



Potential disease interaction reinforced: double-virus-infected escaped farmed Atlantic salmon, *Salmo salar* L., recaptured in a nearby river

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Abstract

The role of escaped farmed salmon in spreading infectious agents from aquaculture to wild salmonid populations is largely unknown. This is a case study of potential disease interaction between escaped farmed and wild fish populations. In summer 2012, significant numbers of farmed Atlantic salmon were captured in the Hardangerfjord and in a local river. Genetic analyses of 59 of the escaped salmon and samples collected from six local salmon farms pointed out the most likely source farm, but two other farms had an overlapping genetic profile. The escapees were also analysed for three viruses that are prevalent in fish farming in Norway. Almost all the escaped salmon were infected with salmon alphavirus (SAV) and piscine reovirus (PRV). To use the infection profile to assist genetic methods in identifying the likely farm of origin, samples from the farms were also tested for these viruses. However, in the current case, all the three farms had an infection profile that was similar to that of the escapees. We have shown that double-virus-infected escaped salmon ascend a river close to the likely source farms, reinforcing the potential for spread of viruses to wild salmonids.

Keywords: aquaculture, escapees, piscine reovirus, salmon alphavirus, virus transmission.

Introduction

The most significant challenges associated with the commercial farming of Atlantic salmon, *Salmo salar* L., in marine net pens are escapees and disease outbreaks. In Norway, the annual reported numbers of farmed Atlantic salmon escapees have been in the hundreds of thousands for most years in the period 2000–2011 (Anonymous 2012). However, the true annual number of escapees has been estimated to be in the millions due to underreporting (Saegrov & Urdal 2006). Escaped Atlantic salmon can disperse over long distances (Hansen 2006; Skilbrei & Jorgensen 2010; Skilbrei *et al.* 2010), may enter rivers (Fiske, Lund & Hansen 2006) and can display a range of ecological (Jonsson & Jonsson 2006) and genetic interactions (Crozier 1993; Clifford, McGinnity & Ferguson 1998; Skaala, Wennevik & Glover 2006; Glover *et al.* 2012, 2013a) with wild conspecifics. Genetic interactions have received considerable scientific attention over the past 2–3 decades, and it is now accepted that escapees represent a threat to the genetic integrity of native populations. In contrast to genetic interactions, potential disease interactions between escaped farmed and wild fish have so far been poorly documented. One exception to this is a recent investigation, which demonstrated that piscine reovirus (PRV)-infected Atlantic salmon that were either discarded dead or escaped from a fish farm in Northern Norway were consumed by wild

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Atlantic cod *Gadus morua* L. (Glover *et al.* 2013b). Although the subsequent infection status in the cod population was not investigated, the study clearly demonstrated the potential for disease interactions between aquaculture fish and native fish in the natural environment.

Viral diseases represent a serious problem in fish farming, causing huge economical losses. Pancreas disease (PD), caused by salmonid alphavirus (SAV), is a major health problem for fish farming in Norway with 137 registered outbreaks in 2012 (Johansen 2013). Most of the disease outbreaks occur in Western Norway, and especially in the Hardangerfjord. Heart and skeletal muscle inflammation (HSMI) is another disease that is associated with a recently discovered virus, piscine reovirus (PRV). The role of this virus in HSMI is still unclear. The disease is a problem in fish farming in Norway with 142 outbreaks registered in 2012. Most of the disease outbreaks occur in middle and northern Norway. The virus has been detected in wild salmon and sea trout, *Salmo trutta* L., as well as certain marine fish species by real-time PCR (Wiik-Nielsen *et al.* 2012; Garseth *et al.* 2013).

Genetic methods to identify the farm of origin for aquaculture escapees have been successfully

developed for Atlantic salmon (Glover, Skilbrei & Skaala 2008; Glover 2010), rainbow trout (*Oncorhynchus mykiss*) (Glover 2008) and Atlantic cod (Glover *et al.* 2010). These methods are now routinely implemented by the management authorities in Norway to identify the farm of origin for escapees in unreported escape events (Glover 2010). Genetic methods are often able to identify a single farm of origin for the unreported escapees. However, in some cases, two or more farms under suspicion may contain fish that are genetically very similar. Consequently, there is also a need for alternative, non-genetic identification methods to supplement the analyses. Given that infection histories for groups of fish being reared on separate farms may differ, 'infection profiling' has the potential to supplement genetic analysis to help identify the farm of origin.

