Annual Report

Health monitoring of wild anadromous salmonids in freshwater in Norway 2019

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Health monitoring of wild anadromous salmonids in freshwater in Norway 2019

Content

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Introduction

Since 2012, the Norwegian Veterinary Institute (NVI) and the Institute of Marine Research (IMR) have been commissioned by the Norwegian Food Safety Authority (NFSA) to carry out an annual health monitoring of wild anadromous salmonids in Norway. NVI coordinates the programme in freshwater and publishes the overall results in annual reports available on https://www.vetinst.no/overvaking/sykdom-hos-villfisk.

Viral diseases associated with cardiac pathology is a significant challenge in farming of salmonids in Norway [1]. Several infections may occur simultaneously at the same aquaculture site and in the same fish [2]. Piscine myocarditis virus (PMCV) causes cardiomyopathy syndrome (CMS), a severe disease affecting Atlantic salmon (*Salmo salar* L). CMS was first recognized in farmed salmon in Norway in 1985 [3], and PMCV was detected and described in 2010 and 2011 [4, 5]. Cardiac pathology resembling CMS has been described from wild Atlantic salmon [6] and screening of wild salmonids have revealed low prevalence's of the virus in wild salmon [7-9]. Phylogenetic analyses show that PMCV in wild salmon cluster with virus from farmed salmon, indicating viral exchange between farmed and wild salmon [7, 10].

Piscine orthoreovirus-1 (PRV-1) causes heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon [11, 12]. Several studies have shown that PRV-1 occurs in wild salmon and seatrout (*Salmo trutta*) [13-15], but there is little evidence of a freshwater reservoir of the virus in Norway [16]. Pathology associated with PRV-1 infection has not been detected in wild salmonids. Piscine orthoreovirus-3 cause a disease resembling HSMI in rainbow trout (*Oncorhynchus mykiss)* [17], but is also prevalent in seatrout [18]. PRV-3 can also infect Atlantic salmon, but is less adapted to this species [19].

Salmonid alphavirus (SAV) cause pancreas disease (PD) in salmonids. In Norway, two genotypes are present in farmed salmon and rainbow trout. SAV 2 occurs in Mid-Norway, and SAV 3 along the west coast. Despite several studies [14, 15, 20], SAV has only been detected in wild salmon once [20]. Wild salmonids are thus unlikely to represent a reservoir for these viruses.

Atlantic salmon calicivirus (ASCV) has a high prevalence in farmed salmon in Norway, and is detected in fish suffering several different diseases as well as in presumable healthy fish [21]. Previous investigations have shown that ASCV occurs as a co-infection with salmon-pathogenic viruses that cause cardiac pathology [2], but unlike caliciviruses infecting other species, ASCV has not been linked to a specific disease entity [22]. The presence of this virus in wild salmonids has not been studied previously; ASCV was thus included in the health-monitoring programme to investigate the presence of a wild reservoir of the virus, and possible role in co-infections.

While Atlantic salmon and rainbow trout are the dominating aquaculture species, Atlantic salmon, seatrout and Arctic char (*Salvelinus alpinus*) are the native wild anadromous salmonids in Norway. Rainbow trout is an alien species, intentionally introduced for aquaculture purposes. Pink salmon (*Oncorhynchus gorbuscha*) is an invasive alien species with increasing significance in the Norwegian fauna [23]. Self-sustained population have established in North-western Russia and after intentional releases in the White Sea. The occurrence of pink salmon in Norway is a result of secondary spreading from Russia.

Aim

In 2019, the objective of the health-monitoring program was to investigate occurrence and co-infections of PMCV, PRV-1, PRV-3, SAV and ASCV in wild Atlantic salmon and seatrout captured in the sea. The prevalence of pathogens in wild salmonids before they enter freshwater is an important reference point in the investigation of disease interaction between wild and farmed salmonids.

Invading pink salmon were included in the programme to increase the knowledge about their role in introduction and spread of fish pathogens in Norway.

Materials and methods

Sampling

Atlantic salmon and seatrout in the sea

The study sample comprises 171 Atlantic salmon and 16 seatrout obtained from coastal fisheries at locations along the coast of Norway (Table 1). In 2018, authorised fish health personnel performed tissue sampling at three locations (35 samples). In 2019, coastal fishermen at 23 locations received written instructions and disposable equipment enabling them to perform the sampling themselves.

