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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 2020

REPORT 46/2021

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 salmon and *G. salaris* population, the Norwegian Food Safety Authority commissioned NVI to carry out surveillance in river Drammenselva, starting from 2020.

The surveillance program was carried out as a combination of environmental DNA (eDNA) monitoring and electrofishing. The combined results from the eDNA survey and electrofishing show that the closure of the fishing ladder in Hellefoss has had the desired effect. Only few salmon were found above Hellefossen and none of these were young of the year (0+). If Hellefossen has been an absolute barrier to upstream migration of salmon, the expectation is that even fewer or no salmon will be found in 2021. The results from eDNA analyses and the combined electrofishing and parasitological examination corresponded well, as there was a general increase in eDNA concentration of both *G. salaris* and Atlantic salmon downstream with the absolute highest concentration in the lower most station.

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 salmon parr in the river Drammenselva. The infection had probably reached this river via escaped infected 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. This especially concerns the lakes Tyrifjorden, Randsfjorden and Strondafjorden [3]. To substantiate the likely absence of *G. salaris* from these areas, the Norwegian Veterinary Institute has carried out several studies on behalf of the Norwegian Food Health Authorities 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 here 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 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 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 Norwegian Food Safety Authority made a decision to close the fish ladder in Hellefossen. From the 2020 season onwards, 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 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 salmon and *G. salaris* population. The Norwegian Food Safety Authority 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. 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 seven localities in the river (Station 1 – 7, see fig. 1); one upstream of the anadromous stretch, i.e. above Embretsfoss, four on the stretch between Hellefossen and Embretsfoss, and two below Hellefossen. The sample above Embretsfoss was taken as a negative control sample (no presence of Atlantic salmon and *G. salaris*) and the two below Hellefoss as positive control samples (confirmed presence of *G. salaris*). The chosen locations had previously been used as locations 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, environmental DNA

From station 2 to station 7, 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]. At station 1, one control sample (1 x 5 l) was filtered on site. Filters were placed in separate 15 ml Falcon tubes containing ATL buffer. DNA was isolated in the 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 [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 water course 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 upstream of Hellefossen.

Electrofishing and parasitological examination

Electrofishing was carried out following standard protocols with the aim to catch any fish that was present in the localities chosen. The fish were euthanised following the strict codes of practice in force in Europe, preserved intact in 96% ethanol and later examined for the presence of *Gyrodactylus spp*. using a stereo microscope (Leica MZ 7.5, Leica microsystems, St. Gallen, Switzerland). Only Atlantic salmon were euthanised and taken back to the lab for examination, while the presence of brown trout and other fish species were noted.

Parasite diagnostics

A selection of *Gyrodactylus* specimens from each location were picked off the fish, both from brown trout upstream Embretsfoss (station 1) and from Atlantic salmon downstream Embretsfoss (stations 2 – 6.). DNA was extracted using standard protocols and specimens identified to species by PCR and sequencing of the Internal Transcribed Spacer 2 (ITS2) as outlined in the OIE Manual of Diagnostic Tests for Aquatic Animals. http://www.oie.int/index.php?id=2439&L=0&htmfile=chapitre_gyrodactylus_salaris.htm

Results and discussion

Electrofishing

In total, 52 Atlantic salmon were caught by electrofishing and brought back to the lab. No salmon were caught upstream Embretsfoss and only 10 salmon in total between Hellefoss and Embretsfoss (see table 1 and figure 2). No young of the year (0+) were caught between Hellefoss and Embretsfoss. The density of salmon on the locality in the main river below Hellefoss was much higher than above and 0+ and 1x salmon were caught. Brown trout were only caught in station 1, 5 and 7.

Other fish observed were minnows (*Phoxinus phoxinus*), lampreys (*Lampetra* sp.), ruffe (*Acerina cernua*), perch (*Perca fluviatilis*), three-spined sticklebacks (*Gasterosteus aculeatus*), and European flounder (*Platichthys flesus*).

