

Annual report on health monitoring of wild anadromous salmonids in Norway

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Since 2012, the Norwegian Veterinary Institute (NVI) and the Institute of Marine Research (IMR) have been commissioned by the Norwegian Food Safety Authority to carry out an annual health monitoring of wild anadromous salmonids in Norway.

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Health monitoring of migrating smolts from western Norway

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1. Introduction

Viral diseases represent a serious problem in Atlantic salmon (*Salmo salar* L.) farming in Norway, often leading to substantial economic losses. Disease outbreaks in salmon farms may lead to increased infection and disease risks at neighbouring farms and in wild fish populations [1]. There is increasing public concern of this impacting wild salmonids in Norway. Today, there is limited data on the prevalence of pathogens in wild salmonid populations. It is difficult to quantify disease incidence in wild fish because sick individuals in nature may be less catchable or may disappear unnoticed (e.g. due to predation). Therefore, it is challenging to evaluate the impact of disease in wild stocks since we normally are only able to collect infected but non-diseased fish such as individuals that has recently acquired or has survived an infection (carriers). There are evidences for pathogen transmission from farmed to wild fish [2-5]. However, the frequency and the consequence of transmission of many viral disease agents are largely unknown.

Pathogens that cause disease in farmed salmon can also infect wild salmon. The effect of fish farming on the infection status of wild salmon stocks may be evaluated by comparing pathogen prevalence in wild fish populations captured from coastal areas that have different fish farming intensities and disease outbreak profile.

Pancreas disease (PD), caused by salmonid alphavirus (SAV), is a major health problem for fish farming in Norway with 99–142 annual cases in the last 5 years [6]. Two subtypes of SAV occur in Norway, SAV3 and the more recently detected SAV2 [7]. Most of the disease outbreaks due to SAV3 occur in the western part of the country, especially in Hordaland county, while SAV2 cases are mostly restricted to an area along Mid-Norway.

Heart and skeletal muscle inflammation (HSMI) is another disease that is caused by piscine orthoreovirus (PRV). High PRV viral loads are found in both fish developing HSMI and in healthy fish. The disease is an increasing problem in fish farming in Norway with 101–181 annual registered cases of HSMI in the period 2010–2016 [6]. PRV has been detected in wild salmon and sea trout, as well as certain marine fish species by real-time rt-PCR [8-10]. It has previously been shown that there was no regional pattern in virus genotypes isolated from wild and farmed salmon, suggesting prolonged and extensive spread due to aquaculture activities (fish transport) and frequent exchange of the virus types between farmed to wild fish [3]. However, little is known about the life cycle of the virus. Modelling has suggested that fish farming intensity in a region is a major risk factor for HSMI outbreaks [11], implying that water borne transmission may be important.

Wild salmon may be infected by viruses prevalent in salmon farming; in rivers as parr by virus-infected farm escapees and spawning wild salmon or from salmon farms in the fjord when migrating as smolt or returning as adults. Therefore, infection status in migrating smolt may represent a direct indicator of infection pressure from salmon farming during their migration routes.

2. Aim

The aim of the current program is to investigate the occurrence of SAV and PRV infections in migrating Atlantic salmon smolt originated from Osterfjord, western Norway.

3. Materials and methods

Institute of Marine Research and Uni Research Environment have smolt release projects in the rivers Dale and Vosso where thousands of cultivated smolts are released every year (for further information see [12, 13]). The smolts were adipose fin clipped, tagged and towed in small pens and released at different locations between the rivers and the coast. Part of the released smolt from rivers Vosso and Dale and wild smolt originated from rivers located in the area are captured in a specially designed smolt trap in the Herdlefjord area [14](Fig. 1).