Table 1. Overview of study sample including species, year and number of counties, watercourses and fish.

	No. Fish	Production areas*	Sampling Years
Atlantic salmon	171	1, 4-10, 13	2019, 2018
Seatrout	68	1,3-10	2017-2019, 2009-2010
Pink salmon	60	10	2019
Rainbow trout (escaped farmed)	1	4	2019

* Defined in the traffic light system that regulates growth in the aquaculture industry [24]

Gills, myocardium and kidney tissue were sampled in RNAlater for PCR-analyses, and gills, heart, kidney, liver, spleen and pancreas tissue were sampled in formaldehyde for histopathology. Scales were sampled in scale sample envelopes (Figure 1). Relevant information regarding the individual fish, including species, weight, body-length, sex, presence of the adipose fin, wounds etc. was provided on the scale sample envelopes. Samples were returned to the Norwegian Veterinary Institute (NVI) for a preliminary quality control and preparation of samples before PCR-analyses.

Among the samples from coastal fishery catches, there was one escaped rainbow trout. This was included in the program due to the overarching aim to study interaction between wild and farmed salmonids.

Additional samples from seatrout

Due to the low number of samples from seatrout in the sea catches, additional samples were included from previous years. These comprised wild caught broodfish for stock enhancement in River Eira and the genebank in Hardanger, three seatrout from an anadromous lake (Straumsvatnet) and 11 postsmolt captured in Sunndalsfjorden.

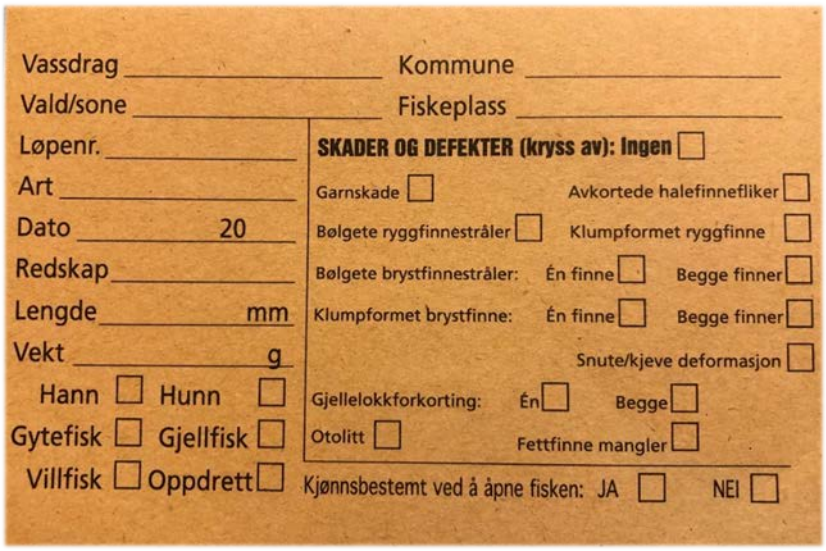

Figure 1: Displays the front of a scale sample envelope.

Pink salmon

The sample comprised 60 pink salmon (19 female and 41 male) captured in River Karpelva in the County of Troms and Finnmark (Figure 2). The local hunting and angling association, Sør-Varanger Jeger og Fiskeforening (SVJFF), installed traps in several rivers in the area and reported catching 1314 pink salmon in River Karpelv during the period July 6th to August 28th 2019 and similar figures in nearby rivers. All pink salmon were euthanized with a blow to the head and subsequent bleeding from cuts in the gill arches.

In addition to the pink salmon, 98 salmon, 94 Sea trout and 40 Arctic char were captured but released from the traps. A local veterinarian autopsied 60 pink salmon (19 female and 41 male) and collected samples of gills, myocardium, kidney and spleen in RNAlater and formaldehyde for PCR-analyses and histopathology respectively. The material was sent to NVI and included in the health monitoring program for wild anadromous salmonids. The pink salmon were also included the Norwegian Food Safety Authority's monitoring and control program for infectious haematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia (VHSV) [25] and also examined for other relevant infectious agents.

