

Introgression of non-native mitochondrial haplotypes from farmed to wild Atlantic salmon

Sebastian Wacker , Geir H. Bolstad , Ola H. Diserud, Kjetil Hindar, and Sten Karlsson

Norwegian Institute for Nature Research, Trondheim, Norway

Corresponding author: Sebastian Wacker (email: sebastian.wacker@nina.no)

Abstract

Farmed salmon escape and interbreed with wild Atlantic salmon on a large scale. We studied introgression of mitochondrial haplotypes from farmed Atlantic salmon originating from the Eastern Atlantic phylogenetic group to wild salmon of the Barents-White Sea (BWS) phylogenetic group. We find that farmed genetic introgression introduced novel, non-native haplotypes into the BWS phylogenetic group. The mitochondrial genome has important functional effects and is inherited as a haploid from the mother. Hence, the observed introgression across natural genetic barriers is expected to cause long-lasting functional maladaptation of the hybrids in the maternal line. As the use of farmed Atlantic salmon from non-native phylogenetic groups is widespread in aquaculture, the impact on wild Atlantic salmon may be more severe than previously recognized. Our results highlight the ecological risks of releasing non-native wild and domesticated animals.

Key words: Atlantic salmon, aquaculture, genetic introgression, *Salmo salar*, phylogeny, haplotypes

Introduction

Human-mediated gene flow is widespread and has detrimental effects on wild populations (Crispo et al. 2011; Ottenburghs 2021). Important and growing causes of human-mediated gene flow are escaped domesticated animals (Nijman et al. 2003; Kidd et al. 2009; Anderson et al. 2019) and intentionally or unintentionally translocated wild animals (Seabra et al. 2019; Faust et al. 2021) that interbreed with native wild conspecifics. The resulting changes in the gene pool may reduce the fitness and evolutionary potential of wild populations (Rhymer and Simberloff 1996; Tufto 2010). Escaped domesticated animals can differ genetically from local wild populations not only due to domestication but also due to the origin of their founder populations. Genetic differences are particularly large when the founder populations of domesticated escapees and local wild populations belong to different phylogenetic groups (Bourret et al. 2013; Bradbury et al. 2022). Gene flow from domesticated escapees may in such cases not only change frequencies of gene variants (alleles) in wild populations but also introduce novel, non-native gene variants (Anderson et al. 2009; Hertwig et al. 2009). Despite its large detrimental potential, there is limited evidence for this process.

In Norway, farmed Atlantic salmon (*Salmo salar*) outnumber their wild conspecifics 1000-fold (ICES 2023), and despite only a small fraction escaping, they sometimes make up a large proportion of the spawners in wild populations (Fiske et al. 2006; Diserud et al. 2019; Glover et al. 2019). Genetic introgression from farmed salmon into wild populations (Table 1) is widespread along the coast of Norway (Glover

et al. 2013; Karlsson et al. 2016). Introgressed salmon (Table 1) show changes in important life history traits (Bolstad et al. 2017, 2021; Besnier et al. 2022), and farmed and hybrid individuals show lower survival in the wild than wild conspecifics (Fleming et al. 2000; McGinnity et al. 2003; Wringe et al. 2018; Skaala et al. 2019; Wacker et al. 2021).

Farmed Atlantic salmon are genetically different from their wild origin due to selective breeding for commercially important traits and due to the phylogenetic origin of their founder populations (Gjedrem and Baranski 2009). The effect of introgression on wild populations appears to depend on the phylogenetic origin (Table 1) of the wild populations (Fraser et al. 2010; Bolstad et al. 2017; Islam et al. 2022). Atlantic salmon is geographically sub-divided into several distinct phylogenetic groups (Verspoor et al. 2005; Bourret et al. 2013; Rougemont and Bernatchez 2018; Wennevik et al. 2019). In Norway, farmed Atlantic salmon strains originate from populations in the west and mid-coast of Norway (Gjedrem et al. 1991), which belongs to the Eastern Atlantic (EA) phylogenetic group. These strains are being used in northern Norway, where Atlantic salmon from the Barents-White Sea (BWS) phylogenetic group inhabit the rivers (Wennevik et al. 2019). The same strains are also being used in North America, where Atlantic salmon belong to the Western Atlantic phylogenetic group (Bradbury et al. 2022), and in Ireland, Scotland, and Iceland, each with genetically distinct populations of wild Atlantic salmon within the EA phylogenetic group (Bourret et al. 2013; Rougemont and Bernatchez 2018). Therefore, Atlantic salmon aquaculture represents a large-scale experiment in nature from release of domesticated an-

Table 1. Terms used to categorise Atlantic salmon and mitochondrial haplotypes in this study.

Wild salmon	Individuals hatched in the wild; pre-introgression individuals and contemporary individuals with low farmed genetic ancestry
Farmed salmon	Individuals hatched in aquaculture facilities; breeding lines (from three breeding companies) and escapees
Introgressed salmon	Individuals hatched in the wild, with farmed genetic ancestry
Pre-introgression	Defined by history of escaped farmed salmon (reported from c. 1986); adult samples treated as pre-introgressed until 1992 for Barents-White Sea and until 1990 for Eastern Atlantic
Ancestral populations	Wild salmon populations (collected 1971–1974) that gave rise to the breeding lines of farmed salmon
Phylogenetic origin	Native (indigenous) phylogenetic group, or non-native (exogenous) phylogenetic group
Non-native haplotypes	Haplotypes that were absent in a phylogenetic group before farmed genetic introgression

imals into wild populations of same and different phylogenetic origin. The extensive knowledge on magnitude and effect of farmed gene flow on wild populations has made Atlantic salmon a model system for understanding this problem (Glover et al. 2017).

