ANNUAL REPORT ON HEALTH MONITORING OF WILD ANADROMOUS SALMONIDS IN NORWAY 2020

Screening of wild Atlantic salmon (Salmo salar) postsmolts for viral infections

Abdullah Sami Madhun, Ørjan Karlsen, Rune Nilsen and Bjørn Olav Kvamme (IMR)

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Authors:

Abdullah Sami Madhun, Ørjan Karlsen, Rune Nilsen and Bjørn Olav Kvamme (IMR)

Reasearch group leader(s): Bjørn Olav Kvamme (Smittespredning og sykdom) Approved by: Forskningsdirektøre(r) en: Geir Lasse Taranger Program leader(s): Terje Svåsand

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Summary (English):

The Institute of Marine Research has investigated the prevalence of infectious salmon anaemia virus (ISAV) and salmonid alphavirus (SAV, PD-virus) infections in migrating wild Atlantic salmon postsmolts captured in 2019 in three fjord systems located in three aquaculture production areas (PO2-4). These areas have had a sporadic case of ISA and a stable high incidence rate of PD, as well as a very high aquaculture intensity. The fish were collected as part of the national salmon lice monitoring program in the outer parts of the Bokna (N=132), Hardanger (N=110) and Sogne (N=110) fjords by trawling during the period May-June. ISAV was detected in two postsmolts from Hardanger fjord and SAV in one smolt from Sogne fjord. The Ct-value of SAV-positive fish was very high (38.9) which may suggest a false positive result. The findings from the current report indicate a very low prevalence of these viruses in wild migrating postsmolts. These findings complement and corroborate our previously reported data and may suggest that prevalence of ISAV or SAV infections in wild salmon postsmolts are not significantly influenced by the occurrence of these infections in fish farming.

Summary (Norwegian):

Havforskningsinstituttet har undersøkt utbredelsen av infeksiøs lakseanemi virus (ILAV) og salmonid alfavirus (SAV, PD-virus) infeksjoner i utvandrende vill Atlantiske postsmolt fanget i 2019 i tre fjordsystemer lokalisert i tre akvakultur produksjonsområder (PO2- 4). Disse områdene har hatt sporadisk tilfeller av ILA og en stabil høy forekomst av PD, samt en veldig høy oppdrettsintensitet. Fisken ble samlet inn som en del av NALO i de ytre delene av fjordene; Bokna (N = 132), Hardanger (N = 110) og Sogne (N = 110) ved tråling i perioden mai-juni. ILAV ble påvist i to postsmolt fra Hardangerfjorden og SAV i en smolt fra Sognefjorden. Høy Ct-vardien (38,9) i SAV-positiv fisk kan tyder på et falskt positivt resultat. Funnene fra den nåværende rapporten indikerer en svært lav forekomst av disse virusene i villmigrerende postsmolt. Disse funnene utfyller og støtter våre tidligere rapporterte data, og kan antyde at utbredelsen av ILAV- eller SAV-infeksjoner i villaks postsmolt ikke er signifikant påvirket av forekomsten av disse infeksjonene i fiskeoppdrett.

Content

1 - Introduction

Viral infections are one of the major challenges facing Atlantic salmon farming in Norway, often leading to disease outbreaks and to substantial economic losses. The most common viral diseases in salmon farming the last five years are; pancreas disease (PD), caused by salmonid alphavirus (SAV), heart and skeletal muscle inflammation (HSMI), caused by a piscine orthoreovirus 1 (PRV1), cardiomyopathy syndrome (CMS) caused by piscine myocarditis virus (PMCV), infectious salmon anaemia (ISA) caused by ISA virus (ISAV) and infectious pancreatic necrosis (IPN) caused by IPN virus (IPNV).

PD is one of the major diseases in fish farming and much of the fish in endemic areas are believed to become infected through a production cycle. In Norway, SAV3 was detected for a long time, but in 2011 SAV2 was also detected in salmon. Since then, PD outbreaks have been dominated by SAV2 in central Norway and SAV3 in western Norway [1].