The present study is based upon a case investigation of unreported escape of Atlantic salmon from the Hardangerfjord located in Western Norway in 2012. Following reports of large numbers of small escaped farmed Atlantic salmon in the middle area of the fjord (Fig. 1), the Norwegian Directorate of Fisheries (NDF), responsible for

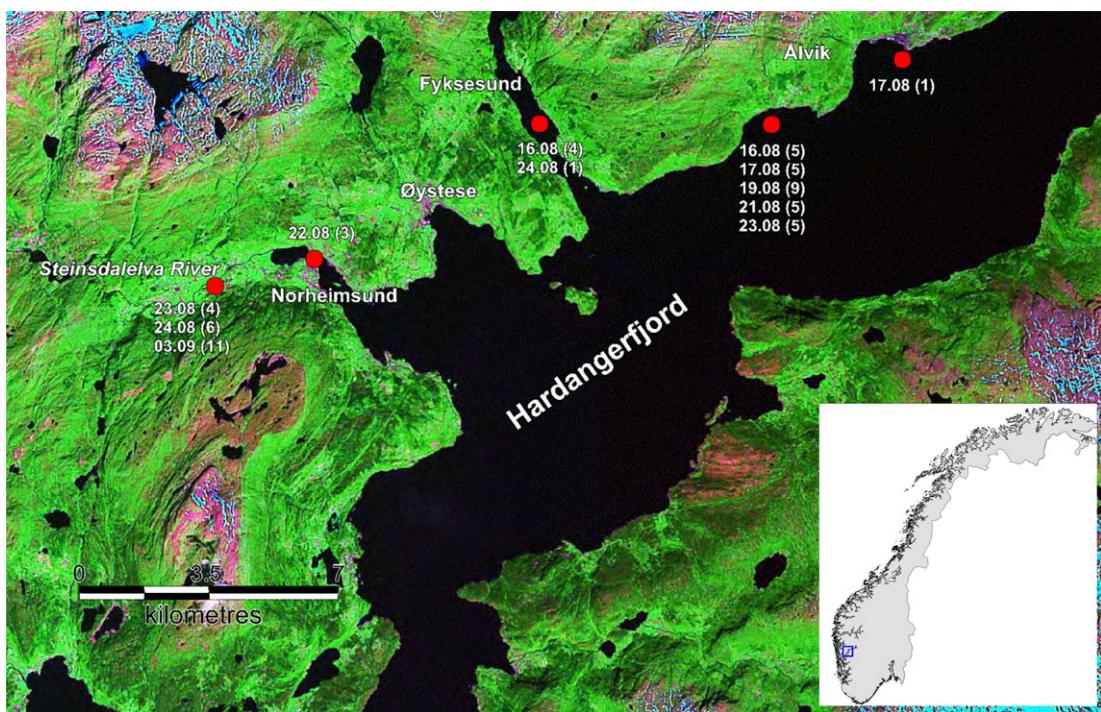


Figure 1 Map showing the sites where escaped salmon were captured. The date of capture and the number of fish (in brackets) are also shown. The locations of suspected farms were kept anonymous.

implementation of fisheries and aquaculture regulation in Norway, decided to collect samples of escapees and from salmon farms in the local area. These samples were given to the Institute of Marine Research (IMR) who conducted genetic analyses in order to identify the farm of origin according to established protocols. In addition to the standard genetic analyses, the escapees and fish from potential source farms were tested for three of the most prevalent viruses in Norwegian aquaculture.

Materials and methods

In the period between 16.08.2012 and 03.09.2012, a total of 59 escaped salmon were captured by local fishermen in the area around Alvik–Norheimsund in West of Norway (Fig. 1). Twenty-one of these salmon were captured in a nearby river, Steinsdalselva. All river-captured salmon were caught by angling, while seven of the 38 sea-caught fish were angled (all in estuaries, Norheimsund, Fyksesund). The rest of the salmon were caught with passive equipment, that is, 3 in 45-mm gill-nets and 28 in a custom-made trap net (Barlaup *et al.* 2013).

