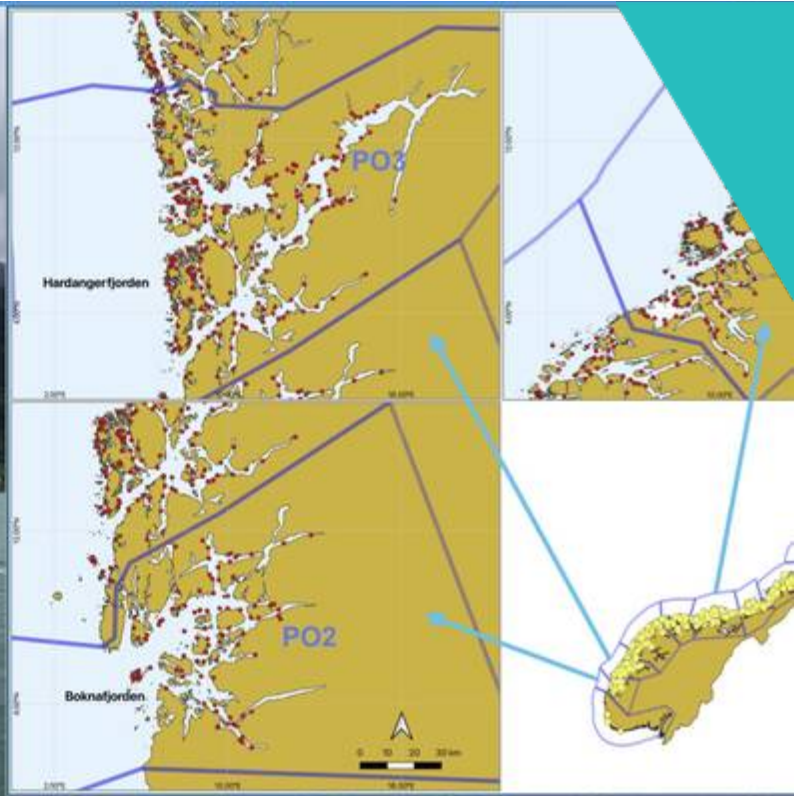




ANNUAL REPORT ON HEALTH MONITORING OF WILD ANADROMOUS SALMONIDS IN NORWAY 2024

Screening of Atlantic salmon postsmolts from Boknafjorden, Hardangerfjorden and Trondheimsfjorden as well as sea trout from Hitra for pathogen infections



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Annual report on health monitoring of wild anadromous salmonids in Norway 2024

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Screening of Atlantic salmon postsmolts from Boknafjorden, Hardangerfjorden and Trondheimsfjorden as well as sea trout from Hitra for pathogen infections

Patogenscreening av atlantisk laks postsmolt fra Boknafjorden og Hardangerfjorden, Trondheimsfjorden og sjøørret fra Hitra

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Summary (English):

The Institute of Marine Research has investigated the occurrence of infectious piscine orthoreovirus 1 (PRV-1), piscine myocarditis virus (PMCV) and bacterial kidney disease (BKD) infections in wild Atlantic salmon postsmolt and sea trout caught in 2024 in three aquaculture production areas (PO2, 3 and 6). The fish were collected as part of the national salmon lice surveillance program (NALO). Post-smolt were captured in the outer parts of Boknafjorden (N = 50), Hardangerfjorden (N = 50) and Trondheimsfjorden (N = 50) by trawling during the period May-June. Sea trout (N = 21) were also caught by trawling in Hitra area during the same period. In addition, five escaped farmed salmon caught in the same area (Hitra) were tested for these pathogens. Low concentrations of PRV1-RNA were detected in two post-smolts from Boknafjorden and two from Trondheimsfjorden. PRV1 was also detected in one sea trout and one escaped farmed salmon from Hitra area. Low to moderate PMCV-RNA levels were detected in one post-smolt from Boknafjorden, three from Hardangerfjorden and two from Trondheimsfjorden. PMCV was not detected in sea trout, but moderate amounts of PMCV-RNA were detected in all five escaped farmed salmon. *Renibacterium salmoninarum* (BKD agent) was not detected in any of the tested postsmolt, sea trout or escaped salmon. The results in this report showed that both postsmolt and sea trout from the studied fjords had very low occurrence of pathogen infections that are common in Norwegian aquaculture. These findings complement and confirm our previously reported data and may indicate that the prevalence of pathogen infections in wild salmon postsmolt and sea trout is not significantly affected by the incidence of these infections in fish farming.