ISA is a serious disease that has led to serious episodes in aquaculture in Faroe Islands, Norway and Chile, with enormous challenges for industry and a high risk of spreading to susceptible hosts. Since the 1980s, there have been relatively few ISA outbreaks in Norway. However, an increase in the number of ISA outbreaks in recent years along the entire Norwegian coast indicates an increasing problem in the salmon farming and an increasing risk of the spread of infection from infected farms to wild fish. There are two variants of the virus, one that causes disease (virulent, HPR-del) and one that does not cause disease (avirulent, HPR-0). There are indications that avirulent ISAV can mutate to virulent ISAV, but what triggers this is not known.

PD and ISA are notifiable diseases and available data on outbreaks provide a good basis for assessing the possibility for the spread of infection from farming to wild salmonids in the sea phase (Table 1).

Table 1: The number of registered PD and ISA outbreaks in fish farming in the years 2016-2020 [1]

	2016	2017	2018	2019	2020
PD	138	176	163	152	158
ISA	12	14	13	10	23

It is believed that pathogen exchange between farmed and wild salmon occurs and that disease outbreaks in salmon farms may lead to increased infection pressure on wild fish populations. There is an increasing public concern of this negatively impacting wild salmonids in Norway. However, there are limited data on the prevalence of pathogens in wild salmonid populations [2]. It is difficult to quantify disease incidence and its impact in wild fish since sick individuals may be less catchable or may disappear unnoticed (e.g. due to predation). Therefore, it is challenging to evaluate the impact of pathogens on individuals as well as stocks in nature, 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).

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

Wild salmon may be infected by viruses prevalent in salmon farming; in rivers as fry or parr by virus-infected farmed escapees and spawning wild salmon, or from salmon farms in the fjord when migrating as smolts or returning as adults. Therefore, infection status in migrating smolts may represent a direct indicator of infection pressure from salmon farming during their migration routes. Studying viral infection during all life stages of salmon life cycle is necessary to assess the impact of diseases in fish farming on the salmon wild 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). These activities lie under a program that is part of prioritized research area at IMR which addresses the environmental impact of disease transmission from Norwegian fish farming to wild fish. The program aims to evaluate the virus transmission from farmed fish to wild salmonids by monitoring and identifying changes in the prevalence of selected viruses in wild salmonids as a result of fish farming activities. In addition, the program will help to increase the knowledge base on pathogens in wild salmonids in general, as well as establish a biobank that can be used when new disease challenges arise.

Part of the research activities in the program aim to generate data about:
- Virus prevalence in fry, parr, postsmolt and returning adult salmon,
- Prevalence of viruses in sea trout
- Prevalence of infectious escaped salmonids
- Genotyping of detected viruses

The virus screening is based on selected materials obtained through monitoring of virus infections in wild salmonids project and other associated projects at IMR, such as:
- Salmon lice monitoring program (NALO)
- Escaped salmon monitoring program
- Etne research station (fish trap)
- Dale research project
- Atlantic salmon at sea (Seasalar) project

The program aims to investigate the occurrence of virus 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.

2 - Aim

The aim of the current study was to investigate the occurrence of SAV and ISAV infections in migrating wild Atlantic salmon postsmolts captured in 2019 in three fjord systems located in three aquaculture production areas (PO2-4). These areas have had a sporadic case of ISA and a stable high incidence rate of PD, as well as a very high aquaculture intensity.

3 - Materials and methods

To provide data about the prevalence of different viruses in different salmon life stages and different geographical regions, we selected fish from our available material for analysis in 2020. These materials were migrating postsmolts captured, as part of the national salmon lice monitoring program [3], in the outer parts of the Bokna (N=132), Hardanger (N=110) and Sogne (N=110) fjords by trawling during the period May-June 2019 (Table 2). The postsmolt collection areas had 83 PD outbreaks and only one ISA outbreak (Fig. 1).

