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The surveillance programme to document absence of Atlantic salmon (*Salmo salar*) and *G. salaris* in the River Drammenselva upstream of Hellefossen in Norway 2021

REPORT 17/2022

A surveillance programme to document absence of Atlantic salmon (*Salmo salar*) and *G. salaris* in the River Drammenselva upstream of Hellefossen.

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Content

Summary

In 2019, the Norwegian Food Safety Authority made a decision to close the fish ladder in Hellefossen. This was done to exclude the stretch upstream of Hellefossen in a future treatment of the river to get rid of *Gyrodactylus salaris*. Provided that Hellefossen functions as an absolute barrier to fish migration, the area upstream will in the long run be free of Atlantic salmon and *G. salaris*. To document if the closure of the fish ladder in Hellefossen has had the desired reducing effect on the Atlantic salmon and *G. salaris* population, the Norwegian Food Safety Authority commissioned the Norwegian Veterinary Institute to carry out surveillance in river Drammenselva, starting from 2020.

The surveillance program is carried out as a combination of environmental DNA (eDNA) monitoring and electrofishing. The combined results from the eDNA survey and electrofishing in 2020 demonstrated that the closure of the fishing ladder in Hellefoss seemed to have had the desired effect. Only a few Atlantic salmon were found above Hellefossen and none of these were young of the year (0+). The results from the eDNA analyses and the combined electrofishing and parasitological examination also corresponded well in 2020. There was a general increase in eDNA concentration, going downstream, of both *G. salaris* and Atlantic salmon with the absolute highest concentration in the lower most station. For the locations upstream of Hellefossen in 2021, no Atlantic salmon were caught by electrofishing, while the environmental DNA analyses for Atlantic salmon were positive for two samples, indicating that Atlantic salmon is still present, however in low densities. *Gyrodactylus salaris* eDNA was only detected in the water sample from below Hellefossen and this corresponded well to the finding of high intensities of parasites on the Atlantic salmon parr in the same location.

Introduction

The parasite *Gyrodactylus salaris* is considered one of the main threats to Atlantic salmon (*Salmo salar*) populations [1] and the policy of the Norwegian Authorities is to eradicate *G. salaris* from infected watersheds and farms [2]. In 1987, *G. salaris* was detected on Atlantic salmon parr in the river Drammenselva. The infection had probably reached this river via escaped infected Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) from a fish farm in Lake Tyrifjorden situated upstream of the anadromous stretch of river Drammenselva (i.e. upstream Embretsfoss, see Fig. 1). Eradicating the parasite from this infected river is a considerable challenge, mostly due to the size of the river with a high water flow, high fish species diversity and an estuary with brackish water (the Drammensfjord) covering a large area.

There has been uncertainty regarding the infection status for *G. salaris* upstream of the anadromous part of River Drammenselva as the parasite was present on rainbow trout farms in this area earlier. This uncertainty especially concerns the lakes Tyrifjorden, Randsfjorden and Strondafjorden [3]. To substantiate the likely absence of *G. salaris* from these areas, the

Norwegian Veterinary Institute (NVI) has carried out several studies on behalf of the Norwegian Food Safety Authority (NFSA) in the period 2014-2018 [4, 5, 6, 7] and these studies did not find any evidence for the presence of *G. salaris*.

In 2015, the Norwegian Environment Agency appointed a working group with a mandate to investigate whether it is possible to eradicate *G. salaris* from the Drammen region by using chemical treatment. In 2018, the report from the group was presented and they concluded that *G. salaris* could be eradicated from the region [8]. It was pointed out that the probability of succeeding with a chemical treatment would increase by closing the fish ladder in Hellefossen (see fig. 1). A closure of this barrier would result in reduced upstream migration of salmon, and thus reduced recruitment of Atlantic salmon juveniles on the stretch between Hellefoss and Embretsfoss, which would subsequently lead to a reduction in population size of *G. salaris*. At best, the closure could eradicate Atlantic salmon and *G. salaris* on the river stretch upstream of the waterfall, if Hellefossen functions as an absolute barrier to migration. Excluding the stretch upstream of Hellefossen in a possible eradication measure will reduce the complexity and size of the task greatly and increase the chance of succeeding.