Summary (Norwegian):

Havforskningsinstituttet har undersøkt forekomsten av infeksiøs piscine orthoreovirus 1 (PRV-1), piscine myokardittvirus (PMCV) og bakteriell nyresyke (BKD) infeksjoner i vill atlantisk laks postsmolt og sjøørret fanget i 2024 i tre akvakulturproduksjonsområder (PO2, 3 og 6). Fisken ble samlet inn som en del av det nasjonale overvåkingsprogrammet for lakselus (NALO). Postsmolten ble tatt i ytre deler av Boknafjorden (N = 50), Hardangerfjorden (N = 50) og Trondheimsfjorden (N = 50) ved tråling i perioden mai-juni. Sjøørreten (N = 21) ble også fanget ved trål i Hitra område i samme periode. I tillegg, ble fem rømt oppdrettslaks som var fanget i samme område (Hitra) testet for disse patogenene. Lave konsentrasjoner av PRV1-RNA ble påvist i to postsmolt fra Boknafjorden og to fra Trondheimsfjorden. PRV1 ble også påvist i én sjøørret og en rømt laks fra Hitra område. Lav til moderat PMCV-RNA mengde ble påvist i én postsmolt fra Boknafjorden, tre fra Hardangerfjorden og to fra Trondheimsfjorden. PMCV ble ikke påvist i sjøørret, men moderat mengde PMCV-RNA var detektert i all fem rømte oppdrettslaksene. *Renibacterium salmoninarum* (BKD agens) ble ikke påvist i noen av postsmolten, sjøørreten eller rømte laksen. Resultatene i den rapporten viste at både postsmolt og sjøørret fra de undersøkte fjordene hadde svært lav forekomst av patogensmitte som er vanlig i norsk oppdrett. Disse funnene utfyller og bekrefter de tidligere rapporterte dataene våre og kan tyde på at prevalensen av patogeninfeksjoner i villaks postsmolt og sjøørret ikke påvirkes signifikant av forekomsten av disse infeksjonene i fiskeoppdrett.

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Introduction

Infectious diseases in fish farming in Norway is a problem which often leads to serious economic losses, reduced fish welfare and increased mortality (Table 1) [1]. The most frequently reported viral diseases in salmon farming are pancreas disease (PD), caused by salmonid alphavirus (SAV), infectious salmon anaemia (ISA), caused by ISA virus (ISAV), heart and skeletal muscle inflammation (HSMI), caused by piscine orthoreovirus 1 (PRV1) and cardiomyopathy syndrome (CMS), caused by piscine myocarditis virus (PMCV).

Heart and skeletal muscle inflammation (HSMI) is the most abundant viral disease in salmon farming. The disease is an increasing problem in fish farming in Norway with 79–188 annual registered cases of HSMI in recent years [1,2]. High PRV-1 viral loads are found in fish developing HSMI but may also occur in healthy fish. Cardiomyopathy syndrome (CMS) is a growing problem in Norwegian salmon farming with 82 to 155 annual outbreaks in the last few years [1,3].

Bacterial kidney disease (BKD) is a serious chronic systemic infection in salmonids that is caused by *Renibacterium salmoninarum*. It is a notifiable disease and has been sporadically reported in farmed and wild salmonids in Norway in the last 20 years. However, 12 BKD-outbreaks were reported in production areas (PO) 4 (N=3) and PO6 (N=9) in 2023. Furthermore, 8 new outbreaks were reported in PO5 (N=2) and PO6 (N=6) in 2024 [1]. The sudden increase in outbreaks is alarming and needs further attention from the fish farming industry as well as the management authorities. In May 2024, 8400 farmed salmon escaped from one of the farms that have been diagnosed with BKD in PO6. The occurrence of such an escape event may lead to increased infection pressure on wild salmonids in the surrounding area. Previous studies have shown that farmed escapees represent a continuous source of infectious agents which could potentially be transmitted to wild fish populations both in rivers and at sea [4,5].