Results from 375 migrating smolt captured in May-June 2013 and 2014 were used in the current

report. The fish weight, length, sex and origin (wild or released smolt) were determined (released smolt were adipose fin clipped or/and tagged). The fish were frozen (-20 °C) as soon as possible after capture. At autopsy, tissues from the heart were aseptically taken out from the fish while still frozen and transferred to tubes on dry ice. Heart samples were sent on dry ice to an accredited commercial laboratory for RNA extraction and virus testing (PatoGen Analyse AS). Analyses for SAV and PRV viruses (for detection viral RNA) were performed by PatoGen using their in-house real-time PCR assays. The SAV assay used detects both SAV2 and SAV3. Samples with C_t (cycle-threshold) value below 37.0 were considered positive. Fisher's exact tests were used to compare the prevalence of virus in different fish groups.

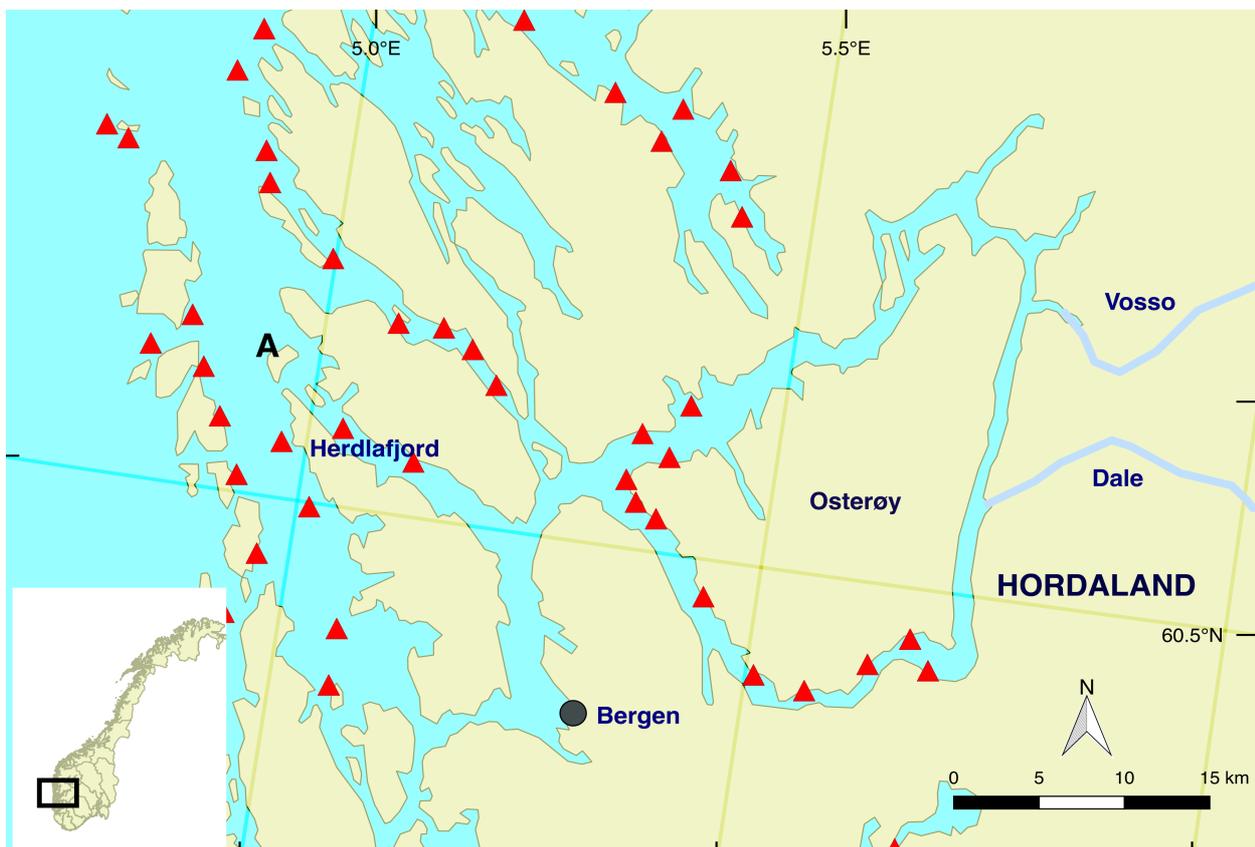


Figure 1: A map showing smolt collection site (A), fish farms (red triangle) and Osterfjord basin.