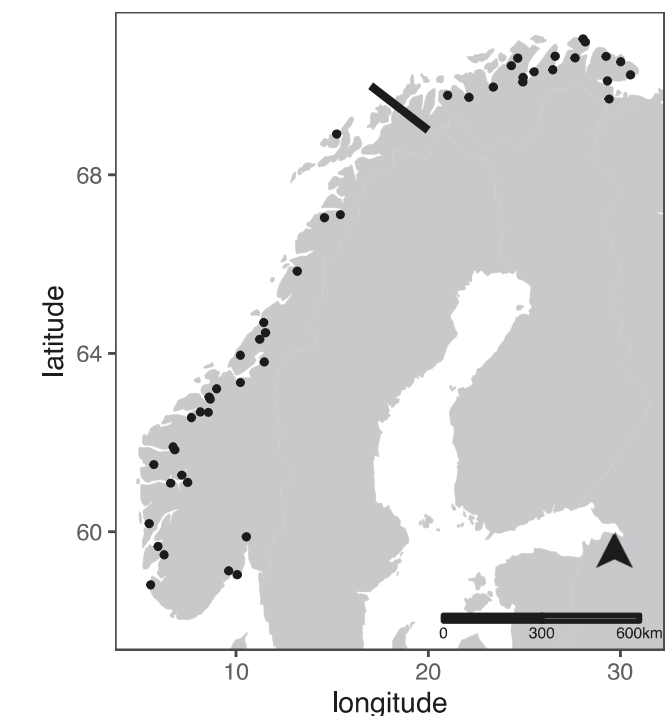
Molecular genetic analyses of farmed genetic introgression have focused on changes in autosomal gene frequencies that are explained by genetic differences between domesticated strains and their wild origin (Glover et al. 2012; Karlsson et al. 2016; Wringe et al. 2018). Despite its potential importance, introgression of non-native mitochondrial haplotypes has largely been ignored. The mitochondrial genome is entirely passed on from mother to offspring without recombination, and different haplotypes therefore represent different maternal lines with genetic variation accumulated in the evolutionary history of the species. New haplotypes from a non-native source can therefore involve genetic introgression of very different mitochondrial genomes. Such introgression is expected to have significant fitness consequences (da Fonseca et al. 2008; Christie et al. 2011; Foote et al. 2011), since the mitochondrial genome is of functional importance in a range of biological processes (e.g., metabolism, thermal tolerance, and aging). High levels of variation in the mitochondrial genome are found among populations and phylogenetic groups of Atlantic salmon (Karlsson et al. 2010; Verspoor et al. 2012). Here, we explore introgression of novel, non-native mitochondrial haplotypes from farmed Atlantic salmon escaping into wild populations of a different phylogenetic origin. Specifically, we compare mitochondrial haplotype frequencies in Atlantic salmon in samples taken before and after large-scale salmon farming.

Methods

Data

Scale samples of Atlantic salmon were collected by means of recreational angling in Norwegian rivers (Fig. 1) encompassing two phylogenetic groups: the EA group and the BWS group (Bourret et al. 2013; Wennevik et al. 2019). We analysed mitochondrial haplotypes in samples of the BWS phylogenetic group collected before and after large-scale Atlantic salmon farming could influence these populations. Atlantic salmon farming in Norway started in the 1970s and grew rapidly from the mid-1980s (Glover et al. 2017). Escaped

farmed salmon were found to be numerous in rivers in southern Norway some years before becoming numerous in northern Norway (Gausen and Moen 1991; Diserud et al. 2019). The analysed material included pre-introgression (Table 1) BWS samples ($N = 520$) from 9 rivers in the period 1978–1992 and contemporary BWS samples ($N = 1982$) from 21 rivers in the period 1994–2019 (Fig. 1; Fig. S1; Table S1).



As a reference for farmed Atlantic salmon mitochondrial haplotypes, we included samples from the major breeding companies in Norway, escaped farmed salmon, and pre-introgression samples from the EA phylogenetic group (ancestor phylogenetic group of Norwegian farmed Atlantic salmon). The pre-introgression EA samples ($N = 1895$) were

from 35 rivers and were collected in the period 1949–1990 (Fig. 1; Fig. S1; Table S1). Among those were home rivers of major ancestral populations (Table 1) for Norwegian farmed Atlantic salmon (Suldalslågen, Vosso, Årøyelva, Driva, Surna, Gaula, and Namsen; Table S1) (Gjedrem et al. 1991; Gjølven and Bentsen 1997). Samples of farmed Atlantic salmon came from the breeding lines of the major breeding companies in Norway (AquaGen, SalmoBreed, and Mowi) ($N = 732$; Table S1). The escaped farmed Atlantic salmon samples ($N = 272$) were from 19 rivers and 2 fjords (Sunndalsfjord and Bolstadfjord) in the EA region collected in the period 1986–2018 (Table S1). Experienced scale readers identified the escaped farmed Atlantic salmon by analysis of growth pattern (Lund and Hansen 1991).

Ethical approval

The majority of scale samples were collected from Atlantic salmon captured by angling. Collection and handling of fish were in accordance with local regulations.

Mitochondrial haplotypes

DNA was extracted from scale samples using the DNEASY tissue kit (QiAGEN) and genotyped at 81 nuclear and 15 mitochondrial single-nucleotide polymorphisms (SNPs) using an EP1™ 96.96 Dynamic array IFCs platform (Fluidigm). Forty-eight of the nuclear SNPs were used to estimate farmed introgression (described below), while the remaining nuclear SNPs were not used in this study. The 15 mitochondrial SNPs (Table S2) are described in Karlsson et al. (2010). Mitochondrial SNPs were combined into multi-locus mitochondrial haplotypes. Four SNPs had a low minor allele frequency (<0.01) and were excluded from further analysis. This reduced the number of detected mtDNA haplotypes from 23 ($N = 5217$) to 16 ($N = 5401$) (Table S3). Five haplotypes (P, Q, U, Y, and Z) were very rare (1–4 out of 5401 individuals; Table S1; Table S3) and were excluded from further analysis.

Estimation of genome-wide farmed introgression

For quantifying genetic introgression, we used 48 of the nuclear SNPs identified by Karlsson et al. (2011) as showing large differences between Norwegian farmed and wild salmon. Data on these markers were used in the programme STRUCTURE (Pritchard et al. 2000) to estimate the likelihood of an individual to belong to a wild versus a farmed reference, as described by Karlsson et al. (2014). We hereafter refer to this likelihood as $P(\text{wild})$. The wild reference were fish caught in rivers belonging to the BWS phylogenetic group between 1978 and 1992, at an early stage of Atlantic salmon farming in Norway. The farmed reference comprised fish from the major breeding lines in Norway (Karlsson et al. 2016). The proportion of farmed genetic ancestry was calculated by scaling individual $P(\text{wild})$ estimates to averages in pre-introgression BWS samples (0.984) and farmed Atlantic salmon (0.0178) (Karlsson et al. 2014).

For the BWS contemporary samples, we compared mitochondrial haplotypes between individuals classified as either wild or admixed (introgressed). We classified individuals with

a $P(\text{wild})$ estimate > 0.8315 as wild and the rest as admixed. This threshold is the 5-percentile of $P(\text{wild})$ estimates in pre-introgression BWS samples (Karlsson et al. 2014). Note that on the individual level the $P(\text{wild})$ is estimated with uncertainty. Therefore, we do expect error in the classification. The error is asymmetrical and fewer wild-origin fish are wrongly classified as introgressed than introgressed fish wrongly classified as wild (Karlsson et al. 2014). This error leads to an underestimate of the differences between the groups, and we therefore consider our analysis and conclusions as conservative.

Statistical analysis

We calculated haplotype frequencies for six groups of samples: (i) pre-introgression BWS samples, (ii) BWS Atlantic salmon classified as introgressed, (iii) BWS Atlantic salmon classified as wild, (iv) pre-introgression EA samples, (v) escaped farmed salmon, and (vi) samples of farmed Atlantic salmon breeding lines. We present haplotype frequencies for each group across all populations pooled. Haplotype frequencies for each population are available in the Supplementary material (Table S1). In the Supplementary material, we also present haplotype frequencies calculated with equal weight on each river and excluding samples with small sample size ($N < 20$; Fig. S2). Haplotype richness based on the smallest sample ($N = 271$) was estimated by rarefaction (Heck et al. 1975) using the R package vegan (Oksanen et al. 2020).