Table 1: The number of registered viral disease outbreaks in fish farming in the years 2020-2024 [1]

	2020	2021	2022	2023	2024
PD	158	100	98	58	48
ISA	23	25	15	18	13
HSMI	161	188	147	184	115
CMS	154	155	131	129	78
Total	496	468	391	389	254

Disease outbreaks in salmon farms may negatively impact the wild salmonids in Norway. It is difficult to quantify disease incidence and its impact on wild fish since sick individuals may be less catchable or may disappear in nature unnoticed (e.g. due to predation). Therefore, it is challenging to evaluate the impact of pathogens on individuals as well as stocks, since we normally are only able to collect infected but non-diseased fish such as individuals that have recently contracted or survived an infection (carriers).

The effect of fish farming on the infection status of wild salmon stocks can be evaluated by comparing pathogen prevalence in wild fish populations originating from areas with different fish farming intensities and disease outbreak profiles.

Wild salmon can be infected by pathogens prevalent in salmon farming; in rivers as fry or parr by virus-infected farmed escapees or spawning wild salmon, or from salmon farms in the fjord when migrating as postsmolts or

returning as adults. Therefore, infection status in migrating postsmolts may represent a direct indicator of infection pressure from salmon farming during their migration routes. However, the study of pathogen infection during all the life stages of salmon is necessary to assess the overall impact of diseases in fish farming on the wild salmon stocks.

Since 2012, the Institute of Marine Research (IMR) has been commissioned by the Norwegian Food Safety Authority (NFSA) to carry out an annual health monitoring of wild anadromous salmonids in Norway. The current monitoring activities are financed by both NFSA and the Norwegian Ministry of Trade, Industry and Fisheries (NFD). The activities lie within a prioritized research area at IMR which addresses the environmental impact of disease transmission from Norwegian fish farming to wild fish. The surveillance activities aim to evaluate the virus transmission from farmed fish to wild salmonids by monitoring and identifying changes in the prevalence of selected pathogens in wild salmonids as a result of fish farming activities. In addition, the surveillance aims to increase the knowledgebase about pathogens in wild salmonids in general, as well as establish a biobank that can be used when new disease challenges arise. Furthermore, the surveillance consolidates with the other activities in the larger strategic research effort on diseases and disease transmission in wild fish.

Part of the research activities in the surveillance program aims to generate data about:

- Pathogen prevalence in fry, parr, postsmolt and returning adult salmon.
- Prevalence of pathogens in sea trout.
- Prevalence of infections in escaped farmed salmonids.
- Genotypes and characteristics of detected pathogens.

The virus screening is based on selected materials obtained through monitoring of pathogen infections in wild salmonids project and other associated projects at IMR, such as:

- National salmon lice monitoring program (NALO).
- National escaped salmon monitoring program.
- Etne research platform (fish trap).

The current monitoring program aims to investigate the occurrence of pathogen infections in wild salmonids captured from different Norwegian coastal areas with different farming intensities and disease outbreak frequencies. Each year selected sets of fish are analysed in order to complement or complete our data and time series. Part of the results from pathogen screening are used in an annual health monitoring of wild anadromous salmonids in Norway commissioned by NFSA. The generated knowledge from the program contributes to the institute's main goal/strategy in providing advice and further development of sustainable management of aquaculture and is utilized in the IMR's annual risk assessment of Norwegian fish farming [6,7].

