ICES Journal of Marine Science

ICES Journal of Marine Science (2017), 74(5), 1371–1381. doi:10.1093/icesjms/fsw243

Orginal Article

The ecological profile of Atlantic salmon escapees entering a river throughout an entire season: diverse in escape history and genetic background, but frequently virus-infected

Abdullah S. Madhun[1,*], Vidar Wennevik[1], Ove T. Skilbrei[1,†], Egil Karlsbakk[1], Øystein Skaala[1], Ingrid U. Fiksdal[1], Sonnich Meier[1], Yongkai Tang[1], and Kevin A. Glover[1,2]

[1]*Institute of Marine Research, Nordnesgaten 50, N-5005 Bergen, Norway*
[2]*Institute of Biology, University of Bergen, Bergen, Norway*

**Corresponding author: tel: +47 947 85 883; fax: +47 55 23 85 31; e-mail: abdullah.madhun@imr.no*
[†]Deceased.

Madhun, A. S., Wennevik, V., Skilbrei, O. T., Karlsbakk, E., Skaala, Ø., Fiksdal, I. U., Meier, S., Tang, Y., and Glover, K. A. 2016. The ecological profile of Atlantic salmon escapees entering a river throughout an entire season: diverse in escape history and genetic background, but frequently virus-infected. – ICES Journal of Marine Science, 74: 1371–1381.

Received 27 June 2016; revised 29 November 2016; accepted 8 December 2016; advance access publication 20 January 2017.

In 2014, 129 farmed salmon escapees captured in an upstream-migration trap located in the river Etne, western Norway, were investigated for viral infections, age at escape, size, and genetic composition. The frequency of escapees positive for salmonid alphavirus (SAV), piscine orthoreovirus (PRV), and infectious salmon anaemia virus (ISAV) was 12, 79, and <1%, respectively. Fatty acid analysis demonstrated that the individuals had escaped from farms at different stages of the production cycle, although the majority had probably escaped from farms in the same year as their capture in the river. Genetic analyses demonstrated that the escapees originated from multiple farms. This was also supported by the distribution of fish size and timing of entry into the river. A combination of genetic, fatty acid and viral infection analyses showed that in the river Etne in 2014: (i) most of the fish entering the river were infected with one or more viruses, (ii) the majority of them had escaped in the same year that they entered the river, (ii) they originated from multiple farm sources, and (iv) two of the identified genetic groups likely originated from two recent and distinct escape events. This is the first study to integrate results from multiple analytical methods in order to reveal the ecological and genetic diversity of escaped farmed fish entering a river with native salmon population throughout an entire season.

Keywords: aquaculture, escaped farmed salmon viral diseases, fatty acid, genetics.

Introduction

In Norway, the annual reported numbers of farmed Atlantic salmon (*Salmo salar* L.) escapees have been in the hundreds of thousands for most years in the period 2000–2015 (Anonymous, 2016a,b). However, the true annual numbers of escapees have been estimated to be in the millions due to underreporting (Saegrov and Urdal, 2006; Skilbrei *et al.*, 2015a). Escaped Atlantic salmon can disperse over long distances (Hansen, 2006; Skilbrei *et al.*, 2010; Skilbrei and Jorgensen, 2010), may enter rivers (Fiske *et al.*, 2006), and can display a range of ecological (Jonsson and Jonsson, 2006) and genetic interactions with wild conspecifics (Crozier, 1993; Clifford *et al.*, 1998; Skaala *et al.*, 2006; Glover *et al.*, 2012, 2013a).

In contrast to other ecological and genetic effects, limited studies have specifically addressed the potential disease interactions between escaped farmed and wild fish (Garseth *et al.*, 2013a,b; Madhun *et al.*, 2015). In one study, analysis of presumed salmon post-smolts found inside the stomachs of wild Atlantic cod (*Gadus morhua*) in Northern Norway revealed that these originated from a fish farm in the vicinity where the cod were captured, and that the post-smolts were infected with piscine

orthoreovirus (Glover *et al.*, 2013b). A more recent investigation has demonstrated that virus-infected escaped salmon entered a river close to the farm of origin (Madhun *et al.*, 2015). While it was not specifically investigated, these escapees could therefore potentially transmit virus to the wild salmonids inhabiting that river. Nevertheless, despite the fact that there have been investigations addressing farmed escapees in rivers, including monitoring programs (Fiske *et al.*, 2006; Anonymous, 2016a; Svenning *et al.*, 2016), in most cases where escaped farmed salmon are observed ascending rivers, their origin, escape history, and infection status are completely unknown.

Pancreas disease (PD) and heart and skeletal muscle inflammation (HSMI) were the most frequently diagnosed viral diseases in Norwegian aquaculture in 2014, with 142 and 181 cases respectively (Hjeltnes et al., 2016). PD is caused by salmonid alphavirus (SAV), which is considered enzootic in Atlantic salmon aquaculture in mid and western Norway. Piscine orthoreovirus (PRV) is the cause of HSMI in Atlantic salmon (Palacios *et al.*, 2010) and occurs in farmed salmon in all farming areas along the Norwegian coast (Kongtorp *et al.*, 2006). Infectious salmon anaemia (ISA) was a major problem in Norwegian aquaculture in the late 1980s, but is now infrequent (1–15 annual cases in the last 5 years). The disease is caused by virulent infectious salmon anaemia virus (ISAV) types (HPRΔ), that are believed to originate by mutations in some genes of the avirulent ISAV (HPR0) variant. Infections with HPR0 ISAV are widespread in Norwegian aquaculture (Lyngstad *et al.*, 2012).

Farmed salmon may escape at different stages during the production cycle. The time of escape influences post-escape behaviour, survival, and ultimately the dispersal patterns of the escapees. Fish that escape during their first summer in net-pens at sea (post-smolt) normally migrate rapidly to the open sea (Skilbrei, 2010) and a small percentage of these return to the coast and enter rivers as maturing adults after 1–3 years on the oceanic feeding areas (Skilbrei *et al.*, 2015a). The migration motivation can be less developed in salmon that escape at an older age. These fish may reside in the fjord in the vicinity of fish farms for weeks and sometimes for several months (Olsen and Skilbrei, 2010; Skilbrei and Jorgensen, 2010). Farmed salmon feed has a high content of lipids of terrestrial origin. These terrestrial lipids are low in typical marine long-chain polyunsaturated fatty acids (PUFAs) and high in medium-chain PUFAs such as 18:2n − 6 (Olsen *et al.*, 2013). Fatty acid profiling is a newly developed and reliable method to distinguish between farmed salmon that have escaped early in the production-cycle as post-smolts, or later on as adults (Skilbrei *et al.*, 2015b). These authors demonstrated that escaped fish with a concentration of 18:2n − 6 fatty acid > 7% are likely to have had escaped within the same year as their capture (Skilbrei *et al.*, 2015b). In contrast, escapees with a concentration of 18:2n − 6 fatty acid < 7% are likely to have had escaped > 1–3 years before their capture. These fish have migrated to the open sea and have been feeding on natural marine food. Thus, the concentration of 18:2n − 6 fatty acid in farmed escapees depends on their age at escape and the time elapsed since their escape (Skilbrei *et al.*, 2015b). Fatty acid analysis of escaped farmed fish has revealed that the majority of escapees entering Norwegian rivers escaped in the same year as their entry to freshwater (Skilbrei *et al.*, 2014, 2015b).