4. Results

Smolt characteristics

More than half (64 %) of smolts caught in Herdlafjord were released smolt (adipose fin clipped or/and tagged). Of the released smolt, 26 % were from river Vosso, 68 % from river Dale and 6 % from unknown origin. The remaining fish were wild smolt likely to originate from rivers in the Osterfjord basin, of which Vosso and Dale rivers are the largest.

SAV was not detected.

SAV was not detected in any of the hearts from the tested smolt.

The prevalence of PRV in returning salmon

PRV was detected in 5% of the migrating smolt (Table 1). PRV prevalence was significantly higher in 2013 smolt (7 %) compared to smolt captured in 2014 (2 %). Infected smolt had low to moderate viral load (C_t values; 22.2-36.7). In 2013, the wild smolt had a significantly higher (14 %) PRV prevalence than released smolt (1 %). However, there was no

significant difference in PRV prevalence between wild and released smolt in 2014 (Table 1).

Table 1: The numbers of tested smolt and percentages of PRV-positive fish in migrating smolt from Osterfjord basin.

	Catch Year		Total
	2013	2014	
Wild	93 (14 %)	43 (2 %)	136 (10 %)
Released	106 (1 %)	133 (2 %)	239 (2 %)
Total	199 (7 %)	176 (2 %)	375 (5 %)

5. Discussion and Conclusion

SAV3 is endemic in western Norway where fish in the majority of salmon farms become infected during production cycle [15, 16]. PD outbreaks in western Norway are common in May-June [6]. In the Osterfjord and smolt migration route there were two PD outbreaks in June 2013 and five in June-July 2014. It is likely that migrating smolt were exposed to virus released from the farms before (subclinical infection) and during PD outbreaks. However, we could not detect SAV in any of the tested smolt. The concentration of virus released from farms with clinical PD outbreaks or with subclinically infected salmon is currently unknown. A recent report has shown that a small number of smolts (3 of 24) that were exposed experimentally to low concentration of SAV3 in water was virus-positive in heart samples one-three weeks after exposure [17]. The absence of SAV infection in the tested migrating smolt is consistent with previous findings that SAV infections are uncommon in wild salmonids irrespective of farming intensity or the frequency of PD outbreaks at the locations examined [10, 18-20]. These observations may indicate that wild salmon are exposed to a low infection pressure from fish farming. However, the possibility that SAV infection may lead to rapid disappearance of the infected wild fish cannot be ruled out.

In contrast to SAV, PRV infection was detected in 5 % of the migrating smolt. There is no available data about HSMI outbreaks in fish farms located in the area during the 2013-2014 period [21]. However, PRV infection is very abundant in fish farming in Norway [8]. In cohabitant experiments, PRV was detected in the blood and heart-samples of infected smolt 4-6 weeks after infection [22]. The time between river descent and arrival time at Herdla (smolt capture site) for smolt originated from rivers located in outer and inner parts of the Osterfjord was estimated to be 3.0 and 6.5 days, respectively [23]. Consequently, it is likely that most of the PRV-positive smolts in the current study were infected in the rivers of origin and not from fish farms located in the migration routes. Indeed, PRV infection was detected in parr from a river in western Norway (A. S. Madhun, unpublished data). The observation that wild smolt had a higher prevalence of PRV than released smolt in 2013 also suggests that PRV transmission may occur naturally in the rivers. Studies of escaped farmed salmon entering rivers show that most of the escapees often are infected with one or more viruses, PRV being the most common. These observations highlight the potential role of escaped salmon as pathogen vectors that may transfer infections to wild salmon populations in rivers [24, 25].

