

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 2013 commissioned by the Norwegian Food Safety Authority to carry out a health monitoring of anadromous salmon, *Salmo salar*, in Norway. IMR was given responsibility for the seawater phase, whereas NVI was given responsibility for the freshwater phase.

Health monitoring of wild anadromous salmonids in seawater in Norway

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1. Introduction

Viral diseases are 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 elevated infection levels (prevalence) and potentially 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 only are able to collect infected but non-diseased fish such as individuals that has recently acquired or has survived an infection (carriers). There is increasing evidence for pathogen transmission from farmed to wild fish (Costello 2009, Johansen, Jensen et al. 2011). 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 infection status of returning salmon may be used as an indicator of virus transmission from fish farming. 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 75-137 annually registered outbreaks in the last 5 years. Two subtypes of SAV occur in Norway, SAV3 and the more recently detected SAV2 (Hjortaa, Skjelstad et al. 2013). Most of the disease outbreaks due to SAV3 occur in western part of the country especially in Hordaland county, while SAV2 outbreaks are mostly restricted to an area along Mid-Norway (Møre and Trøndelag). There were very few PD outbreaks in the northern counties (Nordland, Troms and Finnmark) in the recent years (Table 1).

Heart and skeletal muscle inflammation (HSMI) is another disease that is associated with a recently discovered virus; piscine orthoreovirus (PRV). The role of this virus in HSMI is not fully understood. Large PRV intensities are found in fish developing HSMI, but the virus may also be found in healthy fish. The disease is an increasing problem in fish farming in Norway with 131-162 annual outbreaks registered in the period 2009-2013 (Hjeltnes 2014). PRV has been detected in wild salmon and sea trout, as well as certain marine fish species by real-time rt-PCR (Wiik-Nielsen, Lovoll et al. 2012, Garseth, Fritsvold et al. 2013). A new report has 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 (Garseth, Ekrem et al. 2013). However, little is known about the mechanism of transmission of the virus. Modelling has suggested that farm-intensity in a region is a major risk factor for HSMI outbreaks (Kristoffersen, Bang Jensen et al. 2012), implying that water borne transmission may be important. Nordland county had the highest number of HSMB outbreaks in northern Norway in 2010-12 (Table 1).

Table 1: The number of the registered outbreak of PD and HSMI in Northern Norway from 2010-2012 ((Taranger 2014).

County/Year	Number of PD outbreaks				Number of HSMI outbreaks			
	2010	2011	2012	Total	2010	2011	2012	Total
Finnmark	0	0	1	1	8	16	14	38
Troms	0	0	1	1	9	7	17	33
Nordland	0	0	0	0	24	47	30	101
Total	0	0	2	2	41	70	61	172

There are limited data on the pathogen prevalence in wild salmon in northern Norway (Garseth, Fritsvold et al. 2013). The fish farming intensities vary, being lowest in Finnmark and highest in Nordland (Table 2). Some of the largest wild salmon populations in Norway are also found in the northern Norwegian counties. Therefore, northern Norway is an interesting area to study the disease interaction between farmed and wild salmon populations.

Table 2: The number (mean) of operative fish farms in the northern counties from 2010-2012.

(source: Fiskeridirektoratet <http://www.fiskeridir.no/statistikk/akvakultur/biomassestatistikk/biomassestatistikk>)

County	The number (mean) of operative farming sites			Mean
	2010	2011	2012	
Finnmark	35	37	39	37
Troms	51	48	48	49
Nordland	106	115	113	111

Our previous disease screening has not detected SAV infections in sea trout (Biering, Madhun et al. 2013). However, we showed that wild sea trout could be naturally infected by PRV, albeit the prevalence and the intensity of infection were low. The prevalence of PRV varied with place and apparently also with year of sampling. In the current report, we further tested 200 sea trout collected in 2013 for SAV and PRV infection in order to expand our previous work (2011-12) by a year to reduce the impact of interannual differences and confirm the previous finding.

2 Aim

The aim of the current program is to investigate the occurrence and distribution of SAV and PRV in wild Atlantic salmon captured in northern Norway, from coastal areas that have different fish farming intensities and disease outbreak profile.

Additionally, we analysed wild sea trout collected in 2013 from Hordaland and Rogaland counties for SAV and PRV infections, in order to substantiate our previous findings (Biering, Madhun et al. 2013).

3. Materials and methods

A total of 422 salmon were caught using nets and fish traps, at five sea locations in Nordland, Troms and Finnmark counties in 2012 (Figure 1). A total of 200 sea trout were collected from Rogaland (Hellvik, Nedstrand) and Hordaland (Etne, Rosendal, Ålvik) as previously described (Biering, Madhun et al. 2013).