It is generally accepted that escapees represent a threat to the genetic integrity and fitness of wild salmon populations. Therefore, minimizing the number of farmed escapees and genetic interactions with wild conspecifics is regarded as a major challenge to a sustainable aquaculture industry (Taranger *et al.*, 2015). Genetic methods to identify the farm of origin for aquaculture escapees have been successfully developed for Atlantic salmon (*Salmo salar* L.) (Glover *et al.*, 2008; Glover, 2010), rainbow trout (*Oncorhynchus mykiss*) (Glover, 2008) and Atlantic cod (*Gadus morhua*) (Glover *et al.*, 2010). These methods have been routinely implemented by the management authorities in Norway since 2007 in order to identify the farm of origin for escapees in unreported escape events (Glover, 2010). These population genetic methods can also be applied to divide the escapees that ascend a river into distinct genetic groups (Quintela *et al.*, 2016).

In 2013, an upstream fish migration trap, using the Resistance Board Weir system, was installed in the river Etne, Western Norway (Skaala *et al.*, 2015). This river supports one of the largest native populations of Atlantic salmon in this region. The salmon population inhabiting this river has experienced genetic changes through introgression of farmed escapees (Glover *et al.*, 2012, 2013a; Karlsson *et al.*, 2016). The trap, which is in operation throughout the entire upstream migration period for this population is located approximately 500 m from the river mouth and permits sampling the vast majority of the ascending salmon. Consequently, the trapping facility on the river Etne provides a new and unique opportunity for in-depth and multidisciplinary studies of farmed escapees entering freshwater. The aim of this study was to establish a detailed ecological, viral infection and genetic profile of the farmed escapees entering the river Etne throughout the entire season in 2014.

Material and methods

Overall design

Escaped farmed fish captured in the river Etne in 2014 were tested for the occurrence of three viruses that are prevalent in fish farming using real-time Polymerase Chain Reaction (RT-PCR). The escape history of the sampled escapees was investigated using fatty acid profiling to determine whether they were "recent" escaped farmed salmon (i.e. same year as capture in Etne trap) or farmed salmon believed to have escaped at an "early" (i.e. escaped at least one year prior to river entry) age (Skilbrei *et al.*, 2015b). Additionally, genetic clustering (grouping) analysis was used in order to investigate the genetic background and the origin of these escapees. Finally, we attempted to establish the ecological profile of the escapees entering the river by combining the above data, together with morphological data and time of capture throughout the season.

Sampling

All samples used in this study originated from the trap located in the river Etne in the Hardangerfjord, Western Norway (Figure 1). The river Etne is one of several national salmon rivers in Norway where legislation provides the native population with protection against anthropogenic threats, including aquaculture. A total of 168 escaped farmed salmon were captured in the trap in the period between the 28th of April and 19th of November in 2014. The captured escapees represent approximately 90% of the escaped farmed salmon ascending the river in that season (Skaala *et al.*, 2015). Fish were initially classified as either wild salmon or escaped farmed salmon based on their appearance, and the classification was later verified by scale analysis (Lund *et al.*, 1991;

Fiske *et al.*, 2005). The most important criteria for the identification of the fish through scale reading are smolt size, smolt age, and transition from fresh to salt water (Lund *et al.*, 1991). Length and weight of the farmed escapees were recorded. Scale samples and adipose fin clips were collected and used for life-history, genetic, and fatty acid analyses. The head of each escapee was cut behind the pectoral fin and transferred to a freezer in individual plastic bags and kept frozen at −20°C until sampling of tissues for virus testing. Complete sets of samples and information from 129 individuals were available for detailed analysis.

Analyses for viral infection

Tissue samples from the heart ventricle and gills were taken from the head of the fish while still frozen and transferred to tubes on dry ice. RNA was extracted from the heart samples and tested for SAV and PRV, while RNA from gills was tested for ISAV (both HPR0 and HPRΔ) at PatoGen Analyse AS using Real-Time PCR.

Real-time PCR

PatoGen Analyse AS is a real-time PCR analysis company accredited according to International Standard ISO17025. Analyses for SAV virus was done by a PCR assay targeting the nsP1 gene (Hodneland and Endresen, 2006). This assay does not differentiate between the different SAV subtypes (2 and 3) found in Norway (Hjortaas *et al.*, 2013). The PRV assay was performed as it has been previously described (Palacios *et al.*, 2010; Glover *et al.*, 2013b). The *in house* ISAV assay used by PatoGen is designed to target the HE gene and validated for detection of both HPRΔ and HPR0 variants based on sequences from NCBIA (Lyngstad *et al.*, 2012).

Fatty acid (FA) analysis

The adipose fin was used for TAG-derived FA-profiling of the salmon in accordance with the recommendation by Olsen *et al.* (2013), but with some modification of the extraction and the separation methods. Lipids were extracted from the adipose fin of individual fish as previously described (Hara and Radin, 1978) and the total lipid samples were dissolved in chloroform to a lipid concentration of 10 mg/mL and stored at −20 °C until analysis. TAGs were separated from the total lipid by High-Performance Liquid Chromatography (HPLC) on a Agilent, 1260 Infinity analytical/semi-preparative system (Agilent Technologies). Chromatography was performed on a cyanopropyl column (ACE 100 é HPLC Column, ACE 5 µm, CN 125 × 4.6 m) (Advanced Chromatography Technologies, Aberdeen, Scotland). A two channel gradient system was used; Hexane (A) and Chloroform:MEOH (1:1; v/v) (B) and the program started with 90% A and 10% B for 3 min, a linear gradient from 10% B to 100% B for 7 min, 100% B for 8 min, followed by linear gradient from 100% B to 10% B for 1 min. The flow rate was 1.0 mL/min and the column temperature was 30 °C. A 1260 Quaternary pump was used connected to a fraction collector (1260 Preparative-scale) and a 1260 Infinity evaporative light scattering detector (Agilent Technologies) with the evaporation tube at 40 °C and gas pressure was 3.5 bar/51 psi (nitrogen). Twenty microlitres of the lipid extract were injected on the HPLC and the TAG fraction was collected between 1.5 min to 2.7 min after injection. All samples were evaporated to dryness with N_2 (g), the fatty acids were methylated, and the respective fatty acid methyl esters (FAME) were analysed on a HP-7890A gas chromatograph (Agilent) with a flame ionization detector (GC-FID) as described previously (Meier *et al.*, 2006). In total, 61 well-defined peaks in the chromatogram were selected and identified by comparing retention times with a FAME standard (GLC-463 from Nu-Chek Prep) and retention index maps and mass spectral libraries (GC-MS) (www.chrombox.org/index.html) performed under the same chromatographic conditions as the GC-FID (Wasta and Mjos, 2013). Chromatographic peak areas were corrected as required by empirical response factors calculated from the areas of the GLC-463 mixture. The chromatograms were integrated using the EZChrom Elite software (Agilent Technologies).

The escapees were classified into two categories based on the percentage of the fatty acid 18:2n − 6 in the sample (Skilbrei *et al.*, 2015b). Escapees with > 7% 18:2n − 6 were classified as recently escaped farmed salmon (i.e. escaped the same year as capture in the Etne trap), while escapees with ≤ 7% 18:2n − 6 were classified as early escapees (i.e. escaped at least 1 year prior to river entry).

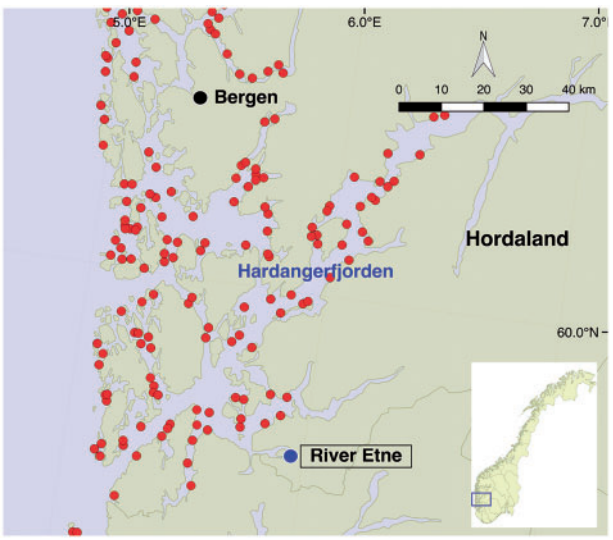

Figure 1. A map showing the location of river Etne (blue circle) and local salmon farms (red circles).