Table 1: Number of Atlantic salmon per station with mean size and size range, followed by notes on other fish observed. BT=brown trout, minnows=M, three-spined stickleback=TS, perch=P, lamprey=L, ruffe=R. European flounder=EF. +=one or a few specimens, ++ = relatively high density

Station	n salmon	mean fish size (mm, range)	fish size range	Other fish observed
1	-	-	-	BT (++), L (+), M (+), TS (+), P (+)
2	1	90	90	-
3	2	110	95-125	-
4	4	98	85-110	R (++)
5	4	84	75-90	BT (+), R (+)
6	37	57	35-100	M (++), TS (+)
7	4	90	40-120	BT (++), L (+), M (+), EF (+)

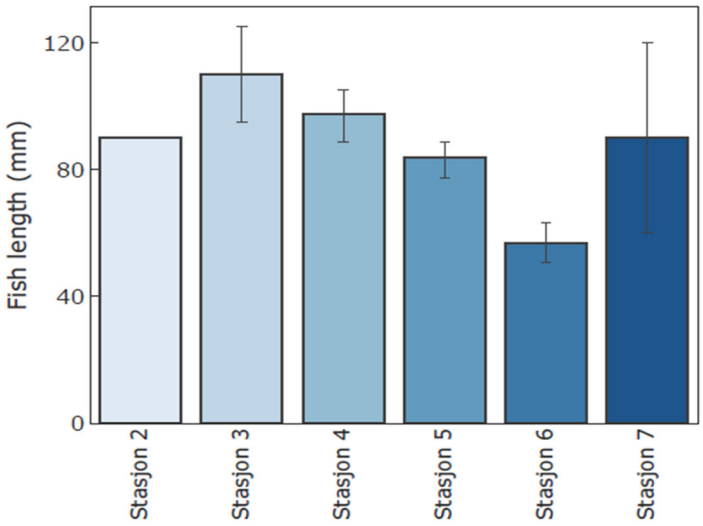

***Figure 2**: Size distribution (mm) of Atlantic salmon in each station. No salmon were caught on station 1 and it is therefore not shown.*

Parasitological examination

The prevalence of infection on all fish was 100%. The intensity of infection was generally high; only ten fish in total had intensities of below 100, while the rest varied from 100 to several hundred (Fig. 3 and Fig 4 (image)). The mean intensity per station is shown in Figure 3. All analysed specimens from Atlantic salmon downstream Embretsfoss (station 2 – 6) were diagnosed as *G. salaris*, while the specimens from brown trout upstream Embretsfoss (station 1) were diagnosed as *G. derjavinoides*.

