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RESEARCH ARTICLE

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Occurrence of salmonid alphavirus and piscine orthoreovirus-1 infections in migrating salmon (*Salmo salar* L.) post-smolt in western Norway

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Abstract

Viral diseases are a serious problem in Atlantic salmon (*Salmo salar* L.) farming in Norway, often leading to reduced fish welfare and increased mortality. Disease outbreaks in salmon farms may lead to spread of viruses to the surrounding environment. There is a public concern that viral diseases may negatively affect the wild salmon populations. Pancreas disease (PD) caused by salmonid alphavirus (SAV) and heart and skeletal muscle inflammation (HSMI) caused by piscine orthoreovirus-1 (PRV-1) are common viral diseases in salmon farms in western Norway. In the current study, we investigated the occurrence of SAV and PRV-1 infections in 651 migrating salmon post-smolt collected from three fjord systems (Sognefjorden, Osterfjorden and Hardangerfjorden) located in western Norway in 2013 and 2014 by real-time RT-PCR. Of the collected post-smolts, 303 were of wild origin and 348 were hatchery-released. SAV was not detected in any of the tested post-smolt, but PRV-1 was detected in 4.6% of them. The C_t values of PRV-1 positive fish were usually high (mean 32.0; range: 20.1–36.8). PRV-1 prevalence in post-smolts from the three fjords was 6.1% in Sognefjorden followed by 4.8% in Osterfjorden and 2.3% in Hardangerfjorden. The prevalence PRV-1 was significantly higher in wild (6.9%) compared to hatchery-released post-smolt (2.6%). The occurrence of PRV-1 infection in the fish was lowest in the Hardangerfjorden which has the highest fish farming intensity. Our results suggest that SAV infection are uncommon in migrating smolt while PRV-1 infection can be detected at low level. These findings suggest that migrating smolts were at low risk from SAV or PRV-1 released from salmon farms located in their migration routes in 2013 and 2014.

KEYWORDS
migration post-smolt, PRV-1, salmon farming, SAV

1 | INTRODUCTION

Viral diseases represent major problems in Atlantic salmon (*Salmo salar* L.) farming in Norway, often leading to reduced fish welfare and increased mortality. Disease outbreak in salmon farms may lead an increased infection pressure not only on fish in neighbouring farms but also on wild fish. There are increased public concerns that diseases in salmon farming may have a negative impact on wild salmon populations. Wild salmon may be exposed to the viruses prevalent in salmon farming in the fjords when migrating as post-smolt or returning as adults. Therefore, the prevalence of infections in migrating post-smolts may increase if impacted by infection pressures from salmon farms (Johnsen et al., 2021; Torrissen et al., 2013). On the contrary, juvenile salmon may be exposed already in the rivers, both from virus-infected farmed escapees and spawning wild salmonids. The annual salmon disease outbreak statistics allows a consideration of qualitative (types of pathogens), quantitative (frequency of outbreaks), and temporal variation in potential exposure (infection pressure) to pathogens. Therefore, 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 profile. However, using pathogen prevalence as indicator of infection pressure has its limitations (McVicar, 1997). Virulent pathogens may cause disease in migrating salmon post-smolt rendering them less catchable due to altered behaviour or/and increased mortality (predation). Therefore, when screening wild stocks for infections we are normally able to collect non-diseased infected fish such as individuals that have recently acquired infections or have survived an infection and become 'carriers'.

Pancreas disease (PD) and heart and skeletal muscle inflammation (HSMI) are among the most frequently diagnosed diseases in Norwegian fish farming in recent years (Sommerset et al., 2023). PD, caused by salmonid alphavirus (SAV) is enzootic in western and in mid-Norway. HSMI caused by PRV-1 is a common problem in salmon farms along the Norwegian coast (Sommerset et al., 2023). The PRV-1 infections have been detected in wild salmonid populations in Norway and other countries (Garseth, Fritsvold, et al., 2013; Madhun et al., 2016; Madhun, Isachsen, et al., 2018; Polinski et al., 2020; Siah et al., 2021; Teffer et al., 2020), normally at low prevalence.

