

Annual report on health monitoring of wild anadromous salmonids in Norway

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The Norwegian Veterinary Institute (NVI) and the Institute of Marine Research (IMR) were in 2014 commissioned by the Norwegian Food Safety Authority to carry out a health monitoring of anadromous salmon, *Salmo salar*, in Norway.

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Health monitoring of anadromous salmonids from western Norway

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1. Introduction

Viral diseases are a serious problem in fish farming in Norway that leads to huge economical losses. Disease outbreaks in fish farms may lead to a substantial increased infection pressure on neighbouring farms and on wild fish. This may cause increased infection levels (prevalence) and potentially also disease in susceptible wild stocks. Today, there is limited data on the prevalence of pathogens in wild salmonid populations in Norway. 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 [1-3]. 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 88–142 annual cases in the last 5 years (Bornø, Lie Linaker 2015). Two subtypes of SAV occur in Norway, SAV3 and the more recently detected SAV2 [4]. 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 (Møre and Trøndelag).

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 131–181 annual registered cases of HSMI in the period 2010–2014 [5]. PRV has been detected in wild salmon and sea trout, as well as certain marine fish species by real-time rt-PCR [6, 7]. 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 transmission of the virus types from farmed to wild fish [8]. 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 [9], implying that water borne transmission may be important.

2. Aim

The aim of the current program is to investigate the occurrence and distribution of SAV and PRV infections in returning Atlantic salmon originated from Vosso and Dale rivers located in the 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 [10, 11]). The smolts were tagged and

towed in small pens to release sites at different locations between the rivers and the coast (Fig. 1).

