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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 2012 commissioned by the Norwegian Food Safety Authority to carry out a health monitoring of anadromous salmonids in Norway (salmon, *Salmo salar*, and sea trout , *Salmo trutta*). IMR was given responsibility for the seawater phase whereas NVI was given responsibility for the freshwater phase (returning brood fish).

Health monitoring of wild anadromous salmonids in fresh water in Norway

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Contents

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 a piece of head kidney that is removed 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 also choose to test for infectious pancreatic necrosis virus (IPNV), the virus causing infectious pancreatic necrosis (IPN). Brood fish intended for the gene bank program shall in addition to the two mentioned agents also be tested for *Aeromonas salmonicida* which causes furunculosis. 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 current program was to investigate the occurrence and distribution of *R. salmoninarum*, IPNV, *A. salmonicida*, SAV, ISAV, PRV and PMCV in returning wild brood fish of the species *Salmo salar* and *Salmo trutta* collected from different geographical areas along the Norwegian coastline.

3 Materials and methods

The Norwegian Veterinary Institute undertook to analyze wild caught salmonid brood fish for salmonid alphavirus (SAV), infectious salmon anemia virus (ISAV), piscine reovirus (PRV) and piscine myocarditis virus (PMCV). Results from *Renibacterium salmoninarum*, IPNV and *Aeromonas salmonicida* analyses that were already done on the samples were also to be reported. 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, *A. salmonicida,* and PRV were analyzed on kidney, SAV, ISAV and PMCV were analyzed on a mix of heart and gill. All autopsies and samplings were performed by authorized fish health personnel (veterinary 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 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.

4 Results

Table 1 gives an overview of all samples and analyses.

Table 1. *Salmo salar* and *Salmo trutta*: Total number of analyses for each agent. PRV+ shows the number of PRV positives.[1)] 7 positive for *R. salmoninarum* in Hordaland (one wild and six released smolts), [2)] 1 positive for SAV in Hordaland (released smolt), [3)] 1 positive for ISAV in Møre og Romsdal (wild), [4)] 1 positive for PMCV in Sogn og Fjordane (wild), [5)] 1 positive for PMCV in Vestfold (escaped farmed).

PCR analysis	R. salmoninarum	IPNV	A. salmonicida	SAV	ISAV	PRV	PRV+	PMCV
Salmon								
County								
Finnmark	41	41	41	41	41	41	1	41
Troms	2	2	2	2	2	2	0	2
Nord-Trøndelag	19	19	0	19	19	19	18	19
Sør-Trøndelag	36	36	36	36	36	36	11	36
Møre og Romsdal	108	108	90	49	49 [3)]	108	11	49
Sogn og Fjordane	85	71	25	71	60	85	8	71 [4)]
Hordaland	103 [1)]	81	39	97 [2)]	88	103	38	97
Rogaland	7	0	0	7	7	7	2	7
Vest-Agder	43	43	0	43	43	43	3	43
Vestfold	53	0	0	53	53	53	3	53 [5)]
Østfold	35	35	35	35	35	35	35	35
Total no. analyses	**532**	**436**	**268**	**453**	**433**	**532**	**130**	**453**
Sea trout								
County								
Møre og Romsdal	95	95	0	95	95	95	2	95
Rogaland	5	0	0	5	5	5	1	5
Total no. analyses	**100**	**95**	**0**	**100**	**100**	**100**	**3**	**100**

In total 632 kidney samples, 553 heart samples and 533 gill samples were tested. The HSMI associated reovirus PRV is the only infectious agent that was found to be highly prevalent in the brood fish material, 24.4 % of the salmon and 3 % of the sea trout were positive for PRV. In addition we found 7 *R. salmoninarum* positive salmon in Hordaland (1 wild and 6 released smolts), 1 SAV positive salmon in Hordaland (released smolt), 1 ISAV positive salmon in Møre og Romsdal (wild), 1 PMCV positive salmon in Sogn og Fjordane (wild), and finally 1 PMCV positive salmon in Vestfold (escaped farmed). No positives were found for IPNV or *A. salmonicida*.

5 Discussion and conclusion

The findings of PRV positive fish mainly agrees with our previous work on a similar brood fish material sampled in 2007, 2008 and 2009 (Garseth et al. 2012a) that showed a PRV prevalence of 13,4 % in wild brood fish and 24.0 % in brood fish released from stock enhancement hatcheries. In the presented results we do not distinguish between truly wild salmon and salmon released from stock enhancement hatcheries. It should be noted that 3 hatcheries localized in Nord-Trøndelag, Hordaland and Østfold respectively, had 95-100% prevalence of PRV. We have no indications that wild salmonids will develop HSMI as a consequence of PRV infection (Garseth et al. 2012a). However, as the virus is newly identified, it would be premature to conclude about the effects on wild fish at the present time.

The 7 *R. salmoninarum* positive salmon from Hordaland all originated from the same hatchery. The finding was confirmed as BKD by the NVI both by PCR and pathological investigation, and the Norwegian Food Safety Authority has been informed of the findings. *R. salmoninarum* has previously been found occasionally in this river system, and the infection pressure may have been amplified by extensive cultivation of smolts in cages in lake Evanger during the last years. However, this is just speculations.