This is the first study to investigate the prevalence of SAV and PRV-1 infections in migrating post-smolt collected in three different fjords located in western Norway.

2 | MATERIALS AND METHODS

2.1 | Fish collection

A total of 651 migrating salmon post-smolt were caught in 2013 and 2014 in outer parts of three fjords (Sognefjorden, Osterfjorden and Hardangerfjorden) in western Norway by using either a trawl (Holst & McDonald, 2000), or a specially designed smolt trap (Barlaup et al., 2013; Figure 1). In all, 148 fish were collected from Sognefjorden, 375 from Osterfjorden and 128 from Hardangerfjorden (Table 1). The total length and the weight of the fish were recorded at the collection site. The fish was classified as wild or hatchery-released based on the presence of adipose fin (hatchery-released smolt is adipose fin clipped). The captured fish were deep frozen (−20°C) as soon as possible after capture and kept frozen until sampling.

2.2 | Tissue sampling

Tissue samples were taken aseptically from the frozen heart (ventricle) and transferred to tubes on dry ice. The samples were sent frozen (dry ice) to an accredited commercial laboratory for RNA extraction and virus testing (PatoGen Analyse AS). After tissue sampling, the fish were thawed and visually inspected for tags, external and internal gross pathology or signs of disease and for sex determination.

2.3 | Detection of viruses

Testing for SAV and PRV-1 viruses were performed by PatoGen Analyse AS using their in-house real-time RT-PCR assays, as previously described (Madhun et al., 2015). PCR assay targeting the nsP1 gene was used to detect SAV infection (Hodneland & Endresen, 2006). This assay detects both the SAV subtypes (2 and 3) found in Norway (Hjortaas et al., 2013). The PRV-1 assay used was as previously described (Glover et al., 2013; Palacios et al., 2010), based on previously published sequences. Samples with C_t (cycle threshold) value below 37.0 were considered positive.

2.4 | Statistical analysis

Fisher's Exact test and Students *t*-test were used to compare the groups using Stata 14 software.

3 | RESULTS

3.1 | Characteristics of the studied post-smolt

None of the 651 captured post-smolt showed external or internal lesions or other signs of disease. The collected fish contained 303 wild and 348 hatchery-released (adipose fin clipped) post-smolt (Table 1). The average weight and length of the post-smolt was 35.4 g (SEM = 0.8) and 15.7 cm (SEM = 0.1) respectively (Table 1). The hatchery-released post-smolts were significantly larger (17.4 cm and 48.9 g) than wild smolt (13.7 cm and 19.2 g), a pattern evident in all the three fjords (Figure 2). Of the hatchery-released post-smolt, there were 224 fish from known releases in Osterfjorden. These hatchery-produced fish originated from wild parents from the rivers Vosso ($N = 61$) and Dale ($N = 163$) draining into Osterfjorden.