The captured fish were deep frozen (-20 °C) as soon as possible after capture. At autopsy, tissues from the heart and head kidney were aseptically taken out from the fish while still frozen and transferred frozen to tubes on dry-ice. In addition, gill samples were taken. Heart samples were sent frozen (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-



Fig. 1: Map of salmon sampling sites (red circles) in northern Norway.

house real-time rt-PCR assays. The SAV assay used detects both SAV2 and SAV3. Samples with C_t (cycle-threshold) value below 37.0 were considered positive. Length, weight and the sex of the fish were recorded. Scales from fish were used to determine if the salmon was wild or farmed (i.e. escaped) and to reveal their sea age.

4. Results

Sea Trout

SAV and PRV were not detected in any of the tested sea trout irrespective of the capture area. These results substantiate our previous findings (Biering, Madhun et al. 2013), that analysis of sea trout for these viral infections is unsuitable as indicator of infection pressure from fish farming.

Salmon from Northern Norway

One in ten captured salmon were escaped farmed fish

The escaped farmed fish constituted 10% of the analysed captured fish (Table 3). The highest proportion of farmed fish was found in Nordland, followed by Troms and Finnmark counties.

No SAV virus was detected in salmon from northern Norway

SAV was not detected in any of the hearts from the tested salmon, wild or farmed (escaped). The tested fish were caught in areas with no or very low number of PD outbreaks in the last three years (see Table 1), and they likely migrated from the area as smolts at a time with no (registered) PD outbreaks. Therefore the present observations represent baseline data on prevalence of SAV in wild salmon populations in northern Norway.

Prevalence of PRV in wild salmon

Piscine orthoreovirus infections were detected in 67 (16%) of the 422 salmon (Table 3). The real-time rtPCR C_t -values ranged from (23.0-36.7) indicating a variable amount of virus present. The prevalence of PRV was significantly higher in escaped farmed fish (85%) than in wild salmon (8%). In wild salmon, the prevalence of PRV was significantly higher in Nordland (21%) than in Finnmark (6%).

Table 3: The numbers and percentages of escaped farmed salmon and the prevalence of PRV virus in returning salmon collected from different geographical areas in northern Norwegian coast.

County	Number and origin of captured salmon			Prevalence of PRV positive salmon	
	Total	Wild	Farmed	Wild	Farmed
Finnmark	261	251	10 (4%)	15 (6%)	8 (80%)
Troms	105	87	18 (17%)	8 (9%)	15 (83%)
Nordland	56	43	13 (23%)	9 (21%) ^a	12 (92%)
Total	422	381	41 (10%)	32 (8%)	35 (85%) ^b

^aSignificantly higher than in Finnmark.

^bSignificantly higher than in wild salmon

Among the wild fish the two sea-winter (2010 smolt) salmon had significantly higher prevalence (19%) of PRV than one sea-winter (2011 smolt) salmon (6%). In the three counties, the total number of HSMB outbreaks was 41 and 70 in 2010 and 2011 respectively. Therefore, PRV prevalence in wild salmon appears to be unrelated to the number of HSMI outbreaks in the year of smolt migration. There was no difference in PRV prevalence between male and female salmon.

5. Discussion and Conclusion

We investigated the possibility of using sea trout as indicator of infection pressure from fish farming for certain viral infections. The lack of SAV detection in sea trout from farming areas with reoccurring PD outbreaks may suggest that this species is more resistant to the virus. Since the discovery of the SAV virus, there has not been any challenge experiments performed with sea trout. However, sea trout challenged with tissue homogenates from pancreas-diseased salmon did not become sick, nor did they develop pancreatic lesions (Boucher, Raynard et al. 1995). Our previous and current results suggest that infection status of sea trout is not a suitable indicator of infection pressure from fish farming for SAV and PRV viruses.

SAV was not detected in any the captured salmon, either farmed or wild. The fish were captured in areas with very low numbers of PD outbreaks (2010-12). Therefore, the probability of exposure to the virus both when migrating as smolts (2010-11) and when returning in 2012 was very low. The current data, our unpublished data and the available literature suggest that the prevalence of SAV in wild salmonid populations is very low (Biering, Madhun et al. 2013), irrespective of the area of capture, farming intensity and the number of PD outbreaks in fish farming. Salmon that survive SAV infection may become a carrier of the virus for months (Andersen, Bratland et al. 2007, Graham, Fringuelli et al. 2010). However, whether the host will be a life-long carrier or the virus will 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.

In contrast, PRV was detected in both wild and escaped farmed fish. The escaped farmed salmon collected in this study had a high PRV prevalence (85%). Therefore, it is evident that escaped salmon may constitute mobile pathogen reservoirs, that may act as a source of infections in wild salmon (Madhun, Karlsbakk et al. 2014).