Figure 2. Map of Northern Norway, indicating the location of River Karpelva. (Ill. Roar Sandodden, NVI)

PCR-analyses

Myocard and/or kidney samples fixed in RNAlaterTM were sent to PatoGen AS for specific real-time RT-PCR analysis for PMCV, PRV-1, PRV-3 and SAV. The University of Life Science (NMBU), Faculty of Veterinary Medicine received extracted nucleic acids from PatoGen AS and performed specific real-time RT-PCR analysis for ASCV. Some of the samples had previously been tested for PRV-1 or PRV-3, in which case only the remaining analyses were performed.

Scale analyses and genetic tests

Scales from all salmonids captured in the coastal fisheries were analysed at NVI. Atlantic salmon were classified as of wild, farmed or uncertain origin based on scale analyses. All but one of the virus-positives and a selection of virus-negatives were classified by genetic tests (farmed vs. wild origin) by the Norwegian Institute for Nature Research (NINA) [26-30]. Genetic tests were also used to confirm species (salmon vs. seatrout) in individuals where we, from inspection of scales or results from PCR-analyses, suspected misclassification.

Results and discussion

Atlantic salmon

Based on the scale analyses, two Atlantic salmon were classified as escaped from aquaculture. Seven of the salmon were released from stock enhancement hatcheries, and genetic analyses of a subsample comprising 47 salmon revealed three farmed-wild hybrids.

Table 2. Results from PCR-analyses from Atlantic salmon.

	No. tested	PMCV	PRV-1	PRV-3	SAV	ASCV
Wild	158	0	2	1	0	0
Hatchery reared	6	0	0	0	0	0
Uncertain	2	1	1	0	0	0
Farmed-wild hybrid	3	0	0	0	0	0
Escaped farmed salmon	2	0	2	0	0	0

The PMCV-positive salmon, a female, 100 cm and 9.2 kg, was captured in production area 5. Scale analyses classified the salmon as of uncertain origin, either released from a stock enhancement hatchery or escaped from aquaculture as a smolt. Unlike other salmon released from stock enhancement hatcheries in this area, this salmon had an intact adipose fin. Genetic tests revealed that the salmon was of wild origin (P(wild) = 0,964), which means that it has been released from a stock enhancement hatchery. However, the genetic tests also revealed that it was not an offspring of broodfish used by the local stock enhancement hatchery.

Only two wild salmon (1.3 %) were PRV-1 positive, these were captured in production area 5 and 9. Both of the farmed escapees, captured in production area 8, were PRV-1 positive. One salmon, captured in production area 6 was of uncertain origin, hatchery reared or a farmed salmon escaped as smolt. Unfortunately the latter salmon has not been through genetic tests to establish the P(wild)-value. A previous study of salmon from coastal fisheries found PRV-1 in 8 % of the wild salmon and in 86% of the farmed escapees [15].

One Atlantic salmon from production area 7 was PRV-3 positive. This low prevalence in Atlantic salmon is at par with a previous study of PRV-3 in wild salmonids [18], and transmission studies indicating that the virus is less adapted to Atlantic salmon [19].

ASCV and SAV was not detected, and none of the salmon were PCR-positive for more than one of the selected viruses.

Rainbow trout

The farmed escaped rainbow trout was captured in production area 4 and was PRV-3 positive.

Sea trout

The most prevalent virus in seatrout was PRV-3 (Table 3). All subsamples in the study were small and only suitable to detect very prevalent pathogens. For instance, in the coastal fisheries the six PRV-3 positive samples were obtained from the four production areas 1, 7, 8 and 10, despite sample sizes comprising only 1, 3, 2 and 4 seatrout respectively. This is in accordance with our previous study [18].

Table 3. Results from PCR-analyses from seatrout. The sample collection comprised wild caught broodfish for stock enhancement in River Eira in 2009 and the genebank in Hardanger in 2017, three seatrout from Lake Straumsvatnet (2017) and 11 postsmolt captured in Sunndalsfjorden in 2010.

Virus	Coastal fisheries 2019, n=16	Broodfish Hardanger 2017, n=29	Broodfish Eira 2009 n=6	Sunndalsfjord Postsmolt 2010 n=14	Straumsvatn 2017, n=3
PMCV	0	0	0	0	0
PRV-1	0	0	0	0	0
PRV-3	6	10*	2	0	1
SAV	0	0	0	0	0
ASCV	0	0	1	0	0

* Prevalence of PRV-3 in original material from Hardanger was 15 %.

