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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 2022

REPORT 22/2023

A surveillance programme to document absence of Atlantic salmon (*Salmo salar*) and *G. salaris* in the River Drammenselva upstream of Hellefossen in Norway 2022.

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Content

Summary

In 2019, the Norwegian Food Safety Authority decided 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 over time become free of Atlantic salmon and *G. salaris*. To document if the closure of the fish ladder in Hellefossen has had the desired effect on reducing the Atlantic salmon and *G. salaris* population, the Norwegian Food Safety Authority, NFSA, commissioned the Norwegian Veterinary Institute, NVI, to carry out surveillance in River Drammenselva, starting from 2020. In 2022, NFSA further commissioned NVI to carry out surveillance for *G. salaris* in lakes draining into the River Drammenselva catchment upstream of the anadromous part of the river.

The surveillance program in River Drammenselva is carried out as a combination of environmental DNA (eDNA) monitoring and electrofishing. The combined results from the eDNA survey and electrofishing have for the last two years demonstrated that the closure of the fishing ladder in Hellefoss have had the desired effect, although there were still indications of a continued presence of Atlantic salmon in the stretch between Hellefoss the absolute migration barrier at Embretsfoss in 2021 [1, 2]. Environmental DNA from *G. salaris* was not detected above Hellefoss in 2021. The results from the eDNA analyses and the combined electrofishing and parasitological examination were found to correspond well in 2020 and 2021.

As for 2021, no Atlantic salmon were caught by electrofishing in the locations upstream of Hellefossen in 2022, while all three locations between Hellefoss and Embretsfoss tested positive for Atlantic salmon eDNA. The results thus still indicate that Atlantic salmon is present, but in low densities. *Gyrodactylus salaris* eDNA was only detected in the water sample from below Hellefossen.

The surveillance for *G. salaris* in the lakes draining into River Drammenselva for 2022 focussed on Lake Randsfjorden (Innlandet and Viken counties) and was carried out as a combination of eDNA-monitoring and parasitological examination of fins of Arctic char, *Salvelinus alpinus*, for the presence of *G. salaris*. For 2022 eDNA- and fish samples for parasitological examination were analysed for the presence of *G. salaris*. *Gyrodactylus salaris* was not detected in any of the eDNA- or fish samples obtained from Lake Randsfjorden.

Introduction

The parasite *Gyrodactylus salaris* is considered one of the main threats to Atlantic salmon (*Salmo salar*) populations [3] and the policy of the Norwegian Authorities is to eradicate *G. salaris* from infected watersheds and farms [4]. 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 Figure. 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 [5]. Rainbow trout and arctic char are the only hosts present in this area known to be susceptible to *G. salaris* [6, 7, 8]). To substantiate the likely absence of *G. salaris* from these areas, the Norwegian Veterinary Institute (NVI) has previously carried out several studies on behalf of the Norwegian Food Safety Authority (NFSA) in the period 2014-2018 [9, 10, 11, 12] and these studies did not find any evidence for the presence of *G. salaris*.

In 2018, a working group appointed by the Norwegian Environment Agency concluded that *G. salaris* could be eradicated from the region Drammen infection region by chemical treatment [13]. 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*. If Hellefossen functions as an absolute barrier to migration, the closure would result in an eradication of the Atlantic salmon and thus *G. salaris* on the river stretch upstream of the waterfall. Excluding the stretch upstream of Hellefossen in a possible eradication measure will substantially reduce the complexity and size of the task 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. 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.

As the preparation for future eradication measures in the Drammen infection region, NFSA further commissioned NVI to carry out surveillance for *G. salaris* in lakes draining into River Drammen. This was done to further substantiate the likely absence of *G. salaris* from these areas, following up on the previous surveillance efforts [9, 10, 11, 12]. In addition, the NFSA has expressed an interest in testing eDNA monitoring as a tool for surveillance. Therefore, the surveillance program in the lakes is carried out as a combination of eDNA monitoring and classical parasitological examination of fish for the presence of *G. salaris*.

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.

The second aim was to further substantiate the likely absence of *G. salaris* infection in lakes draining into the River Drammenselva catchment, upstream of the anadromous part of the river.

