction of new farmed strains to Norway will make it harder to trace genetic introgression. New genetic markers that differentiate between wild salmon and farmed salmon from all farmed strains in use will then have to be found and implemented.

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