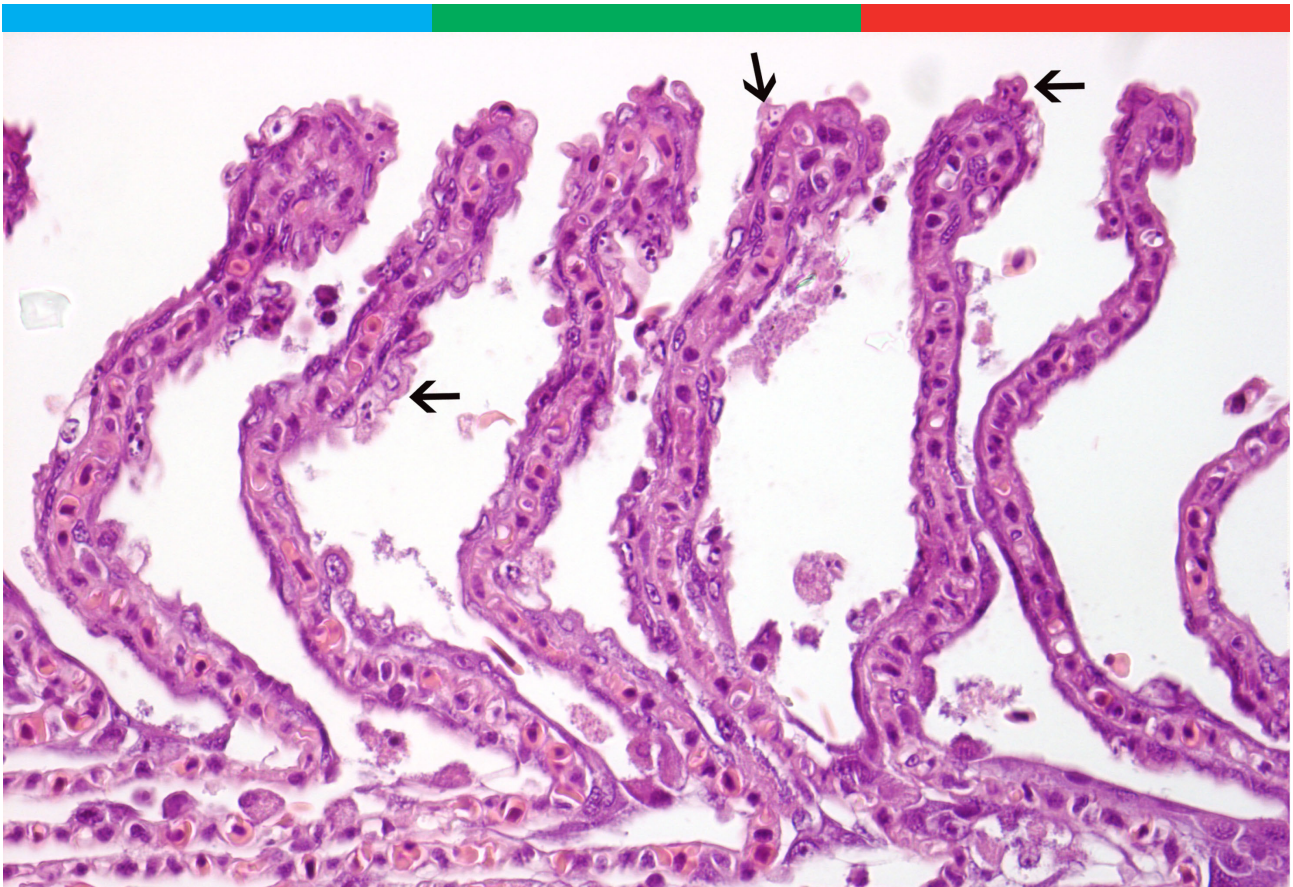




Health monitoring of wild anadromous salmonids in freshwater in Norway 2020



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Cover design: Reine Linjer

Cover photo: Mona C. Gjessing. Histological sections of gills from wild-caught broodfish of Atlantic salmon infected with salmon gill poxvirus. Arrows indicating apoptotic cells about to be shed.