After having contacted all farms in the region that reared salmon overlapping in size with the escapees, the source of the escaped fish was not revealed. Therefore, the NDF collected samples of the escapees captured by the local fishermen and samples from farms in the region to identify the source of the escapees using DNA-based method (Glover *et al.* 2008; Glover 2010). In total, samples were collected from 13 cages located on six commercial farms. Each cage sample consisted of adipose fin clips from approximately 47 fish. For farms with more than one genetic group or type of fish, a cage sample representing each group was taken. Samples were given the numbers 1A for the first sampled cage on farm 1, and 1B for the second cage sampled on farm one, etc. The preliminary results showed that fish from cages 4A and 4B were genetically identical, and therefore, the results from these two cages were combined (assigned as 4A/B). The samples are collectively referred to as the baseline samples and represent the potential sources of the escapees. For legal reasons, the exact location of the local fish farms from which the different samples were taken is kept anonymous.

Molecular genetic analyses of fish

DNA extraction was conducted in 96-well format using a commercially available kit (Qiagen DNeasy[®] 96 Blood & Tissue Kit). Each 96-well plate included two blank wells as negative controls. Routine genotyping control plays a standard role in genotyping in the laboratory at IMR (Glover 2010; Haaland *et al.* 2011). Thus, each of the individual escapees was isolated twice to control genotyping consistency.

All fish samples were subject to genotyping with a set of 18 microsatellites that are routinely used in the laboratory at IMR for Atlantic salmon genetics projects (Glover 2010; Zhang *et al.* 2013). These loci were amplified in three multiplexes, using standard protocols (full genotyping conditions available from authors upon request): *SSsp3016* (GenBank no. AY372820), *SSsp2210*, *SSspG7*, *SSsp2201*, *SSsp1605*, *SSsp2216* (Paterson *et al.* 2004), *Ssa197*, *Ssa171*, *Ssa202* (O'Reilly *et al.* 1996), *SsaD157*, *SsaD486*, *SsaD144* (King, Eackles & Letcher 2005), *Ssa289*, *Ssa14* (McConnell *et al.* 1995), *SsaF43* (Sanchez *et al.* 1996), *SsaOsl85* (Slettan, Olsaker & Lie 1995), MHC I (Grimholt *et al.* 2002) and MHC II (Stet *et al.* 2002). PCR products were analysed on an ABI 3730 Genetic Analyser and sized by a 500LIZTM size-standard. The raw data were controlled manually twice before export for statistical analysis. No genotyping inconsistencies were observed among these re-analysed samples.

Analyses for viral infections

The captured escaped fish were frozen (-20°C) as soon as possible and kept frozen until sampling day. A tissue sample from the heart ventricle was aseptically taken out from 58 fish while still frozen and transferred to tubes on dry ice (one river-caught fish was excluded because it was received gutted and the heart had been removed). After thawing, the fish was visually inspected for external or internal pathologies or signs of disease. Additionally, length, weight, sex of the fish was recorded, and stomach and intestine contents were examined.

After obtaining the results of genetic analyses from the escaped fish and the suspected farms, we received permission to access the management data of three of the fish farms. These fish farms were selected based on the genetic analysis (see above and results). The management data have

shown that the three farms had signs of pancreas disease (lethargy, appetite drop and altered swimming behaviour) and increased fish mortality and that samples from fish were sent to the Norwegian Veterinary Institute for routine disease diagnosis. The samples were taken at site by authorized fish health personnel. Tissues taken from recently dead fish or fish that had clinical signs of disease were collected in RNAlater® (Ambion® Inc.) or in formalin. The source of farm samples, the organs collected and the date of sampling are shown in Table 1. The cage numbers where samples were taken were not specified in the management reports. The RNAlater® samples were used in real-time PCR, and formalin-fixed tissues were used for histopathological examination. After a request from the Norwegian Directorate of Fisheries, these RNAlater® samples were further tested for piscine reovirus (PRV) and piscine myocarditis virus (PMCV) at the Norwegian Veterinary Institute using real-time PCR.

The frozen heart samples from the escapees were tested for SAV, PRV and PMCV 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 ISO 17025. The Norwegian Veterinary Institute is a national reference and accredited laboratory.