To test for differences in haplotype frequencies between groups, we used generalised linear mixed models (GLMMs). In the first model, we tested whether the occurrence of novel, non-native haplotypes (Table 1) in the BWS phylogenetic group differed between Atlantic salmon classified as introgressed and Atlantic salmon classified as wild. The response variable was the occurrence (absence/presence) of one of the non-native haplotypes in a given individual in the BWS phylogenetic group. In the second model, we tested for differences in frequency in a haplotype that was present in all six groups of samples listed above. We chose the most common haplotype (A) for this test, and the response variable of the model was the occurrence (absence/presence) of haplotype A in a given individual. Both models had a binomial error link function. Random effects were sampling location (river or fjord) for Atlantic salmon caught in the wild and breeding line and year for samples from breeding companies, respectively (Table S1).

Using GLMMs with individual farmed genetic ancestry as an explanatory variable, we tested for (i) an effect on the occurrence of non-native haplotypes in the BWS phylogenetic group and (ii) an effect on the frequency of the most common haplotype (A) in the BWS phylogenetic group. These analyses allowed us to attribute introgression of non-native haplotypes and changes in haplotype frequencies to gene flow from escaped farmed Atlantic salmon, as opposed to other sources of gene flow between the EA and BWS phylogenetic groups. Response variables were the occurrence (absence/presence) in a given individual of one of the non-native haplotypes or haplotype A, respectively. Random effects were sampling locations (river or fjord). All analyses were carried out in R (R Development Core Team 2019).

Fig. 2. Frequencies of mitochondrial haplotypes in pre-introgression Barents-White Sea (BWS) samples, contemporary BWS samples of wild and genetically introgressed individuals, pre-introgression Eastern Atlantic (EA) samples, escaped farmed salmon collected in EA rivers, and samples obtained from the breeding lines of Norwegian farmed salmon. See Table S1 for the within-river frequencies for the different groups. Mitochondrial haplotypes non-native to BWS are indicated with asterisks.

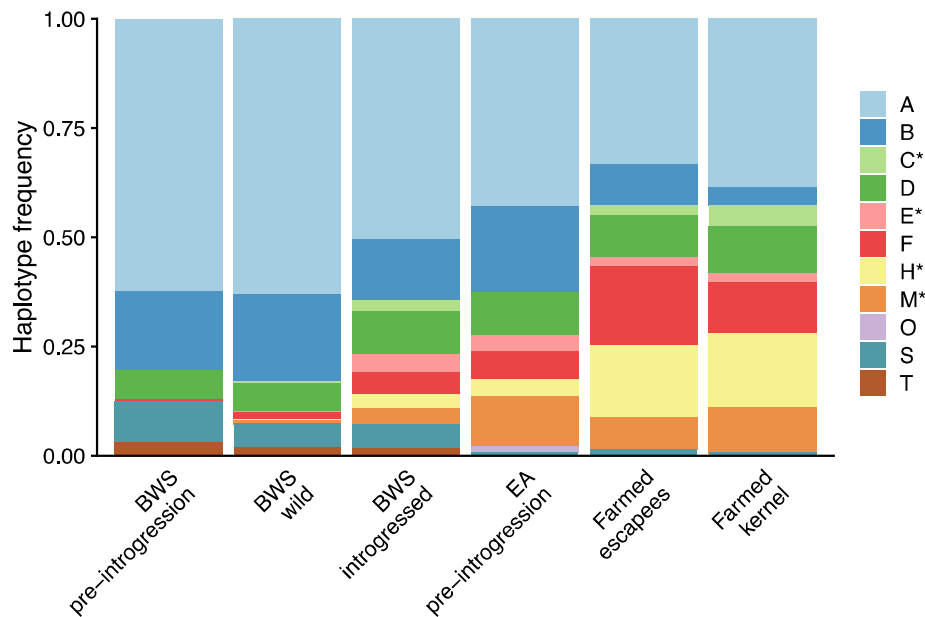
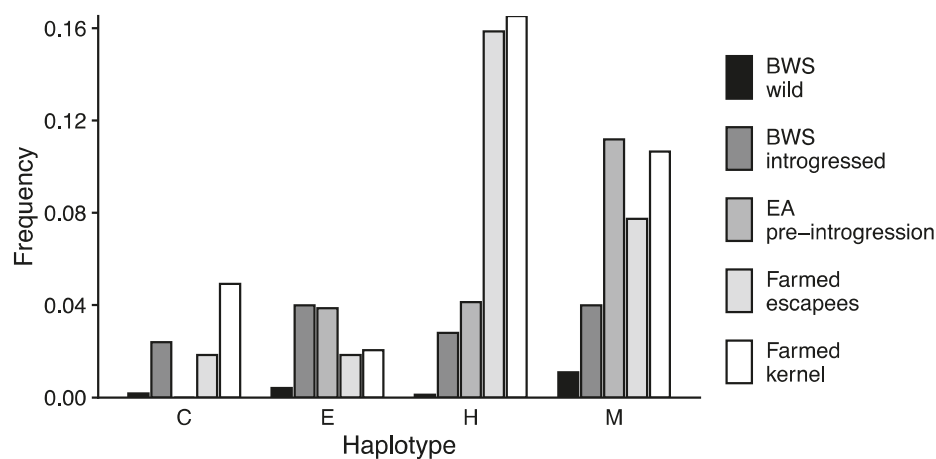


Fig. 3. Frequencies of four mitochondrial haplotypes present in contemporary Barents-White Sea (BWS) samples and farmed Atlantic salmon samples but not in pre-introgression BWS samples. Frequencies are shown for breeding lines of Norwegian farmed salmon (farmed kernel), escaped farmed salmon collected in Eastern Atlantic (EA) rivers, pre-introgression EA samples, and contemporary BWS samples of genetically introgressed and wild individuals.