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Introduction

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

The overall purpose of the programme is to investigate the sources and occurrence of disease-causing agents in wild anadromous salmonids, including Atlantic salmon *Salmo salar*, anadromous brown trout (sea trout) *Salmo trutta* and Arctic char *Salvelinus alpinus*.

In the implementation of the programme, NVI has chosen to study different infectious agents every year instead of generating time series of a few selected infectious agents.

In farmed salmonids, gill diseases are widespread with complex, multifactorial causation [1]. Gill diseases may be caused by specific infectious agents alone or by an interplay of various infectious agents, host factors and the environment. In both Atlantic salmon and rainbow trout, gill disease causes increased mortality, and reduced growth and welfare [2].

Gills have a large surface and only a thin layer of epithelial and endothelial cells separate the blood in vessels from the environment. This facilitates the multiple functions of the gills including gas exchange and regulation of osmotic, ionic and acid-base balances. On the other hand, the large surface and close contact with the environment makes the gills a major port of entry for infectious and toxic agents.

One of the major threats facing the global environment is the changing climate. Warmer water will change the distribution of both hosts and infectious agents. In that context, the gill health of both farmed and wild fish should receive special attention. Rising water temperatures will increase the metabolic rate of fishes and hence the demand for oxygen. At the same time, the oxygen holding capacity of the water will decline. Monitoring of gill health in wild and farmed species will be important in the years to come.

Aim

In 2020, the health monitoring programme expanded on the findings made in the 2016 and 2018 health monitoring programmes for wild anadromous salmonids [3, 4]. The overarching aim was to get a more complete picture of the occurrence of gill disease-associated infectious agents in wild salmonids in the sea, in rivers and in waterbodies that only house freshwater resident salmonids. The selected gill associated infectious agents are salmon gill poxvirus (SGPV), Atlantic salmon paramyxovirus (ASPV), *Ca. Branchiomonas cysticola*, *Ca. Piscichlamydia salmonis*, *Desmozoon lepeophtherii* and *Paramoeba perurans*.

The health of salmonids in the sea and in freshwater resident salmonids are important reference points in the investigation of disease interaction between wild and farmed salmonids.

This year the programme also investigated the presence of infectious agents in brown trout captured in large lakes, including the lakes Selbusjøen, Femunden and Snåsavatnet. Information about the fish health in these ecosystems is useful in risk assessments, but also as baseline information in the context of climate change and other man made sources of impact on fish communities.

Materials and methods

Sampling

Table 1 displays an overview of the samples included in the 2020 health monitoring programme.

Table 1: Overview of study sample including species, number of samples, location and year.

	No. Fish	Locations	Sampling Years
Atlantic salmon in the sea	152	Production areas* 1, 4, 5, 7-10, 13	2019
Sea trout in the sea	27	Production areas 1, 5, 7-10	2019,2020
Rainbow trout (escaped farmed)	1	Production area 4	2019
Atlantic salmon in rivers	112	Production areas 1, 5, 6	2019, 2020
Brown trout in large lakes	91	Femunden, Selbusjøen, Snåsavatnet	2020

* Defined in the traffic light system that regulates growth in the aquaculture industry [5]

Atlantic salmon, sea trout and rainbow trout from coastal fisheries

The study sample comprised of 152 Atlantic salmon, 27 sea trout and one escaped farmed rainbow trout obtained from coastal fisheries at locations along the coast of Norway in 2019 (salmon, sea trout and one rainbow trout) and 2020 (sea trout).

Coastal fishermen performed the sampling in accordance with illustrated, written instructions. Gills, myocardium and kidney tissue were sampled in RNAlater for PCR-analyses. Tissue samples from gills, myocardium, kidney, liver, spleen and pancreas were also collected in formaldehyde for histopathology. Scales were sampled in scale sample envelopes (Figure 1). On these envelopes, information regarding the individual fish including geographical location, species, weight, body-length, sex, presence of adipose fin and lesions was recorded. Samples were returned to NVI for a preliminary quality control and preparation of samples for PCR-analyses.

