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Report 22 – 2025

A surveillance programme to document absence of Atlantic salmon (*Salmo salar*) and *G. salaris* in the River Drammenselva upstream of Hellefossen in Norway 2023 and 2024

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Content

Summary

In 2019, the Norwegian Food Safety Authority decided to close the fish ladder in Hellefossen in river Drammenselva. This was done to exclude the stretch of river upstream of the fish ladder in a future treatment to get rid of the parasite *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 conducted as a combination of environmental DNA (eDNA) monitoring and electrofishing. The current report concerns the results from the surveillance conducted in 2023 and 2024.

In 2023, no electrofishing was performed due to severe flooding of the river, but eDNA monitoring was conducted as planned. Two stations between Hellefossen and Embretsfoss (approx. 14 km upstream of Hellefossen) tested positive for Atlantic salmon eDNA, and even more unexpectedly, there were also positive results for Atlantic salmon DNA upstream of the absolute migration barrier of Embretsfoss. To follow up on these unexpected results, eDNA monitoring was extended for 2024 and samples were taken at three dates. All samples taken from stations upstream of Embretsfoss in 2024 were found negative for Atlantic salmon. As for the samples obtained from stations between Hellefossen and Embretsfoss, a few samples demonstrated inconclusive results for Atlantic salmon. Individual technical replicates appeared positive for Atlantic salmon, but either the amplifications were above the cut-off value for a positive analyses or the analysis did not meet the requirement for the number of positive replicates. As for 2021 and 2022, no eDNA from G. salaris was detected above Hellefossen in 2023 and 2024. In addition, no Atlantic salmon were caught by electrofishing in the stations upstream of Hellefossen in 2024.

For 2023 and 2024, the surveillance for G. *salaris* in large lakes draining into River Drammenselva focused on Lake Tyrifjorden and Lake Krøderen, respectively. The surveys were carried out as a combination of eDNA monitoring and parasitological examination of fins of gill-netted Arctic char (*Salvelinus alpinus*), for the presence of G. *salaris*. Both eDNA and fish samples showed no detection of G. *salaris* from Lake Tyrifjorden in 2023 and Lake Krøderen in 2024.

In summary, all results from eDNA monitoring for G. *salaris* upstream of Hellefossen have been negative since 2020, the year after the closure of the fish ladder. In addition, no Atlantic salmon have been caught upstream of Hellefossen in the same period. The combined results from the eDNA monitoring and electrofishing have since the start of the programme demonstrated that the closure of the fishing ladder in Hellefossen have had the desired effect. Based on environmental DNA monitoring alone, the results are inconclusive. Thus, based on this method alone, we cannot conclude that Atlantic salmon is absent upstream of Hellefossen.

Sammendrag

I 2019 bestemte Mattilsynet seg for å stenge fisketrappen i Hellefossen i Drammenselva. Dette ble gjort for å ekskludere elvestrekningen oppstrøms fisketrappen i en fremtidig behandling for å bli kvitt parasitten *Gyrodactylus salaris*. Forutsatt at Hellefossen fungerer som en absolutt barriere for fiskemigrasjon, vil området oppstrøms over tid bli fritt for atlantisk laks og G. *salaris*. For å dokumentere om stengingen av fisketrappen i Hellefossen har hatt ønsket effekt på å redusere bestanden av atlantisk laks og G. *salaris*, ga Mattilsynet oppdrag til Veterinærinstituttet om å gjennomføre overvåking i Drammenselva fra 2020. I 2022 ga Mattilsynet videre oppdrag til Veterinærinstituttet om å overvåke G. *salaris* i innsjøer som renner inn Drammenselva (oppstrøms den anadrome strekningen). Overvåkingsprogrammet i Drammenselva gjennomføres som en kombinasjon ved bruk av miljø-DNA (eDNA) og elektrofiske. Denne rapporten omhandler resultatene fra overvåkingen utført i 2023 og 2024.

I 2023 ble det ikke utført elektrofiske på grunn av kraftig flom i elva, men miljø-DNA overvåking ble gjennomført som planlagt. To stasjoner mellom Hellefossen og Embretsfoss (ca. 14 km oppstrøms Hellefossen) testet positivt for eDNA fra atlantisk laks, men i tillegg ble det testet positivt for miljø-DNA fra atlantisk laks oppstrøms den absolutte migrasjonsbarrieren ved Embretsfoss. For å følge opp disse uventede resultatene ble miljø-DNA overvåkingen utvidet for 2024, og det ble tatt prøver på tre ulike datoer. Alle prøver tatt fra stasjoner oppstrøms Embretsfoss i 2024 var negative for atlantisk laks, men i strekningen mellom Hellefoss og Embretsfoss viste noen få prøver inkonklusive resultater; individuelle tekniske replikater viste seg positive for atlantisk laks, men enten var amplifikasjonene over grenseverdien for en positiv analyse, eller analysen oppfylte ikke kravet til antall positive replikater. Som for 2021 og 2022 ble det ikke påvist miljø-DNA fra G. *salaris* over Hellefossen i 2023 og 2024. I tillegg ble ingen atlantisk laks fanget ved elektrofiske i stasjonene oppstrøms Hellefossen i 2024.