The finding of 2 PMCV positive salmon is on par with our previous findings from 2007, 2008 and 2009 (Garset et al. 2012b).

We usually have a few findings of IPNV each year (http://www.vetinst.no/Publikasjoner/Fiskehelserapporten), but no findings will still be within the normal range. There has been a decline in IPNV cases in the aquaculture industry in 2012, indicating a lower infection pressure. However, we do not know if our previous findings of IPNV in wild brood fish are related to salmon farming. They may also be due to virus isolates that are specific for wild salmon.

In conclusion, it appears that viral infectious agents that are highly prevalent within the Norwegian aquaculture industry, in particular IPNV and SAV, are found only in low prevalence in wild brood fish. The obvious question, as raised by McVicar in 1997 (McVicar 1997), is whether this is due to a low infection pressure or if wild fish infected by a virulent agent rapidly die and thus avoid to be sampled. Sequencing of viral agents found in wild fish may show if they are similar to virus found in farmed fish or if they are specific for wild populations, and thus contribute to answer this question.

6 Acknowledgements

Thanks to the Health service for stock enhancement hatcheries, other non-associated hatcheries, and the gene bank program for salmon and sea trout for providing the samples. Also thanks to the fish health personnel that autopsied the fish and secured tissue samples.

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Health monitoring of wild anadromous salmonids in seawater in Norway

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Contents

1 Introduction

Disease is a serious problem in fish farming in Norway that leads to huge economical losses. Disease outbreaks in fish farms may lead to a substantial increase in infection pressure on neighbouring farms and on wild fish. This may change the infection and disease status of susceptible wild stocks. Today, there is limited data on the prevalence of pathogens in wild salmonid populations in Norway. It is difficult to measure the disease incidence in wild fish because sick individuals may disappear in the nature unnoticed. 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 survived an infection. There is increasing evidence for pathogen transmission from farmed to wild fish [1], [2]. However, the frequency and the consequence of transmission of many viral disease agents are largely unknown.

The anadromous sea trout migrate between river and seawater during its lifecycle. During summer, most sea trout are feeding in sea areas that are closed to the river of origin. Therefore, it may have an important role in spreading pathogens. Many pathogens that cause disease in farmed salmon can also infect other salmonids. Sea trout has been used to evaluate the infection pressure of salmon lice from fish farming on wild salmonid populations [3]. The infection status of sea trout may also be used as an indicator of virus transmission from fish farming, if i) sea trout is susceptible and ii) if infections persist after exposure. The effect of fish farming on the infection status of wild sea trout stocks may be evaluated at a local level by comparing pathogen prevalence in the trout before and after a disease outbreak in a farm, or at a larger level by comparing pathogen prevalence in wild fish populations captured from coastal areas that have different fish farming intensities.

Pancreas disease (PD), caused by salmon anaemia virus (SAV), is a major health problem for fish farming in Norway with 89 registered outbreaks in 2011. Most of the disease outbreaks occur in western part of the country especially in Hordaland County.

Heart and skeletal muscle inflammation (HSMI) is another disease that is associated with a recently discovered virus; piscine reovirus (PRV). The role of this virus in HSMI is still unclear, large PRV intensities are found in fish suffering from HSMI, but may also be found in healthy fish. The disease is a problem in fish farming in Norway with 162 outbreaks registered in 2011. PRV has been detected in wild salmon and sea trout, as well as certain marine fish species by real-time rt-PCR ([4], [5]). However it is not clear if marine fish is infected with virus genotype similar to that occur in salmon, since virus levels were low and sequences were not obtained. Little is known about the mechanism of transmission of the virus, but modelling has suggested that farm-intensity in a region is a major risk factor for HSMI outbreaks [6].

2 Aim

The aim of the current program is to investigate the occurrence and distribution of SAV3 and PRV in wild sea trout collected from different geographical areas in Norwegian coastline.

3 Materials and methods

A total of 657 sea trout were caught using net and fish trap from different sea areas in Rogaland, Hordaland, Sogn og Fjordende and Finnmark counties (Fig. 1). Sea trouts used in the current survey were collected as part of the national salmon lice monitoring program. The captured fish were kept in ice in the field and deep frozen (-20^{o}C) as soon as possible the

same day. At autopsy, tissue from heart, head kidney and gills were aseptically taken out from the fish while still frozen and transferred frozen to tubes on dry-ice. Heart samples were sent in dry ice to an accredited commercial laboratory for virus testing (PatoGen Analyse AS). The presence of SAV3 and PRV viruses was determined by PatoGen using their in-house real-time rt-PCR assays. Samples with C_t value below 37.0 were considered to be positive. Length, weight and the sex of the fish were recorded. Scales from selected group of fish were used to determine the age of fish and the number of years spent in river (smolt) and sea. After thawing, the fish was visually inspected for external or internal pathologies or signs of disease.