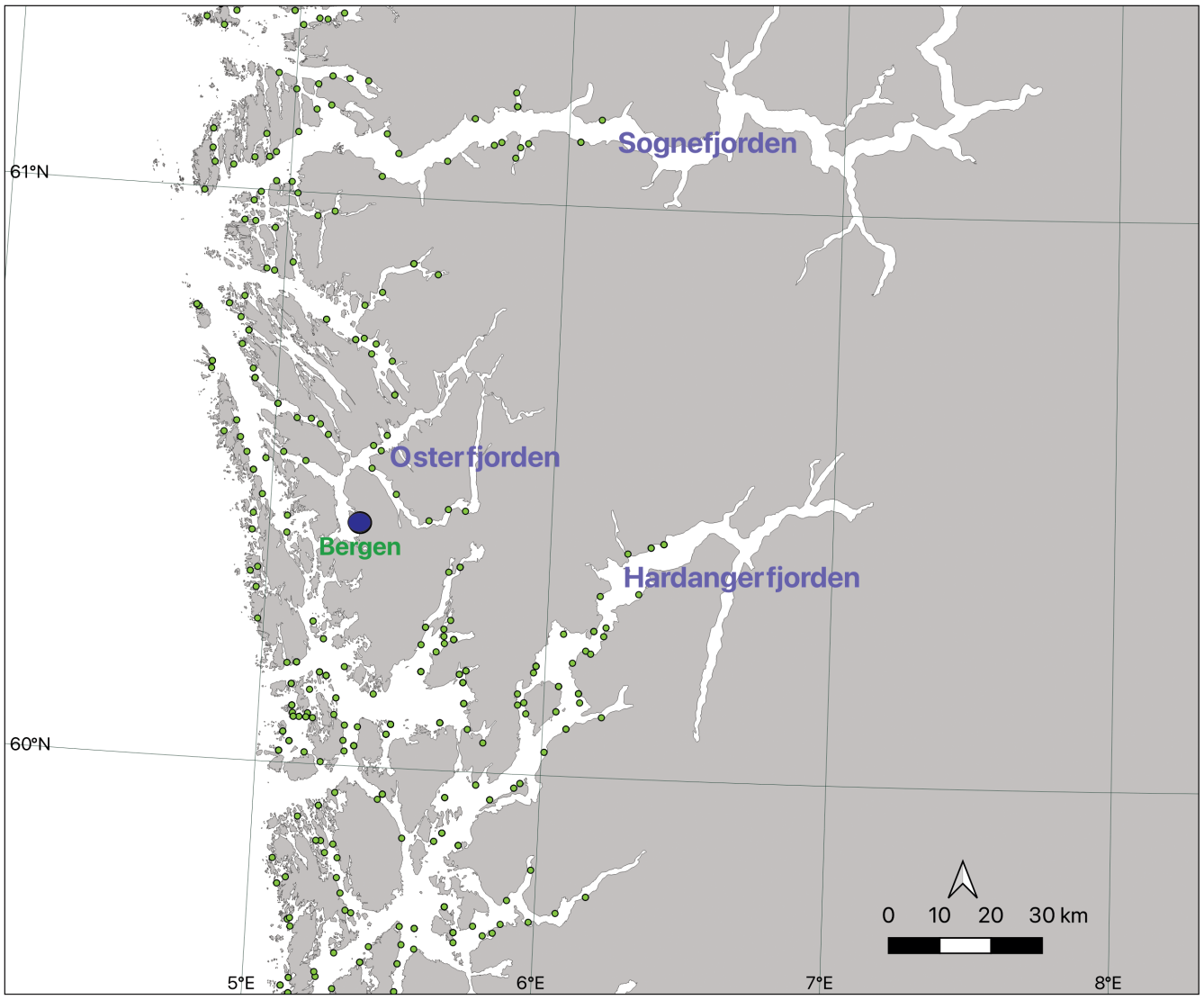

FIGURE 1 Map showing the fjords of post-smolt collection and the salmon farms (green circle) in these fjords.

TABLE 1 Numbers, origin (wild or hatchery-released), total length (TL, cm ± SEM) and weight (*W*, g ± SEM) of post-smolt and the fjord of collection.