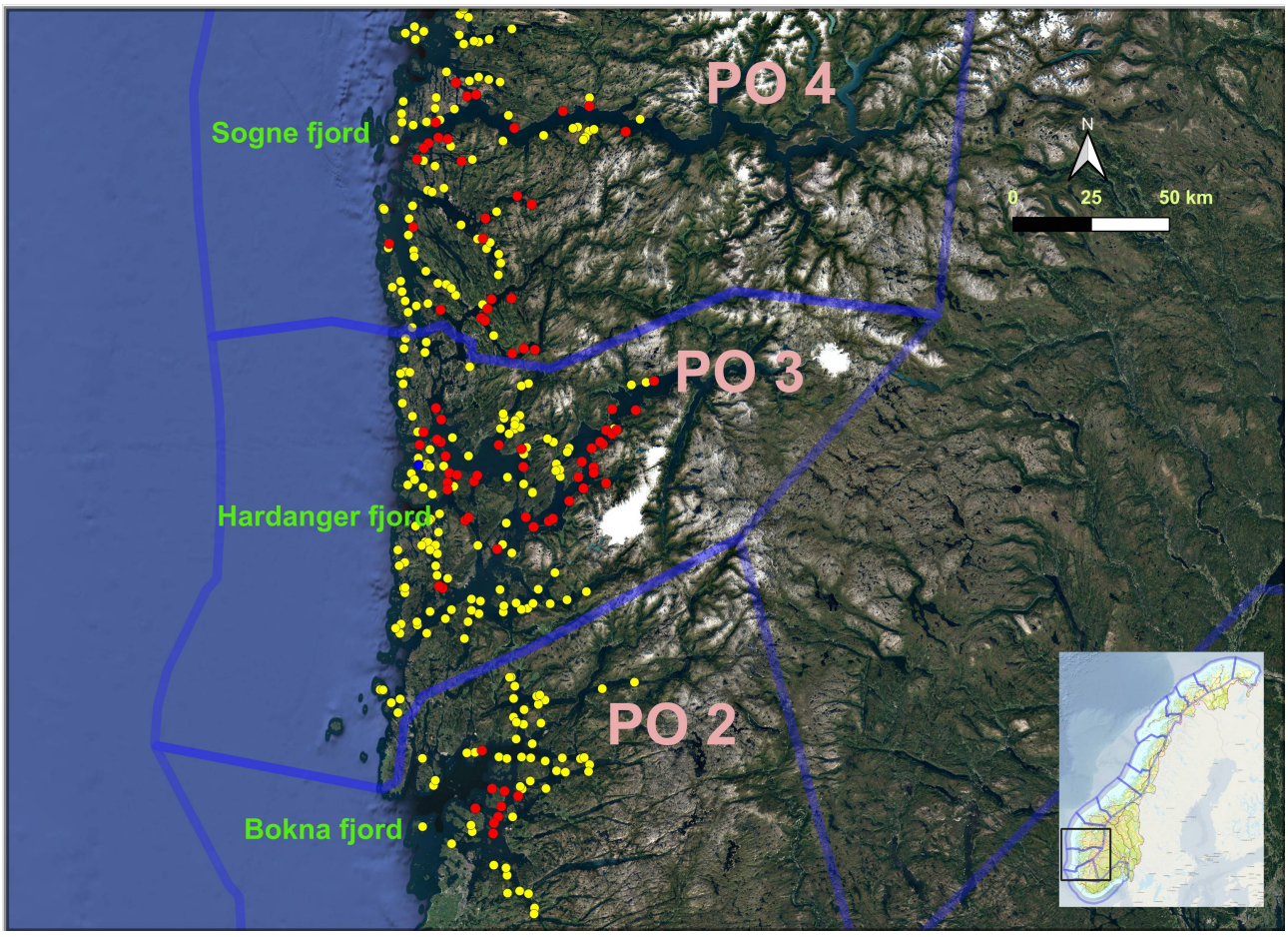

Fig. 1: Map showing postsmolt collection fjords, the fish farming sites (yellow circle) and sites with PD (red circle) and ISA (blue circle) outbreak during the period May-June 2019.