Materials and methods

In the River Drammenselva itself, the surveillance program was carried out as a combination of environmental DNA (eDNA) monitoring and electrofishing, in the same way as in 2020 and 2021 [1, 2]. For 2022, the Lake Randsfjorden (Innlandet and Viken counties), was included in the surveillance program. In this lake, the surveillance was carried out as a combination of environmental DNA (eDNA) monitoring and sampling of fish by gillnetting, followed parasitological examination of fins of Arctic char, *Salvelinus alpinus*.

Environmental DNA 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 [14]. 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* [15] and has previously been applied in field studies in the River Drammenselva and elsewhere [12, 15, 16, 17]. While eDNA monitoring for *Gyrodactylus* parasites has not yet been tested in large lakes, several studies have investigated the use of eDNA for pathogen surveillance in large aquatic systems [see 18 and references therein].

Sampling localities

In the River Drammenselva, fish samples and water filter samples were obtained from five localities in the river (Station 1 – 5, see Figure 1) on the 12th September 2022; 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.) and are the same as included in this programme for the previous years.

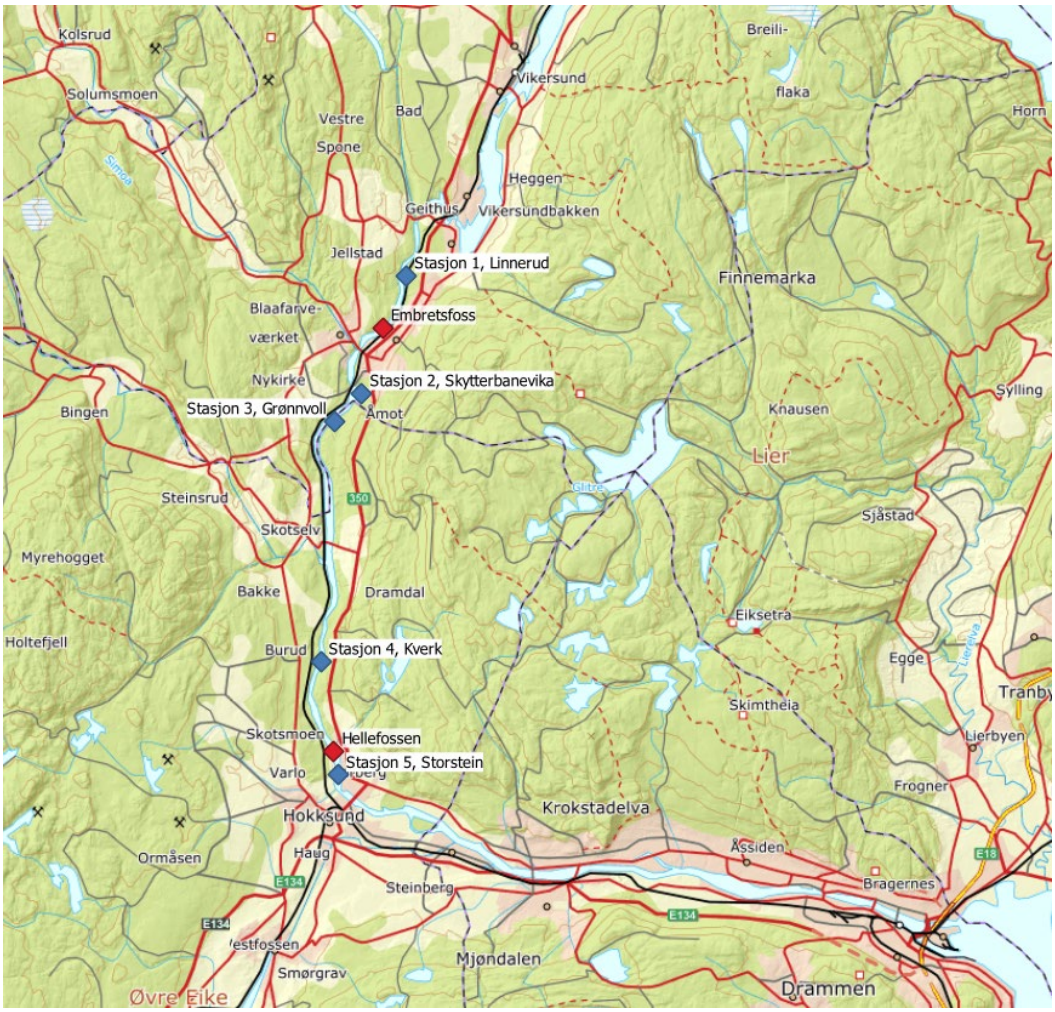

Figure 1: Sampling locations (blue diamonds) for eDNA samples and electrofishing in the River Drammenselva. The barriers for upstream migration of salmon, Hellefoss and Embretsfoss, are shown by red diamonds.