Population genetic analyses of fish

Total genomic DNA was extracted from the adipose fin or alternatively 2–3 fish scales using a 96-well format Qiagen DNeasy blood and tissue kit, following the manufacturer's protocol. Each 96-well plate contained two or more negative controls. The DNA concentration of the extracts was measured for 15 samples on each plate, averaged, and a working dilution for PCR amplification was prepared with a DNA concentration of approximately 15 ng/µL. A total of 31 microsatellite loci were amplified in five different multiplex reactions. PCR products were analysed on an ABI 3730XL Genetic Analyser and sized by a 500LIZ™ size standard. Multiplexes were loaded into the machine in four separate runs, physically mixing PCR products from two of the multiplex reactions before fragment size analysis. Size estimation and scoring of alleles was conducted in GENEMAPPER 5.0, by two persons evaluating the results independently.

Statistical analyses

Analysis of viral infection

Fisher's exact tests were used to compare the prevalence of viruses in different fish groups.

Cluster and kinship analysis of genetic data

We genotyped the escapees in order to determine whether those entering the river Etne in 2014 originated from single or multiple sources. The dataset was explored by cluster (grouping) analysis in the program STRUCTURE 2.3.4 (Pritchard *et al.*, 2000; Falush *et al.*, 2003). For this analysis, only "recently escaped" fish that were determined by fatty acid analysis were used ($N = 118$). This decision was made after pilot analysis of the genetic data for all 129 escapees, in order to split up the fish into genetic groups for the analysis of disease profiles. The separate genetic groups represented by the early escapees had too few individuals in them (only 11 individuals identified as early escapees, see results) in order to compute any quantitatively meaningful comparisons of disease profile. For the STRUCTURE analysis, we used standard settings allowing for admixed individuals and correlated allele frequencies. Burn-in runs were set to 250 000 before conducting 750 000 runs. Ten replicate runs were conducted at each of the K-values explored (1–10), and the results were further analysed in STRUCTURE HARVESTER (Earl and Vonholdt, 2012), before replicate runs were combined in the CLUMPP program (Jakobsson and Rosenberg, 2007). Microsoft Excel was used to construct plots of the merged replicates of individual clustering from CLUMPP. To further explore association between the individual escaped salmon, kinship between individuals was analysed in the program COLONY (Wang, 2004; Jones and Wang, 2010; Wang, 2012), which estimates full- and half-sib relationships between individuals of a sample. Analyses were performed assuming a scoring error rate per locus of 2%, allowing for female and male polygamy, likelihood precision set to "High" and length of run set to "Very long".

Results

General characteristics of the escapees

The 129 escapees examined in the current study were identified as escaped farmed salmon based on body morphology as well as scale analysis. Most of the escaped fish were captured in the upstream fish trap during the period between weeks 33 and 43, with peak in week 42 (Figure 2a). However, they ascended the river in almost all weeks of the entire sampling period (weeks 23–48). The escapees comprised 80 males and 49 females. The average weight and length of the escapees were 3.2 kg (range: 0.6–9.2 kg) and 67.3 cm (range: 40–97 cm), respectively (Figure 2b). The fish had a mean condition factor (CF) of 0.94 (95% CI: 0.90–0.97%), although CF decreased through the sampling period (Figure 2c).

Viral infection status

Of the 129 escapees tested, 81% were positive for one or more viruses. SAV was detected in the heart of 16 (12%), and PRV was detected in 102 (79%) of the escapees. In contrast, avirulent (HPR0) ISAV was detected in the gills in only a single escapee, captured in week 46. Ct-values of SAV-positive escapees ranged from 21.7 to 35.9 (mean 30.4) while the PRV-positive salmon had Ct-values ranging from 22.4 to 36.7 (mean 29.4), indicating low to moderate loads of both viruses (Figure 3a, Supplementary Table S1). The single ISAV positive escapee had a Ct-value of 36.

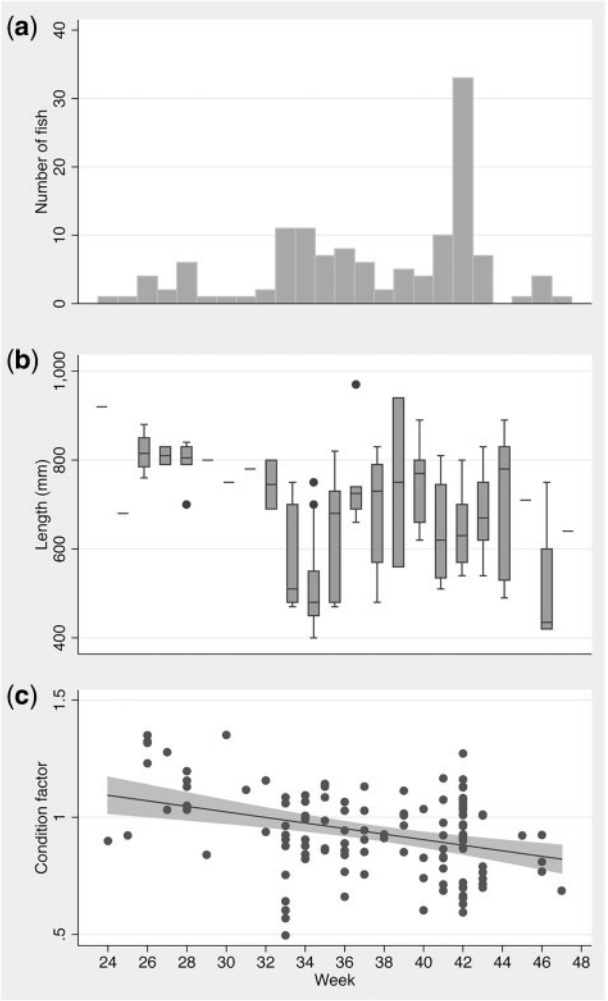

Figure 2. (a) The number, (b) length (graph box with median, 25 and 75 percentile) and (c) condition factor (fitted value and 95% CI) of escaped farmed salmon ascending river Etne, by week of capture.

0. Of the total number of escapees, 24 (19%) were $PRV^{-}SAV^{-}$, 89 (69%) were $PRV^{+}SAV^{-}$, 3 (2%) were $PRV^{-}SAV^{+}$ and 13 (10%) were $PRV^{+}SAV^{+}$. Female escapees had significantly higher prevalence of PRV (90% vs. 73%, $p = 0.025$) and SAV (22% vs. 6%, $p = 0.011$) than male escapees. In contrast, the PRV-positive escapees were captured throughout the trap-operating period (weeks 24–47), the SAV-positive escapees were captured mainly during two periods (weeks 26–28 and 40–42) (Figure 3a).

Fatty acid profile and link to infection status

Based on the fatty acid analysis, 118 (91%) of the escapees captured in the trap were classified as recently escaped adults (i.e. escaped in the same year that they entered the trap), while only 11 (9%) of the fish had escaped at an earlier stage (i.e. > 1 year before entering the trap, probably as post-smolts). While the recently escaped salmon were captured during the whole sampling period (weeks 24–47), all early escapees, except one, appeared during weeks 34–43 (Figure 3b). Male fish dominated both early (82%) and the recently (60%) escaped farmed salmon.