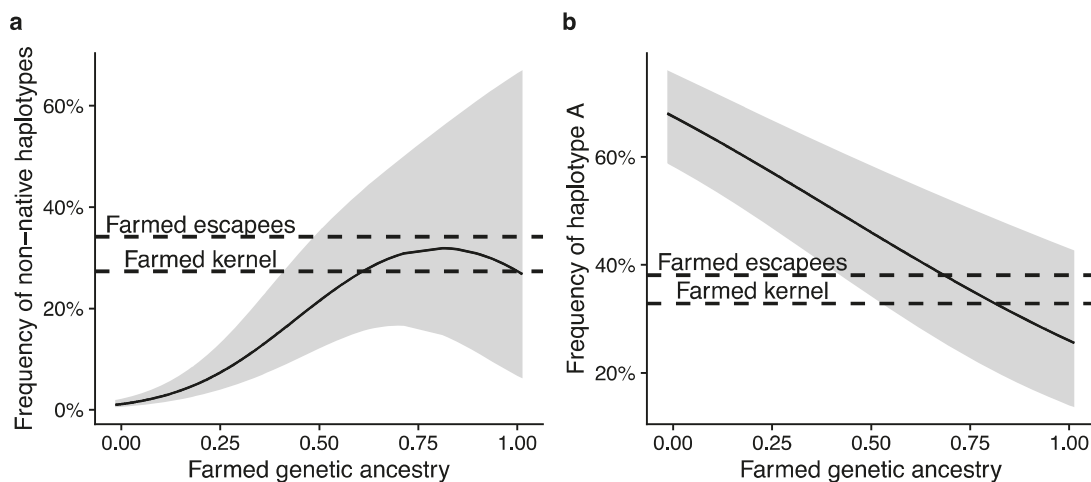
Results

We identified four haplotypes (C, E, H, and M) that were present in farmed Atlantic salmon (escapees and breeding lines) but not in the BWS phylogenetic group before the onset of Atlantic salmon farming. All four haplotypes non-native to the BWS phylogenetic group were present in contemporary BWS samples (Figs. 2 and 3) and at least one was found in 18 out of 21 rivers (Table S1).

The vast preponderance of non-native haplotypes was introduced to the BWS phylogenetic group through gene flow

from escaped farmed Atlantic salmon rather than other types of gene flow from the EA phylogenetic group. This was evident, first because of a higher incidence of non-native haplotypes among contemporary BWS Atlantic salmon classified as introgressed ($N=250$) than among individuals classified as wild ($N=1732$) (difference log odds: 2.239, 95% CI: 1.696–2.781; Fig. 3). Secondly, the frequency of non-native haplotypes in contemporary BWS Atlantic salmon increased with the degree of individual farmed genetic ancestry (Fig. 4a). Genetic introgression from escaped farmed Atlantic

Fig. 4. Effect of genetic introgression on the occurrence of (a) non-native mitochondrial haplotypes (haplotypes C, E, H, and M) and (b) mitochondrial haplotype A in contemporary Barents-White Sea Atlantic salmon. Genetic introgression was estimated as genome-wide farmed genetic ancestry. The black line shows the effect of farmed genetic ancestry on the occurrence of (a) non-native mitochondrial haplotypes (linear term on logit scale: 9.275 ± 1.465 ; quadratic term on logit scale: -5.757 ± 1.927) and (b) mitochondrial haplotype A (linear slope on logit scale: -1.779 ± 0.364) estimated by generalised linear mixed models. Shaded areas represent the 95% confidence intervals. Horizontal dashed lines indicate haplotype frequencies for samples obtained from breeding lines of Norwegian farmed salmon (farmed kernel) and escaped farmed salmon collected in Eastern Atlantic rivers.



salmon also increased haplotype richness in the BWS phylogenetic group from 6.21 ± 0.67 to 9.28 ± 0.73 haplotypes (estimate \pm standard error based on rarefaction using 271 individuals; Table S4).

Rare haplotypes may have been missed in sampling of Atlantic salmon from the various groups, including the haplotypes non-native to the BWS phylogenetic group. However, the likelihood of missing the rare haplotypes in the pre-introgression BWS sample of 520 individuals is low. Assuming that the four non-native haplotypes were equally common in the pre-introgression BWS samples as in contemporary BWS samples (total proportion: 0.032), the likelihood of not encountering any non-native haplotype in the pre-introgression BWS samples, inferred from a binomial distribution (number of trials: 520; likelihood of success: 0.032), is 3.87×10^{-8} .

Farmed genetic introgression also affected the frequency of haplotypes native to the BWS phylogenetic group. Haplotype A was the most common haplotype in all groups, but the frequency differed between groups (Fig. 2). Haplotype A was more frequent in pre-introgression BWS samples than in pre-introgression EA samples (difference in log odds: 0.749, 95% CI: 0.254–1.245), in farmed escapees samples (1.047, 95% CI: 0.484–1.611), and in farmed breeding lines samples (2.004, 95% CI: 1.322–2.686) (Fig. 2). In contemporary BWS samples, haplotype A was more common in individuals classified as wild than in individuals classified as introgressed (difference in log odds: 0.485, 95% CI: 0.202–0.767; Fig. 2). The frequency of haplotype A decreased with the proportion of farmed genetic ancestry in contemporary BWS Atlantic salmon (Fig. 4b).

The four non-native haplotypes introduced to BWS Atlantic salmon were all present in the farmed escapees and breeding lines, and all but one (haplotype C) were present in the EA phylogenetic group (Fig. 2). Haplotype C differed in only one SNP (out of eleven SNPs) from haplotype B (Table S2), which was common in both phylogenetic groups (Fig. 2). Haplotype frequencies of farmed Atlantic salmon (escapees and breeding lines) were more similar to the EA phylogenetic group than to the BWS phylogenetic group before the onset of Atlantic salmon farming (Fig. 2). The frequency of haplotype A in farmed escapees was lower than that in pre-introgression BWS samples (difference log odds: 1.047, 95% CI: 0.484–1.611) but not different from pre-introgression EA samples (difference log odds: 0.298, 95% CI: -0.028 –0.623) (Fig. 2).

Discussion

Analysis of more than 5000 wild and farmed Atlantic salmon revealed that gene flow from farmed escapees has introduced non-native mitochondrial haplotypes into wild populations of the BWS phylogenetic group. Using individual estimates of genetic introgression from nuclear genetic markers, we show that at least the vast preponderance of introgression of non-native haplotypes is a result of gene flow from escaped farmed Atlantic salmon rather than natural gene flow between phylogenetic groups. This occurred after the buildup of large-scale aquaculture based on Atlantic salmon originating from the EA phylogenetic group and provides evidence that gene flow from escaped farmed Atlantic salmon not only changes frequencies of gene variants in wild popu-

lations but also introduces novel gene variants when farmed and wild fish are of different phylogenetic origin.