In the lake Randsfjorden, Arctic char was caught from seven different locations from the southern part of the lake using gillnets (Figure 2). Sampling in the autumn is considered the best period as these fish spawn in the autumn and thus gather at the spawning grounds. This makes it easier to catch sufficient numbers of fish, but it is also likely that parasite transmission happens in the spawning period and therefore the intensity of parasites are likely higher, increasing the chances of detecting an infection [19]. Water filter samples were collected close to five of the locations used for gillnetting, covering the entire fishing area. (Station 1, 3, 5, 6 and 7, see Figure 2) on the 20th October 2022.

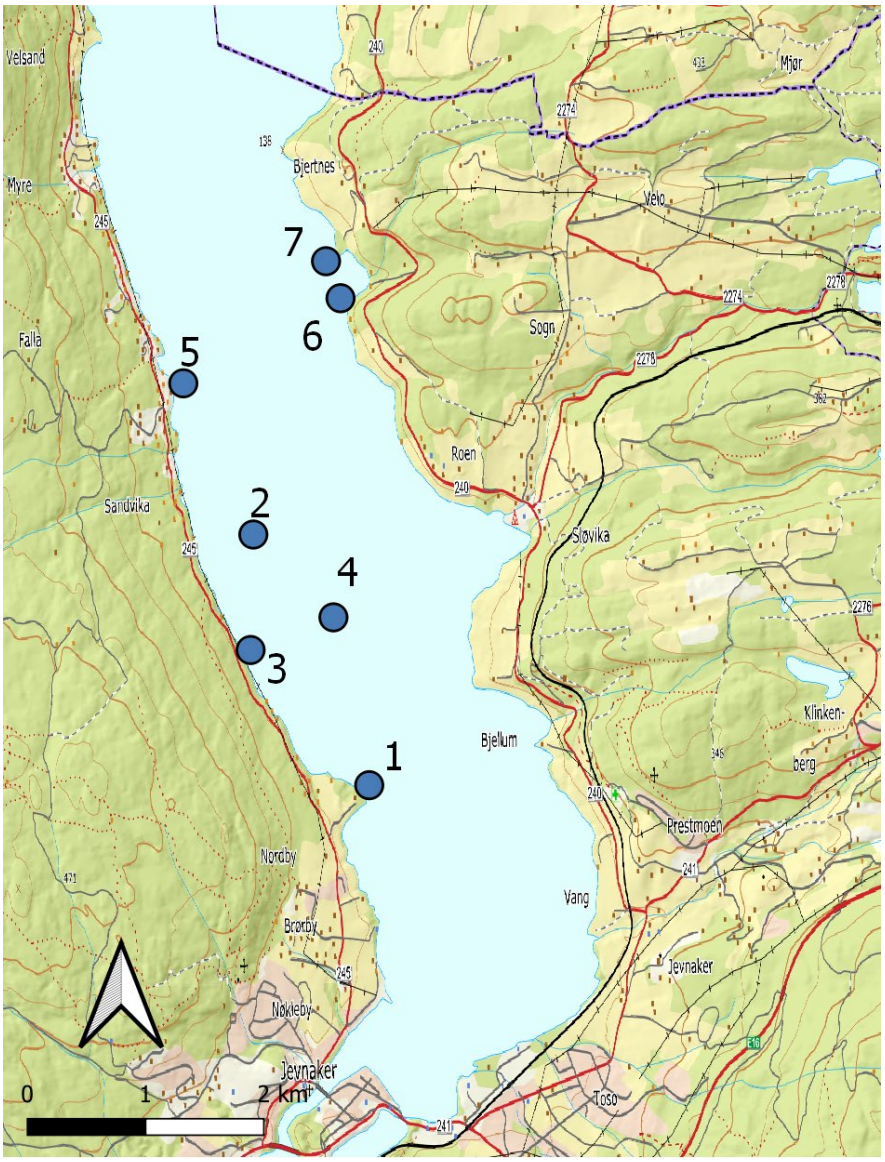

Figure 2: Sampling locations (blue dots) for gillnetting in the lake Randsfjorden. eDNA samples were obtained downstream from location 1, 3, 5, 6 and 7.