Garseth et al. [3] have suggested that extensive transmission of PRV from fish farms to wild salmon has occurred. The impact of PRV infections on the fitness and mortality of wild salmon populations is currently unknown, although the ability of mature salmon to ascent rivers may be

affected [26].

Time series of samples of migrating smolt are necessary to better evaluate the effect of infection pressure from salmon farming on the virus prevalence in wild salmon populations. The studies on migrating smolt will continue in the coming years. From 2017, it will be trawled for migrating salmon smolts in 6 fjord systems from south to north, covering larger parts of the Norwegian coastline.

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A survey of salmon gill pox virus (SGPV) in wild salmonids in Norway

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1. Introduction

Gill diseases and associated pathogens in farmed fish have received increasing attention in recent years. In the 1990s, fish pathologists suspected that a specific manifestation of gill disease was caused by a pox virus infection (O.B. Dale and A. Kvellestad, NVI, unpublished data). Nylund and co-workers detected pox particles by transmission electron microscopy in association with these lesions in 2008 [1], and in 2015, the link between lesions and the etiological agent was confirmed by the characterisation of salmon gill pox virus (SGPV) [2, 3]. In farmed salmon, SGPV is present at all stages of the production cycle and associated with disease and mortality in both juvenile salmon in freshwater and in adult fish after seatransfer [4]. The occurrence of SGPV in wild salmonids has so far not been reported. Accordingly, there is a need generate knowledge about SGPV as a potential threat to wild salmonid fish health and to reveal the role of wild reservoirs in the epidemiology of the virus.

2. Aim

An important objective of the health monitoring program for wild anadromous salmonids is to examine and monitor the occurrence and distribution of virus that are common and cause diseases within the salmon farming industry. The aim of this study was thus to investigate the occurrence of SGPV in wild anadromous and non-anadromous salmonids in Norway.

3. Materials and methods

A qPCR based survey of anadromous Atlantic salmon, non-anadromous (landlocked) salmon (*Salmo salar* L.), brown trout and sea trout (*Salmo trutta* L.) and Arctic char (*Salvelinus alpinus* L.) was conducted.

The main source of material was adult salmonids captured and used as broodfish by stock enhancement hatcheries and the Norwegian Genebank for wild Atlantic salmon. Other sources of material were fish captured during population regulation, research, and in addition fish killed during rotenone treatments of rivers – a measure issued by the Norwegian environment agency (Miljødirektoratet) to eradicate *Gyrodactylus salaris*. An overview of the analysed material is presented in Table 1. All qPCRanalyses were performed at NVI.

Table 1. Overview of analysed material.

Lifeform and species		Counties	Watercourses	No. Fish
Anadromous	Atlantic salmon (<i>Salmo salar</i>)	7	26	276
	Sea trout (<i>Salmo trutta</i>)	4	14	205
	Arctic char (<i>Salvelinus alpinus</i>)	1	2	26
Non-anadromous	Brown trout (<i>Salmo trutta</i>)	2	3	79
	Landlocked salmon (<i>Salmo salar</i>)	2	2	71

There are two major sources of sampling bias that should be presented. First, broodfish are kept together in tanks from a few days and up to 6-7 weeks prior to stripping and sampling. Secondly, some stocks of sea trout broodfish are kept in tanks with Atlantic salmon prior to sampling. Accordingly, cohabiting of broodfish in tanks may have caused both inter- and intraspecific pathogen transmission prior to sampling.

4. Results

SGPV was detected in Atlantic salmon from all counties and in 25 of 26 watercourses (Table 2). Altogether 205 of 244 tested broodfish were SGPV-positive, while 12 of 26 salmon that were killed during rotenone treatment were positive.

A proportion of sea trout that was cohabiting with SGPV-positive salmon were found to be virus-positive. In contrast, sea trout that were not kept in tanks with salmon were virus negative. All Arctic char, landlocked salmon and brown trout were virus-negative.

Table 2. Overview of results from qPCR analyses.