Given the functional importance of mitochondrial DNA, introgression of mitochondrial haplotypes across phylogenetic boundaries may have substantial biological consequences. Mitochondrial DNA plays an important role in metabolic functions related to life history (Salin et al. 2015), thermal tolerance (Bize et al. 2018), and aging (Austad 2018). Natural selection on regions of the mitochondrial DNA has been found in several taxa (da Fonseca et al. 2008; Foote et al. 2011), including Pacific salmon (Garvin et al. 2011). Introgression of non-native haplotypes is therefore expected to interfere with the adaptation of wild populations to their local environment and to reduce their fitness (da Fonseca et al. 2008; Christie et al. 2011; Foote et al. 2011). Human-mediated introgression of non-native haplotypes may be widespread when wild and domesticated animals are translocated across phylogenetic groups, such as in the fast-growing aquaculture industry (Naylor et al. 2001; Bostock et al. 2010; Naylor et al. 2021). Human-mediated introgression of non-native mitochondrial haplotypes has earlier also been found to occur from escaped domesticated mammals to wild conspecifics (Hertwig et al. 2009; Godinho et al. 2011; Anderson et al. 2019) and from translocated wild to local populations in insects (Seabra et al. 2019) and fishes (Takamura and Nakahara 2015; Kitanishi et al. 2018). In Atlantic salmon, human-mediated introgression of non-native mitochondrial haplotypes has previously been documented in populations that were stocked with non-local wild fish (Ciborowski et al. 2007; Campos et al. 2008; Gabian and Moran 2019). Introgression from escaped farmed Atlantic salmon of Norwegian origin has been found to introduce a non-native mitochondrial haplotype, or altered its frequency, in wild salmon populations in Northwest Ireland (Clifford et al. 1998a; Clifford et al. 1998b). Our results, together with previous evidence of introgression of non-native haplotypes in other species, highlight the ecological risks of commercial use of non-native wild and domesticated animals. Future work is needed to understand the relative contribution of introgression of mitochondrial and nuclear DNA to fitness effects in wild Atlantic salmon populations.

The large genetic divergence of farmed Atlantic salmon to wild strains of the BWS phylogenetic group consists not only in differences in frequencies of the same gene variants but also in gene variants in the farmed Atlantic salmon that are non-native to the wild strains and is expected to increase maladaptation and thereby reduce fitness for introgressed individuals and populations (Huisman and Tufto 2012; Baskett et al. 2013; Glover et al. 2017). Farmed introgression has been found to change important life-history traits, and this effect was different and for some aspects larger in populations of the BWS phylogenetic group than in populations of the EA phylogenetic group (Bolstad et al. 2017). Farmed introgression strongly reduced freshwater survival of juvenile Atlantic salmon in an Atlantic salmon population of the BWS phylogenetic group (Wacker et al. 2021). Future work is needed to quantify and disentangle the fitness consequences of farmed genetic introgression on wild populations resulting from changes in gene variant frequencies and the introgression of novel, non-native gene variants, respectively.

Our results revealed that the introgression of non-native mitochondrial haplotypes in the BWS phylogenetic group was a result of gene flow from escaped farmed Atlantic salmon but do not exclude a minor contribution of natural gene flow to those changes. Natural introgression of mitochondrial haplotypes has occurred between different phylogenetic groups of Atlantic Salmon (Asplund et al. 2004; Makhrov et al. 2005; King et al. 2007; Bradbury et al. 2015). The BWS and EA phylogenetic groups reflect different post-glacial colonisation lineages, with a clear genetic divide, which may be maintained by divergent selection regimes (Wennevik et al. 2019) and that is in line with our results that several haplotypes of the EA group were absent in the BWS group before Atlantic salmon farming. The short time span between pre-introgression samples and recent samples in our study (few decades), relative to the timespan since post-glacial colonisation, makes it unlikely that natural gene flow has contributed to the observed introgression of non-native haplotypes.

Our findings of introgression of non-native haplotypes, as well as previous findings on fitness consequences, may be transferable to other regions with larger genetic differentiation between wild populations and farmed Atlantic salmon; Iceland, Ireland, and Scotland are mainly or fully using farmed salmon of Norwegian origin. Norwegian-origin farmed salmon has also been used in North America (O'Reilly et al. 2006), and genetic introgression in North American wild Atlantic salmon populations has been documented (Bradbury et al. 2022). Human-mediated gene flow across boundaries of phylogenetic groups may have profound effects on the genetic population structure of the species, and the negative genetic consequences of introgression from non-native phylogenetic origin are expected to add an extra genetic load on the wild local populations.

Acknowledgements

We thank G. Østborg, J.G. Jensås, and K. Urdal for scale reading and I.P.Ø. Andersskog, T. Balstad, H. Brandsegg, L.B. Eriksen, and M. Spets for DNA extraction and genotyping. We thank colleagues at the Norwegian Institute for Nature Research, Rådgivende Biologer AS, the Norwegian Veterinary Institute, County Fishery Officers, NORCE Norwegian Research Centre, and the Natural Resources Institute Finland (Luke) for providing samples. The Norwegian Environment Agency provided funding for scale collection.

Article information

History dates

Received: 16 February 2023

Accepted: 21 June 2023

Version of record online: 31 July 2023

Copyright

© 2023 The Author(s). This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/) (CC BY 4.0), which permits unrestricted use, distribution, and reproduc-

tion in any medium, provided the original author(s) and source are credited.

Data availability

Data analyzed during this study are available in the Dryad repository, <https://doi.org/10.5061/dryad.280gb5mv7>.

Author information

Author ORCIDs

Sebastian Wacker <https://orcid.org/0000-0002-3035-8615>

Geir H. Bolstad <https://orcid.org/0000-0003-1356-8239>

Author contributions

Conceptualization: SK

Data curation: SW, SK

Formal analysis: SW, GHB, OHD

Funding acquisition: GHB, KH, SK

Methodology: SW, SK

Project administration: SW, SK

Supervision: SK

Validation: SW, SK

Visualization: SW, GHB

Writing – original draft: SW

Writing – review & editing: SW, GHB, OHD, KH, SK

Competing interests

The authors declare there are no competing interests.

Funding information

This research was supported by the Norwegian Environment Agency, the Research Council of Norway (QuantEscape 254852 and EcoEvoGene 275862), and the Norwegian Institute for Nature Research.

Supplementary material

Supplementary data are available with the article at <https://doi.org/10.1139/cjfas-2023-0044>.

References

- Anderson, T.M., vonHoldt, B.M., Candille, S.I., Musiani, M., Greco, C., Stahler, D.R., et al. 2009. Molecular and Evolutionary History of Melanism in North American Gray Wolves. *Science*, **323**: 1339–1343.
- Anderson, D., Toma, R., Negishi, Y., Okuda, K., Ishiniwa, H., Hinton, T.G., et al. 2019. Mating of escaped domestic pigs with wild boar and possibility of their offspring migration after the Fukushima Dai-ichi Nuclear Power Plant accident. *Sci. Rep.* **9**: 11537. doi:10.1038/s41598-019-47982-z. PMID: 31395920.
- Asplund, T., Veselov, A., Primmer, C.R., Bakhmet, I., Potutkin, A., Titov, S., et al. 2004. Geographical structure and postglacial history of mtDNA haplotype variation in Atlantic salmon (*Salmo salar* L.) among rivers of the White and Barents Sea basins. *Ann. Zool. Fenn.* **41**: 465–475.
- Austad, S.N. 2018. The comparative biology of mitochondrial function and the rate of aging. *Integr. Comp. Biol.* **58**: 559–566. doi:10.1093/icb/icy068. PMID: 29939249.
- Baskett, M.L., Burgess, S.C., and Waples, R.S. 2013. Assessing strategies to minimize unintended fitness consequences of aquaculture on wild populations. *Evol. Appl.* **6**: 1090–1108. doi:10.1111/eva.12089. PMID: 24187590.