The SAV assay, targeting the nsP1 gene, was conducted as previously described (Hodneland & Endresen 2006). The current SAV assay does not differentiate between the different salmon alphavirus subtypes (2 and 3) found in Norway (Hjortaas *et al.* 2013). The PRV assay used was as previously described (Palacios *et al.* 2010; Glover *et al.* 2013b). The PMCV PCR analyses of samples were carried out as previously published Løvoll *et al.* (2010), (Haugland *et al.* 2011). A cut-off C_t -value of 37.0 was used for all the assays conducted at PatoGen Analyse AS.

Normalized expression (NE) of viral RNA in escaped salmon was calculated based on the C_t -values for virus and elongation factor (Elf1 α), and the efficacy (E) of the real-time PCR assays using the formula: $NE = E^{C_t}(\text{Elf1}\alpha)/E^{C_t}(\text{virus})$. The standard curves of real-time PCR based on tenfold dilutions in triplicates were produced. The curves had slopes of -3.26 ($R^2 0.986$), -3.25 ($R^2 0.998$) and -3.49 ($R^2 0.996$) for SAV, PRV and PMCV, respectively. The efficiencies ($E = 10^{-1/\text{slope}}$) were 2.028 (SAV), 2.030 (PRV) and 1.934 (PMCV).

Statistical analyses

Analyses to identify farm of origin. The statistical analyses upon which the farm of origin is identified for farmed escapees have been extensively documented (Glover *et al.* 2008, 2013b; Glover 2010; Zhang *et al.* 2013), and technical details surrounding these statistical analyses are therefore not repeated in detail here. Focal elements of the method include comparing the genetic profile of each individual escapee with the genetic profiles for each of the baseline samples to identify which of the potential sources each escapee resembles the most and to identify which of the potential sources the escapee can be excluded from at a given statistical probability. To achieve this, a complementary range of population genetics-based statistical approaches are implemented using several programs including MSA (Dieringer & Schlotterer 2003), Genepop v3.3 (Raymond & Rousset 1995), ParallelStructure (Besnier & Glover 2013) STRUCTURE 2.2 (Pritchard, Stephens & Donnelly 2000; Falush, Stephens & Pritchard 2003) and GeneClass2 (Piry *et al.* 2004). However, only analyses arising from the latter two programs are presented in the results of this study and are therefore described below.

First, the escapees were identified using a statistical approach known as Bayesian cluster analysis as implemented in STRUCTURE. This program assigns individual fish into genetic groups without taking into consideration the population or

Table 1 Samples collected from the local salmon farms

Source	Date	Organ tested by PCR	Organs used for histopathology
Farm 2	07.09.2012	Heart	Heart, pancreas, kidney, gill, muscles
Farm 4	13.07.2012	Kidney	Heart, pancreas, liver, kidney, spleen, gill
Farm 6	19.07.2012	Heart	Heart, pancreas, liver, kidney, spleen, gill, muscles

sample from which each individual originated. Correlated allele frequencies, an admixture model, and no data prior were assumed. Each run of the program consisted of a burn-in of 50 000 MCMC steps, followed by 250 000 steps. The program was run with all baseline and escapee samples included, with the number of populations set between $k = 1\text{--}7$. Escapees were also identified to source using genetic assignment as conducted in the program GeneClass2 (Piry *et al.* 2004). A specific computation method was used within the program (Rannala & Mountain 1997) to first directly assign each escapee to a specific cage sample. This method places each escapee into the baseline sample (i.e. cage sample) that it genetically resembles most. This assignment is conducted irrespective of the absolute degree of similarity. In addition, genetic exclusion was conducted in this program. Here, each escapee is assigned a probability value to belong to each of the potential sources. Rejection of each source was conducted at a predetermined threshold ($P < 0.01$). A combination of assignment results from these two programs was hereunder used to identify the potential source of the escapees.

Analysis of viral infections. Normalized expression (NE) of viral RNA was calculated for infected (positive) escaped salmon. Fold-change was calculated from the mean NE (MNE) values. As it has been found that the expression of *Elf1αB* may be reduced during SAV infection (Løvoll *et al.* 2011), the SAV analyses were also performed using Log (C_t^{-1}). The relationships between NE of a virus, host parameters, mode of capture and river- vs. sea-caught fish were examined using analysis of covariance (ANCOVA) in the GLM module of Statistica 64 (Statsoft Inc.). NE values were log₁₀-transformed to stabilize variance. Correlations were examined using Spearman's rank correlation coefficients (R_s).