Aim

The aim of the current study was to investigate the occurrence of PRV1, PMCV and *Renibacterium salmoninarum* (BKD) infections in migrating wild Atlantic salmon postsmolts captured in 2024 in three fjord systems located in three aquaculture production areas (PO2, PO3 and PO6) with differing risk for pathogen infection. Additionally, the occurrence of PRV1/3, PMCV and *Renibacterium salmoninarum* infections were tested in sea trout from PO6 (Hitra). Five escapees that were captured in the same area (Hitra) were also tested for these pathogens.

Materials and methods

To provide data about the prevalence of different pathogens in different salmon life stages and different geographical regions, we randomly selected fish caught by trawl as part of the national salmon lice monitoring program (NALO) [8]. The salmon postsmolts were caught in the outer parts of Boknafjorden, Hardangerfjorden and Trondheimsfjorden during the period May-June 2024 (Fig. 1 and Table 2). The trawl data are available at nmdc.no. Twenty-one sea trout and 5 escaped farmed salmon were caught in the outer part of Trondheimsfjorden (Hitra area) and were also used in the current study.

Salmon lice infestation (count), weight and length of all postsmolts and sea trout were recorded, and the fish were then frozen (-20 °C) as soon as possible. At autopsy, tissues from the gills, head kidney and heart were taken from the fish while still frozen and stored at -80 °C. Samples for analysis were sent on dry ice to an accredited commercial laboratory for RNA extraction and pathogen testing (Pharmaq Analytiq AS; <https://www.pharmaq.com/en/analytiq>). All fish were tested for PRV1/3, PMCV and Renibacterium salmoninarum (Table 2) by real-time RT-PCR assays (for detection of pathogen RNA). Samples with Ct (cycle threshold) value below 37.0 were considered positive. A total of 528 real-time RT-PCR test were performed on 176 fish and included in the current report.