SAV infections were not detected in the early escaped salmon, but occurred in 14% the recently escaped fish. Furthermore, PRV

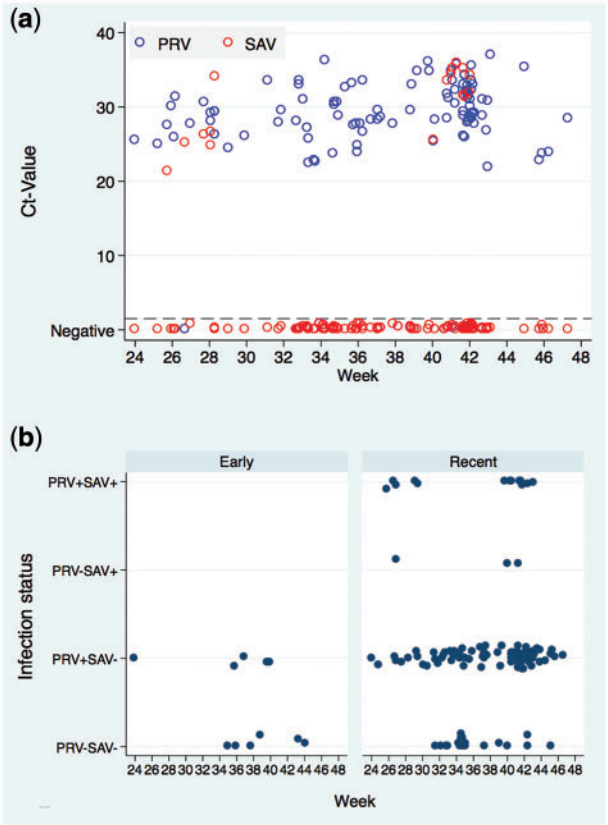

Figure 3. (a) SAV and PRV infections in the escaped farmed salmon represented by the Ct-values from the real-time PCR assay and (b) infection status of the early and recently escapees during collection period. PRV-: PRV-negative, PRV+: PRV-positive, SAV-: SAV-negative, SAV+: SAV-positive.

prevalence was significantly lower in the early (45%) than in the recently (82%) escaped salmon ($p = 0.011$). Whilst the recently escaped salmon included fish from all the infection categories ($PRV^{-}SAV^{-}$, $PRV^{+}SAV^{-}$, $PRV^{-}SAV^{+}$, or $PRV^{+}SAV^{+}$), early escaped fish were either $PRV^{-}SAV^{-}$, $PRV^{+}SAV^{-}$ (Figure 3b, Supplementary Table S1).

Genetic background and link to infection status

The clustering analysis (i.e. placing escapees into genetic groups) performed in STRUCTURE, and subsequent evaluation of the number of genetic groups by Evanno's method implemented in STRUCTURE HARVESTER indicated that two genetic groups ($K = 2$) provided the best fit for the data. However there was also support for $K = 3$ and $K = 4$. Through visual inspection of the generated plots from the replicates merged in CLUMPP, and the distribution of the different genetic groups through the sampling period, we found that $K = 4$ (i.e. dividing the escapees into a minimum of four genetic groups) would provide the most informative representation of the genetic profile of the escapees entering the river (Figure 4). Furthermore, examining the average degree of individual admixture for different values of K, the average admixture was lowest for $K = 4$ (data not shown). Individuals were assigned to the four different groups based on the respective values of q, accepting membership in a cluster only when $q > 0.75$ for that group. Individuals with a value of q below 0.75 in all group were designated as being of uncertain origin, and were assigned to a fifth group for simplicity. The individuals in this group probably originated from a number of different sources, and were not necessarily linked to each other.

Looking at the time distribution of catches of fish classified in genetic groups 1, 4, and 5, we find that they were caught throughout the sampling period (weeks 25–47). Although most of fish from group 3 were captured in the period week 33–35, five individuals from this group were sporadically caught during the following weeks (Figure 5a). In contrast, all the fish assigned to group 2 were caught in weeks 41 and 42. Additionally, the length of fish showed that both groups 2 and 3 generally had a homogeneous length distribution compared to the other groups (Figure 5a).

The results from the kinship analyses in COLONY revealed the presence of a number of full-siblings among the escaped salmon, as well as a high number of likely half-siblings (Supplementary Figure S1). The number of full-sibling pairs varied between the different genetic groups identified by STRUCTURE, and was highest in the groups 2 and 3 with nine and six pairs respectively (Supplementary Figure S2).

The condition factor (CF) of the fish from all the genetic groups except fish from group 2 decreased through the sampling time (Figure 5b).

Although the infection profile varied among the genetic groups, all the genetic groups included virus-infected fish (Figure 6, Supplementary Table S1). Genetic group 1 arrived in the trap during more than 5 months (weeks 26–46). This group contained both PRV (82%) and SAV (14%) infected fish, and a single escapee that was infected with ISAV. Group 1 included escapees

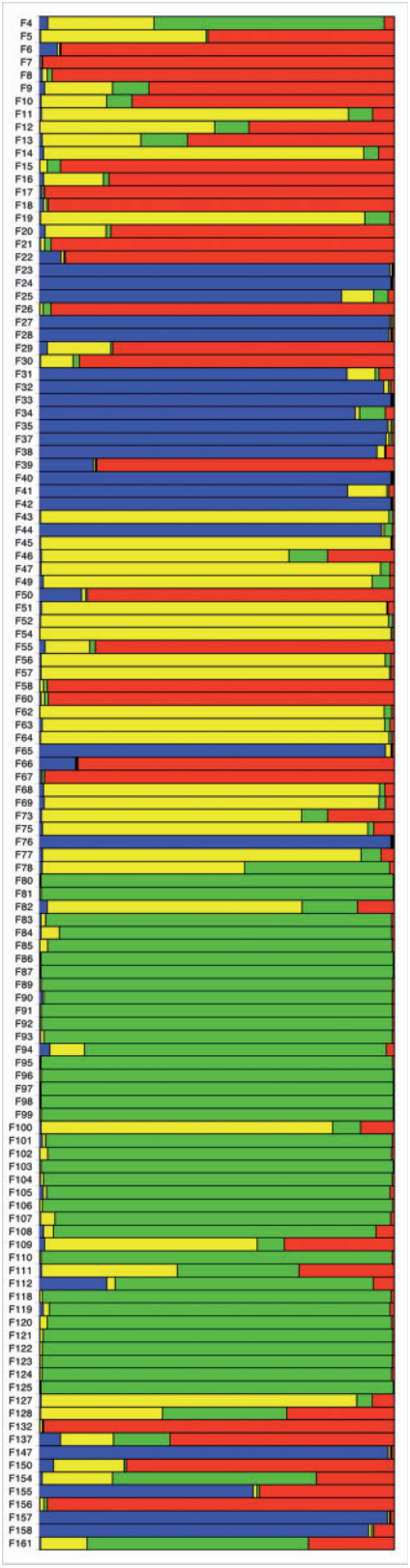

Figure 4. Cluster plots at putative K4 of 118 recently escaped farmed salmon captured in the river Etne. Each individual is represented by a column divided into four colours where each colour represents a genetic group. The genetic groups 1, 2, 3 and 4 are shown in red, green, blue and yellow, respectively.

that were either PRV^{-}SAV^{-}, PRV^{+}SAV^{-}, or PRV^{+}SAV^{+}. The escapees that were assigned to genetic group 2 were only captured in the upstream trap in weeks 41 and 42. This group had fish from the infection categories; PRV^{-}SAV^{-}, PRV^{+}SAV^{-}, PRV^{-}SAV^{+}, or PRV^{+}SAV^{+}. The prevalence of SAV and PRV infection was 9% and 96% respectively. Most of the salmon in genetic group 3 arrived in the trap in weeks 33 and 34. They were either PRV^{-}SAV^{-} or PRV^{+}SAV^{-} (none of them was SAV-positive). PRV prevalence in this group was low (33%) compared to the other groups. The escapees assigned to group 4 arrived in the trap during a period of approximately 4 months (weeks 28–43). The individuals of the group were either PRV^{+}SAV^{-} or PRV^{+}SAV^{+}. All fish were PRV infected, while the prevalence of SAV infections was 14%. The escapees that did not belong to any specific genetic group were placed into the "unassigned cluster", i.e. group 5. These escapees were captured throughout the period (week 25–47). All of the fish in group 5 were virus-infected and were either PRV^{+}SAV^{-}, PRV^{-}SAV^{+}, or PRV^{+}SAV^{+}. The prevalence of PRV and SAV was 89% and 22%, respectively.