Results

The escapees

Fifty-nine escaped salmon were captured in the area between Ålvik and Norheimsund in the Hardangerfjord using rod, traps and nets. The average weight and length of the fish were 415 g and 55.5 cm, respectively. The fish were thin with a mean condition factor of 0.80 (0.61–1.13).

Most of the fish had an empty stomach and intestines. All fish were immature, except one sea-caught male fish, which was maturing (stage IV). Twenty-one of the 59 escapees were captured in the Steinsdalselva River. Visual inspection of the fish did not reveal any external or internal pathologies or signs of disease.

Genetic identification of the farm of origin

A combination of various summary statistics (not presented), Bayesian cluster analysis (Fig. 2) and genetic assignment (Fig. 3) demonstrated that nearly all of the farmed escapees were genetically highly similar to cage samples 2A, 4A/B and 6B, and genetically distinct to all other baseline samples. This is, for example, demonstrated by the fact that samples 2A, 4A/B and 6B were almost exclusively depicted by the blue genetic cluster, which was also shared by escapees, but none of the other baseline samples (Fig. 2). Furthermore, almost all of the escapees were directly assigned to these three samples, but rejected from most of the other samples (Fig. 2). Thus, the source of the escapees was quickly narrowed down from the 13 potential cage samples down to three.

A large degree of genetic overlap was observed between cage samples 2A, 4A/B and 6B. The ability to conduct genetic assignment and degree of genetic differentiation between baseline samples is tightly linked. Thus, while direct assignment (Fig. 3) indicated that cage sample 4A/B was the best genetic match for most of the escapees (i.e. 34 of the 58 escapees directly assigned to this sample), the sources 2A and 6A were so genetically similar that these could not be statistically rejected as the potential source of the escapees. Thus, based upon genetic analyses in isolation, it was not possible to unequivocally identify which of the three cage samples identified above represented the primary source of the escapees, and furthermore, it was not possible to unequivocally demonstrate whether the escapees originated from one or more of these sources. To help the investigation, infection profiling was performed as a second non-genetic supplementary method to assist in the identification of the escapees' source.

Infection status of salmon

Escapees. Real-time PCR analyses detected SAV virus RNA in the heart of 57 of the 58 tested