Fjord of collection	No.	Year of collection		Origin of fish		Size of fish	
		2013	2014	Wild	Released	TL	*W*
Sognefjorden	148	66	82	118	30	14.2 (0.2)	22.2 (1.0)
Osterfjorden	375	199	176	105	270	16.6 (0.1)	40.3 (1.0)
Hardangerfjorden	128	101	27	80	48	14.7 (0.3)	36.5 (2.6)
Total	651	366	285	303	348	15.7 (0.1)	35.4 (0.8)

3.2 | Virus infections in the migrating post-smolt

SAV was not detected in any of the tested post-smolt irrespective of the fjord or the year of collection. In contrast to SAV, PRV-1 was detected in 4.6% of the captured fish (Table 2). The mean C_t value of PRV-1 positive fish was 32.0 (range: 20.1–36.8). The numbers of PRV-infected post-smolt in relation to year, origin and the collection site is shown in detail in Table 2. There were no significant differences in PRV-1 prevalence between the years (4.6% both years). Overall, the prevalence of PRV-1 infection did not vary significantly between the three fjords: Sognefjorden (6.1%), Osterfjorden (4.8%) and Hardangerfjorden (2.3%). PRV-1 prevalence was significantly higher ($p < .05$) in Osterfjorden in 2013 and lower in 2014 compared to the other two fjords. On the contrary, the prevalence of PRV-1 was significantly higher in Sognefjorden in 2014 compared with the other two fjords

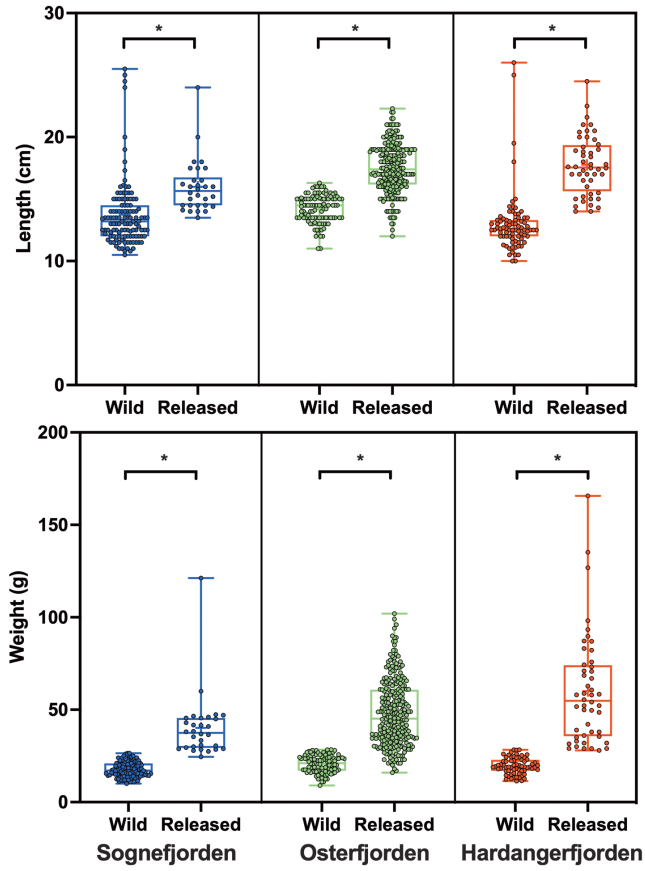

FIGURE 2 Box and whiskers plot showing the length and weight in relation to origin of fish (wild or hatchery-released) and the fjord of collection. * p⟨ .05.

($p < .05$). When considering only wild post-smolt, the prevalence was significantly lower in the Hardangerfjorden compared with Osterfjorden ($p < .01$) and occurrence of PRV-1 infection was lowest in Hardangerfjorden both collection years.

The prevalence of PRV-1 was significantly ($p < .02$) higher in the wild (6.9%) compared with the hatchery-released post-smolt (2.6%) across the years and irrespective of the fjord of origin.