Lake Randsfjorden is a large lake covering an area of 140.7 km2 and with a maximum depth of 131 m. In addition to Arctic char, the fish fauna in the lake consists of brown trout (*Salmo trutta*), whitefish (*Coregonus lavaretus*), European smelt (*Osmerus eperlanus*), roach (*Rutilus rutilus*), minnows (*Phoxinus phoxinus*), crucian carp (*Carassius carassius*), pike (*Esox lucius*), ninespine stickleback (*Pungitus pungitus*), three-spined stickleback (*Gasterosteus aculeatus*), perch (*Perca fluviatilis*) and river lamprey (*Lampetra fluviatilis*) [20]).

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 (Alexis peristaltic pump, Proactive Environmental Products, Florida, USA), tygon tubing and an in-line filter holder (Millipore) according to Strand et al. [17]. In the river, sampling was performed from the river bank, while the samples in Lake

Randsfjorden were taken from a boat. These latter samples were obtained from near the bottom of the lake to be as close to the actual sites were Arctic char were caught in gillnets.

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. [16].

The eDNA extracted from the River Drammenselva was analysed with qPCR assays designed to detect the following five targets; *G. salaris* [15], *Gyrodactylus derjavinoides* [21], *Gyrodactylus* spp. [21], Atlantic salmon [22], and brown trout (*Salmo trutta*) [23]. The assays for brown trout, *G. derjavinoides* and *G.* spp. 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.

From the Lake Randsfjorden, DNA extracted from the filters was analysed with qPCR assays designed to detect four targets; *G. salaris* [15], *Gyrodactylus* spp. [21], brown trout [23], and Arctic char (*Salvelinus alpinus*) [24]. The assays for both brown trout and arctic char were included as positive controls as both fish species are found in lake Randsfjorden, but were also applied to test the detection of fish DNA in such a large lake. As *G. derjavinoides*, to our knowledge, is not confirmed present in Lake Randsfjorden, the assay for *G.* spp. was applied as positive control for the detection of *Gyrodactylus* eDNA in the lake. For both River Drammenselva and Lake Randsfjorden, the assay for *Gyrodactylus* spp. was also included as a test of its general applicability for eDNA monitoring.

Fish sampling and parasitological examination

In the River Drammenselva, fish were sampled by electrofishing following standard protocols. The aim was 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).

In the Lake Randsfjorden, Arctic char was caught using gillnets (mesh size 35 to 45 mm) from seven different locations from the south side of the lake by a local fisherman in the autumn 2022. All fins except the adipose fin were cut off with a pair of scissors and preserved directly in 96% ethanol. The fins were transported to the NVI laboratory and examined for the presence of *Gyrodactylus* spp. as explained above. All *Gyrodactylus* spp. on the caudal fins were counted and 376 specimens were isolated from caudal fins for species identification.

Parasite species identification

DNA was extracted from all the 376 isolated specimens using the Extracta DNA Prep for PCR kit (Quantabio, Hilden, Germany). The DNA extracts were analysed by qPCR specific for *G. salaris* (see above). As a confirmation of negative qPCR results, a random selection of specimens that tested negative in the qPCR analyses, was subjected to PCR and DNA sequencing of the ribosomal internal transcribed spacer 2 following standard protocols [25, 26].

Results and discussion

River Drammenselva

Electrofishing
No Atlantic salmon were caught by electrofishing at the four stations (1-4) upstream Hellefoss. In total, 14 Atlantic salmon were caught by electrofishing at station 5, below Hellefossen. The Atlantic salmon varied in size between 41 and 105 mm. Other fish species observed in the River Drammenselva were brown trout (*Salmo trutta*), minnows (*Phoxinus phoxinus*), ruffe (*Acerina cernua*), pike (*Esox Lucius*) and european flounder (*Platichthys flesus*).

Parasitological examination
The prevalence of infection of *Gyrodactylus* spp. on the fish sampled downstream of Hellefossen (Station 5) was 100%. The intensity of infection was generally high and varied from 40 to more than a thousand. As *G. salaris* has been confirmed present at this location several times [see e.g 1], no *Gyrodactylus* specimens were identified to species.