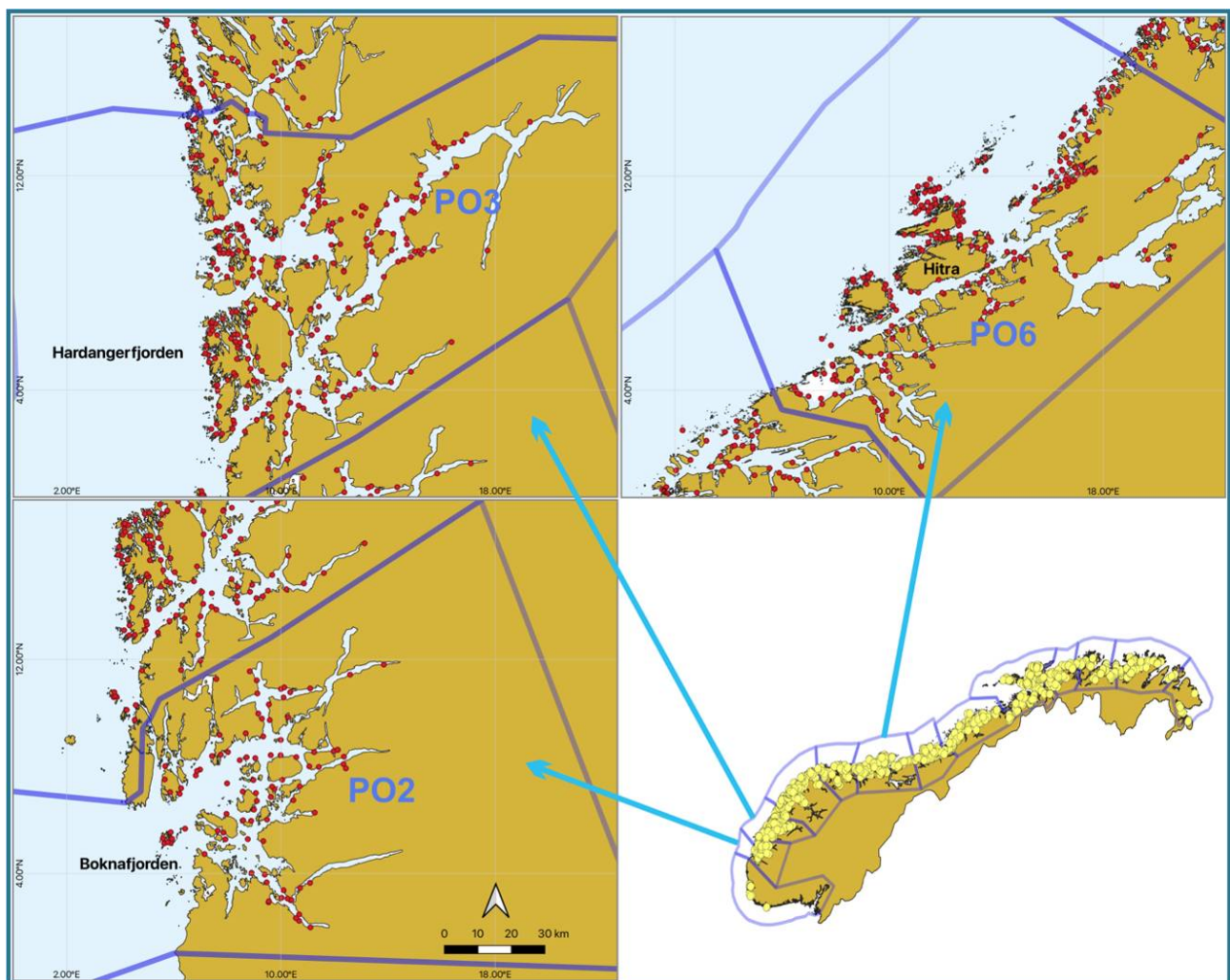


Fig. 1: Map showing postsmolt and sea trout collection fjords and fish farms (red circle) in these fjords. PO refers to production areas.

Results

PRV1 was detected in four postsmolts; two from Boknafjorden and two from Trondheimsfjorden (Table 2). PRV1 was also detected in one sea trout as well as one escaped farmed salmon from Hitra (PO6). The Ct-values of PRV1-positive samples ranged from 22 to 36.

Low to moderate concentrations of PMCV (Ct-values: 25-37) were detected in 6 postsmolts; one from Boknafjorden, three from Hardangerfjorden and two from Trondheimsfjorden. None of the sea trout were positive for PMCV. However, all the five escaped farmed fish were infected with moderate concentrations of PMCV (Ct-values: 23-28). *Renibacterium salmoninarum* was not detected in any of the tested fish.

Table 2: The numbers (N) and the collection sites of tested fish and the numbers (percentage) of pathogen-positive postsmolt.

Collection Site (production area)	N	PRV1	PMCV	R. Sal.*
<i>Postsmolt Boknafjorden (PO2)</i>	50	2 (4%)	1 (2%)	0 (0%)
<i>Postsmolt Hardangerfjorden (PO3)</i>	50	0 (0%)	3 (6%)	0 (0%)
<i>Postsmolt Trondheimsfjorden (PO6)</i>	50	2 (4%)	2 (4%)	0 (0%)
<i>Sea trout Hitra (PO6)</i>	21	1 (5%)	0 (0%)	0 (0%)
<i>Escaped farmed salmon (PO6)</i>	5	1 (20%)	5 (100%)	0 (0%)

* *Renibacterium salmoninarum*

Discussion and conclusion

PRV1 infections are abundant in fish farming in production areas PO2-13. Low concentrations of PRV1 were detected in two wild salmon postsmolt from production area PO2 and two postsmolt and one sea trout from PO6. Our earlier reports have shown low prevalence of PRV1 in sea trout, returning adult salmon, postsmolt and juveniles from Norwegian rivers [6,7,10-15]. Previous reports have also 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 and wild fish [10,16]. However, little is known about the mechanism of transmission of the virus. The low prevalence of PRV1 infections in wild salmonids [9-14] may suggest that the prevalence of virus infections in wild salmonids is not significantly affected by fish farming intensities considering the fish farming activities in the three production areas used in the current study.