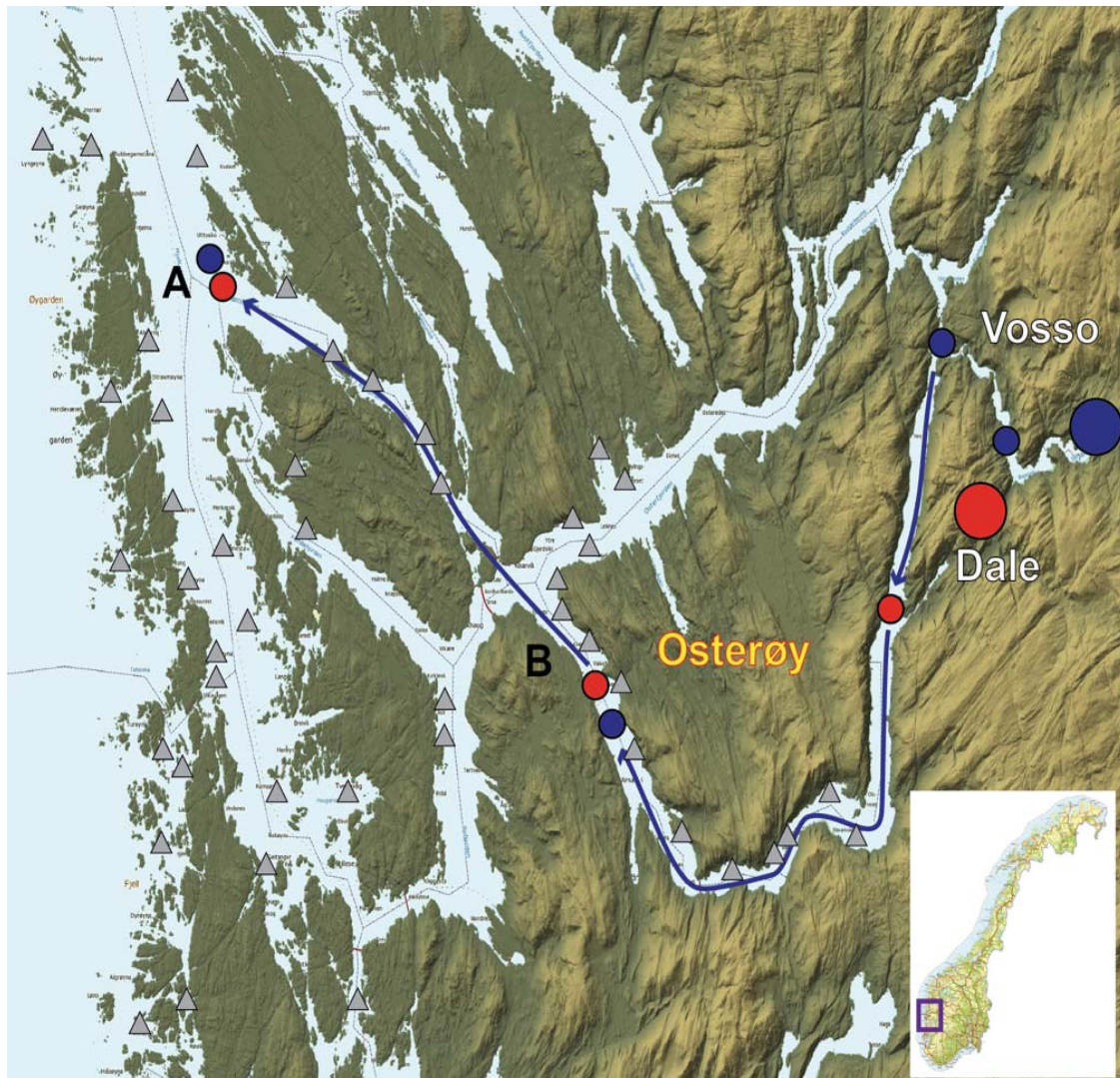


Fig. 1: A map showing the Osterfjord system. Circles are showing the release sites for smolt from Vosso (blue) and Dale (red). Grey triangles are showing approved fish farming sites. The returning salmon were captured in inner part of fjord (sea), the river mouth (estuary) or in the rivers (fresh

A total of 460 returning salmon were captured by angling in the rivers or by bag net in the fjord. The salmon were caught at three areas (sea, estuary and rivers) in the Osterfjord system in 2012 and 2013 (Fig. 1).

A total of 460 returning salmon were captured by angling in the rivers or by bag net in the fjord. The salmon were caught at three areas (sea, estuary and rivers) in the Osterfjord system in 2012 and 2013 (Fig. 1). The fish weight, length and sex were determined. Tags were read to determine release site, release date and the river of origin. The head of captured fish was cut off behind the pectoral fin and deep 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 were performed by PatoGen using their in-house real-time PCR assays (for detection viral RNA). The SAV assay used detects both SAV2 and SAV3. Samples with C_t (cycle-threshold) value below 37.0 were considered positive.

4. Results

SAV was not detected.

SAV was not detected in any of the hearts from the tested salmon originating from the rivers Vosso and Dale.

The prevalence of PRV in returning salmon

Piscine orthoreovirus (PRV) were detected in 10% of the returning salmon. Males had a higher prevalence (13%) than females (5%). PRV prevalence was higher in one sea winter (1-SW) salmon (18%) than in the 2- and 3-SW fish (3 and 6% respectively), but males predominate the 1-SW group. The highest prevalence of PRV was found in salmon that migrated as smolts in 2010 (8%) and 2012 (28%) (Table 1).

PRV prevalence was higher in fish that had been released in Sjørfjorden (12%, Site B) than in Hjeltefjorden (8%, Site A). There were no differences in prevalence of PRV between the returning salmon captured in sea (9%), in brackish water (12%) or in rivers (11%).

Table 3: The numbers and percentages of PRV-positive fish in returning salmon from Vosso and Dale rivers.