Discussion

This is the first study to implement a multidisciplinary approach in order to provide an extensive ecological and genetic profile of farmed Atlantic salmon escapees entering a river throughout an entire migration season. The results can be summarised as follows: (i) 81% of the escapees were infected with one or more viruses, (ii) fatty acid analysis showed that the majority of the fish escaped in the same year that they were captured (recently escaped), (iii) genetic analysis demonstrated that the escapees originated from multiple farm sources, and (iv) the time of capture, size distribution, kinship analysis, and virus infection profile indicated that genetic groups 2 and 3 have probably originated from two recent and distinct escape events.

Collectively, these data illustrate the ecological, genetic and viral-infection diversity of escapees entering a single river in one season. Importantly, these results reinforce our earlier findings (Madhun *et al.*, 2015) that farmed salmon escapees entering rivers represent a source of infectious agents which could potentially lead to transmission of diseases to wild salmonid populations.

The upstream migration trap was installed in the river Etne as a mitigation strategy to reduce the potential for interactions between farmed escapees and wild salmon. This system is effective in capturing the majority of fish entering the river, and provides a powerful sampling platform for multidisciplinary studies on wild salmon and escapees. Most of the escapees ascended the river between weeks 33 and 43 (Figure 2a), however, escapees entered the river in almost all weeks of the entire sampling period (weeks 23–48). Of note is the fact that the condition factor of escapees entering the river decreased with time of ascendance (Figure 2b). This was observed in escapees belonging to almost all of the genetic groups identified from the clustering analysis. This observation may reflect either a natural stop in feeding prior to spawning, or the fact that following escape, farmed escapees typically struggle to find food and will therefore gradually lose weight (Olsen and Skilbrei, 2010; Abrantes *et al.*, 2011).

Most of the escaped salmon (81%) were infected with one or more of the tested viruses; SAV occurred in 12%, PRV in 79% and ISAV (HPR0) in <1% of the fish. Transmission of these viruses may occur among individuals in both sea and freshwater (Nylund *et al.*, 2003; Løvoll *et al.*, 2012; Lyngstad *et al.*, 2012; Cano *et al.*, 2015). It is possible that the escapees studied here

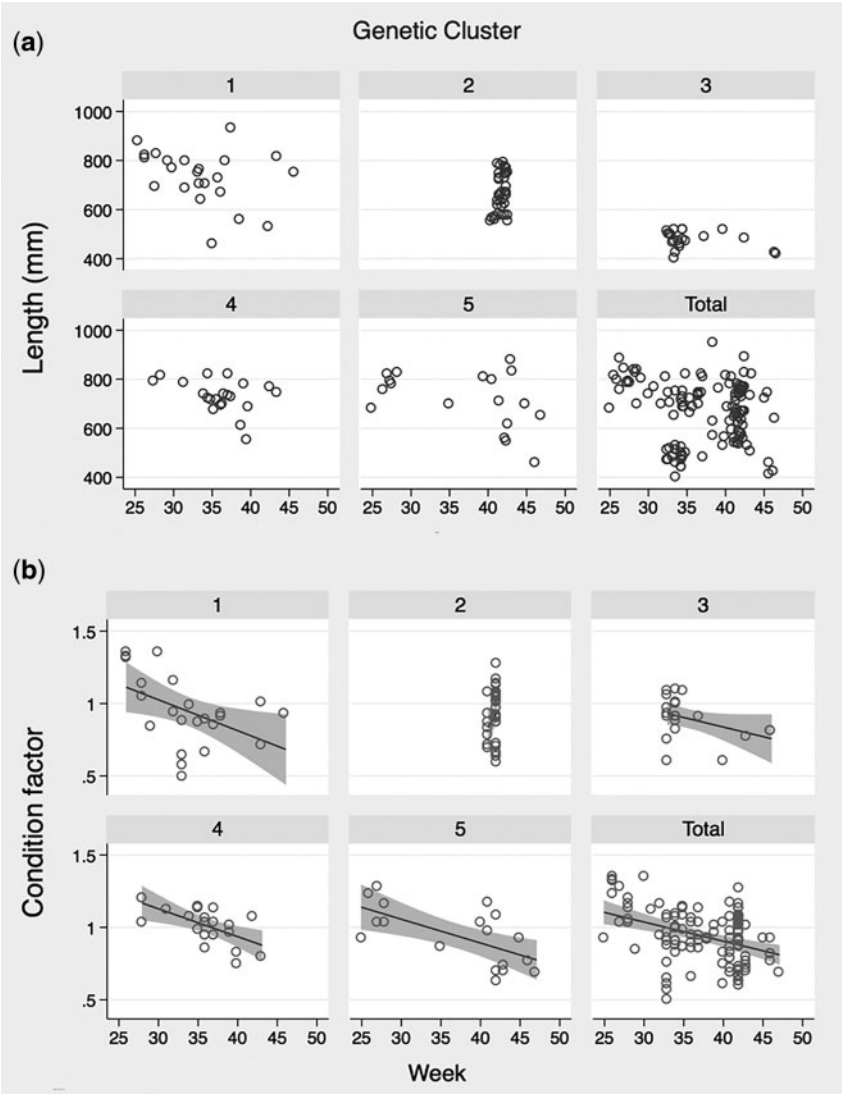

Figure 5. (a) The length distribution of the different genetic clusters and (b) the condition factor (individual CF, the mean fitted value and the 95% confidance interval of the fitted value) of salmon from different genetic clusters.

may also have been infected with other infectious agents that are prevalent in Norwegian salmon farming. While the salmon trap in the river Etne served as an effective installation to remove most of escaped salmon (both infected an non-infected), most rivers have no such trap, and the escaped fish may therefore interact with native salmonids that inhabit these rivers. PRV infection is widespread in wild Atlantic salmon in Norway (Garseth *et al.*, 2013b). Phylogenetic analysis of the sequences of the PRV-positive wild and escaped farmed salmon showed no regional pattern in virus genotypes isolated from the wild and the farmed salmon (Garseth *et al.*, 2013a), suggesting a frequent transmission of the virus between farmed and wild fish. On the other hand, all data gathered so far have not demonstrated the presence of SAV infection in wild salmonid populations in Norway, irrespective of the area of capture, farming intensity or the number of PD outbreaks in fish farming (Biering *et al.*, 2013; Plarre and Nylund, 2014; Garseth *et al.*, 2015; Madhun *et al.*, 2016). Garseth *et al.* (2015) have reported that SAV was detected in one salmon released for stock enhancement. Whether SAV-infected salmon become a life-long carrier of the virus is currently unknown. A major concern is the potential pathogen release in rivers where the survival of potentially exposed juvenile salmonids may be negatively influenced. Therefore, a prolonged river entry by escaped salmon could affect natural recruitment through disease interactions, an area that needs more research.