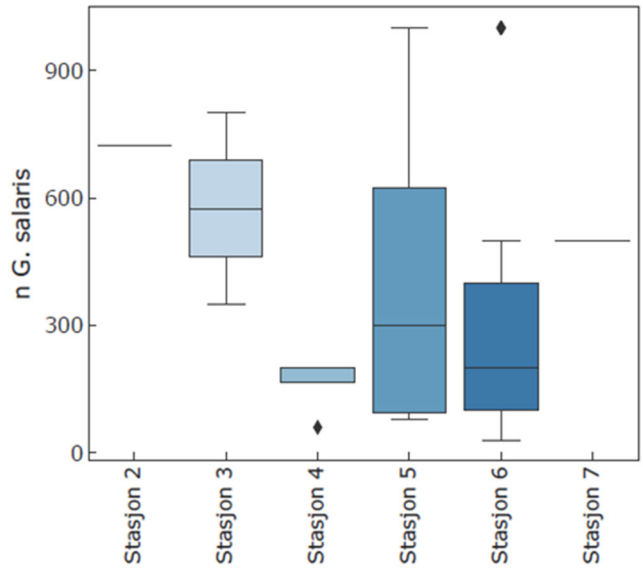

***Figure 3**: Mean intensity of Gyrodactylus salaris per fish per station.*

***Figure 4**: Atlantic salmon, size 45 mm, highly infected by Gyrodactylus salaris.*

Environmental DNA analyses

A total of 19 eDNA samples of 5 l was collected and analysed. No eDNA from Atlantic salmon and *G. salaris* was detected at station 1, the negative control site above Embretsfoss (fig. 5). Below Embretsfoss, eDNA from Atlantic salmon and *G. salaris* was detected at all stations. At station 2, only one of three replicate samples were positive for *G. salaris* eDNA, while all three replicate samples were positive for station 3 to 7. Brown trout eDNA was detected in all stations while *G. derjavinoides* eDNA was detected in all stations except station 6 (fig. 6). In general, there were higher eDNA concentrations of both *G. salaris* and Atlantic salmon at station 6 and 7, the two positive control sites downstream of Hellefoss (fig. 5). For brown trout, the eDNA concentrations were quite similar at the different stations while the eDNA concentrations for *G. derjavinoides* were generally low in all stations, but with the highest concentration where most brown trout was observed and caught with electrofishing (station 1 and 7, see Fig. 6).

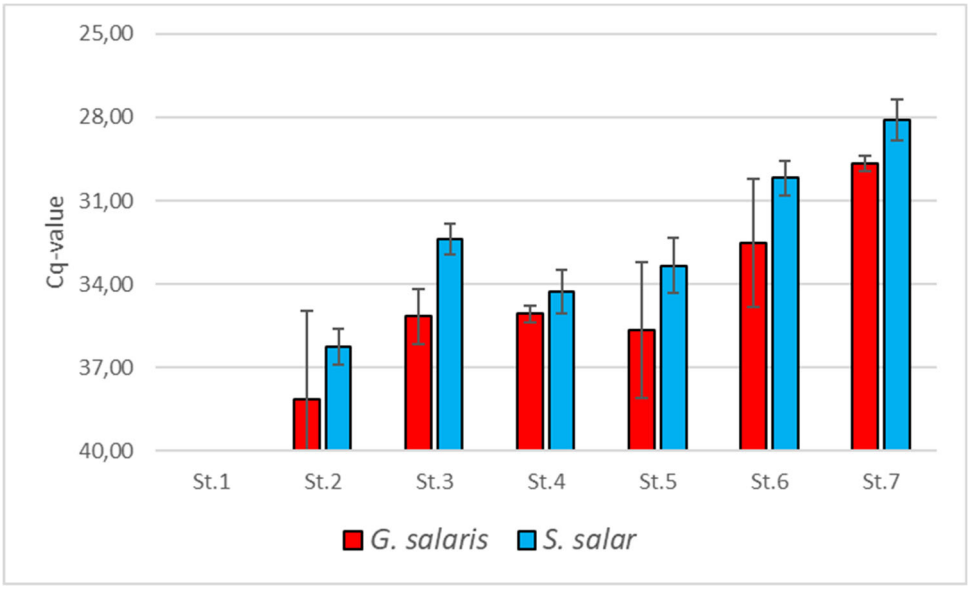

***Figure 5**: Bar plot showing the average Cq-value (±SD) of G. salaris and Atlantic salmon, S. salar, eDNA per station. The Cq-value reflects the level of target DNA in the sample where lower Cq-value indicates higher DNA content in the sample.*