- Besnier, F., Ayllon, F., Skaala, Ø., Solberg, M.F., Fjeldheim, P.T., Anderson, K., et al. 2022. Introgression of domesticated salmon changes life history and phenology of a wild salmon population. *Evol. Appl.* **15**: 0–12.
- Bize, P., Lowe, I., Hurlimann, M.L., and Heckel, G. 2018. Effects of the mitochondrial and nuclear genomes on nonshivering thermogenesis in a wild derived rodent. *Integr. Comp. Biol.* **58**: 532–543. doi:10.1093/icb/icy072. PMID: 29945248.
- Bolstad, G.H., Hindar, K., Robertsen, G., Jonsson, B., Sægvog, H., Diserud, O.H., et al. 2017. Gene flow from domesticated escapes alters the life history of wild Atlantic salmon. *Nat. Ecol. Evol.* **1**: 0124.
- Bolstad, G.H., Karlsson, S., Hagen, I.J., Fiske, P., Urdal, K., Sægvog, H., et al. 2021. Introgression from farmed escapees affects the full life cycle of wild Atlantic salmon. *Sci. Adv.* **7**: eabj3397. doi:10.1126/sciadv.abj3397. PMID: 34936452.
- Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., et al. 2010. Aquaculture: global status and trends. *Philos. Trans. R. Soc. B Biol. Sci.* **365**: 2897–2912. doi:10.1098/rstb.2010.0170.
- Bourret, V., Kent, M.P., Primmer, C.R., Vasemagi, A., Karlsson, S., Hindar, K., et al. 2013. SNP-array reveals genome-wide patterns of geographical and potential adaptive divergence across the natural range of Atlantic salmon (*Salmo salar*). *Mol. Ecol.*, **22**: 532–551.
- Bradbury, I.R., Hamilton, L.C., Dempson, B., Robertson, M.J., Bourret, V., Bernatchez, L., and Verspoor, E. 2015. Transatlantic migration contact in Atlantic Salmon, comparing microsatellites, a single nucleotide polymorphism array and restriction-site associated DNA sequencing for the resolution of complex spatial structure. *Mol. Ecol.* **24**: 5130–5144. doi:10.1111/mec.13395. PMID: 26407171.
- Bradbury, I.R., Lehnert, S.J., Kess, T., Van Wyngaarden, M., Duffy, S., Messmer, A.M., et al. 2022. Genomic evidence of recent European introgression into North American farmed and wild salmon. *Evol. Appl.* **15**: 1436–1448. doi:10.1111/eva.13454. PMID: 36187183.
- Campos, J.L., Posada, D., and Morán, P. 2008. Introgression and genetic structure in northern Spanish Atlantic salmon (*Salmo salar* L.) populations according to mtDNA data. *Conserv. Genet.* **9**: 157–169. doi:10.1007/s10592-007-9318-y.
- Christie, J.S., Picornell, A., Moya, A., Ramon, M.M., and Castro, J.A. 2011. Mitochondrial DNA effects on fitness in *Drosophila subobscura*. *Heredity*, **107**: 239–245. doi:10.1038/hdy.2011.8. PMID: 21364694.
- Ciborowski, K.L., Consuegra, S., de Leaniz, C.G., Wang, J., Beaumont, M.A., and Jordan, W.C. 2007. Stocking may increase mitochondrial DNA diversity but fails to halt the decline of endangered Atlantic salmon populations. *Conserv. Genet.* **8**: 1355–1367. doi:10.1007/s10592-007-9286-2.
- Clifford, S.L., McGinnity, P., and Ferguson, A. 1998a. Genetic changes in an Atlantic salmon population resulting from escaped juvenile farm salmon. *J. Fish Biol.* **52**: 118–127. doi:10.1111/j.1095-8649.1998.tb01557.x.
- Clifford, S.L., McGinnity, P., and Ferguson, A. 1998b. Genetic changes in Atlantic salmon (*Salmo salar*) populations of northwest Irish rivers resulting from escapes of adult farm salmon. *Can. J. Fish. Aquat. Sci.* **55**: 358–363. doi:10.1139/f97-229.
- Crispo, E., Moore, J.S., Lee-Yaw, J.A., Gray, S.M., and Haller, B.C. 2011. Broken barriers: human-induced changes to gene flow and introgression in animals. *BioEssays*, **33**: 508–518. doi:10.1002/bies.201000154. PMID: 21523794.
- da Fonseca, R.R., Johnson, W.E., O'Brien, S.J., Ramos, M.J., and Antunes, A. 2008. The adaptive evolution of the mammalian mitochondrial genome. *BMC Genomics*, **9**: 119. doi:10.1186/1471-2164-9-119. PMID: 18318906.
- Diserud, O.H., Fiske, P., Sægvog, H., Urdal, K., Aronsen, T., Lo, H., et al. 2019. Escaped farmed Atlantic salmon in Norwegian rivers during 1989–2013. *ICES J. Mar. Sci.* **76**: 1140–1150. doi:10.1093/icesjms/fsy202.
- Faust, E., Jansson, E., Andre, C., Halvorsen, K.T., Dahle, G., Knutsen, H., et al. 2021. Not that clean: aquaculture-mediated translocation of cleaner fish has led to hybridization on the northern edge of the species' range. *Evol. Appl.* **14**: 1–16.
- Fiske, P., Lund, R.A., and Hansen, L.P. 2006. Relationships between the frequency of farmed Atlantic salmon, *Salmo salar* L., in wild salmon populations and fish farming activity in Norway, 1989–2004. *ICES J. Mar. Sci.* **63**: 1182–1189. doi:10.1016/j.icesjms.2006.04.006.
- Fleming, I.A., Hindar, K., Mjølnerød, I.B., Jonsson, B., Balstad, T., and Lamberg, A. 2000. Lifetime success and interactions of farm salmon in-