The salmon that had escaped early were SAV-negative and had a lower prevalence of PRV than the recently escaped adult salmon. This observation may have several explanations: (i) the salmon which escaped early are less likely to be infected with viruses prevalent in salmon farming, (ii) the early escaped fish may have the possibility to clear virus infection later in life, and (iii) the infected salmon (especially SAV-infected) have a lower survival rate than non-infected fish and hence may disappear in nature before they can return to freshwater. Earlier studies have shown that the risk for farmed fish to be infected with viruses increases with increased time in net-pens (Jansen *et al.*, 2010; Jensen *et al.*, 2013; Kristoffersen *et al.*, 2013). Therefore, fish escaping early in the production cycle are less likely to be infected with viruses

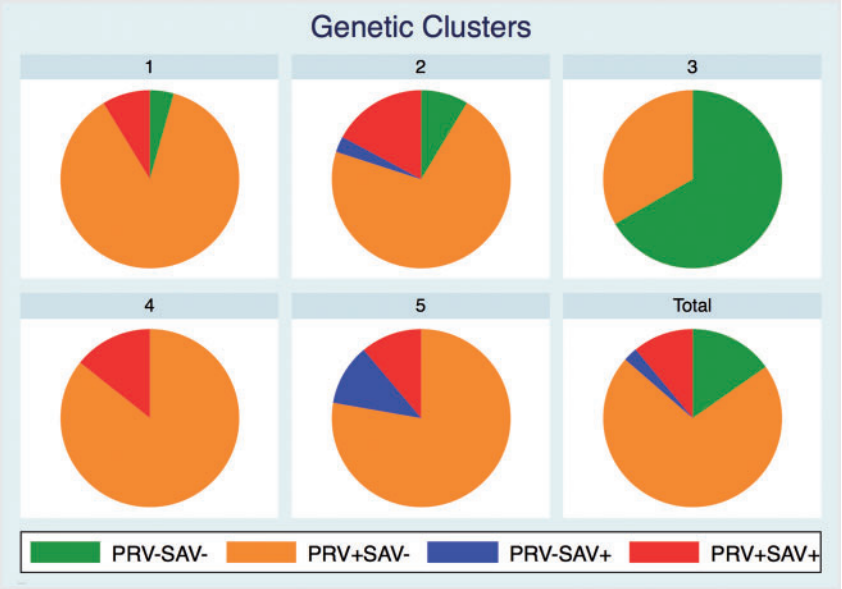

Figure 6. The infection status of the escaped farmed salmon in the genetic clusters. PRV-: PRV-negative, PRV+: PRV-positive, SAV-: SAV-negative, SAV+: SAV-positive.

prevalent in salmon farming. Salmon that survive SAV or PRV infection may become carriers of the virus for several months (Andersen *et al.*, 2007; Graham *et al.*, 2010). However, whether SAV infection in juvenile (post-smolt) escaped salmon can be detected by real-time PCR when they become adults is currently unknown. Further studies to investigate the persistence and longevity of viral infections in salmon are therefore needed.

The recently escaped salmon had a higher PRV prevalence (82%) compared to SAV (14%) despite that both viruses are enzootic in fish farming in the surrounding areas (Kristoffersen *et al.*, 2009; Jansen *et al.*, 2010). That observation, and both the absence of SAV infection and the low prevalence of PRV in the early escaped salmon, suggest that SAV (and to a less extent PRV) infection may have a stronger impact on the ability of escaped salmon to survive in nature and therefore infected fish are less likely to be sampled compared to uninfected fish.

Measuring the level of the fatty acid 18:2n − 6 in escaped fish made it possible to classify farmed salmon into "early" and "recent" escapees as previously described (Skilbrei *et al.*, 2015b). This analytical method was applied to the escapees captured in the trap in Etne throughout the season, and thus enabled us to demonstrate that most of the escapees (91%) analysed in the current study were recently escaped salmon (i.e. they escaped from farms in the same year that they ascended the river). This is consistent with previous reports using this method in other rivers in Norway (Skilbrei *et al.*, 2014, 2015b).

Classification of farmed salmon into "early" and "recent" escapees is important in revealing the ecological background of escapees entering rivers, which in turn has potential implications for interbreeding with wild salmon. Based upon the fact that fish that have experienced most of their life cycle in the wild, as opposed to salmon released from a hatchery or farm, potentially display higher reproductive success (Fleming *et al.*, 1996, 1997), it is likely that early escapees have in general better chances to successfully reproduce with wild salmon than salmon escape from net-pens as adults. Genetic changes in native salmon populations as a consequence of interbreeding between farmed and wild salmon has been documented in a number of rivers in Norway and Ireland (Crozier, 1993; Clifford *et al.*, 1998; Glover *et al.*, 2012, 2013a; Karlsson *et al.*, 2016).

The genetic analysis of the recently escaped salmon revealed that those entering the river Etne in 2014 originated from multiple farm sources. This is similar to results from an investigation of farmed escapees capture in a coastal netting station in Norway where escapes from multiple farms were identified (Zhang et al., 2013), and to a genetic investigation of farmed escapees entering the trapping facility on the river Etne in 2013 (Quintela *et al.*, 2016).

In the present study, recently escaped fish were assigned to four distinct genetic groups, which are likely to represent four or more sources. In addition, 18 of the escapees did not belong to any of the four genetic groups. These 18 fish probably originated from multiple sources, with varying degrees of overlapping genetic compositions, and were therefore not identified as belonging to any single group. A combination of the timing of entry to freshwater, genetic clustering data, kinship analysis, fish size, and infection status of the recently escaped salmon entering the river Etne, indicate that the escapees identified to groups 2 and 3 were from two distinct recent escape events (i.e., two farms have lost fish, and this resulted in two distinct "pulses" of escapees into the river Etne, Figure 5a). In contrast, escapees identified to groups 1 and 4 appeared to be more heterogeneous, and may reflect fish from multiple sources with similar genetic backgrounds (i.e. multiple farms rearing the same breed of fish). The current results show the extent of genetic diversity of farmed escaped salmon ascending a river and may contribute to the observation reported earlier that introgression of farmed salmon tends to increase the microsatellite allelic diversity of the native population in the short-term (Glover *et al.*, 2012).

Based upon this multi-disciplanary study, we conclude that the escapees entering the river Etne in 2014 were diverse in escape history and genetic background, and were frequently

virus-infected. These findings have potential policy and management implications.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Acknowledgements

We would like to dedicate this paper in the honour of Ove T. Skilbrei who tragically died while this work was being completed. Through his work, he provided us with unique and important insights into salmon ecology, and the post-escape behaviour of farmed escapees. We acknowledge the efforts of the technicians operating the trap facility in the River Etne, and the local river owners association for their support. This specific study was jointly-funded using resources from the Ministry of Trade, Industry and Fisheries, Norwegian fish farmers, the Hordaland County Governor, the Norwegian Environment Agency, and the Directorate of Fisheries.

References

Abrantes, K. G., Lyle, J. M., Nichols, P. D., and Semmens, J. M. 2011. Do exotic salmonids feed on native fauna after escaping from aquaculture cages in Tasmania, Australia? Canadian Journal of Fisheries and Aquatic Sciences, 68: 1539–1551.

Andersen, L., Bratland, A., Hodneland, K., and Nylund, A. 2007. Tissue tropism of salmonid alphaviruses (subtypes SAV1 and SAV3) in experimentally challenged Atlantic salmon (*Salmo salar* L.). Archives of Virology, 152: 1871–1883.

Anonymous. 2016a. Escaped farmed salmon i rivers (in Norwegian). Report of the national monitoring program 2015. Institute of Marine Research. 2b-2016. Available at http://www.imr.no/filarkiv/2016/04/sernummer_2b_-web.pdf_1/nb-no.