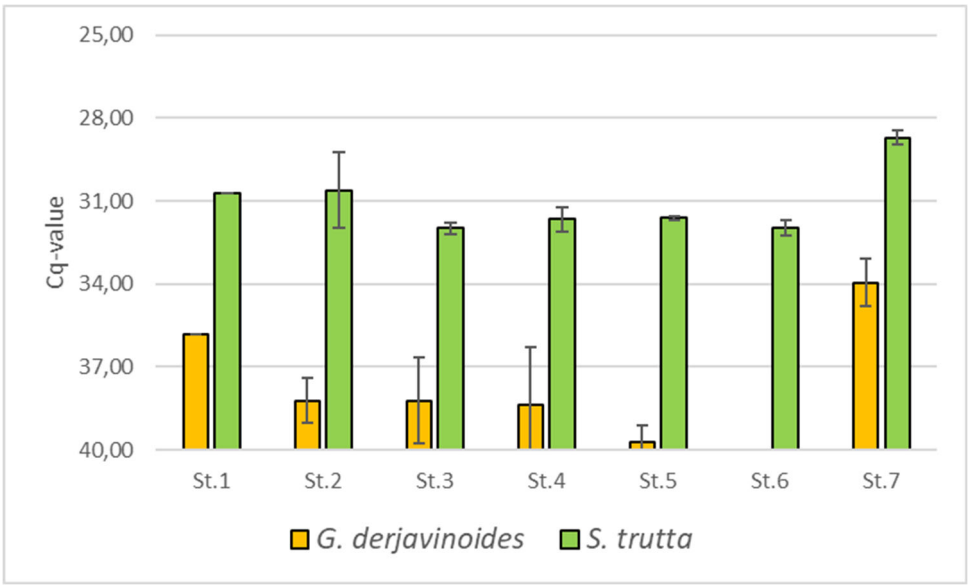

***Figure 6**: Bar plot showing the average Cq-value (±SD) of G. derjavinoides and* brown trout, *S. trutta, eDNA per station. The Cq-value reflects the level of target DNA in the sample where lower Cq-value indicates higher DNA content in the sample.*

Conclusions

The combined results from the eDNA survey and electro fishing show that the closure of the fishing ladder in Hellefoss has had the desired effect as only few salmon were found above Hellefossen and none of these were young of the year (0+). If Hellefossen has been an absolute barrier to upstream migration of salmon, the expectation is that even fewer or no salmon will be found in 2021. The results from eDNA analyses and the combined electrofishing and parasitological examination corresponded well, with a general increase in the eDNA concentration of both *G. salaris* and Atlantic salmon downstream and the absolute highest concentration in the lower most station.