Atlantic salmon in rivers

Samples from fingerlings, smolt and returning adult salmon in River Vigda, County of Trøndelag were obtained during sampling for the EU-financed project CIRCLES-Controlling microbiomes Circulations for better food systems (<https://Circlesproject.eu>). Samples of fingerlings of Atlantic salmon from River Enningdalselva, County of Viken were obtained during investigation of red skin disease in this river [1]. Samples from adult salmon in River Måna and Innfjord, County of Møre & Romsdal were obtained in conjunction with a programme that evaluates the restoration of Atlantic salmon stocks after eradication of *Gyrodactylus salaris*.

Vassdrag _____	Kommune _____
Vald/soner _____	Fiskeplass _____
Løpenr. _____	SKADER OG DEFEKTER (kryss av): Ingen <input type="checkbox"/>
Art _____	Garnskade <input type="checkbox"/>
Dato _____ 20 _____	Avkortede halefinnefliker <input type="checkbox"/>
Redskap _____	Bølgete ryggfinnestråler <input type="checkbox"/>
Lengde _____ mm	Klumpformet ryggfinne <input type="checkbox"/>
Vekt _____ g	Bølgete brystfinnestråler: Én finne <input type="checkbox"/>
Hann <input type="checkbox"/> Hunn <input type="checkbox"/>	Begge finner <input type="checkbox"/>
Gytetisk <input type="checkbox"/> Gjellfisk <input type="checkbox"/>	Klumpformet brystfinne: Én finne <input type="checkbox"/>
Villfisk <input type="checkbox"/> Oppdrett <input type="checkbox"/>	Begge finner <input type="checkbox"/>
	Snute/kjeve deformasjon <input type="checkbox"/>
	Gjellelokkforkorting: Én <input type="checkbox"/>
	Begge <input type="checkbox"/>
	Otolitt <input type="checkbox"/>
	Fettfinne mangler <input type="checkbox"/>
	Kjønnsbestemt ved å åpne fisken: JA <input type="checkbox"/>
	NEI <input type="checkbox"/>

Figure 1: Displays the front of a scale sample envelope used to gather scales and individual information about salmon and trout captured in the coastal fisheries.

Brown trout in Lakes Selbusjøen, Snåsavatnet and Femunden.

Samples from Selbusjøen comprised wild-caught brown trout used as broodfish by a stock enhancement hatchery. After capture, these brown trout were held up to one week in a net cage (1 x1 x2 m) in the outlet of River Nea before stripping and immediate post mortem examination.

Brown trout from Femunden and Snåsavatnet were captured by the Norwegian Institute for Nature Research (NINA) as part of fish monitoring in large lakes under the EU Water Framework. These fish were captured by trawling and net fishing (NordicBG series), then frozen and thawed before sampling.

Scale analyses and genetic tests

All Atlantic salmon captured in the coastal fisheries were classified as of wild, farmed or uncertain origin based on scale analyses at NVI. All salmon that were not classified as farmed were further classified by combining information from scale analyses, presence or absence of the adipose fin and genetic tests performed by NINA [6-10]. Genetic tests were also used to confirm species (salmon versus sea trout) in individuals where we, from inspection of scales or results from PCR-analyses, suspected misclassification.

PCR-analyses

Gill samples in RNAlater™ were sent to PatoGen AS or Pharmaq Analytiq for specific real-time RT-PCR analysis for salmon gill poxvirus (SGPV), Atlantic salmon paramyxovirus (ASPV), *Ca. Branchiomonas cysticola*, *Ca. Piscichlamydia salmonis*, *Desmozoon lepeophtherii* and *Paramoeba perurans* (the latter only in salmonids captured in the sea).

Statistics

Due to low sample sizes and geographical differences between and within groups, all statistics are and should be interpreted with caution. The presence of an infectious agent is given more attention than the absence of infectious agents. Results are presented as proportion of positive fish among tested. Infection loads are presented as the mean and range cycle threshold values (Ct-values) delivered by the laboratories.