In 2019, the NFSA made a decision to close the fish ladder in Hellefossen. From the 2020 season onwards, Atlantic salmon would thus to a large extent be prevented from reaching the spawning areas between Hellefoss and Embretsfoss, a stretch of approx. 14 km. Provided that Hellefossen functions as an absolute barrier to fish migration, the area upstream will in the long run be free of *G. salaris*. Monitoring of the Atlantic salmon and *G. salaris* population is imperative to document if closure of the fish ladder in Hellefossen has had the desired reducing effect on the Atlantic salmon and *G. salaris* population. The NFSA therefore commissioned NVI to carry out surveillance for *G. salaris* and Atlantic salmon upstream of Hellefossen, starting from 2020

Aims

The aim of the surveillance program is to document if the Atlantic salmon population, and subsequently the *G. salaris* population, is reduced and eventually eradicated upstream of Hellefossen after the closure of the fish ladder. This surveillance program thus aims to document if the decision to close the ladder has had the intended effect.

Materials and methods

The surveillance program was carried out as a combination of environmental DNA (eDNA) monitoring and electrofishing, in the same way as in 2020. eDNA monitoring is a tool that can detect minute amounts of DNA in water samples using a combination of water filtering and molecular detection. All organisms in water shed cells containing DNA into the environment [9]. By using species-specific primers and probes and sensitive PCR-methods, it is possible to detect and identify the presence of DNA from specifically targeted species in water samples.

This method is also developed for detecting *G. salaris* [10] and has previously been applied in field studies in the river Drammen and elsewhere [4, 10, 11, 12].

Sampling localities

Fish samples and water filter samples were obtained from five localities in the river (Station 1 – 5, see Figure 1) on the 29th September 2021; one upstream of the anadromous stretch, i.e. above Embretsfoss, three on the stretch between Hellefossen and Embretsfoss, and one below Hellefossen. The sample above Embretsfoss was taken as a negative control sample (no presence of Atlantic salmon and *G. salaris*) and the one below Hellefoss as a positive control sample (confirmed presence of *G. salaris*). The chosen locations are locations previously used for density assessment of Atlantic salmon in River Drammenselva (Odin Kirkemoen, Naturrestaurering AS, pers. comm.).