CMS is a growing problem in Norwegian salmon farming with frequent annual outbreaks in salmon farming along the Norwegian coastal areas [1,3]. PMCV (CMS-virus) was detected in 6 wild fish from the three production areas. The Ct-values ranged between 25 and 37 indicating a low to moderate viral load. Previous reports have shown low prevalence of PMCV infections in wild salmon and that infection was not associated with fish farming activities [6,13,17].

There was no known BKD-outbreak in PO2 and PO3 during 2023-2024. Meanwhile, 9 BKD-outbreaks were reported in PO6 in this period. All salmonids (including sea trout) are susceptible to infection by *Renibacterium salmoninarum* which is the causative agent of BKD. However, *Renibacterium salmoninarum* was not detected in any of the tested fish irrespective of collection area or the number of BKD-outbreaks. Our previous report and unpublished results did not reveal any *Renibacterium salmoninarum* infection in wild salmonids collected from areas with or without BKD-outbreaks. These findings suggest that despite the increased number of BKD-outbreaks in PO6, *Renibacterium salmoninarum* was not detected in wild salmonids collected from the area. However, infection by *Renibacterium salmoninarum* progresses slowly and it may take long time before the bacterium is detectable in infected wild fish. Therefore, the negative result should be interpreted with caution. Screening of wild salmonids for *Renibacterium salmoninarum* will continue in the coming years.

Five escaped farmed fish were captured as a bycatch during postsmolt trawling conducted as part of the national salmon lice monitoring program in PO6. Based on the size of fish (average weight: 8.5 kg) and the location of capture, it is likely that the escapees originated from the escape event reported in May 2024. The likely origin farm was diagnosed with both PD and BKD. Therefore, it was interesting to test the escapees for *Renibacterium salmoninarum*, PRV1, PMCV and SAV and include the results in the current report. All the five tested escapees were infected with PMCV and one of the escaped farmed salmon was infected with PRV1. The Ct-values of PMCV positive samples ranged between 23 and 28 indicating a moderate viral load. However, *Renibacterium salmoninarum* was not detected in the escapees and only one of the fish was SAV positive (data not shown). Our previous reports have shown that most of escaped farmed fish are infected with one or more viruses that are common in fish farming. Therefore, these findings have shown that farmed escapees represent a continuous source of infectious agents which could potentially be transmitted to both farmed and wild fish populations both in rivers and at sea. Additionally, these results also highlight the need for more knowledge about the impact of escapees in rivers on the prevalence of pathogens, common in fish farming, in vulnerable wild juveniles in these rivers.

The farming intensities in PO2, PO3 and PO6 were approximately 44.9 and 48.5 and 25.3 tonnes/km² respectively [7]. Hardangerfjorden is located in PO3 which is one of the areas with the highest fish farming intensity in Norway. Our results indicate that the prevalence of pathogen infections in postsmolt collected from these production areas was not associated with high fish farming intensity. The current findings are in line with our previous reports that showed no apparent relationship between the prevalence of pathogen infection in wild salmon and the fish farming intensity or the frequency of disease outbreaks in collection areas [9-15]. These observations may indicate that wild salmon are exposed to a low infection pressure from fish farming. However, the possibility that infection may lead to rapid disappearance or altered behaviour of the infected fish, and therefore potentially affect the results, cannot be ruled out. Other explanations for the low prevalence of pathogens in postsmolts is the time needed after pathogen exposure (incubation time) before the pathogen can be detected in tissues of fish.

These findings complement and corroborate our previously reported data and suggest that it is unlikely that wild salmonids are the main reservoir for pathogens that are common in fish farming and therefore an unlikely source of spill over for these pathogens (mainly viruses) to fish farming. There are still gaps in our knowledge about diseases in wild fish and the interaction between farmed and wild fish [6,7]. Time series of samples of all life stages of wild salmonids from areas with different salmon farming intensity are necessary to better evaluate and understand the long-term effect of infection pressure from aquaculture on the virus prevalence in wild salmon populations. Such series will also enable us to assess changes in the prevalence due to increased fish farming activities, increased pathogen virulence, the emergence of new diseases and climate change.

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