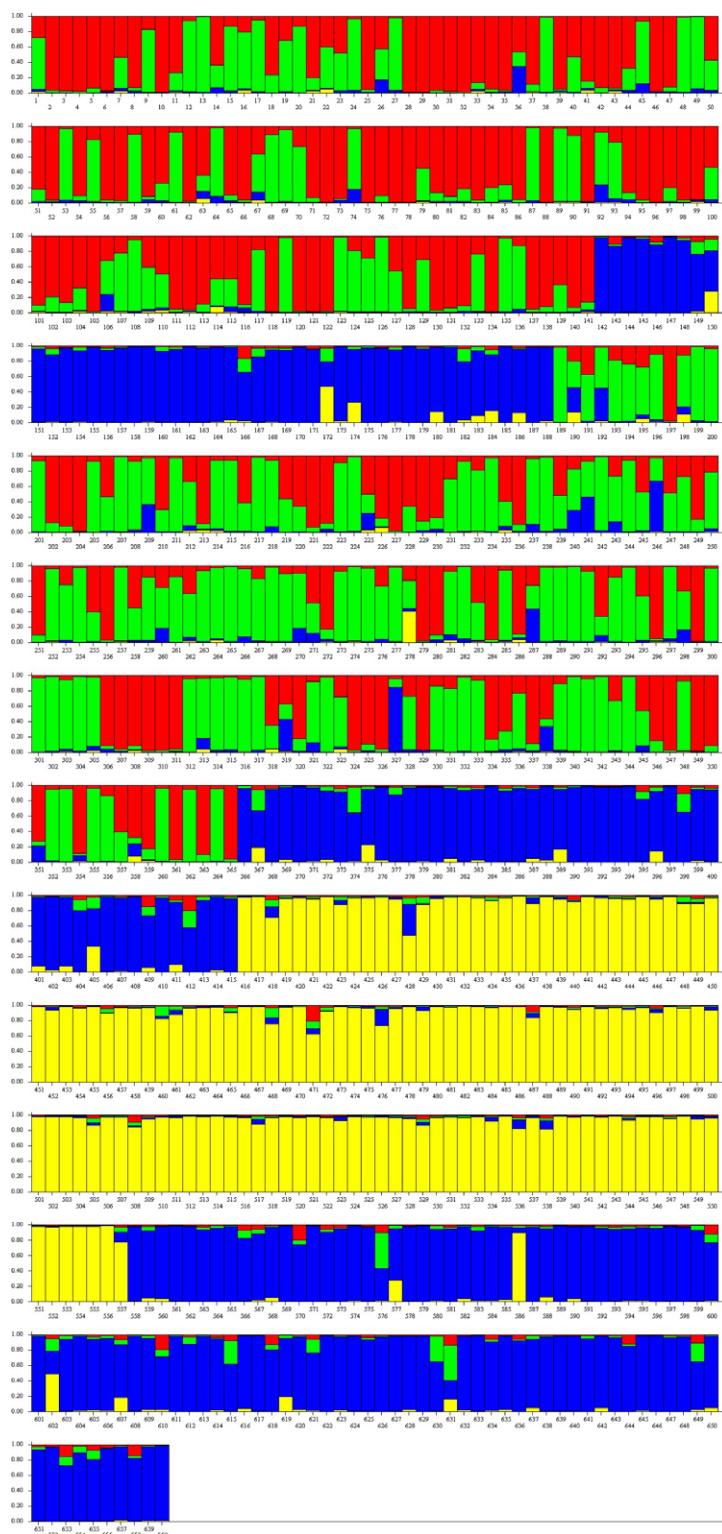


Figure 2 Bayesian cluster analysis depicting the genetic relationship between fish from each of the 14 baseline samples and the 58 escapees. Each vertical line represents an individual, and each colour a genetic cluster. An individual can be a mixture of genetic clusters. The results are presented for four genetic clusters. Results from other numbers of genetic clusters gave similar results (not presented). 1–47 = farm 1A, 48–94 = 1B, 95–141 = 1C, 142–188 = 2A, 199–232 = 3A, 233–271 = 3B, 272–318 = 3C, 319–365 = 3D, 366–390 = 4A, 391–415 = 4B, 416–462 = 5A, 463–509 = 5B, 510–556 = 6A, 557–602 = 6B, 603–660 = escapees.

escaped fish (Table 2). One fish was considered SAV negative (C_t -value = 40). C_t -values of SAV-positive fish ranged from 20.7 to 31.1 (mean

26.2), indicating moderate to high virus load. The C_t -value ranges were 24.7–29.7 (mean 26.9) and 20.7–31.1 (mean 25.9) in river-caught and

sea-caught fish, respectively. PRV virus was detected in all the escaped farmed fish with C_t -values ranging from 21.5 to 34.9 (mean 28.3). The fish generally had a moderate virus load with few fish having a high amount of virus. The C_t -values ranges were 24.8–34.5 (mean 28.5) and 21.5–34.9 (mean 28.2) in river-caught and sea-caught fish, respectively. None of the escaped salmon were positive for PMCV virus.

The main finding from ANCOVAs was that the river-caught salmon had significantly ($P < 0.01$) lower SAV and PRV NE compared with sea-caught salmon (Data S1). Mean NE of SAV and PRV were, respectively, 9.5- and 15-fold

higher in sea-caught fish compared with river-caught fish. There was no correlation between the NE of SAV and PRV ($R_s = -0.05$).

Samples from salmon farms. Samples from 2 of the 3 suspected farms (identified by genetics) were collected several weeks before the capture of the escapees, while samples from the third farm were collected afterwards (Fig. 1 and Table 1). In this period, all three farms had an increased mortality and clinical signs, suggesting pancreas disease outbreak. Therefore, samples were analysed for SAV and examined for histopathology. Most of the fish showed cardiomyopathy (infiltration, degeneration and necrosis in heart) and necrosis of exocrine pancreatic tissues (typical pancreas disease pathology). Most of the fish collected from the farms 2 and 6 were positive for SAV by real-time PCR with C_t -values, indicating moderate to high virus load in the heart (Table 2). Only kidney samples were available from farm 4, these showed mostly high C_t -values (moderate to low viral loads). Based on real-time PCR results and histopathological examination, all the three farms were diagnosed as having PD outbreak.