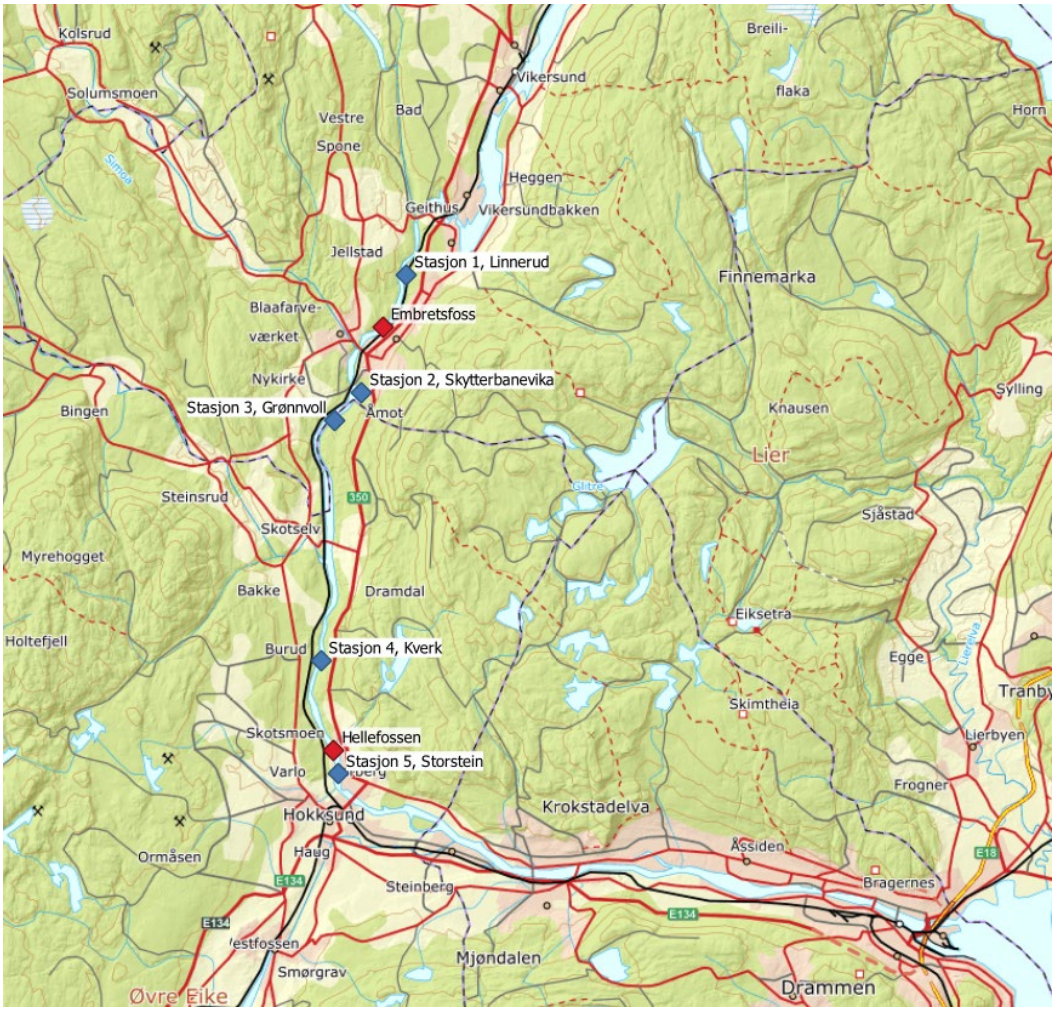

Figure 1: Sampling locations (blue diamonds) for eDNA samples and electrofishing in the River Drammenselva. The barriers for upstream migration of salmon, Hellefoss and Embretsfoss, are shown by red diamonds.

Water sampling and environmental DNA

From all stations, triplicate water samples of 5 l (3 × 5 l) were collected and filtered on site onto glass fibre filters (47 mm AP25 Millipore, 2 µm pore size, Millipore, Billerica, USA) using a

portable peristaltic pump (Masterflex E/S portable sampler, Masterflex, Gelsenkirchen, Germany), tygon tubing (Masterflex) and an in-line filter holder (Millipore) according to Strand et al. [13]. Filters were placed in separate 15 ml Falcon tubes containing ATL buffer. DNA was isolated in the laboratory using a Nucleospin Plant II midi kit and Qiagen buffer according to Fossøy et al. [11]. The DNA extracted from the filters was analysed with qPCR assays designed to detect the following four targets; *G. salaris* [10], *G. derjavinoides* [14], Atlantic salmon [15] and brown trout (*Salmo trutta*) [16]. The assays for brown trout and *G. derjavinoides* were included as positive controls; i.e. brown trout is found on all localities and *G. derjavinoides* is also known from the watercourse, and suspected to be present in most parts of the river. Thus, we would expect amplification of one or both of these targets in all localities.

Electrofishing and parasitological examination

Electrofishing was carried out following standard protocols with the aim to catch any fish present in the localities chosen. The presence of fish species other than Atlantic salmon was only noted and these fish where immediately released. The Atlantic salmon were euthanised following the strict codes of practice in force in Europe, preserved intact in 96% ethanol, transported back to the laboratory where they were examined for the presence of *Gyrodactylus spp*. using a stereo microscope (Leica MZ 7.5, Leica microsystems, St. Gallen, Switzerland).

Results and discussion

Electrofishing

No Atlantic salmon were caught by electrofishing at the four stations (1-4) upstream Hellefoss. In total, 15 Atlantic salmon were caught by electrofishing at station 5, below Hellefossen. The Atlantic salmon varied in size between 40 and 98 mm. Brown trout were caught in station 1, 2, 3 and 5.