4 | DISCUSSION

SAV is endemic in western and mid-Norway where fish in a large number of salmon farms become infected during production cycle (Jensen et al., 2012; Sommerset et al., 2021, 2023). PD outbreaks in western Norway are common in May–June. In the salmon migration route there were PD outbreaks in all three fjords in both 2013 and 2014 (Figure S1). The SAV-infected fish shed virus to the water and therefore it is likely that migrating post-smolt were at risk of being exposed to virus released from the farms before (subclinical infections) and during PD outbreaks (Bernhardt et al., 2021). However, SAV was not detected in any of the tested post-smolt. The absence of SAV infection in the tested migrating post-smolt is consistent with previous findings that SAV infections are uncommon in wild adult salmonids irrespective of farming intensity or the frequency of PD outbreaks at the locations examined (Garseth et al., 2015; Madhun et al., 2016; Madhun, Isachsen, et al., 2018; Madhun, Skaala, et al., 2018). These observations indicate that wild salmon are exposed to a low SAV infection pressure from fish farming. The possibility that SAV infection may lead to a rapid disappearance or altered behaviour of the infected fish and therefore affecting the results, cannot be completely ruled out. A prepatency can be expected, that is some time lag after exposure and before the virus can be detected in tissues of the fish. Challenge studies have shown that SAV can be detected in fish 5–7 days after infection (Andersen et al., 2007; Jarungsriapisit, Moore, Mæhle, et al., 2016; Jarungsriapisit, Moore, Taranger, et al., 2016). Migrating post-smolt may use days to weeks in the fjords before reaching the open sea (Davidsen et al., 2009; Halttunen et al., 2018; Thorstad et al., 2012; Vollset et al., 2016). However, SAV was not detected in 32 wild smolt from Hardangerfjorden that had been kept in laboratory tanks up to 6 weeks after capture in 2013 ($N = 13$) and 2015 ($N = 19$) (data not shown). In sum, these observations suggest that the occurrence of PD in salmon farms is not associated with an increased occurrence of SAV infection in migration post-smolt.

In contrast to SAV, PRV-1 was detected in 4.6% of the captured fish. There is no available data about HSMI outbreaks in fish farms located in the area during the 2013–2014 period (Sommerset et al., 2023). However, PRV-1 infections are very common in Norwegian salmon farming (Løvoll et al., 2012; Sommerset et al., 2023). In cohabitant experiments, PRV-1 was first detected in the blood and heart samples of infected smolt 4–6 weeks after infection (Finstad et al., 2014; Kannimuthu et al., 2023; Wessel et al., 2017). The time between river descent and capture sites at the outer part of the fjords was estimated to be from few days to few weeks (Davidsen et al., 2009; Halttunen et al., 2018; Johnsen et al., 2021; Thorstad et al., 2012; Vollset et al., 2016). Consequently, some PRV-1 positive post-smolts in the current study could have been infected in the rivers of origin and not from fish farms located in the migration routes. Indeed, PRV-1 infections have been detected in wild Atlantic salmon parr from different rivers in western Norway and these observations suggest that PRV-1 transmission may occur in the rivers (A. S. Madhun, unpubl. data).

Studies of escaped farmed salmon entering rivers show that most of the escapees often are infected with one or more viruses, PRV-1 being the most common (Madhun et al., 2015, 2017). The previous observations highlight the potential role of escaped salmon as pathogen vectors that may transfer infections to wild salmon populations in the rivers.

The prevalence of PRV-1 in adult wild salmon is ranging from 8% to 13% (Garseth, Fritsvold, et al., 2013; Madhun et al., 2016). The current results indicate that the prevalence of PRV-1 may be lower in migrating post-smolt than in returning adult salmon. This could be related to that sea-age may increase the likelihood of PRV-1 infection in wild salmon (Garseth, Biering, & Aunsmo, 2013; Madhun,

TABLE 2 Numbers and the (percentage) of piscine orthoreovirus-1 infected smolt in relation to year, collection sites and origin.