Results and discussion

Atlantic salmon

Scale analyses classified two Atlantic salmon as escaped from aquaculture (Farmed escapees). Seven salmon were released from stock enhancement hatcheries (Hatchery reared), and genetic analyses revealed ten farmed-wild hybrids. The remaining 133 salmon are of wild origin, meaning that they have wild salmon in their pedigree, were hatched in a river and have completed their lifecycle as wild (Wild).

With exception of the wild salmon group, the number of fish in each life-history group were low and there were no obvious trends with regards to distribution of infectious agents between groups (Appendix 1). Results from PCR analyses of gill tissue from Atlantic salmon from coastal fisheries and rivers are shown in Table 2.

Absence of Atlantic salmon paramyxovirus and *Paramoeba perurans*

None of the Atlantic salmon were carriers of ASPV or *P. perurans*. This is in line with previous investigations in wild salmonids in Norway [4].

Ca. Branchiomonas cysticola

In Atlantic salmon in the sea, *Ca. B. cysticola* is widespread with a high proportion of PCR-positive fish in all production areas. The overall prevalence was 79 %, with a ranged between 68 and 100 % in the different production areas. The Ct-values for *Ca. B. cysticola* in Atlantic salmon in the sea were between 17.8 and 36.6 (mean 28.9). In this and previous studies the bacteria is shown to be present in salmonids in both freshwater and seawater. Further studies of histopathological samples are needed to assess the health impact of this bacteria in wild salmonids.

Desmozoon lepeophtherii* and *Ca. Piscichlamydia salmonis

Both infectious agents were detected in adult salmon returning from marine migration, while the marine parasite *D. lepeophtherii* was not detected in juvenile salmon.

There seems to be a south and eastward skewed distribution of *D. lepeophtherii* and *Ca. P. salmonis* in Atlantic salmon in the sea, which is partly in line with the southward distribution of these infectious agents in farmed Atlantic salmon [1].

Desmozoon lepeophtherii was originally described as a parasite of salmon lice (*Lepeophtheirus salmonis*), and it is thus noteworthy that the highest proportion of *D. lepeophtherii* was

detected in production area 1 where 13 of the 14 cases were from the County of Viken, a county without aquaculture in open net pens. Altogether 40.6 % of the salmon from this county were PCR-positive for *D. lepeophtherii*.

How the presence of *D. lepeophtherii* in salmon lice affects the occurrence of *D. lepeophtherii* in wild Atlantic salmon is unknown. It is also unknown how frequent eradication measures against salmon lice in aquaculture affect the prevalence of *D. lepeophtherii* in salmon lice in aquaculture dense areas.

Table 2. Results from PCR-analyses of gill tissue from Atlantic salmon for salmon gill poxvirus (SGPV), *Candidatus Branchiomonas cysticola*, *Desmozoon lepeophtherii* and *Candidatus Piscichlamydia salmonis* are shown in the table. Results for Atlantic salmon paramyxovirus (ASPV) and *Paramoeba perurans* are omitted from the table since all samples were PCR-negative. Results for Atlantic salmon in the sea are shown for the different production areas (PA) [5].