Lifeform and species		Positive Watercourses	Positive Fish	Range Ct-values
Anadromous	Atlantic salmon (<i>Salmo salar</i>) Cohabitation in tanks	24/25	205/276	16.8-37.6
	Atlantic salmon (<i>Salmo salar</i>) No Cohabitation in tanks	1/1	12/26	25.3-36.5
	Sea trout (<i>Salmo trutta</i>) Cohabiting with salmon in tanks	3/8	26/109	31.8-37.3
	Sea trout (<i>Salmo trutta</i>) No cohabitation with salmon	0/6	0/96	-
	Arctic char (<i>Salvelinus alpinus</i>)	0/2	0/26	-
Non-anadromous	Brown trout (<i>Salmo trutta</i>)	0/3	0/79	-
	Landlocked salmon (<i>Salmo salar</i>)	0/20	0/71	-

5. Discussion and Conclusion

SGPV is a common finding in wild Atlantic salmon returning from marine migration. The prevalence of SGPV in broodfish is significantly higher than for other viruses in similar material [5]. The high prevalence may partly be due to transmission between salmon during cohabitation in tanks, but even in the river without cohabitation the prevalence exceeded 46 % (CI 28.8-64.5). These results indicate that adult salmon in rivers may constitute a reservoir for the virus and therefore be a source of infection for juvenile wild stages as well as for salmon in aquaculture facilities that use freshwater from anadromous sources. Histopathological lesions consistent with SGPV-infection have been detected in SGPV-positive wild salmon [6], indicating that the virus is capable of causing disease also in wild populations.

The detection of SGPV in sea trout seems to be due to the cohabitation with infected salmon rather than naturally occurring infection. This is supported by the lack of PCR-positives in sea trout that were not cohabiting with salmon and also by the absence of virus in brown trout.

SGPV was not detected in non-anadromous salmonids (i.e. brown trout and landlocked salmon). This may indicate that the virus primarily is associated with the marine environment. SGPV was not detected in Arctic char, but the limited sample size renders the investigation inconclusive. The occurrence of the SGPV in juvenile salmon as well as in brown trout, sea trout and Arctic char should be further studies.

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Gills from a wild salmon. Photo Åse Helen Garseth, Norwegian Veterinary Institute.



The Norwegian Veterinary Institute (NVI) is a nationwide research institute in the fields of animal health, fish health, and food safety. The primary mission of the NVI is to give research-based independent advisory support to ministries and governing authorities. Preparedness, diagnostics, surveillance, reference functions, risk assessments, and advisory and educational functions are the most important areas of operation.

The Norwegian Veterinary Institute has its main laboratory in Oslo, with regional laboratories in Sandnes, Bergen, Trondheim, Harstad and Tromsø, with about 360 employees in total.

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The Institute of Marine Research (IMR) is the largest marine science community in Norway with more than 700 employees. Our main task is providing advice to the Norwegian authorities on aquaculture and on the ecosystems of the Barents Sea, Norwegian Sea, North Sea and the Norwegian coastal zone. Around half of our activities are funded by the Ministry of Trade, Industry and Fisheries.

The Institute of Marine Research has headquarters located in Bergen, but important aspects of our work are done at our department in Tromsø, at our research stations in Matre, Austevoll and Flødevigen and on board our research vessels.

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The Norwegian Food Safety Authority (NFSA) is a governmental body whose aim is to ensure through regulations and controls that food and drinking water are as safe and healthy as possible for consumers and to promote plant, fish and animal health and ethical farming of fish and animals. We encourage environmentally friendly production and we also regulate and control cosmetics, veterinary medicines and animal health personnel. The NFSA drafts and provides information on legislation, performs risk-based inspections, monitors food safety, plant, fish and animal health, draws up contingency plans and provides updates on developments in our field of competence.

The NFSA comprises three administrative levels, and has some 1300 employees. The NFSA advises and reports to the Ministry of Agriculture and Food, the Ministry of Trade, Industry and Fisheries, and the Ministry of Health and Care Services.

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