Other fish species observed were minnows (*Phoxinus phoxinus*), ruffe (*Acerina cernua*), and three-spined sticklebacks (*Gasterosteus aculeatus*).

Parasitological examination

The prevalence of infection of *Gyrodactylus* spp. on all fish from below Hellefossen was 100%. The intensity of infection was generally high and varied from 100 to several hundreds.

Environmental DNA analyses

A total of 15 eDNA samples of 5 l were collected from the 5 stations (Figure 1, three replicate samples per station) and analysed. The results are summarised in figures 2 and 3. No eDNA from Atlantic salmon and *G. salaris* was detected at station 1, the negative control site above Embretsfoss. Environmental DNA from *G. salaris* was only detected in the sample from below

Hellefossen (station 5), while for salmon, two samples from above Hellefossen (station 3 and 4), and the sample below Hellefossen (station 5) were positive. For one of the two positive samples from above Hellefossen (station 3), only one of the three replicates were positive and with a high cq –value, indicating very low eDNA amounts (see Figure 3).

Brown trout eDNA and *G. derjavinoides* eDNA was detected at all stations (Figure 3).

The eDNA concentrations of Atlantic salmon was higher downstream of Hellefossen as compared to upstream, while for brown trout, the concentrations were quite similar at the different stations. The eDNA concentrations for *G. derjavinoides* were generally low in all stations, with the highest concentration in station 1 (see Figure 3).

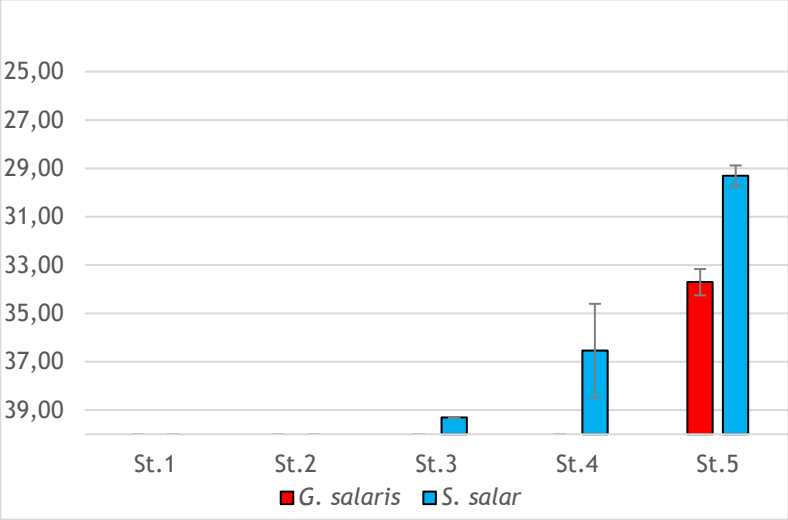

Figure 2: Bar plot showing the average Cq-value (±SD) of Gyrodactylus salaris (red) and Atlantic salmon, Salmo salar (blue), eDNA per station. The Cq-value reflects the level of target DNA in the sample where lower Cq-value indicates higher DNA content in the sample.