The subsample from Sunndalsfjord comprised only postsmolt, which are younger fish that have just left the river. Due to the low sample size, it is not possible to conclude that life-stage and/or age is of importance for the presence of PRV-3, but this should be further investigated.

ASCV detected in one of six wild-caught broodfish used in stock enhancement in River Eira in 2009, represented the only positive PCR-reaction for this virus in the study. It is not possible to know if sampling year is of relevance for the detection of the virus.

In the seatrout material from Hardanger (2017), it was of special interest to investigate presence of co-infections. The original material comprised 68 seatrout, whereof 10 (15%) were PRV-3 positive [18]. None of the seatrout in the subsample selected for this study, comprising the 10 PRV-3 positive and 19 PRV-3 negative fish, were positive for either of the other viruses.

Co-infections were not detected in seatrout.

Pink salmon

PCR-analyses performed on myocardium resulted in four of the 60 pink salmon being positive for PRV-1 (Ct-values 24.2-35.2). None of the pink salmon were positive for any of the other viral agents included in the program. Presence of PRV-1 has previously been described in pink salmon in Northern America [31]. Subsequent PCR-analyses conducted by the NVI revealed that several more of the pink salmon were positive for PRV-1 when testing RNA isolated from a mixture of myocardium, kidney and spleen. Ct-values also tended to be lower in these analyses (lowest value was 13.5).

PRV-1 replicate in erythrocytes. Accordingly, it is evident that blood filled organs such as kidney and spleen contain more red blood cells and are thus more suitable tissue for PRV-1 detection in pink salmon. However, one can not rule out that cutting of the gill arches, as part of the euthanasia procedure, may have led to contamination, including between fishes.

In conclusion, although it was not possible to conclude on the exact number of PRV-1 positive pink salmon, the study represents the first detection of PRV-1 in pink salmon in Norway.

Conclusion

Although it was not possible to conclude on the exact number of PRV-1 positive pink salmon, it is evident that the virus infect and is present in this species in Norway.

In this study the prevalence of PRV-1 in Atlantic salmon returning from the feeding grounds was very low (1.3 %, 95% CI 0.4-4.5). PRV-1 was also present in one salmon that either was released from a stock enhancement hatchery, or had escaped from aquaculture as a smolt.

PRV-3 was detected in Atlantic salmon, rainbow trout and seatrout. This is in accordance with previous studies. The study expands the geographical area where PRV-3 has been detected in seatrout, and it is noteworthy that it was found in seatrout and rainbow trout despite very small sample sizes. This strongly indicate that the virus is common in these species. The impact of the virus in seatrout should be investigated.

PMCV was detected in one salmon that probably was released from a stock enhancement hatchery.

In accordance with previous investigations, SAV was not detected in any of the samples of the different species. This indicates that the virus is not widely distributed in wild salmonids. Hence, wild salmonids are not a reservoir. However, it does not imply that the virus have no impact on wild salmonids.

ASCV was only detected in one seatrout, a broodfish caught in river Eira in 2009. The result may indicate that the virus is present but not widely distributed in wild salmonids in Norway. However, further investigation of salmonids captured and sampled after entering the river may present a different result. Unlike the situation for SAV, ASCV has so far not been assigned a role in disease development in farmed or wild salmonids.

Co-infections were not detected.

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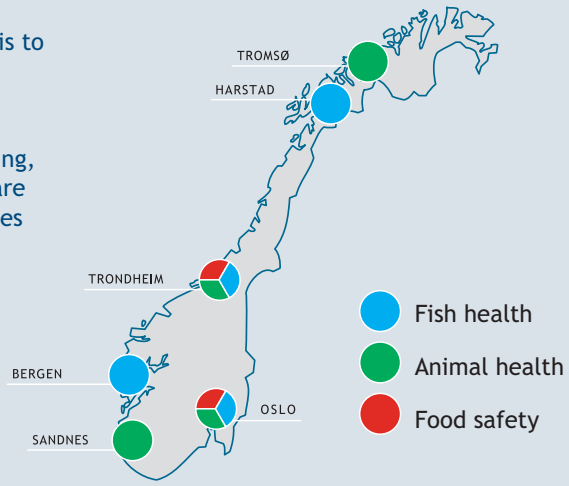

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