The same samples were also tested for PRV and PMCV viruses using real-time PCR. Most of the salmon sampled from the farms were also positive for PRV virus with moderate amounts of virus (Table 2). None of the fish from the three farms was positive for the PMCV virus.

The real-time PCR results indicate that the infection profile of the escaped salmon was similar to the profile in the three suspected farms and therefore did not point out a likely source farm.

Discussion

This is the first study confirming the presence of virus-infected escaped farmed Atlantic salmon in a nearby river shortly after escaping. Nearly all the recaptured escapees were infected with two of the most prevalent viruses in Norwegian fish farming, namely SAV and PRV. SAV belongs to *Togaviridae* family with six known subtypes (i.e. SAV1–SAV6). Salmonid alphavirus subtype 3 (SAV3) is the only subtype present in the Hardangerfjord area and was a long time the only subtype present in Norway. A new subtype, SAV2, has recently been detected in the middle parts of Norway (Hjortaaas *et al.* 2013). SAV3 causes pancreas disease, which is one of the major problems in

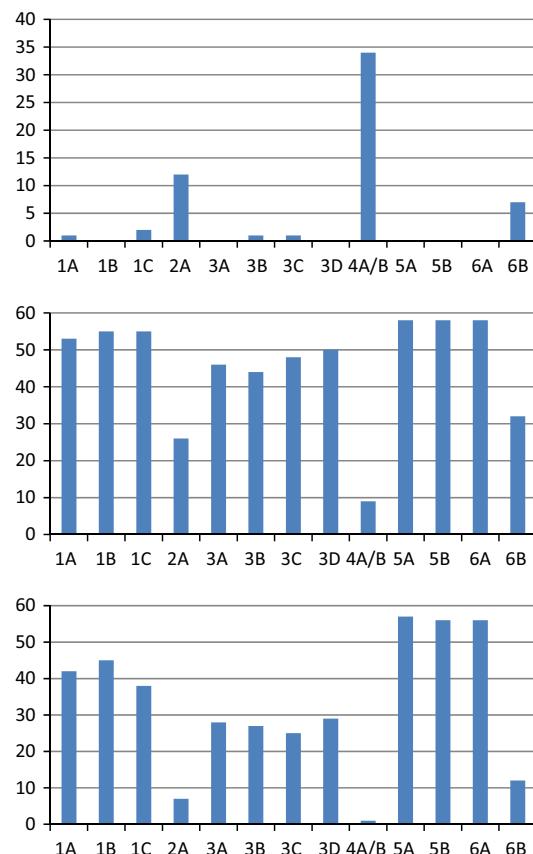


Figure 3 Assignment and exclusion of the farmed escapees. Upper panel = direct assignment of the escapees to each of the baseline samples, middle panel = number of escapees that can be rejected from each baseline sample $P < 0.05$, bottom panel = number of escapees that excluded from each baseline sample at $P < 0.001$. Note that the bottom two panels will not add up to 58 due to the fact that in theory each escapee can be rejected from all sources while the upper panel adds up to 58.

Table 2 The numbers of tested salmon and the results of virus testing

Virus	Escapees Heart (-20°C)			Farm 2 Heart (RNA later) N 9	Farm 4 Kidney (RNA later) N 8	Farm 6 Heart (RNA later) N 7
	All N 58	River N 20	Sea N 38			
SAV +	57	19	38	8	7	7
mean C_t -value (range)	26.2 (20.7–31.1)	26.9 (24.7–29.7)	25.9 (20.7–31.1)	23.3 (19.0–35.0)	33.2 (31.1–37.4)	24.5 (20.6–29.7)
PRV +	58	20	38	8	8	6
mean C_t -value (range)	28.3 (21.5–34.9)	28.5 (24.8–34.5)	28.2 (21.5–34.9)	32.8 (30.8–36.1)	31.0 (28.9–34.2)	33.5 (31.7–36.5)
PMCV +	0	0	0	0		

farmed Atlantic salmon in Western Norway, causing up to 27% mortality (Jansen *et al.* 2010). Although SAV3 has a high prevalence in endemic areas, it is rarely detected in wild salmonids (A.S. Madhun, unpublished data). PRV is a recently discovered virus that is associated with heart and skeletal muscle inflammation (HSMI). The virus has been previously detected in wild salmon, escaped salmon and sea trout by real-time PCR [personal observations and by (Garseth *et al.* 2013)]. The transmission of SAV and PRV may occur in both fresh and sea water as both viruses can be detected in salmonids in fresh and sea water (Nylund *et al.* 2003; Bratland & Nylund 2009; Løvoll *et al.* 2012).