Weight and length of all fish were recorded and postsmolts were then frozen (-20 oC) 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 oC. Samples for analysis were sent on dry ice to an accredited commercial laboratory for RNA extraction and virus testing (Pharmaq Analytic AS). All fish were tested for SAV and ISAV (Table 2) by real-time RT-PCR assays (for detection viral RNA). A total of 704 analyses were performed on 352 fish and included in the current report.

5 - Results and Discussion

Virus infections in wild migrating postsmolts.

ISAV was detected in two postsmolts from Hardanger fjord (Ct-values 34.1 and 36.8) and SAV in one smolt from Sogne fjord (Ct-value 38.9) (Table 2).

Table 2: The numbers and the collection sites of tested fish and the numbers of virus-positive postsmolt.

Collection Site (production area)	Year	Number	SAV	ISAV
Bokna fjord (PO2)	2019	132	0	0
Hardanger fjord (PO3)	2019	110	0	2
Sogne fjord (PO4)	2019	110	1	0
Total/positive		352	352/1	352/2

ISAV was detected in two migrating postsmolt from production area PO3. The high Ct-value of PCR may indicate a very low virus concentration. The virus variant (HPR-0 or HPR-del) could not be confirmed as it was not possible to sequence the virus due to low viral-RNA concentrations in the positive samples. There was one ISA outbreak in the PO3 area as shown in Fig. 1. However, it is unlikely that the virus detected in positive fish was originated from this ISA outbreak as the postsmolt were captured by trawl in an area far away to the south of the outbreak site.

SAV was detected in very low concentration (Ct-value 38.9) in only one postsmolt from Sognefjord. The Ct-value is around the detection limit of the PCR assay (cut-off 37) and may suggest a false positive. There was only one ISA outbreak in the collection areas during postsmolt migration period and therefore the fish exposure to virulent ISAV (HPR-del) was neglectable. On the other hand, avirulent ISAV (HPR-0) is prevalent in Norwegian salmon farming [4] and was detected in wild salmon populations collected in other areas in Norway [5, 6]. Our recent study showed that ISAV (HPR-0) was detected in 7% of wild returning adult salmon from northern Norway [6]. This study showed no apparent relationship between occurrence of ISAV (HPR-0) in wild salmon and to fish farming intensity. Our previous annual heath monitoring reports and the published date confirm that ISAV (HPR-0) infection in wild salmon may occur and that this occurrence is not associated with salmon farming.

Unlike ISA, PD is very prevalent (endemic) in postsmolt collection areas. Therefore, it is likely that migrating postsmolt were exposed to SAV released from the fish farms before (subclinical infections or undetected disease) and during disease outbreaks. The low prevalence of SAV infections in the tested migrating smolt is consistent with previous findings in wild salmonids [6-13]. Our earlier report showed that migrating smolts from Trondheim fjord which has no fish farming activities also had very low occurrence of SAV infection [13]. Similarly, our published data from PD-free areas in northern Norway showed no SAV infection in wild returning adult salmon [14]. Furthermore, SAV was not detected in returning adult salmon collected from river Etne and from Oster fjord (located in production areas PO3 and PO4, respectively).

Our previous and current findings showed no apparent relationship between the prevalence of virus infection in wild salmon and the fish farming intensity or the frequency of disease outbreaks in collection areas [6-15]. 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, altered behaviour or biased sampling of the infected fish and therefore may affect the results, cannot be ruled out. Other explanation for the low prevalence of viruses (especially SAV) in postsmolt is the time needed after virus exposure (incubation time) before the virus can be detected in tissues of fish.

To verify our observation, a large PD-vaccine smolt release study was conducted in 2018 and 2019. In this study, a total of 52 000 (28 000 PD-vaccinated and 24 000 control) smolt were released in rivers Etne and Dale located in production areas PO3 and PO4 which had a high number of PD cases during the release period. The survival rate of returning adult salmon in the subsequent years were determined in both vaccine and control groups and used to estimate the mortality that may be attributed to SAV infection from fish farming in release areas. The current results (unpublished) from the study did not show any statistically significant differences in the mortality rate between the vaccine and the control groups and therefore support our observations that infection pressure of SAV from fish farms to wild salmon is low.