- vading a native population. *Proc. R. Soc. B Biol. Sci.* **267**: 1517–1523. doi:10.1098/rspb.2000.1173.
- Footo, A.D., Morin, P.A., Durban, J.W., Pitman, R.L., Wade, P., Willerslev, E., et al. 2011. Positive selection on the killer whale mitogenome. *Biol. Lett.* **7**: 116–118. doi:10.1098/rsbl.2010.0638. PMID: 20810427.
- Fraser, D.J., Houde, A.L.S., Debes, P.V., O'Reilly, P., Eddington, J.D., and Hutchings, J.A. 2010. Consequences of farmed-wild hybridization across divergent wild populations and multiple traits in salmon. *Ecol. Appl.* **20**: 935–953. doi:10.1890/09-0694.1. PMID: 20597281.
- Gabián, M., and Morán, P. 2019. A genetic tool for evaluating male-mediated stock introgression in Atlantic salmon. *Aquat. Conserv. Mar. Freshwater Ecosyst.* **29**: 142–147. doi:10.1002/aqc.3008.
- Garvin, M.R., Bielawski, J.P., and Gharrett, A.J. 2011. Positive Darwinian selection in the piston that powers proton pumps in complex I of the mitochondria of Pacific salmon. *PLoS ONE*, **6**: e24127. doi:10.1371/journal.pone.0024127. PMID: 21969854.
- Gausen, D., and Moen, V. 1991. Large-scale escapes of farmed Atlantic salmon (*Salmo Salar*) into Norwegian rivers threaten natural populations. *Can. J. Fish. Aquat. Sci.* **48**: 426–428. doi:10.1139/f91-055.
- Gjedrem, T., and Baranski, M. 2009. *Selective breeding in aquaculture: an introduction*. Springer, London.
- Gjedrem, T., Gjøen, H.M., and Gjerde, B. 1991. Genetic-origin of Norwegian farmed Atlantic salmon. *Aquaculture*, **98**: 41–50. doi:10.1016/0044-8486(91)90369-I.
- Gjøen, H.M., and Bentsen, H.B. 1997. Past, present, and future of genetic improvement in salmon aquaculture. *ICES J. Mar. Sci.* **54**: 1009–1014. doi:10.1016/S1054-3139(97)80005-7.
- Glover, K.A., Pertoldi, C., Besnier, F., Wennevik, V., Kent, M., and Skaala, Ø. 2013. Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNPs. *BMC Genet.* **14**. doi:10.1186/1471-2156-14-74.
- Glover, K.A., Quintela, M., Wennevik, V., Besnier, F., Sorvik, A.G.E., and Skaala, Ø. 2012. Three decades of farmed escapees in the wild: a spatio-temporal analysis of Atlantic salmon population genetic structure throughout Norway. *PLoS ONE*, **7**(8): e43129. doi:10.1371/journal.pone.0043129. PMID: 22916215.
- Glover, K.A., Solberg, M.F., McGinnity, P., Hindar, K., Verspoor, E., Coulson, M.W., et al. 2017. Half a century of genetic interaction between farmed and wild Atlantic salmon: status of knowledge and unanswered questions. *Fish Fish.* **18**: 890–927. doi:10.1111/faf.12214.
- Glover, K.A., Urdal, K., Næsje, T., Skoglund, H., Florø-Larsen, B., Otterå, H., et al. 2019. Domesticated escapees on the run: the second-generation monitoring programme reports the numbers and proportions of farmed Atlantic salmon in >200 Norwegian rivers annually. *ICES J. Mar. Sci.* **76**: 1151–1161.
- Godinho, R., Llaneza, L., Blanco, J.C., Lopes, S., Álvares, F., García, E.J., et al. 2011. Genetic evidence for multiple events of hybridization between wolves and domestic dogs in the Iberian Peninsula. *Mol. Ecol.* **20**: 5154–5166. doi:10.1111/j.1365-294X.2011.05345.x. PMID: 22066758.
- Heck, K.L., van Belle, G., and Simberloff, D. 1975. Explicit calculation of the rarefaction diversity measurement and the determination of sufficient sample size. *Ecology*, **56**: 1459–1461. doi:10.2307/1934716.
- Hertwig, S.T., Schweizer, M., Stepanow, S., Jungnickel, A., Böhle, U.R., and Fischer, M.S. 2009. Regionally high rates of hybridization and introgression in German wildcat populations (*Felis silvestris*, Carnivora, Felidae). *J. Zool. Syst. Evol. Res.* **47**: 283–297. doi:10.1111/j.1439-0469.2009.00536.x.
- Huisman, J., and Tufto, J. 2012. Comparison of non-Gaussian quantitative genetic models for migration and stabilizing selection. *Evolution*, **66**: 3444–3461. doi:10.1111/j.1558-5646.2012.01707.x. PMID: 23106709.
- ICES. 2023. Working group on North Atlantic Salmon (WGNAS). *ICES Sci. Rep.* **5**(41): 478.
- Islam, S.S., Xue, X., Caballero-Solares, A., Bradbury, I.R., Rise, M.L., and Fleming, I.A. 2022. Distinct early life stage gene expression effects of hybridization among European and North American farmed and wild Atlantic salmon populations. *Mol. Ecol.* **31**: 2712–2729. doi:10.1111/mec.16418. PMID: 35243721.
- Karlsson, S., Diserud, O.H., Fiske, P., and Hindar, K. 2016. Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. *ICES J. Mar. Sci.* **73**: 2488–2498. doi:10.1093/icesjms/fsw121.
- Karlsson, S., Diserud, O.H., Moen, T., and Hindar, K. 2014. A standardized method for quantifying unidirectional genetic introgression. *Ecol. Evol.* **4**: 3256–3263. doi:10.1002/ece3.1169. PMID: 25473478.
- Karlsson, S., Moen, T., and Hindar, K. 2010. An extended panel of single nucleotide polymorphisms in Atlantic salmon (*Salmo salar* L.) mitochondrial DNA. *Conserv. Genet.* **11**: 1171–1175. doi:10.1007/s10592-009-9910-4.
- Karlsson, S., Moen, T., Lien, S., Glover, K.A., and Hindar, K. 2011. Generic genetic differences between farmed and wild Atlantic salmon identified from a 7 K SNP-chip. *Mol. Ecol. Resour.* **11**: 247–253. doi:10.1111/j.1755-0998.2010.02959.x. PMID: 21429178.
- Kidd, A.G., Bowman, J., Lesbarrères, D., and Schulte-Hostedde, A.I. 2009. Hybridization between escaped domestic and wild American mink (*Neovison vison*). *Mol. Ecol.* **18**: 1175–1186. doi:10.1111/j.1365-294X.2009.04100.x. PMID: 19243512.
- King, T.L., Verspoor, E., Spidle, A.P., Gross, R., Phillips, R.B., Koljonen, M.L., et al. 2007. Biodiversity and population structure. In *The Atlantic salmon: genetics, conservation and management*. Edited by E. Verspoor, L. Stradmeyer and J. Nielsen. Blackwell Publishing Ltd, Oxford. pp. 117–166.
- Kitanishi, S., Onikura, N., and Mukai, T. 2018. A simple SNP genotyping method reveals extreme invasions of non-native haplotypes in pale chub *Opsariichthys platypus*, a common cyprinid fish in Japan. *PLoS ONE*, **13**: e0191731. doi:10.1371/journal.pone.0191731. PMID: 29360868.
- Lund, R.A., and Hansen, L.P. 1991. Identification of wild and reared Atlantic salmon, *Salmo salar* L., using scale characters. *Aquacult. Res.* **22**: 499–508. doi:10.1111/j.1365-2109.1991.tb00763.x.
- Makhrov, A.A., Verspoor, E., Artamonova, V.S., and O'Sullivan, M. 2005. Atlantic salmon colonization of the Russian Arctic coast: pioneers from North America. *J. Fish Biol.* **67**: 68–79. doi:10.1111/j.0022-1112.2005.00840.x.
- McGinnity, P., Prodohl, P., Ferguson, K., Hynes, R., O'Maoileidigh, N., Baker, N., et al. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proc. R. Soc. B Biol. Sci.* **270**: 2443–2450. doi:10.1098/rspb.2003.2520.
- Naylor, R.L., Hardy, R.W., Buschmann, A.H., Bush, S.R., Cao, L., Klinger, D.H., et al. 2021. A 20-year retrospective review of global aquaculture. *Nature*, **591**: 551–563. doi:10.1038/s41586-021-03308-6. PMID: 33762770.
- Naylor, R.L., Williams, S.L., and Strong, D.R. 2001. Aquaculture—a gateway for exotic species. *Science*, **294**: 1655–1656. doi:10.1126/science.1064875. PMID: 11721035.
- Nijman, I.J., Otsen, M., Verkaar, E.L.C., de Ruijter, C., Hanekamp, E., Ochieng, J.W., et al. 2003. Hybridization of banteng (*Bos javanicus*) and zebu (*Bos indicus*) revealed by mitochondrial DNA, satellite DNA, AFLP and microsatellites. *Heredity*, **90**: 10–16. doi:10.1038/sj.hdy.6800174. PMID: 12522420.
- O'Reilly, P.T., Carr, J.W., Whoriskey, F.G., and Verspoor, E. 2006. Detection of European ancestry in escaped farmed Atlantic salmon, *Salmo salar* L., in the Magaguadavic river and Chamcook Stream, New Brunswick, Canada. *ICES J. Mar. Sci.* **63**: 1256–1262. doi:10.1016/j.icesjms.2006.04.013.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGinn, D., et al. 2020. *vegan: community ecology package*. R package version 2.5-6.
- Ottenburghs, J. 2021. The genic view of hybridization in the Anthropocene. *Evol. Appl.* **14**: 2342–2360. doi:10.1111/eva.13223. PMID: 34745330.
- Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, **155**: 945–959. doi:10.1093/genetics/155.2.945. PMID: 10835412.
- R Development Core Team. 2019. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rhymer, J.M., and Simberloff, D. 1996. Extinction by hybridization and introgression. *Annu. Rev. Ecol. Syst.* **27**: 83–109. doi:10.1146/annurev.ecolsys.27.1.83.
- Rougemont, Q., and Bernatchez, L. 2018. The demographic history of Atlantic salmon (*Salmo salar*) across its distribution range reconstructed from approximate Bayesian computations. *Evolution*, **72**: 1261–1277. doi:10.1111/evo.13486. PMID: 29644624.