The prevalence of PRV was significantly lower in wild salmon than in escaped farmed fish. The prevalence was significantly higher in wild salmon from Nordland county (21%) compared to Finnmark (6%). The high prevalence of PRV in wild salmon from Nordland coincides with a high number of the HSMB outbreaks and a high fish farming intensity in that county (Table 1 and 2). In a recent report, Garseth et al. (Garseth, Ekrem et al. 2013) have suggested that extensive transmission of PRV from fish farms to wild salmon has occurred. We are now performing genotyping of PRV from some positive samples (with low C_t) in order to compare genotypes from the wild and farmed fish. The finding that two sea-winter salmon had significantly higher prevalence of PRV than one sea-winter salmon suggest that increased sea-age increases the likelihood of being infected. On the other hand, it is also possible that PRV infection delay the return of salmon by affecting their age at maturity or by increasing mortality in fish that mature early. Further studies are needed to investigate the effect of PRV infection on sexual maturation and behaviour. There is increasing evidence that PRV is the main cause of HSMI. However, the virus is also detected in wild fish with no apparent pathology. The impact of infections on the fitness and mortality of wild salmon populations is currently unknown.

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Health monitoring of wild anadromous salmonids in freshwater 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 we organize mandatory testing of brood stock for infectious agents. The testing is done by PCR on head kidney that is sampled during autopsy after stripping. 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 (<http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-20080617-0822.html>).

2. Aim

The aim of the health monitoring program in 2013 was to investigate the occurrence of salmonid alphavirus (SAV) and infectious salmon anemia virus (ISAV) in returning wild brood fish of the species *Salmo salar* collected from different geographical areas along the Norwegian coastline. Results from prior *R. salmoninarum*, IPNV and *A. salmonicida* analyses from salmon, sea trout and arctic char (*Salvelinus alpinus*) 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. As the main target organ for SAV is heart, and ISAV is most easily detected in gills, an expanded sampling which also included gill and heart was organized. *R. salmoninarum*, IPNV and *A. salmonicida* were analyzed on kidney, SAV and ISAV were analyzed on a mix of heart and gill. In total 660 kidney samples (salmon, sea trout and char) and 418 heart and gill samples (salmon only) were tested. Autopsies and sampling was performed by authorized fish health personnel (veterinarian or fish health biologist) contracted to the individual hatchery or employed by the NVI. 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.

Tissue 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. In addition 420 tests for *R. salmoninarum*, 194 tests for IPNV and 12 tests for *A. salmonicida* were performed on ovarian fluid or milt from salmon, sea trout and arctic char. All PCR assays were performed by PatoGen Analyse AS.

4. Results

In the entire material, one IPNV positive individual was detected. The positive was a confirmed wild salmon caught in Rogaland County. Tables 1 (salmon) and 2 (sea trout and char) provide a county by county listing of the kidney, heart and gill samples.

Table 1 *Salmo salar*: Total number of analyses on kidney, heart and gills for each agent. ¹⁾ One positive for IPNV in Rogaland (wild). All remaining analyses were negative.

PCR analysis	<i>R. salmoninarum</i>	IPNV	<i>A. salmonicida</i>	SAV	ISAV
County					
Finnmark	40	40	-	40	40
Nordland	48	48	-	46	46
Nord-Trøndelag	41	24	-	24	24
Sør-Trøndelag	5	5	-	5	5
Møre og Romsdal	80	51	69	80	80
Sogn og Fjordane	144	68	28	40	40
Hordaland	43	20	20	50	50
Rogaland	66	46 ¹	8	38	38
Vestfold	59	2	-	59	59
Østfold	36	36	-	36	36
Total no. analyses	562	340	125	418	418

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

PCR analysis	<i>Salmo trutta</i> , anadromous sea trout			<i>Salvelinus alpinus</i> , arctic char	
	<i>R. salmoninarum</i>	IPNV	<i>A. salmonicida</i>	<i>R. salmoninarum</i>	IPNV
County					
Nordland	13	13		45	45
Møre og Romsdal	26	26			
Hordaland	14	6	6		
Total no. analyses	53	45	6	45	45

5. Discussion and conclusion

In the entire material, only one individual positive for IPNV was found. All remaining analyses were negative. It appears that some viral infectious agents that are highly prevalent within the Norwegian aquaculture industry, like IPNV and SAV, are found only in low prevalence in wild brood fish. This result is in accordance with our previous report from the 2012 season. The obvious question, as raised by McVicar in 1997 (McVicar 1997), 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 to be sampled. We have previously shown that virus can be transmitted between farmed and wild fish. Piscine orthoreovirus (PRV), the causative agent of heart and skeletal muscle inflammation (HSMI), is a common finding in returning brood fish (Garseth *et al.* 2013a). A recently published phylogenetic analysis shows that PRV is transferred between farmed and wild salmonids (Garseth *et al.* 2013b). It is possible that PRV has less impact on wild fish than for example SAV or IPNV, allowing more positives to be found.

6. Acknowledgements

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7. References

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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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