	No. Fish	Salmon gill poxvirus		<i>Ca. B. cysticola</i>		<i>Ca. P. salmonis</i>		<i>D. lepeophtherii</i>	
		Results	Ct-values	Results	Ct-values	Results	Ct-values	Results	Ct-values
Coastal fisheries									
Production area 1	56	1 (1.8 %)	29.5	38 (68 %)	28.9 (19.2-35.6)	2 (3.6 %)	34.5, 35,7	14 (25 %)	34.2 (31-36.8)
Production area 4	16	2 (12.5 %)	30.9, 35.6	15 (94 %)	27.4 (20.7-35.8)	0		2 (12.5 %)	34.8 (34.3-35.3)
Production area 5	23	3 (13 %)	33.3 (29.6-36.4)	16 (70 %)	28.9 (21.9-35.9)	3 (13 %)	30.1 (27.6-31.6)	4 (16.8 %)	33.5 (30.9-35.4)
Production area 7	7			5 (71 %)	29.1 (27.3-32.0)	1 (14.3 %)	36.7	0	
Production area 8	16			15 (94 %)	28.9 (23.2-36.6)	1 (6.3 %)	32.5	0	
Production area 9	8			8 (100 %)	28.9 (23.6-35.8)	0		0	
Production area 10	6			5 (83 %)	30.5 (27.1-35.4)	0		0	
Production area 13	20			18 (90 %)	29.8 (17.8-36.5)	0		0	
Summarized	152	6 (4 %)	32.7 (29.5-36.4)	120 (79 %)	28.9 (17.8-36.6)	7 (4.6 %)	32.8 (27.6-36.7)	20 (13.2 %)	34.1 (30.9-36.8)
Rivers -juvenile									
Enningdalselva (PA 1)	30	0		0		2 (6.7 %)	29.4 and 32.1	0	
Vigda (PA 6)	14	0		2 (14.3 %)	28.9 and 31.6	12 (85.7 %)	24.1 (20.6-31.0)	0	
Vigda (smolt)	10	0		1 (10 %)	22.7	8 (80 %)	22.8 (18.6-25.0)	0	
Rivers - adult									
Vigda (PA 6)	5	2 (40 %)	22.5 and 25.3	0		3 (60 %)	26.8 (22.1-29.4)	2 (40 %)	22.6 and 22.8
Innfjord (PA 5)	12	1 (8.3 %)	33	10 (83.3 %)	28.7 (22.5-35.2)	1 (8.3 %)	34.7	1 (8.3 %)	27.6
Måna (PA 6)	41	6 (14.6 %)	31.9 (30.4-33.7)	35 (85.3 %)	35.0 (17.7-34.8)	0		8 (19.5 %)	26.3 (22.0-30.2)

Salmon gill poxvirus

In this study, the overall prevalence of SGPV in Atlantic salmon returning from the feeding grounds and captured in the sea was 4 %, and the few detections were in the three production areas 1, 4 and 5. SGPV was also detected in salmon captured in rivers after marine migration, but not in juvenile salmon prior to marine migration.

In our previous investigations of SGPV, the virus was found in adult salmon from 25 of 26 investigated rivers [3, 11]. The sample size in the SGPV-negative river in the mentioned study was only 4 salmon and it was concluded that the virus probably was present but that the sample size was insufficient to detect it [11].

The same study showed that the prevalence in wild-caught broodfish of Atlantic salmon that were held in tanks prior to stripping and sampling of target tissue was 83.7 %, which is significantly higher than in salmon captured in the sea in this study (4 %) and in 2018 (0 %) [3, 4, 11]. It is likely that the high prevalence in cohabiting salmon is caused by transmission of virus from infected to susceptible salmon during the cohabitation period.

The overall data from health monitoring in 2016, 2018 and 2020 point toward low SGPV prevalence in the sea and increasing prevalence after entering the river, and that the prevalence further escalates if wild salmon are captured and held together in tanks. The impact of this virus in wild salmon in their natural environment has not been studied, while characteristic histopathological findings were found in wild-caught broodfish of Atlantic salmon in stock enhancement hatcheries (Figure 2) [11].