Environmental DNA analyses from the river
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 3 and 4. 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, all four stations below Embretsfoss (station 2, 3, 4 and 5) were positive.

Brown trout eDNA was detected at all stations, while *G. derjavinoides* eDNA was only detected at station 1, 2 and 5 (Figure 4). Also, the analyses for *G.* spp. tested positive for all five locations.

The eDNA concentrations of Atlantic salmon was higher downstream of Hellefossen as compared to upstream, while for brown trout, the concentration was higher upstream Embretsfoss. The eDNA concentrations for *G. derjavinoides* were generally low in all stations, with the highest concentration in station 1 (see Figure 4). For *G.* spp., the eDNA concentrations were generally higher than for *G. derjavinoides*, with one filter showing a particularly high concentration (see Figure 4 and 5). This was probably caused by catching a whole or larger part of a *Gyrodactylus* parasite on the filter. The higher concentrations of *G.* spp. as compared to concentrations of *G. derjavinoides* alone, can attributed to the fact that the *G.* spp. assay detects DNA from all *Gyrodactylus* species present in the river, not from just one species. Most fish species are assumed to be hosts for parasites in the genus *Gyrodactylus* [27] and in our own studies we have detected *Gyrodactylus* parasites on several fish species in River Drammenselva.

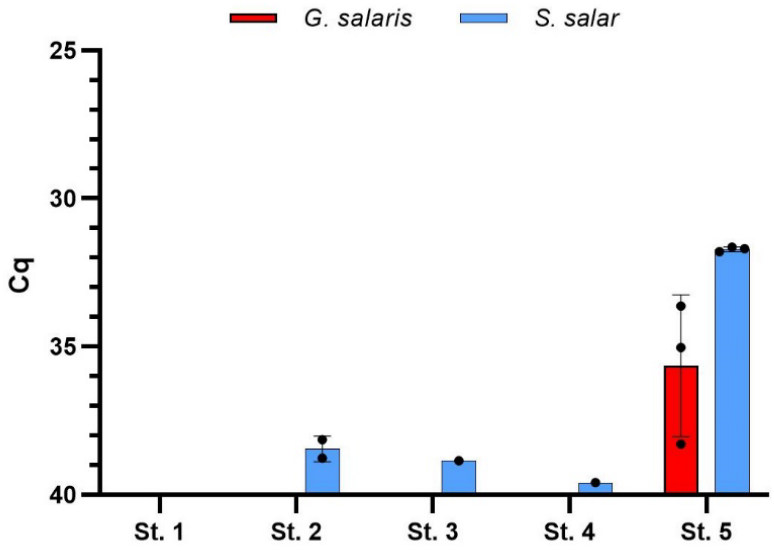

Figure 3: Bar plot showing the average Cq-value (±SD) of Gyrodactylus salaris *(red) and Atlantic salmon,* Salmo salar *(blue), eDNA per station. The Cq-value reflects the level of target DNA in the sample where lower Cq-value indicates higher DNA content in the sample. Each black dot represents a positive sample with correlating Cq-value.*

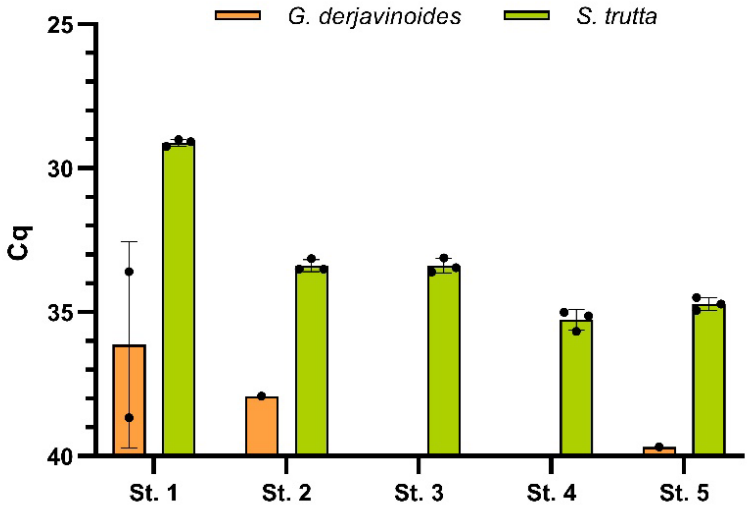

Figure 4: Bar plot showing the average Cq-value (±SD) of Gyrodactylus derjavinoides *(orange) and brown trout,* Salmo trutta *(green), eDNA per station. The Cq-value reflects the level of target DNA in the sample where lower Cq-value indicates higher DNA content in the sample. Each black dot represents a positive sample with correlating Cq-value.*