Acknowledgements

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References

1. Forseth T, Barlaup B, Finstad B, Fiske P, Gjøsæther H, Falkegård M, et al. The major threats to Atlantic salmon in Norway. ICES Journal of Marine Science. 2017;74 6:1496-513; doi: 10.1093/icesjms/fsx020.
2. Anon: Handlingsplan mot lakseparasitten *Gyrodactylus salaris* for perioden 2014-2016. Miljødirektoratet. ; 2014: 1-114.
3. Mo TA: Virksomheten i 1987 og program for virksomheten i 1988. In: *Gyrodactylus*-undersøkelsene ved Zoologisk Museum: Universitetet i Oslo; 1988: 1-29 (in Norwegian).
4. Hytterød S, Rusch J, Darrud M, Mohammad SN, Hansen H: Mapping the occurrence of *Gyrodactylus salaris* upstream of the natural anadromous region of the Drammenselva catchment 2017. Veterinærinstituttet/Norwegian Veterinary Institute; 2018.
5. Hytterød S, Darrud M, Mohammad SN, Rusch J, Hansen H: Mapping the occurrence of *Gyrodactylus salaris* upstream of the natural anadromous region of the Drammenselva catchment 2018. Veterinærinstituttet/Norwegian Veterinary Institute; 2019.
6. Hytterød S, Hansen H, Mo TA: The surveillance programme to map the occurrence of *Gyrodactylus salaris* in the Drammenselva catchment in 2014 – 2015. Veterinærinstituttet/Norwegian Veterinary Institute; 2016: 6.
7. Hytterød S, Rusch J, Darrud M, Mohammad S, Hansen H: Mapping the occurrence of *Gyrodactylus salaris* upstream of the natural anadromous region of the Drammenselva catchment. Veterinærinstituttet/Norwegian Veterinary Institute; 2017: 7.
8. Hindar K, Mo TA, Eken M, Hagen AG, Hytterød S, Sandodden R, et al: Kan *Gyrodactylus salaris* utryddes fra Drammensregionen? - Sluttrapport fra arbeidsgruppen for Drammensregionen. In: NINA Rapport2018: 93.
9. Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MT, et al. Monitoring endangered freshwater biodiversity using environmental DNA. Mol Ecol. 2012;21 11:2565-73; doi: 10.1111/j.1365-294X.2011.05418.x.
10. Rusch JC, Hansen H, Strand DA, Markussen T, Hytterød S, Vrålstad T. Catching the fish with the worm: a case study on eDNA detection of the monogenean parasite *Gyrodactylus salaris* and two of its hosts, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Parasite Vector. 2018;11 333; doi: 10.1186/s13071-018-2916-3.
11. Fossøy F, Brandsegg H, Sivertsgård R, Pettersen O, Sandercock BK, Solem Ø, et al. Monitoring presence and abundance of two gyrodactylid ectoparasites and their salmonid hosts using environmental DNA. Environmental DNA. 2019;2 1:53-62; doi: 10.1002/edn3.45.
12. Hansen H, Rusch J, Degerman E, Jacobsen P-E, Ieshko EP: The application of a modern detection method for the salmon killer *Gyrodactylus salaris* and its two main hosts. In: NASCO Symposium - Managing The Atlantic Salmon In A Rapidly Changing Environment - Management Challenges And Possible Responses. Tromsø, Norway2019.
13. Strand DA, Jussila J, Johnsen SI, Viljamaa-Dirks S, Edsman L, Wiik-Nielsen J, et al. Detection of crayfish plague spores in large freshwater systems. Journal of Applied Ecology. 2014;51 2:544-53; doi: 10.1111/1365-2664.12218.
14. Collins CM, Kerr R, McIntosh R, Snow M. Development of a real-time PCR assay for the identification of *Gyrodactylus* parasites infecting salmonids in northern Europe. Dis AquatOrgan. 2010;90 2:135-42.
15. Matejusová I, Doig F, Middlemas SJ, Mackay S, Douglas A, Armstrong JD, et al. Using quantitative real-time PCR to detect salmonid prey in scats of grey *Halichoerus grypus* and harbour *Phoca vitulina* seals in Scotland - an experimental and field study. Journal of Applied Ecology. 2008;45 2:632-40; doi: 10.1111/j.1365-2664.2007.01429.x.
16. Carim KJ, Wilcox TM, Anderson M, Lawrence DJ, Young MK, McKelvey KS, et al. An environmental DNA marker for detecting nonnative brown trout (*Salmo trutta*). Conservation Genetics Resources. 2016;8 3:259-61; doi: 10.1007/s12686-016-0548-5.

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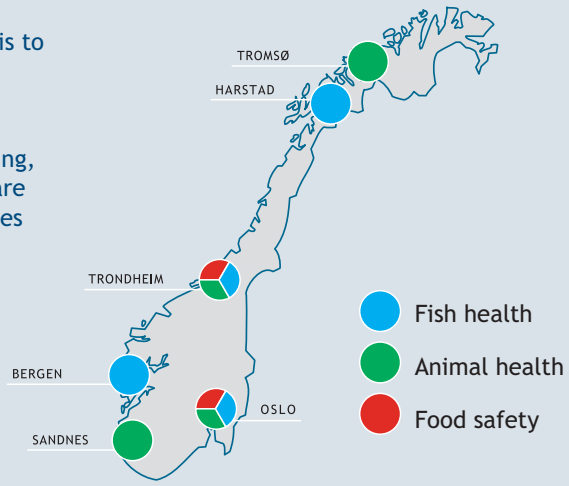

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