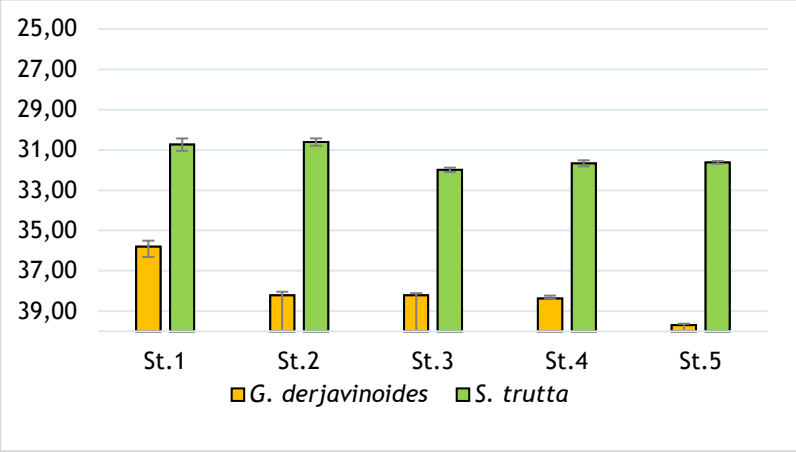

Figure 3: Bar plot showing the average Cq-value (±SD) of Gyrodactylus derjavinoides (yellow) and brown trout, Salmo trutta (green), eDNA per station. The Cq-value reflects the level of target DNA in the sample where lower Cq-value indicates higher DNA content in the sample.

The combined results from the eDNA survey and electrofishing show that the closure of the fishing ladder in Hellefoss seems to have had the desired effect as only a few Atlantic salmon were caught by electrofishing above Hellefossen in 2020 [17] and none in 2021. However, the environmental DNA analyses indicates that Atlantic salmon might still be present, however at a low density. The closure of the migration barrier at Hellefossen was done in spring 2019 and thus the last spawning for the Atlantic salmon occurred in autumn 2018. The offspring from this spawning would thus be 0+ in 2019, 1+ in 2020 and 2+ in 2021. As 3+ smolt is present, although less frequent than 2+, in River Drammenselva [18 and Bjørn Florø-Larsen pers. comm.], the continued presence of Atlantic salmon above Hellefossen as demonstrated by the eDNA monitoring, is not surprising. The lower population size of Atlantic salmon above Hellefossen compared to 2020, as indicated by both electrofishing and eDNA monitoring, corresponds well to the fact that a large proportion of the smolt left the river as 2+ in 2021. The results from eDNA analyses and the combined electrofishing and parasitological examination corresponded well both in 2020 and 2021.

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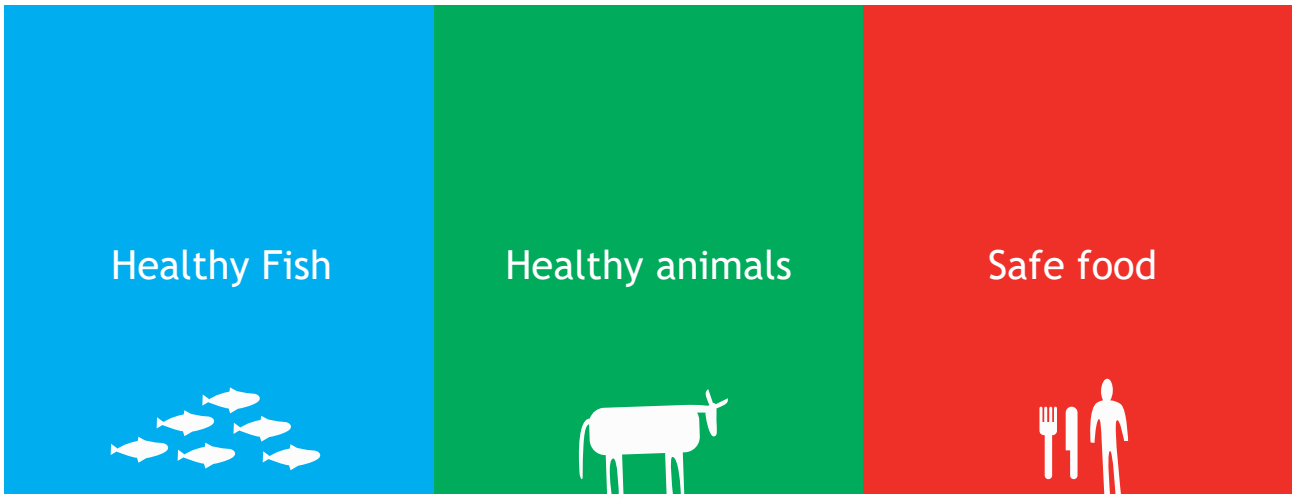

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