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 [2] and none in 2021 [1] and 2022. However, the environmental DNA analyses indicates that Atlantic salmon might still be present, but 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, 2+ in 2021 and 3+ in 2022. 3+ smolt are present, however less frequent than 2+, in River, and the presence of 4+ smolts cannot be completely ruled out either [28 and Bjørn Florø-Larsen pers. comm.]. Thus, the continued presence of Atlantic salmon above Hellefossen as demonstrated by the eDNA monitoring also in 2022, is therefore possible, although maybe surprising. The lower population size of Atlantic salmon above Hellefossen in 2021 and 2022 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 have corresponded well in all three years of surveillance.

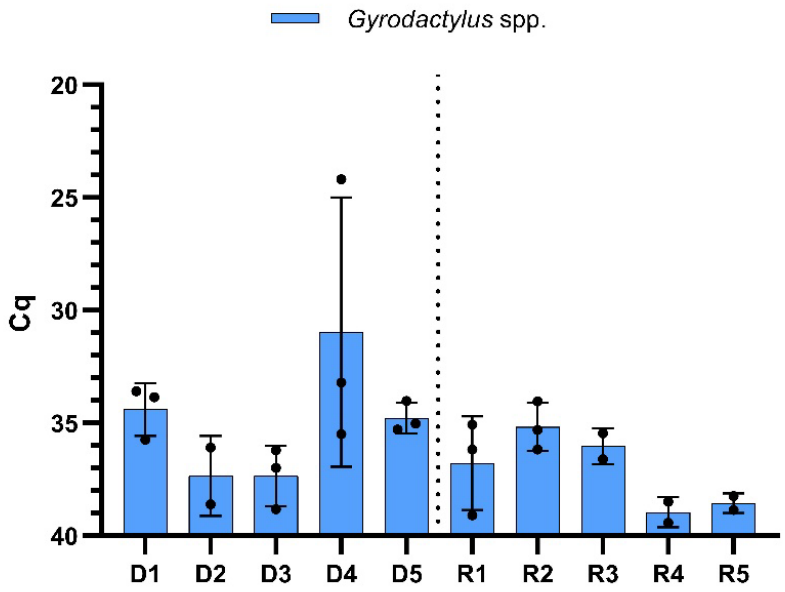

Figure 5: Bar plot showing the average Cq-value (±SD) of Gyrodactylus *spp., eDNA per station. D1-D5 represents station 1-5 from the river Drammenselva, while R1-R5 represents station 1-5 from the lake Randsfjorden. The Cq-value reflects the level of target DNA in the sample where lower Cq-value indicates higher DNA content in the sample. Each black dot represents a positive sample with correlating Cq-value.*

Lake Randsfjorden

Species identification of *Gyrodactylus* specimens

Altogether, 200 arctic char from seven locations in Lake Randsfjorden were collected and the fins were examined. The intensity of infection on individual fish varied from 0 to 55 *Gyrodactylus* specimens per caudal fin.

A total of 376 *Gyrodactylus* specimens were sampled from all infected caudal fins and analysed using the qPCR assay specific for *G. salaris*. All of these tests were negative for *G. salaris*. The subsample of 76 specimens that were subjected to PCR and DNA sequencing, confirmed that *Gyrodactylus salaris* was not present amongst the analysed specimens.

Environmental DNA analyses

qPCR analysis of the water samples yielded no positive results for *G. salaris* in any of the stations in Lake Randsfjorden. The assays for brown trout and arctic char were included as positive controls. Environmental DNA from brown trout was only detected in filter from two of the stations (station 1 and 2), while all filters from all stations were positive for arctic char (Figure 6). These results corresponds well to the fact that samples were taken nearby the spawning ground for Arctic char. A higher concentration of eDNA from Arctic char compared to that from brown trout was thus expected.