	2013		2014		All	
Site/origin	***N***	**PRV+ (%)**	***N***	**PRV+ (%)**	***N***	**PRV+ (%)**
Sognefjorden						
Wild	47	2 (4.3)	71	7 (9.9)	118	9 (7.6)
Released	19	0 (0.0)	11	0 (0.0)	30	0 (0.0)
Total	66	2 (3.0)	82	7 (8.5)	148	9 (6.1)
Osterfjorden						
Wild	76	10 (13.2)	29	1 (3.4)	105	11 (10.5)
Released	123	4 (3.3)	147	3 (2.0)	270	7 (2.6)
Total	199	14 (7.0)	176	4 (2.3)	375	18 (4.8)
Hardangerfjorden						
Wild	68	0 (0.0)	12	1 (8.3)	80	1 (1.3)
Released	33	1 (3.0)	15	1 (6.7)	48	2 (4.2)
Total	101	1 (1.0)	27	2 (7.4)	128	3 (2.3)
All sites						
Wild	191	12 (6.3)	112	9 (8.0)	303	21 (6.9)
Released	175	5 (2.3)	173	4 (2.3)	348	9 (2.6)
Total	366	17 (4.6)	285	13 (4.6)	651	30 (4.6)

Isachsen, et al., 2018). This might be expected for persistent infections such as PRV-1 due to increased exposure of wild salmon to virus from various potential sources as they are ageing.

There were no significant differences in total PRV-1 prevalence between the years (4.6% in both years). However, the prevalence of PRV-1 varied between the fjords across the years ranging from 0% to 13%. These observations and published data (Garseth, Fritsvold, et al., 2013; Madhun et al., 2016; Madhun, Isachsen, et al., 2018) suggest that PRV-1 prevalence in wild salmonids is influenced by time and geographical sites of sampling and therefore point out the importance of time series of samples. The impact of PRV-1 infection on the fitness and mortality of wild salmon populations is currently unknown, although the ability of mature salmon to ascent rivers may be affected (Mordecai et al., 2021).

The prevalence PRV-1 was higher in wild post-smolt (6.9%) compared to hatchery-released fish (2.6%). On the other hand, Garseth, Biering, and Aunsmo (2013) found that in returned maturing salmon (2007–2009) the mean proportion of PRV-1 positives were 13.4% in wild salmon and 24.0% in hatchery salmon released for stock enhancement purposes. This reduction in the prevalence may reflect an increased awareness and an improvement in disinfection procedure of eggs in hatcheries used in stock enhancement at late years (2013–2014).

Hardangerfjorden is Norway's most intensive salmon farming fjord, and the rivers in the fjord have had high numbers of escaped farmed fish (Grefsrud et al., 2023). However, the prevalence PRV-1 in the migrating post-smolt from this fjord was significantly lower than the other two fjords. These results are in accordance with our previous reports (Madhun et al., 2016, 2019; Madhun, Isachsen, et al., 2018; Taranger et al., 2015) and others (Garseth, Biering, & Aunsmo, 2013; Teffer et al., 2020) that did not find an association between fish farming intensity and the occurrence of these viral infections in wild salmonids. It is also suggesting that it is unlikely that wild salmon is the major reservoir that spill over these viruses to fish farming. However, there are still significant gaps in our knowledge about diseases in wild salmon populations and the interaction between farmed and wild fish (Grefsrud et al., 2023; Taranger et al., 2015).

The current study is the first to address the prevalence of SAV and PRV-1 infections in migrating Atlantic salmon post-smolt collected from Norwegian fjords. The results suggest that there is no apparent association between fish farming activities and the occurrence of these viral infection in smolt. Long-time series of samples of all life stages of wild salmonids from areas with different salmon farming intensities 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.

AUTHOR CONTRIBUTIONS

Abdullah Sami Madhun: Investigation; funding acquisition; writing – original draft; project administration; writing – review and editing; conceptualization; formal analysis. **Rune Nilsen:** Methodology; writing – review and editing; resources. **Bjørn T. Barlaup:** Writing – review and editing; resources; methodology. **Ørjan Karlsen:** Methodology; writing – review and editing; resources. **Egil Karlsbakk:** Writing – review and editing; resources; formal analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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