	Smolt migration year				Total
	2009	2010	2011	2012	
PRV+ (%)	1 (4%)	8 (8%)	12 (5%)	24 (28%)	45 (10%)
Total Number	27	98	250	85	460

5. Discussion and Conclusion

We could not detect SAV in any of the tested salmon. SAV is endemic in western Norway where fish in the majority of salmon farms become infected during production cycle [12, 13]. Therefore, the probability of exposure to the virus from salmon farms both when migrating as smolt (2009–2012) and when returning as adult (2012 and 2013) is relatively high. However, the current results, our unpublished data and the available literature suggest that the prevalence of SAV in wild salmonid populations is very low [14, 15].

Salmon that survive a SAV infection may become carriers of the virus for months [16, 17].

However, whether the host will be a life-long carrier or the virus will eventually be cleared by host's immune system is currently unknown. Therefore, it is not clear if infections developed in the oceanic phase of wild salmon can be detectable by real-time PCR when the fish return to the coast after 1 year or more. On the other hand, we could not (our unpublished data) detect SAV in migrating smolt from western Norway in 2013–2014 (including smolt from rivers Vosso and Dale) or in juvenile salmonids from rivers located in the area. These observations could indicate that wild salmon are exposed to a too low infection pressure from fish farming, and therefore, it is difficult to detect SAV infection in wild salmon populations. However, a fatal

outcome of SAV infection that may lead to rapid disappearance of the infected fish cannot be ruled out.

In contrast, PRV was detected in 10% of returning salmon originated from Vosso and Dale rivers. There were few registered HSMB cases in the area during the 2009–2012 period [5]. However, PRV infection is very abundant in fish farming in Norway and is likely to be underreported in western Norway [6]. Our results show a lower PRV prevalence (10%) in the Vosso salmon than previously reported (55%) [6].

We have previously found that 2-SW salmon from northern Norway had significantly higher prevalence of PRV than 1-SW fish. One explanation for this could be that increased sea-age increases the likelihood of being infected [15]. In the current study we found a higher PRV prevalence in 1-SW than in 2-SW salmon. However, males predominated the 1-SW salmon, rendering it unclear whether it is sea-age or gender that influenced the prevalence. On the other hand, a high prevalence of PRV found in salmon that migrated as smolt in 2012 (28%) suggests that smolt migration year is a major factor that influences the prevalence in returning salmon. Similar to PRV infection, the infection pressure of salmon louse from fish farming was also relatively high in 2012 compared to the other years (Barlaup, personal communication). The increased infection pressure of PRV and salmon louse on wild salmon coincide with a high biomass of farmed salmon in the smolt migration route in 2012. The ongoing analyses of salmon captured in 2014 can further verify these findings. Garseth *et al.* [8] have suggested that extensive transmission of PRV from fish farms to wild salmon has occurred. However, the impact of PRV infections on the fitness and mortality of wild salmon populations is currently unknown.

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Health monitoring of wild anadromous salmonids in fresh water in Norway

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1. Introduction

The Norwegian Veterinary Institute organizes the Health service for stock enhancement hatcheries and has also substantial activity in the gene bank program for salmon and sea-trout. In both these projects mandatory testing of brood stock for infectious agents is conducted. The testing is done by PCR on head kidney that is sampled during autopsy after stripping. Alternatively, testing is done on milt and ovarian fluid. For brood stock used in regular cultivation practice it is mandatory to test for *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD). In addition, many hatcheries choose to test for the virus causing infectious pancreatic necrosis (IPNV). Brood fish intended for the gene bank program are tested for *R. salmoninarum*, IPNV and in addition *Aeromonas salmonicida* which causes furunculosis. In most cases an exemption for *A. salmonicida* has been granted by the Norwegian Food Safety Authority. The requirements for testing of wild anadromous brood stock are embodied in the Regulation for the operation of aquaculture facilities [1].

2. Aim

The aim of the health monitoring program in 2014 was to investigate the occurrence of salmonid alphavirus (SAV) and infectious salmon anemia virus (ISAV) in returning wild brood fish of the species salmon (*Salmo salar*), sea trout (*Salmo trutta*) and arctic char (*Salvelinus alpinus*) collected from different geographical areas along the Norwegian coastline. Results from prior *R. salmoninarum*, IPNV and *A. salmonicida* analyses should also be reported.