Samples from all five locations tested positive for *G.* spp. (Figure 5.) and, based on the combined results from both Lake Randsfjorden and River Drammenselva, we conclude that the *G.* spp. assay can be highly suitable as a positive control for eDNA monitoring, both in large lakes and in rivers.

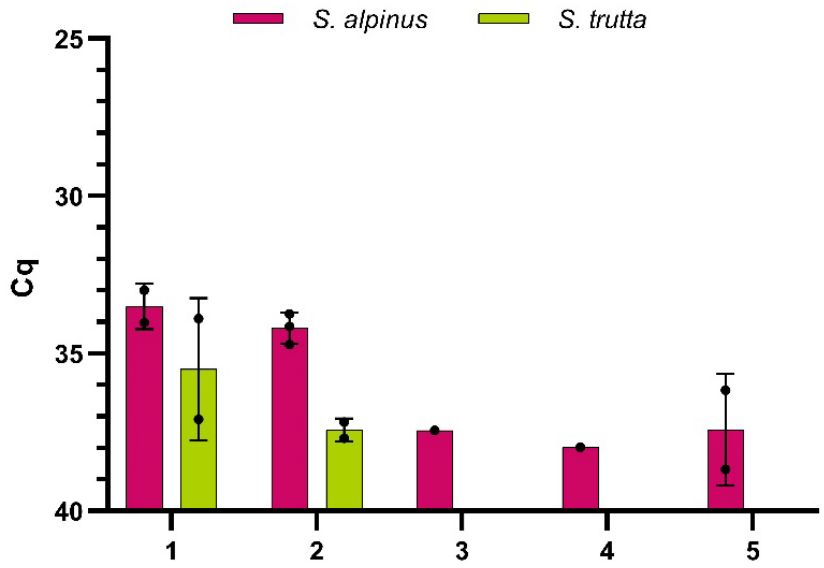

Figure 6: Bar plot showing the average Cq-value (±SD) of arctic char, Salvelinus alpinus *(dark pink) and brown trout,* Salmo trutta *(green), eDNA per station. The Cq-value reflects the level of target DNA in the sample where lower Cq-value indicates higher DNA content in the sample. Each black dot represents a positive sample with correlating Cq-value.*

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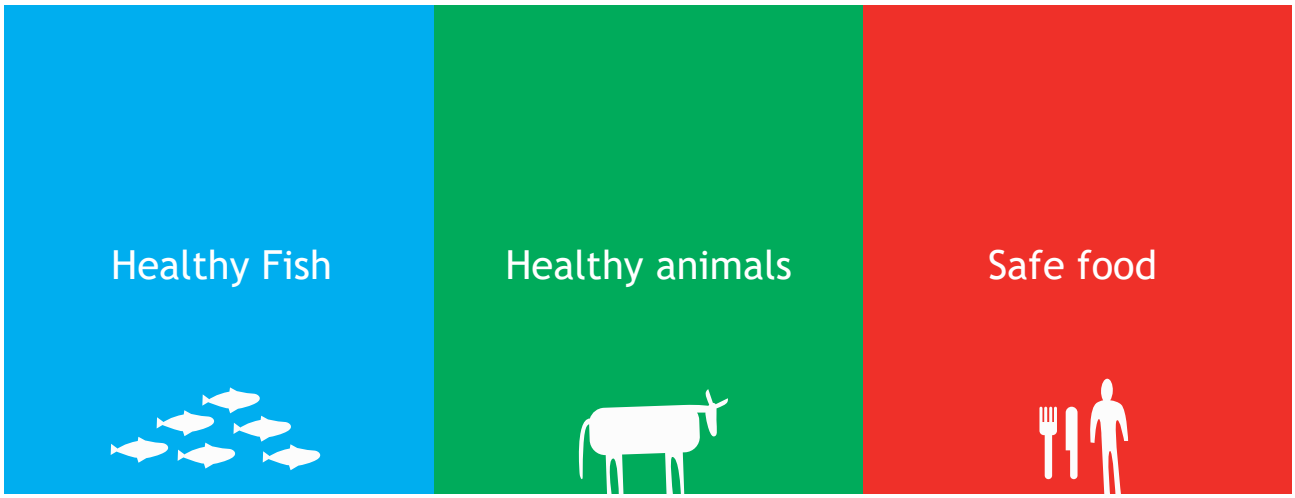
References