Anonymous 2016b. Number of escaped farmed fish (in Norwegian). Directorate of Fisheries: 01.06.2016, Available at http://www.fiskeridir.no/statistikk/akvakultur/oppdaterte-roemmingstall.

Biering, E., Madhun, A., Isachsen, C., Omdal, L., Einen, A., Garseth, é., Bjørn, P., *et al.* 2013. Annual report on health monitoring of wild anadromous salmonids in Norway 2012. Institute of Marine Research. 6-2013: 13 pp. Available at http://www.imr.no/filarkiv/2013/03/annual_report_on_health_monitoring_of_wild_anadromous_salmonids_in_norway_rapport_fra_havforskningen_nr._6-2013_.pdf/nb-no.

Cano, I., Joiner, C., Bayley, A., Rimmer, G., Bateman, K., Feist, S. W., Stone, D., *et al.* 2015. An experimental means of transmitting pancreas disease in Atlantic salmon *Salmo salar* L. fry in freshwater. Journal of Fish Diseases, 38: 271–281.

Clifford, S. L., McGinnity, P., and Ferguson, A. 1998. Genetic changes in Atlantic salmon (*Salmo salar*) populations of northwest Irish rivers resulting from escapes of adult farm salmon. Canadian Journal of Fisheries and Aquatic Sciences, 55: 358–363.

Crozier, W. W. 1993. Evidence of genetic interaction between escaped farmed salmon and wild Atlantic salmon (*Salmo-salar* L) in a northern Irish river. Aquaculture, 113: 19–29.

Earl, D. A., and Vonholdt, B. M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4: 359–361.

Falush, D., Stephens, M., and Pritchard, J. K. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics, 164: 1567–1587.

Fiske, P., Lund, R. A., and Hansen, L. P. 2005. Identifying fish farm escapees. *In* Stock Identification Methods—Applications in Fishery Science, pp. 659–680. Ed. by S. X. Cadrin, K. D. Friedland, and J. R. Waldman. Elsevier Academic Press, San Diego.

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 Journal of Marine Science, 63: 1182–1189.

Fleming, I. A., Jonsson, B., Gross, M. R., and Lamberg, A. 1996. An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo salar*). Journal of Applied Ecology, 33: 893–905.

Fleming, I. A., Lamberg, A., and Jonsson, B. 1997. Effects of early experience on the reproductive performance of Atlantic salmon. Behavioral Ecology, 8: 470–480.

Garseth, Å. H., Ekrem, T., and Biering, E. 2013a. Phylogenetic evidence of long distance dispersal and transmission of piscine reovirus (PRV) between farmed and wild Atlantic salmon. PLOS One, 8: e82202.

Garseth, Å. H., Fritsvold, C., Opheim, M., Skjerve, E., and Biering, E. 2013b. Piscine reovirus (PRV) in wild Atlantic salmon, *Salmo salar* L., and sea-trout, *Salmo trutta* L., in Norway. Journal of Fish Diseases, 36: 483–493.

Garseth, Å. H., Madhun, A. S., Biering, E., Isachsen, C. H., Fiksdal, I., Einen, A. C., Barlaup, B., *et al.* 2015. Annual report on health monitoring of wild anadromous salmonids in Norway 2014. Institute of Marine Research. 7-2015: 14 pp. Available at https://www.imr.no/filarkiv/2015/04/helseovervaking_av_vill_laksefisk_i_norge_2015.pdf/nb-no.

Glover, K. A. 2008. Genetic characterisation of farmed rainbow trout in Norway: intra- and inter-strain variation reveals potential for identification of escapees. BMC Genetics, 9: 87.

Glover, K. A. 2010. Forensic identification of fish farm escapees: the Norwegian experience. Aquaculture Environment Interactions, 1: 1–10.

Glover, K. A., Dahle, G., Westgaard, J. I., Johansen, T., Knutsen, H., and Jorstad, K. E. 2010. Genetic diversity within and among Atlantic cod (*Gadus morhua*) farmed in marine cages: a proof-of-concept study for the identification of escapees. Animal Genetics, 41: 515–522.

Glover, K. A., Pertoldi, C., Besnier, F., Wennevik, V., Kent, M., and Skaala, Ø. 2013a. Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNPs. BMC Genetics, 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: e43129.

Glover, K. A., Skilbrei, O. T., and Skaala, Ø. 2008. Genetic assignment identifies farm of origin for Atlantic salmon *Salmo salar* escapees in a Norwegian fjord. ICES Journal of Marine Science, 65: 912–920.

Glover, K. A., Sørvik, A. G., Karlsbakk, E., Zhang, Z., and Skaala, Ø. 2013b. Molecular genetic analysis of stomach contents reveals wild Atlantic cod feeding on piscine reovirus (PRV) infected Atlantic salmon originating from a commercial fish farm. PLOS One, 8: e60924.

Graham, D. A., Fringuelli, E., Wilson, C., Rowley, H. M., Brown, A., Rodger, H., McLoughlin, M. F., *et al.* 2010. Prospective longitudinal studies of salmonid alphavirus infections on two Atlantic salmon farms in Ireland; evidence for viral persistence. Journal of Fish Diseases, 33: 123–135.

Hansen, L. P. 2006. Migration and survival of farmed Atlantic salmon (*Salmo salar* L.) released from two Norwegian fish farms. ICES Journal of Marine Science, 63: 1211–1217.

Hara, A., and Radin, N. S. 1978. Lipid extraction of tissues with a low-toxicity solvent. Analytical Biochemistry, 90: 420–426.

Hjeltnes, B., Walde, C. S., Jensen, B. B., and Haukaas, A. and (reds). 2016. Fish health report 2015 (in Norwegian). 3/2016: 38 pp.

Available at http://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2016/fiskehelserapporten-2015.

Hjortaas, M. J., Skjelstad, H. R., Taksdal, T., Olsen, A. B., Johansen, R., Bang-Jensen, B., Orpetveit, I., *et al.* 2013. The first detections of subtype 2-related salmonid alphavirus (SAV2) in Atlantic salmon, *Salmo salar* L., in Norway. Journal of Fish Diseases, 36: 71–74.

Hodneland, K., and Endresen, C. 2006. Sensitive and specific detection of salmonid alphavirus using real-time PCR (TaqMan (R)). Journal of Virological Methods, 131: 184–192.

Jakobsson, M., and Rosenberg, N. A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics, 23: 1801–1806.

Jansen, M. D., Taksdal, T., Wasmuth, M. A., Gjerset, B., Brun, E., Olsen, A. B., Breck, O., *et al.* 2010. Salmonid alphavirus (SAV) and pancreas disease (PD) in Atlantic salmon, *Salmo salar* L., in freshwater and seawater sites in Norway from 2006 to 2008. Journal of Fish Diseases, 33: 391–402.

Jensen, B. B., Brun, E., Fineid, B., Larssen, R. B., and Kristoffersen, A. B. 2013. Risk factors for cardiomyopathy syndrome (CMS) in Norwegian salmon farming. Diseases of Aquatic Organisms, 107: 141–150.

Jones, O. R., and Wang, J. L. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources, 10: 551–555.

Jonsson, B., and Jonsson, N. 2006. Cultured Atlantic salmon in nature: a review of their ecology and interaction with wild fish. ICES Journal of Marine Science, 1162–1181.

Karlsson, S., Diserud, O. H., Fiske, P., and Hindar, K. 2016. Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. ICES Journal of Marine Science. doi:10.1093/icesjms/fsw121.

Kongtorp, R. T., Halse, M., Taksdal, T., and Falk, K. 2006. Longitudinal study of a natural outbreak of heart and skeletal muscle inflammation in Atlantic salmon, Salmo salar L. Journal of Fish Diseases, 29: 233–244.