3. Materials and methods

The Norwegian Veterinary Institute undertook to analyze 400 wild caught salmonid brood fish for SAV and ISAV. SAV and ISAV were analyzed on a mix of heart and gill. *R. salmoninarum*, IPNV and *A. salmonicida* were analyzed on kidney, ovarian fluid or milt. Autopsies and sampling of kidney was performed by authorized fish health personnel (veterinarian or fish health biologist) contracted to the individual hatchery or employed by the NVI. Milt and ovarian fluid was sampled by personnel from the hatcheries. Scale-circuli patterns and additional information was used to confirm that the brood fish was truly wild and not escaped farmed salmon. All PCR assays were performed by PatoGen Analyse AS (<http://www.patogen.no>). PatoGen Analyse is an ISO 17025 accredited laboratory.

Kidney, heart and gill samples were fixed in RNAlater™ and shipped chilled to analysis immediately after autopsy, or alternatively stored in the refrigerator for at least 24 hours for fixation before freezing and shipping. Milt and ovarian fluid samples were not fixed and were shipped chilled immediately after sampling. All PCR assays were performed by PatoGen Analyse AS.

4. Results

In 2014, there was a reduction in the number of submitted heart and gill samples. This is partly due to a gradual transition towards use of milt and ovarian fluid (where autopsy is not required) instead of head kidney samples (where autopsy is required) in IPNV and *R. salmoninarum* testing. Hence, health monitoring for ISAV and SAV was based on samples from 306 individuals (260 anadromous salmon, 26 non-anadromous salmon and 20 sea trout). Tables 1 (salmon) and 2 (sea trout and char) provide a county by county listing of the results from 2014. Table 3 provides a summary of results from the period 2012 to 2014.

In 2014, four *R. salmoninarum* positive individuals were detected in the county of Sogn og Fjordane. All remaining analyses were negative.

Table 1 *Salmo salar*: Total number of analyses for each agent in 2014.

¹⁾ Four positive for *R. salmoninarum* in Sogn og Fjordane (wild). All remaining analyses were negative.

PCR analysis	<i>R. salmoninarum</i>	IPNV	<i>A. salmonicida</i>	SAV	ISAV
County					
Troms	14	14			
Nordland	24	24		24	24
Nord-Trøndelag, anadrom	53	28		28	28
Nord-Trøndelag ikke anadrom	24	24		26	26
Sør-Trøndelag	26	26	9	33	33
Møre og Romsdal	123	103	103		
Sogn og Fjordane	123 ¹⁾	83	41	42	42
Hordaland	63	20	20	31	31
Rogaland	46	32		36	36
Vestfold	62	4		66	66
Telemark	72				
Buskerud	12				
Østfold	39		5		
Total no. analyses	681	358	178	286	286

Table 2 *Salmo trutta* and *Salvelinus alpinus*: Total number of analyses for each agent in 2014. All analyses were negative.

PCR analysis	<i>R. salmoninarum</i>	IPNV	<i>A. salmonicida</i>	SAV	ISAV
<i>Salmo trutta</i>, anadromous					
Troms	96	96			
Nordland	116			20	20
Sogn og Fjordane	22				
Hordaland	25	25	9		
<i>Salmo trutta</i>, non-anadromous					
Finnmark	35	35			
Total no. analyses	294	156	9	20	20
<i>Salvelinus alpinus</i>, anadromous					
Troms	155	155			
Total no. analyses	155	155			

Table 3 *Salmo salar*, *Salmo trutta* and *Salvelinus alpinus*: Summary of results from the period 2012-2014.