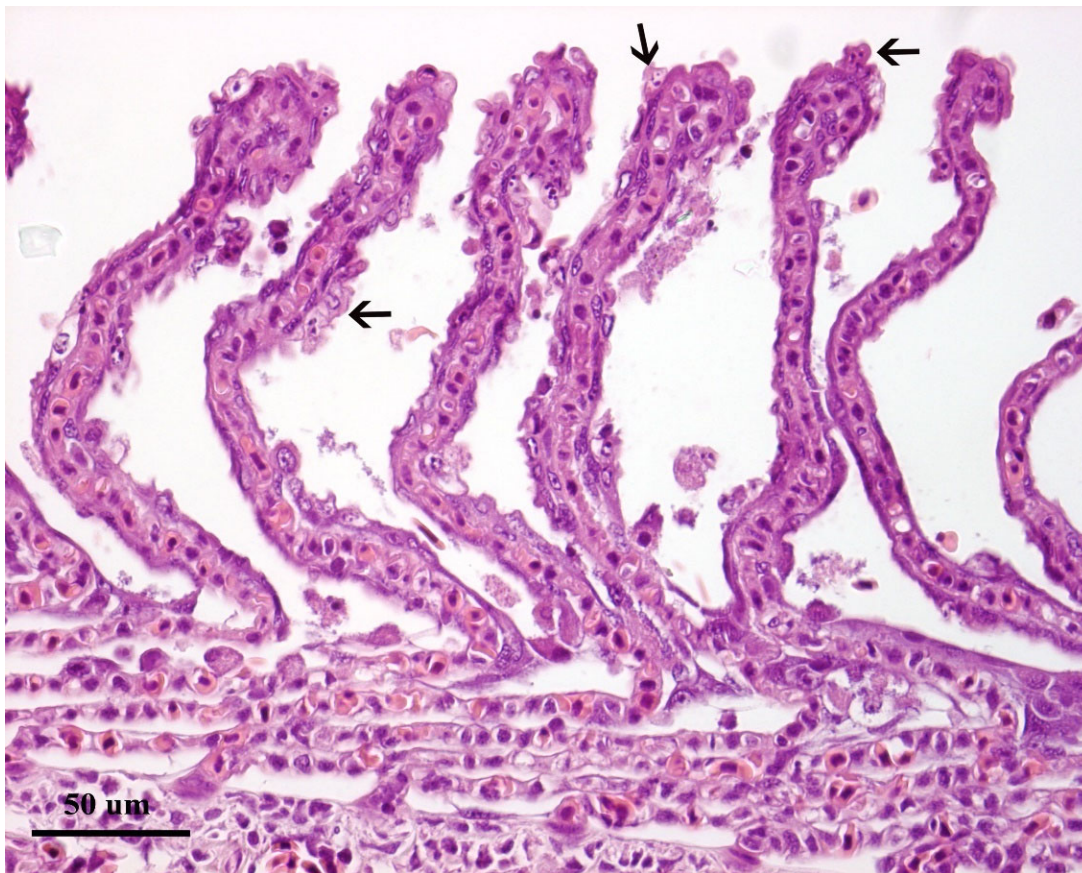


Figure 2: Histological sections of gills from wild-caught broodfish of Atlantic salmon infected with salmon gill poxvirus. Eosinophilic granular cells are observed in the central part of filament and lamellae. Arrows indicate apoptotic cells about to be shed from the lamella [11]. Photo: Mona C. Gjessing, Norwegian Veterinary Institute.

The high prevalence of SGPV in wild-caught broodfish in stock enhancement hatcheries (80-90 %) and the associated gill pathology should draw attention. It is now common practice to release broodfish in their native river after stripping instead of killing them, and it is likely that these broodfish have reduced gill health and possibly also can serve as reservoirs for spread of the virus to other wild salmon in the rivers.

The role of wild salmon in the sea as a reservoirs for SGPV and possible source for farmed salmon is assessed to be negligible, whereas spillover of SGPV from farmed to wild salmon in the sea is more likely and should be further studied by the use of novel tracing tools [12].

Sea trout (anadromous brown trout)

Only a limited number of sea trout were captured in the coastal fisheries in 2019 and 2020. Accordingly, the absence of specific infectious agents is not a conclusive finding. ASPV has previously been detected in sea trout in the health monitoring programme, but was absent in this study. *Paramoeba perurans*, *D. lepeophtherii* and SGPV were also absent. SGPV has been detected in brood fish of sea trout that were cohabiting with infected Atlantic salmon, but has never been found in sea trout in their natural environment [3, 11].

Candidatus *Branchiomonas cysticola* and *Ca. P. salmonis* were present both years (Table 3). There is a statistically significant difference in prevalence of *Ca. B. cysticola* between years since the 2020 prevalence was 7.7 %, (95 % CI: 1.4-33.3 %) and the 2019 prevalence was 78.6 % (95 % CI: 52.4-92.4), but this could for instance be caused by differences in sampling locations between years.

Table 3. Results from PCR-analyses for *Candidatus* *Branchiomonas cysticola* and *Candidatus* *Piscichlamydia salmonis* in of gill tissue of sea trout captured in coastal fisheries in 2019 and 2020.