Kristoffersen, A. B., Bang Jensen, B., and Jansen, P. A. 2013. Risk mapping of heart and skeletal muscle inflammation in salmon farming. Preventive Veterinary Medicine, 109: 136–143.

Kristoffersen, A. B., Viljugrein, H., Kongtorp, R. T., Brun, E., and Jansen, P. A. 2009. Risk factors for pancreas disease (PD) outbreaks in farmed Atlantic salmon and rainbow trout in Norway during 2003-2007. Preventive Veterinary Medicine, 90: 127–136.

Løvoll, M., Alarcon, M., Jensen, B. B., Taksdal, T., Kristoffersen, A. B., and Tengs, T. 2012. Quantification of piscine reovirus (PRV) at different stages of Atlantic salmon *Salmo salar* production. Diseases of Aquatic Organisms, 99: 7–U5.

Lund, R. A., Okland, F., and Hansen, L. P. 1991. Farmed Atlantic Salmon (*Salmo-Salar*) in Fisheries and Rivers in Norway. Aquaculture, 98: 143–150.

Lyngstad, T. M., Kristoffersen, A. B., Hjortaas, M. J., Devold, M., Aspehaug, V., Larssen, R. B., and Jansen, P. A. 2012. Low virulent infectious salmon anaemia virus (ISAV-HPR0) is prevalent and geographically structured in Norwegian salmon farming. Diseases of Aquatic Organisms, 101: 197–206.

Madhun, A. S., Isachsen, C. H., Omdal, L. M., Bardsgjaere Einen, A. C., Bjørn, P. A., Nilsen, R., and Karlsbakk, E. 2016. Occurrence of salmonid alphavirus (SAV) and piscine orthoreovirus (PRV) infections in wild sea trout *Salmo trutta* in Norway. Diseases of Aquatic Organisms, 120: 109–113.

Madhun, A. S., Karlsbakk, E., Isachsen, C. H., Omdal, L. M., Eide Sorvik, A. G., Skaala, O., Barlaup, B. T., *et al.* 2015. Potential disease interaction reinforced: double-virus-infected escaped farmed Atlantic salmon, *Salmo salar* L., recaptured in a nearby river. Journal of Fish Diseases, 38: 209–219.

Meier, S., Mjos, S. A., Joensen, H., and Grahl-Nielsen, O. 2006. Validation of a one-step extraction/methylation method for determination of fatty acids and cholesterol in marine tissues. Journal of Chromatography A, 1104: 291–298.

Nylund, A., Plarre, H., Hodneland, K., Devold, M., Aspehaug, V., Aarseth, M., Koren, C., *et al.* 2003. Haemorrhagic smolt syndrome (HSS) in Norway: pathology and associated virus-like particles. Diseases of Aquatic Organisms, 54: 15–27.

Olsen, R. E., and Skilbrei, O. T. 2010. Feeding preference of recaptured Atlantic salmon Salmo salar following simulated escape from fish pens during autumn. Aquaculture Environment Interactions, 1: 167–174.

Olsen, R. E., Taranger, G. L., Svåsand, T., and Skilbrei, O. T. 2013. Improved method for triacylglycerol-derived fatty acid profiling by various non-lethal and lethal sampling techniques in Atlantic salmon. Aquaculture Environment Interactions, 4: 251–261.

Palacios, G., Lovoll, M., Tengs, T., Hornig, M., Hutchison, S., Hui, J., Kongtorp, R. T., *et al.* 2010. Heart and Skeletal Muscle Inflammation of Farmed Salmon Is Associated with Infection with a Novel Reovirus. PLoS One, 5: e11487.

Plarre, H., and Nylund, A. 2014. Project Report: "The occurance of SAV 2 in wild salmonids in mid-Norway" (in Norwegian). University of Bergen, 2013/7835-25062013: 25. pp.

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945–959.

Quintela, M., Wennevik, V., Sørvik, A. G. E., Skaala, Ø., Skilbrei, O. T., Urdal, K., Barlaup, B. T., *et al.* 2016. Siblingship tests connect two seemingly independent farmed Atlantic salmon escape events together. Aquaculture Environment Interactions, 8: 497–509.

Saegrov, H., and Urdal, K. 2006. Escaped farmed salmon in the sea and rivers; abundance and origin (in Norwegian). Rådgivende Biologer, 947: 26.

Skaala, Ø., Knutar, S., Østebø, B. I., Holmedal, T. E., Skilbrei, O. T., Madhun, A. S., Barlaup, B., *et al.* 2015. Experiances with Resistance Board Weir salmon capture system in river Etne 2013–2014 (in Norwegian). Institute of Marine Research, 6-2015: 22. pp. Availabe at http://www.imr.no/filarkiv/2015/06/nr._6-2015_erfaringar_med_rbw-fangstsystem_etne.pdf/nb-no.

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

Skilbrei, O. T. 2010. Adult recaptures of farmed Atlantic salmon post-smolts allowed to escape during summer. Aquaculture Environment Interactions, 1: 147–153.

Skilbrei, O. T., Heino, M., and Svåsand, T. 2015a. Using simulated escape events to assess the annual numbers and destinies of escaped farmed Atlantic salmon of different life stages from farm sites in Norway. ICES Journal of Marine Science, 72: 670–685.

Skilbrei, O. T., Holst, J. C., Asplin, L., and Mortensen, S. 2010. Horizontal movements of simulated escaped farmed Atlantic salmon (*Salmo salar*) in a western Norwegian fjord. ICES Journal of Marine Science, 67: 1206–1215.

Skilbrei, O. T., and Jorgensen, T. 2010. Recapture of cultured salmon following a large-scale escape experiment. Aquaculture Environment Interactions, 1: 107–115.

Skilbrei, O. T., Normann, E., Meier, S., and Olsen, R. E. 2015b. Use of fatty acid profiles to monitor the escape history of farmed Atlantic salmon. Aquaculture Environment Interactions, 7: 1–13.

Skilbrei, O. T., Skulstad, O. F., and Hansen, T. 2014. The production regime influences the migratory behaviour of escaped farmed Atlantic salmon. Aquaculture, 424: 146–150.

Svenning, M. A., Lamberg, A., Dempson, B., Strand, R., Hanssen, Ø. K., and Fauchald, P. 2016. Incidence and timing of wild and

escaped farmed Atlantic salmon (*Salmo salar*) in Norwegian rivers inferred from video surveillance monitoring. Ecology of Freshwater Fish. doi: 10.1111/eff.12280.

Taranger, G. L., Karlsen, O., Bannister, R. J., Glover, K. A., Husa, V., Karlsbakk, E., Kvamme, B. O., *et al.* 2015. Risk assessment of the environmental impact of Norwegian Atlantic salmon farming. ICES Journal of Marine Science, 72: 997–1021.

Wang, J. L. 2004. Monitoring and managing genetic variation in group breeding populations without individual pedigrees. Conservation Genetics, 5: 813–825.

Wang, J. L. 2012. Computationally efficient sibship and parentage assignment from multilocus marker data. Genetics, 191: 183–194.

Wasta, Z., and Mjos, S. A. 2013. A database of chromatographic properties and mass spectra of fatty acid methyl esters from omega-3 products. Journal of Chromatography A, 1299: 94–102.

Zhang, Z., Glover, K. A., Wennevik, V., Svåsand, T., Sørvik, A. G. E., Fiske, P., Karlsson, S., *et al.* 2013. Genetic analysis of Atlantic salmon captured in a netting station reveals multiple escapement events from commercial fish farms. Fisheries Management and Ecology, 20: 42–51.

Handling editor: Fabrice Pernet