The results in the current report showed very low prevalence of viral infections in migrating postsmolts in fjords for two viruses occurring in Norwegian aquaculture. These findings complement and corroborate our previously reported data and may suggest that prevalence of ISAV or SAV infections in wild salmon postsmolts are not significantly influenced by the occurrence of these infections in fish farming. Time series of samples of all life stages of wild salmonids from areas with different salmon farming intensities are needed to better evaluate 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, the emergence of new diseases and climate change.

6 - References

1. Sommerset, I., et al. (2021). Fish health report 2020 (in Norwegian). 2021, Norwegian Veterinary Institute: 41a/2021.
2. Taranger, G.L., et al. (2015). Risk assessment of the environmental impact of Norwegian Atlantic salmon farming. *ICES Journal of Marine Science*, 72(3):997-1021.
3. Nilsen, R., et al. (2017). Lakselusinfestasjon på vill laksefisk langs norskekysten i 2016; med vekt på modellbasert varsling og tilstandsbekreftelse. Havforskningsinstituttet: 1-2017.
4. Lyngstad, T.M., et al. (2012). Low virulent infectious salmon anaemia virus (ISAV-HPR0) is prevalent and geographically structured in Norwegian salmon farming. *Diseases of Aquatic Organisms*,101(3):197-206.
5. Nylund, A., et al. (2019). Wild and farmed salmon (Salmo solar) as reservoirs for infectious salmon anaemia virus, and the importance of horizontal- and vertical transmission. *Plos One,* 14(4):https://doi.org/10.1371/journal.pone.0215478.
6. Madhun, A.S., et al. (2019) Prevalence and genotypes of infectious salmon anaemia virus (ISAV) in returning wild Atlantic salmon (Salmo salar L.) in northern Norway. *Journal of Fish Diseases*,*42*(8):1217-1221.
7. Madhun, A.S., et al. (2014). Annual report on health monitoring of wild anadromous salmonids in Norway 2013. Havforskningsinstituttet: 17-2014.
8. Garseth, Å.H., et al. (2015). Annual report on health monitoring of wild anadromous salmonids in Norway 2014. Havforskningsinstituttet: 7-2015.
9. Madhun, A.S., et al. (2016). Annual report on health monitoring of wild anadromous salmonids in Norway 2015. Havforskningsinstituttet: 22-2016.
10. Garseth, Å.H., et al. (2017) Annual report on health monitoring of wild anadromous salmonids in Norway 2016. Havforskningsinstituttet:15-2017.
11. Madhun, A.S., et al. (2018) Prevalence of piscine orthoreovirus and salmonid alphavirus in sea-caught returning adult Atlantic salmon (Salmo salar L.) in northern Norway. *Journal of Fish Diseases*, 41(5):797-803.
12. Madhun, A.S., et al. (2018). Annual report on health monitoring of wild anadromous salmonids in Norway 2017: Health monitoring of returning adult salmon from river Etne, western Norway. Havforskningsinstituttet: 26-2018.
13. Madhun, A.S., et al. (2019), Annual report on health monitoring of wild anadromous salmonids in Norway 2018; Screening of migrating Atlantic salmon (Salmo salar) postsmolts from the Trondheim fjord for viral infections. Havforskningsinstituttet: 2019-28.
14. Madhun, A.S., et al. (2016). Occurrence of salmonid alphavirus (SAV) and piscine orthoreovirus (PRV) infections in wild sea trout Salmo trutta in Norway. *Diseases of Aquatic Organisms*, *120*(2):109-113.
15. Biering, E., et al. (2013) , Annual report on health monitoring of wild anadromous salmonids in Norway 2012. Havforskningsinstituttet: 6-2013.

HAVFORSKNINGSINSTITUTTET

Postboks 1870 Nordnes
5817 Bergen
E-post: post@hi.no
www.hi.no