1. Hansen H, Amundsen MM, Mohammad SN, Strand DA: The surveillance programme to document absence of Atlantic salmon (*Salmo salar*) and *G. salaris* in the River Drammenselva upstream of Hellefossen in Norway 2021. In: *Surveillance program report*: Norwegian Veterinary Institute; 2022.
2. Hansen H, Mohammad SN, Strand DA: The surveillance programme to document absence of Atlantic salmon (*Salmo salar*) and *G. salaris* in the River Drammenselva upstream of Hellefossen in Norway 2020. In: *Surveillance program report*: Norwegian Veterinary Institute; 2021.
3. 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.
4. Anon: Handlingsplan mot lakseparasitten *Gyrodactylus salaris* for perioden 2014-2016. Miljødirektoratet. ; 2014: 1-114.
5. 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).
6. Bakke TA, Jansen PA, Kennedy CR. The host specificity of *Gyrodactylus salaris* Malmberg (Platyhelminthes, Monogenea): susceptibility of *Oncorhynchus mykiss* (Walbaum) under experimental conditions. Journal of Fish Biology. 1991;39 1:45-57.
7. Hytterød S, Adolfsen P, Aune S, Hansen H: *Gyrodactylus salaris* funnet på røye (*Salvelinus alpinus*) i Fustvatnet (Nordland); patogen for laks (*Salmo salar*)? In: *Veterinærinstituttets rapportserie*, vol. 11: Veterinærinstituttet (Norwegian Veterinary Institute) (in Norwegian with English summary); 2011.
8. Mo TA. Variations of opisthaptoral parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) in a fish farm, with comments on the spreading of the parasite in south-eastern Norway. Syst Parasitol. 1991;20:1-9.
9. 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.
10. Hytterød S, Hansen H, Mo TA: The surveillance programme to map the occurrence of *Gyrodactylus salaris* in the Drammenselva catchment in 2014 – 2015. In: *Surveillance program report*: Veterinærinstituttet/Norwegian Veterinary Institute; 2016: 6.
11. 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.
12. 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.
13. 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 Rapport*2018: 93.
14. 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.
15. 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;11 333:333; doi: 10.1186/s13071-018-2916-3.

16. 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.
17. 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.
18. Bass D, Christison KW, Stentiford GD, Cook LSJ, Hartikainen H. Environmental DNA/RNA for pathogen and parasite detection, surveillance, and ecology. Trends Parasitol. 2023; doi: 10.1016/j.pt.2022.12.010.
19. Mo TA, Hansen H, Hytterød S. Occurrence and seasonality of *Gyrodactylus salaris* and *G. salmonis* (Monogenea) on Arctic char (*Salvelinus alpinus* (L.)) in the Fustvatnet lake, Northern Norway. J Fish Dis. 2023;46 4:395-403; doi: 10.1111/jfd.13752.
20. Gjelland KØ, Eikland KA: Faktaark Randsfjorden. https://www.nina.no/%C3%98kosystemer/Elver-og-innsj%C3%B8er/Fisk-i-store-innsj%C3%B8er/Randsfjorden (2020).
21. 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.
22. 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.
23. 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.
24. Hernandez C, Bougas B, Perreault‐Payette A, Simard A, Côté G, Bernatchez L. 60 specific eDNA qPCR assays to detect invasive, threatened, and exploited freshwater vertebrates and invertebrates in Eastern Canada. Environmental DNA. 2020;2 3:373-86; doi: 10.1002/edn3.89.
25. Matejusová I, Gelnar M, McBeath AJA, Collins CM, Cunningham CO. Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei). Int J Parasitol. 2001;31 7:738-45; doi: 10.1016/s0020-7519(01)00176-x.
26. WOAH: OiE Manual of Diagnostic Tests for Aquatic Animals. Chapter 2.3.3. Infection with *Gyrodactylus salaris*. https://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/2.3.03_G_salaris.pdf (2021).
27. Bakke TA, Cable J, Harris PD. The biology of gyrodactylid monogeneans: The "Russian-Doll Killers". Adv Parasitol. 2007;64:161-460.
28. Jensen AJ: Geografisk variasjon og utviklingstrekk i norske laksebestander. In: *NINA fagrapport*. Trondheim: Norsk Institutt for Naturforskning; 2004: 79.



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