In the present study, no data were available about the total number of escapees because the escape event was not reported. However, a significant number (21 of the 59) of the escaped fish were recaptured in a local river (Steinsdalselva) that has native trout and salmon populations. The escaped fish in the present case also showed moderate to high viral loads, as indicated by the C_t -values from the real-time PCR analyses. Interestingly, the river-captured fish displayed lower mean NE values for SAV and PRV than sea-captured fish. This suggests that the fish ascending the river had generally lower virus loads. This agrees well with the generally observation that SAV-affected salmon has an impaired swimming ability [reviewed in (McLoughlin & Graham 2007)]. High viral load may also suggest active virus replication and virus shedding to the river water (Andersen *et al.* 2007; Andersen, Hodneland & Nylund 2010; Løvoll *et al.* 2012). Demonstration of viral spread from the escapees following the present event would require relevant baseline data on SAV and PRV infections in wild salmonids, particularly juveniles. However, baseline data from the river regarding these viral infections in salmonids are lacking. Therefore, it is

important to establish baseline data on the occurrence of important disease agents of salmon in parr and smolt in rivers, both in areas with intensive fish farming and in farm-free areas. So far, only the occurrence of infectious salmon anaemia virus infections has been examined in a few populations of juvenile salmonids from western Norway (Plarre *et al.* 2005).

The recapture of the infected escaped salmon in nearby marine sites highlights the potential contribution of escapees in virus transmission to other salmon farms in the area. It has been suggested previously that virus-infected wild salmonids and other marine fish may act as vector for viruses and have a role in spreading of pathogens between fish farms (Nylund & Jakobsen 1995; Nylund *et al.* 1997; Snow *et al.* 2001, 2010; Wiik-Nielsen *et al.* 2012). A similar role can be ascribed to pathogen-infected escaped salmon.

Sea lice infections, farmed escapees and viral diseases are the major challenges that salmon farming is facing today. It is well documented that both sea lice infestation and escapees have negative impact on the wild salmon populations where farmed and wild populations coexist [reviewed in (Costello 2009; Torrisen *et al.* 2013) and (Glover *et al.* 2012, 2013a)]. On the other hand, little is known about the effect of viral disease outbreaks in aquaculture on the wild salmonid populations [reviewed in (Johansen *et al.* 2011)]. Disease outbreaks in salmon farms may lead to a substantial increase in infection pressure on wild fish in the surrounding area. However, escaped salmon may disperse over long distances, may enter rivers and may interact with wild conspecifics in their habitats. Therefore, an infected escapee may spread pathogens from the sea to wild fish populations in both sea and rivers distant from a disease outbreak.

In addition to viral testing of the 58 escaped salmon, we were able to examine material from three likely source salmon farms. The initial idea was to

use infection profiles to supplement the genetic analyses in order to identify a likely source of the escapees. However, in all the three farms that had fish genetically similar to the escapees, the infection profile was also similar (positive for both SAV and PRV and negative for PMCV). Thus, in this specific case, disease profiling was not able to provide more information regarding the farm source of the escapees than already provided by fish genetics (Glover *et al.* 2008; Glover 2010). Nevertheless, the use of disease profiling, as supplement to the DNA identification method, could be useful in other escape episodes. This will require that the pathogen repertoire differs qualitatively or quantitatively between the potential source farms.

The annual reported number of escapees from Norwegian fish farms has been in hundreds of thousands or even millions (Saegrov & Urdal 2006; Anonymous 2012), and in some years higher than the annual number of wild salmon returning to the Norwegian coastline to reproduce in the same period. At the same time, there has been between 473 and 509 annual viral disease outbreaks in aquaculture in Norway in the last 5 years (2007–2012) (Johansen 2013). Considering the number of escapees and disease outbreaks in commercial fish farming, escapees represent a significant threat to the native salmonid populations as pathogen vectors. Therefore, escaped salmon should be monitored for pathogens as well as for ecological and genetic interactions.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Results of analyses for covariance.