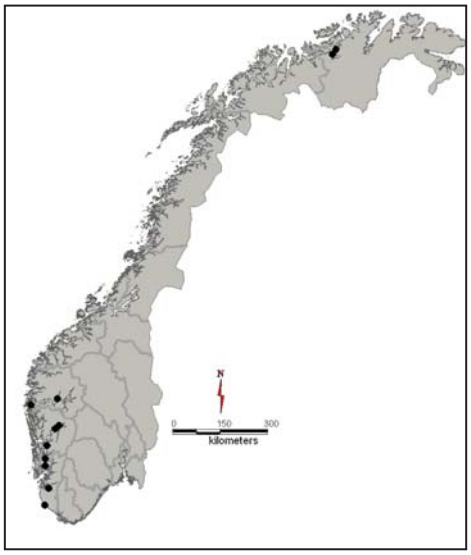

Fig. 1 Map of Norway showing the areas where sea trout were captured

4 Results and discussion

No SAV3 virus were detected in sea trout

SAV3 was not detected in any of the hearts from the tested sea trout (table 1). Significant numbers of the tested fish were caught in areas where SAV3 is endemic with frequent outbreaks of PD. Our results and other published reports [7, 8] may indicate that the sea trout is not a natural host for SAV3.

Table 1: The prevalence of PRV and SAV3 viruses in sea trout collected from different geographical sites.

County	Site	Year	No.	PRV+ (%)	SAV3+ (%)
Finnmark	Skillefjord	2012	30	0	0
	Talvik	2012	29	0	0
Sogn og Fjordene	Dingja	2012	52	0	0
	Balestrand	2012	48	0	0
Hordaland	Etne	2011-2012	132	0	0
	Rosendal	2011-2012	109	0	0
	Ålvik	2012	48	0	0
	Granvin	2011	17	0	0
Rogaland	Vikedal	2011-2012	42	0	0
	Hellvik	2011-2012	71	6 (8.5)	0 (0)
	Forsand	2011-2012	79	5 (6.3)	0 (0)
Total			657	11 (1.7)	0

No.: number of fish, PRV+: number of PRV positive, SAV3+: number of SAV3 positive.

Prevalence of PRV in wild sea trout

Piscine reovirus was detected in 11 of the 657 sea trout (1.7%). The PCR C_t-values ranged from 34 to 36.9 indicating a very low amount of virus present. All the positive sea trout were caught in Rogaland County. Additionally, all the positive fish except one (10 of 11) were caught in 2011.

In Rogaland County, the fish were captured from three areas; Forsand, Hellvik and Vikedal. Hellvik is an open costal area with no salmon farming activities and hence may be considered a control area with respect to pathogen transmission from salmon farms (See fig. 2).

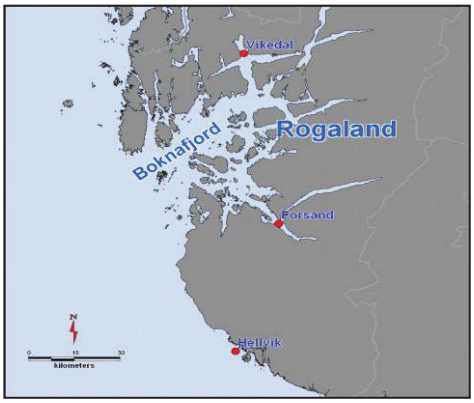

Fig. 2: Map of sea trout sampling sites (red circles) in Rogaland County.

On the other hand, both Forsand and Vikedal areas are located in the inner part of Boknafjord system with high density of salmon farms. There was no association between PRV prevalence and salmon farming or the occurrence of HSMI outbreaks in the studied areas. The data set from Rogaland (Forsand and Hellvik) were further analysed for patterns of infection that may associate with sex, age, weight or time of sampling. There was no correlation between the infection status and sex of fish. One can expect that older fish may have higher prevalence of PRV due to increased probability of exposure to the virus. However, the age of fish or the number of years at sea or at river (years as smolt) does not seem to correlate with the frequency of infection. The higher prevalence of PRV in the Forsand and Hellvik areas in 2011 compared to 2012 or other sampling sites cannot be explained. A recent screening of PRV in sea trout sampled between 2007 and 2009 [4] also found that PRV was detected in sea trout only in a particular year (2008).

5 Conclusion

SAV3 was not detected in any of 657 sea trout tested although significant numbers of the fish were caught in PD endemic areas with many PD outbreaks. This finding and the available literature suggest that sea trout may be not a natural host for SAV3. Similar to the finding in a recent report [4], wild sea trout can be found naturally infected by low levels of PRV. However, the occurrence of the infection is dependent on the place and time of sampling. Although we could not establish an association between the occurrence of PRV infection in sea trout and the fish farming activities or disease outbreaks, transmission of PRV from farmed fish to wild sea trout can not be excluded. So far, no infections detected in sea trout has been so intense that sequencing and hence examination of the PRV genotype has been possible. Time series of samples are necessary to better understand PRV transmission, PRV exchange between wild and farmed fish and the suitability of sea trout as indicator of infection pressure.

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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 aquacultureand 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 fi sh 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, fi sh 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 Affaires and the Ministry of Health and Care Services.

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