	No. Fish	<i>Ca. B. cysticola</i>		<i>Ca. P. salmonis</i>	
		Results	Ct-values	Results	Ct-values
Sea trout 2019	14	11	27.9 (19.3-36.7)	5	30.8 (24.3-35.3)
Sea trout 2020	13	1	25.9	2	31.8 and 33.8

Escaped farmed rainbow trout

The escaped farmed rainbow trout (PA 4) was the only fish in the health monitoring programme that was PCR-positive for *Paramoeba perurans* (Ct-value 31.8) the causative agent of amoebic gill disease (AGD). In addition, this rainbow trout was infected with *Ca. B. cysticola* (Ct-value 21.7) and *D. lepeophtherii* (Ct-value 25.5). *P. perurans* and AGD was first detected in farmed Atlantic salmon in Tasmania the mid-1980. In Norway the first cases were recorded in 2006, but significant losses were not recorded until 2012 [2]. AGD is primarily detected in farmed Atlantic salmon in Norway, but also in rainbow trout and the marine species turbot, lump sucker and in wrasses [2].

Brown trout in large lakes

Results from PCR analyses of gill samples from brown trout from three large lakes are shown in Table 4, although SGPV was omitted since all samples were PCR-negative for this virus. Despite testing of a large number of fish in many locations, the virus has never been detected in brown trout or sea trout sampled in their natural environment.

Atlantic salmon paramyxovirus was detected in one brown trout captured in Snåsavatnet. In the health monitoring programme, this virus has previously been detected in five adult sea trout in rivers in the County of Nordland [4]. Unfortunately, organs for histopathological investigation were not available from any of these trout. The health impact of this virus could thus not be assessed.

Candidatus Branchiomonas cysticola was present in trout from all locations. The prevalence ranged from 6.3 % to 50 % in the three large lakes, with the highest prevalence in Selbusjøen. The bacterial loads, judged by Ct-values were from moderate to low. There is no connectivity and upstream migration of anadromous salmonids to these lakes. In the 2018 programme, the bacteria was found in European whitefish *Coregonus lavaretus* [4].

Candidatus Piscichlamydia salmonis was present in trout from all locations. The prevalence ranged from 18.8 % to 68.1 % in the three lakes, with the highest prevalence in Lake Snåsavatnet. The bacterial loads, judged by Ct-values were from moderate to low.

Co-infections were not detected in brown trout from Femunden. Two of 28 in Selbusjøen, and nine of 47 in Snåsavatnet were infected by both *Ca. B. cysticola* and *Ca. P. salmonis*.

Table 4: Results from PCR-analyses of gill tissue from brown trout captured in Femunden, Selbusjøen and Snåsavatnet. Table show number of fish tested, number of PCR-positive fish, Ct-value as mean and range (in parenthesis). Salmon gill poxvirus is omitted from the table since all fish were PCR-negative for this virus.

Location	No. Fish	Atlantic salmon paramyxovirus		Ca. <i>B. cysticola</i>		Ca. <i>P. salmonis</i>	
		Results	Ct-values	Results	Ct-values	Results	Ct-values
Femunden	16	0		1	24	3	28.9 (20.1-33.1)
Selbusjøen	28	0		14	27.4 (22.7-34.1)	9	34.2 (32.7-36.5)
Snåsavatnet	47	1	27.9	10	24.2 (20.6-34.0)	32	23.3(18.9-33.2)

Conclusion

In this study, the overall prevalence of salmon gill poxvirus (SGPV) in Atlantic salmon in the sea was 4 %. Compiled data from health monitoring in 2016, 2018 and 2020 point toward low SGPV prevalence in Atlantic salmon in the sea and increasing prevalence after entering the river. In addition, the compiled data indicate that the prevalence further escalates when wild-caught Atlantic salmon are held together in tanks in stock enhancement hatcheries.

It is now common practice to release wild-caught broodfish back into their native river after stripping instead of killing them. The high prevalence of SGPV in wild-caught broodfish in stock enhancement hatcheries (80-90 %) and the associated gill pathology indicate that these broodfish are released into the wild with reduced gill health and possibly, can serve as reservoirs for spread of the virus to other wild salmon.