Total number of analyses for each agent and the total number of positive individuals: ¹⁾ 7 positive for *R. salmoninarum* in Hordaland (2012) and 4 in Sogn og Fjordane (2014) ²⁾ 1 positive for IPNV in Rogaland (2013) ³⁾ one SAV-positive in Hordaland (released, 2012), ⁴⁾ 1 positive for ILAV in Møre og Romsdal (wild, 2013), ⁵⁾ 1 positive for PMCV in Sogn og Fjordane (wild), ⁵⁾ and 1 positive for PMCV in Vestfold (escaped farmed).

PCR analysis	R. salmoninarum	IPNV	A. salmonicida	SAV	ISAV	PRV	PMCV
Salmon							
Total no. positives	11 ¹⁾	1 ²⁾	0	1 ³⁾	1 ⁴⁾	130	2 ⁵⁾
Total no. analyses	1775	1134	571	1157	1137	532	453
Sea trout							
Total no. positives	0	0	0	0	0	3	0
Total no. analyses	447	296	15	120	120	100	100
Arctic char							
Total no. positives	0	0	-	-	-	-	-
Total no. analyses	200	200	-	-	-	-	-

5. Discussion and conclusion

In 2014, four individuals from the same river in Sogn og Fjordane were *R. salmoninarum* positive while all other analyses were negative.

The *R. salmoninarum* infected brood fish were kept together for a prolonged period before stripping and it is not unlikely that one fish was the original source and has infected the other three. All eggs from all brood fish in the plant were destroyed after the detection and in addition, smolt in the adjacent facility will be monitored with regards to detecting transmitted infection.

R. salmoninarum has previously been detected in sea-trout in the same river (1986), and in 1991 the hatchery experienced BKD in smolt. BKD has also been detected in neighboring rivers in Sogn og Fjordane and in stock enhancement hatcheries associated with these rivers. In 2012, *R. salmoninarum* was detected in 7 brood fish from a river in Hordaland. Hence, the results from the period 2012-2014 show that it is appropriate and important to maintain *R. salmoninarum* testing of all wild anadromous brood stocks. In commercial aquaculture, a few (<5) cases of BKD are usually detected every year. However, in 2014, BKD was not detected in commercial aquaculture.

In 2014, none of the tested brood fish were virus-positive. As displayed in Table 3 very few wild brood fish are positive for IPNV, ISAV, SAV and PMCV. With the exception of ISAV, these viral infectious agents are highly prevalent within the Norwegian aquaculture industry. As discussed in previous reports, the obvious question is whether this absence of positives is due to a low infection pressure or if wild fish infected by a virulent agent rapidly die and thus avoid being sampled [2]. In the latter case, transmission from farmed to wild populations would mean that aquaculture related infections could lead to disease and mortality in wild stocks. The population level impact of such transmission is however unknown.

In contrast to IPNV, ISAV, SAV and PMCV, piscine orthoreovirus (PRV), the causative agent of heart and skeletal muscle inflammation (HSMI), is a common finding in returning brood fish [3]. Phylogenetic analysis shows that the PRV is transferred between farmed and wild salmonids [4]. By the end of 2013, a total of 379 million farmed salmon were kept at approximately 600 active sea sites along the Norwegian coast. In contrast, 440 000 wild salmon returned from marine migration during the following summer. In farmed salmon, PRV is highly prevalent and HSMI is the most frequently diagnosed viral disease. It is therefore likely that the main reservoir of PRV is in farmed salmon and that the main direction of viral transmission is from farmed to wild salmon. The consequence of this transmission is largely unknown. It is possible that PRV leads to a prolonged infection state and therefore has a higher probability of spreading among wild salmon. It could also be that PRV has less impact on wild fish than for example SAV or IPNV, allowing more positives to be found. However, the detection of PRV transmission from farmed to wild salmon demonstrates the existence of transmission pathways that could be used by other viruses.

6. Acknowledgements

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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 og Tromsø, with about 360 employees in total.

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The Institute of Marine Research (IMR) is 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 therefore funded by the Ministry of Fisheries and Coastal Affairs.

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 Fisheries and Coastal Affairs and the Ministry of Health and Care Services.

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