- Salin, K., Auer, S.K., Rey, B., Selman, C., and Metcalfe, N.B. 2015. Variation in the link between oxygen consumption and ATP production, and its relevance for animal performance. *Proc. R. Soc. B Biol. Sci.* **282**: 14–22. doi:[10.1098/rspb.2015.1028](https://doi.org/10.1098/rspb.2015.1028).
- Seabra, S.G., Silva, S.E., Nunes, V.L., Sousa, V.C., Martins, J., Marabuto, E., et al. 2019. Genomic signatures of introgression between commercial and native bumblebees, *Bombus terrestris*, in western Iberian Peninsula—implications for conservation and trade regulation. *Evol. Appl.* **12**: 679–691. doi:[10.1111/eva.12732](https://doi.org/10.1111/eva.12732). PMID: [30976302](https://pubmed.ncbi.nlm.nih.gov/30976302/).
- Skaala, Ø., Besnier, F., Borgstrøm, R., Barlaup, B.T., Sørvik, A.G., Normann, E., et al. 2019. An extensive common-garden study with domesticated and wild Atlantic salmon in the wild reveals impact on smolt production and shifts in fitness traits. *Evol. Appl.* **12**: 1001–1016. doi:[10.1111/eva.12777](https://doi.org/10.1111/eva.12777). PMID: [31080511](https://pubmed.ncbi.nlm.nih.gov/31080511/).
- Takamura, K., and Nakahara, M. 2015. Intraspecific invasion occurring in geographically isolated populations of the Japanese cyprinid fish *Zacco platypus*. *Limnology*, **16**: 161–170. doi:[10.1007/s10201-015-0450-y](https://doi.org/10.1007/s10201-015-0450-y).
- Tufto, J. 2010. Gene flow from domesticated species to wild relatives: migration load in a model of multivariate selection. *Evolution*, **64**: 180–192. doi:[10.1111/j.1558-5646.2009.00807.x](https://doi.org/10.1111/j.1558-5646.2009.00807.x). PMID: [19663990](https://pubmed.ncbi.nlm.nih.gov/19663990/).
- Verspoor, E., Beardmore, J.A., Consuegra, S., de Leaniz, C.G., Hindar, K., Jordan, W.C., et al. 2005. Population structure in the Atlantic salmon: insights from 40 years of research into genetic protein variation. *J. Fish Biol.* **67**: 3–54. doi:[10.1111/j.0022-1112.2005.00838.x](https://doi.org/10.1111/j.0022-1112.2005.00838.x).
- Verspoor, E., Consuegra, S., Fridjonsson, O., Hjørleifsdottir, S., Knox, D., Olafsson, K., et al. 2012. Regional mtDNA SNP differentiation in European Atlantic salmon (*Salmo salar*): an assessment of potential utility for determination of natal origin. *ICES J. Mar. Sci.* **69**: 1625–1636. doi:[10.1093/icesjms/ffs029](https://doi.org/10.1093/icesjms/ffs029).
- Wacker, S., Aronsen, T., Karlsson, S., Ugedal, O., Diserud, O.H., Ulvan, E.M., et al. 2021. Selection against individuals from genetic introgression of escaped farmed salmon in a natural population of Atlantic salmon. *Evol. Appl.* **14**: 1450–1460. doi:[10.1111/eva.13213](https://doi.org/10.1111/eva.13213). PMID: [34025778](https://pubmed.ncbi.nlm.nih.gov/34025778/).
- Wennevik, V., Quintela, M., Skaala, Ø., Verspoor, E., Prusov, S., and Glover, K.A. 2019. Population genetic analysis reveals a geographically limited transition zone between two genetically distinct Atlantic salmon lineages in Norway. *Ecol. Evol.* **9**: 6901–6921. doi:[10.1002/ece3.5258](https://doi.org/10.1002/ece3.5258). PMID: [31380023](https://pubmed.ncbi.nlm.nih.gov/31380023/).
- Wringe, B.F., Jeffery, N.W., Stanley, R.R.E., Hamilton, L.C., Anderson, E.C., Fleming, I.A., et al. 2018. Extensive hybridization following a large escape of domesticated Atlantic salmon in the Northwest Atlantic. *Commun. Biol.* **1**: 108. doi:[10.1038/s42003-018-0112-9](https://doi.org/10.1038/s42003-018-0112-9). PMID: [30271988](https://pubmed.ncbi.nlm.nih.gov/30271988/).