SGPV was neither present in brown trout nor in sea trout. SGPV has been found in broodfish of sea trout after cohabitation with infected broodfish of Atlantic salmon [11], but despite testing of a large number of fish at many locations over several years, the virus has never been detected in brown trout or sea trout sampled in their natural environment.

Atlantic salmon paramyxovirus was present in one salmonid, a brown trout captured in Snåsavatnet. In conjunction with the health monitoring programme, this virus has previously been detected in five sea trout in the County of Nordland [4]. The result indicate that the virus is present in trout in both freshwater and seawater. The significance for the gill health of wild trout is unknown.

Candidatus Branchiomonas cysticola seem to be ubiquitous in wild salmonids. In this study, the bacteria was detected, often with high prevalence, in juvenile and adult anadromous salmonids in the sea and rivers, and in freshwater resident brown trout in the large lakes.

Desmozoon lepeophtherii was originally described as a parasite of salmon lice, and it is thus noteworthy that the highest proportion of *D. lepeophtherii* was detected in production area 1 where 13 of the 14 cases were from the County of Viken, a county without aquaculture in open net pens. Altogether 40.6 % of the salmon from this county were PCR-positive for *D. lepeophtherii*. The amoeba *Paramoeba perurans* was only detected in an escaped farmed rainbow trout.

The infection status in the studied wild fish is less complex than the status described in farmed fish [1, 2]. Due to the overall high proportion of *Ca. B. cysticola* and/or *Ca. P. salmonis* carriers, co-infection with two infectious agent is not uncommon, while the presence of three infectious agents in the same fish is uncommon. Nevertheless, fish that are PCR-negative for all agents are also detected. The relatively high prevalence and load of infectious agents detected in some wild fish rise important questions. What impact do these infectious agents have on gill health in wild fish? How do wild fish cope with this burden? Will the burden increase with the changing climate, increased eutrophication of freshwater sources or other man made impacts? These issues should be further studied. Monitoring of gill health in wild and farmed species will be important in the years to come.

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Appendix

Appendix 1.

Atlantic salmon in the sea. Results from PCR-analyses of gill samples from Atlantic salmon with different life-histories.

	Wild	Hatchery reared	Farmed/wild hybrids	Farmed escapees
Number tested	133	7	10	2
ASPV	0	0	0	0
SGPV	5	0	1	0
<i>Ca. B. cysticola</i>	106	5	7	2
<i>Ca. P. salmonis</i>	6	0	1	0
<i>D. lepeophtherii</i>	17	2	1	0
<i>P. perurans</i>	0	0	0	0

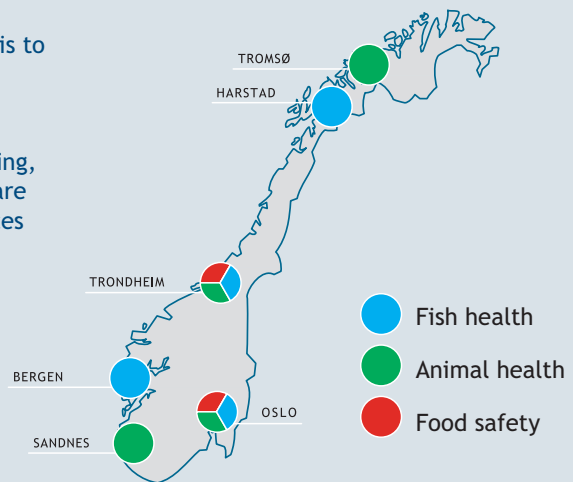
*Scientifically ambitious, forward-looking and cooperatively oriented
– for integrated health*

The Norwegian Veterinary Institute is a national research institute that operates in the fields of animal and fish health, food safety and feed hygiene; its primary task is to provide the authorities with independently generated knowledge.

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The Norwegian Veterinary Institute's central laboratory and administration lie in Oslo, and we operate regional laboratories in Sandnes, Bergen, Trondheim, Harstad and Tromsø.

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Animal health



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