For 2023 og 2024 ble henholdsvis Tyrifjorden og Krøderen inkludert i overvåkingen av G. *salaris* da disse er store innsjøer som renner inn i Drammenselva. Undersøkelsene ble utført som en kombinasjon av miljø-DNA overvåking og parasitologisk undersøkelse av finner fra garnfanget røye for tilstedeværelse av G. *salaris*. Både miljø-DNA og fiskeprøver viste ingen påvisning av G. *salaris* fra Tyrifjorden i 2023 og Krøderen i 2024.

Oppsummert har alle resultater fra miljø-DNA overvåking for G. *salaris* oppstrøms Hellefossen vært negative siden 2020, året etter stengingen av fisketrappen. I tillegg har ingen atlantiske laks blitt fanget oppstrøms Hellefossen i samme periode. De kombinerte resultatene fra miljø-DNA overvåking og elektrofiske har siden programmets start vist at stengingen av fisketrappen i Hellefossen har hatt ønsket effekt. Basert på miljø-DNA overvåking alene er resultatene inkonklusive. Dermed kan vi ikke konkludere med at atlantisk laks er fraværende oppstrøms Hellefossen basert på denne metoden alene.

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 Figure 1 and 2). 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]. Rainbow trout and arctic char are the only hosts present in this area known to be susceptible to G. *salaris* [4, 5, 6]. To substantiate the likely absence of G. *salaris* from these areas, the Norwegian Veterinary Institute (NVI) has previously conducted several studies on behalf of the Norwegian Food Safety Authority (NFSA) in the period 2014-2018 [7, 8, 9, 10] 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 [11]. It was pointed out that the probability of succeeding with a chemical treatment would increase by closing the fish ladder in Hellefossen (see Figure 2). 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 Hellefossen 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 Hellefossen 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 [7, 8, 9, 10]. In addition, the NFSA expressed an interest in testing eDNA monitoring as a tool for surveillance. Therefore, the surveillance program in the lakes is counducted as a combination of eDNA monitoring and classical parasitological examination of fish for the presence of G. *salaris*. 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 12 and references therein].

The reports from the current surveillance program for the years from 2015 to 2022 can be found on the web site of the Norwegian Veterinary Institute: https://www.vetinst.no/overvaking/gyrodactylus-salaris-kartlegging-drammensregionen

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 in 2019.

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

The surveillance program is conducted as a combination of parasitological examination and environmental DNA (eDNA) monitoring. 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 [13]. 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* [14] and has previously been applied in field studies in the River Drammenselva and elsewhere [10, 14, 15, 16].

For 2023 and 2024, the surveillance program included, in addition to eDNA monitoring and electrofishing in River Drammenselva, the two large lakes, Lake Tyrifjorden and Lake Krøderen, both draining into River Drammenselva (see Figure 1).

In 2023, the surveillance program in the River Drammenselva was conducted exclusively using environmental DNA due to severe flooding, which prevented electrofishing. Environmental DNA samples were collected from six stations in the river on the November 7th, 2023 (Figure 2 and Table 1). Three of these stations (Linnerud, Skytterbanevika, and Storstein) were consistent with previous years, while three new station were added (Oppstrøms Døvikfoss, Skotselv, and Oppstrøms Hellefoss).

For 2024, the surveillance program was conducted as planned, combining eDNA monitoring and electrofishing. Environmental DNA samples were obtained on the April 24th, May 16th, and October 31th 2024, from several stations. On October 31th 2024, electrofishing was also performed at six stations in the river (the details can be seen in Table 1 and Figure 2). Five of these stations were identical to those where eDNA samples were collected in 2023, except for Strømbo which replaced Skotselv due to better electrofishing conditions (Figure 2 and Table 1). As in previous years, the eDNA sample at station Linnerud, was collected as a negative control sample (no expected presence of Atlantic salmon and G. *salaris*), while the sample at station Storstein below Hellefossen served as a positive control sample (expected presence of G. *salaris*).

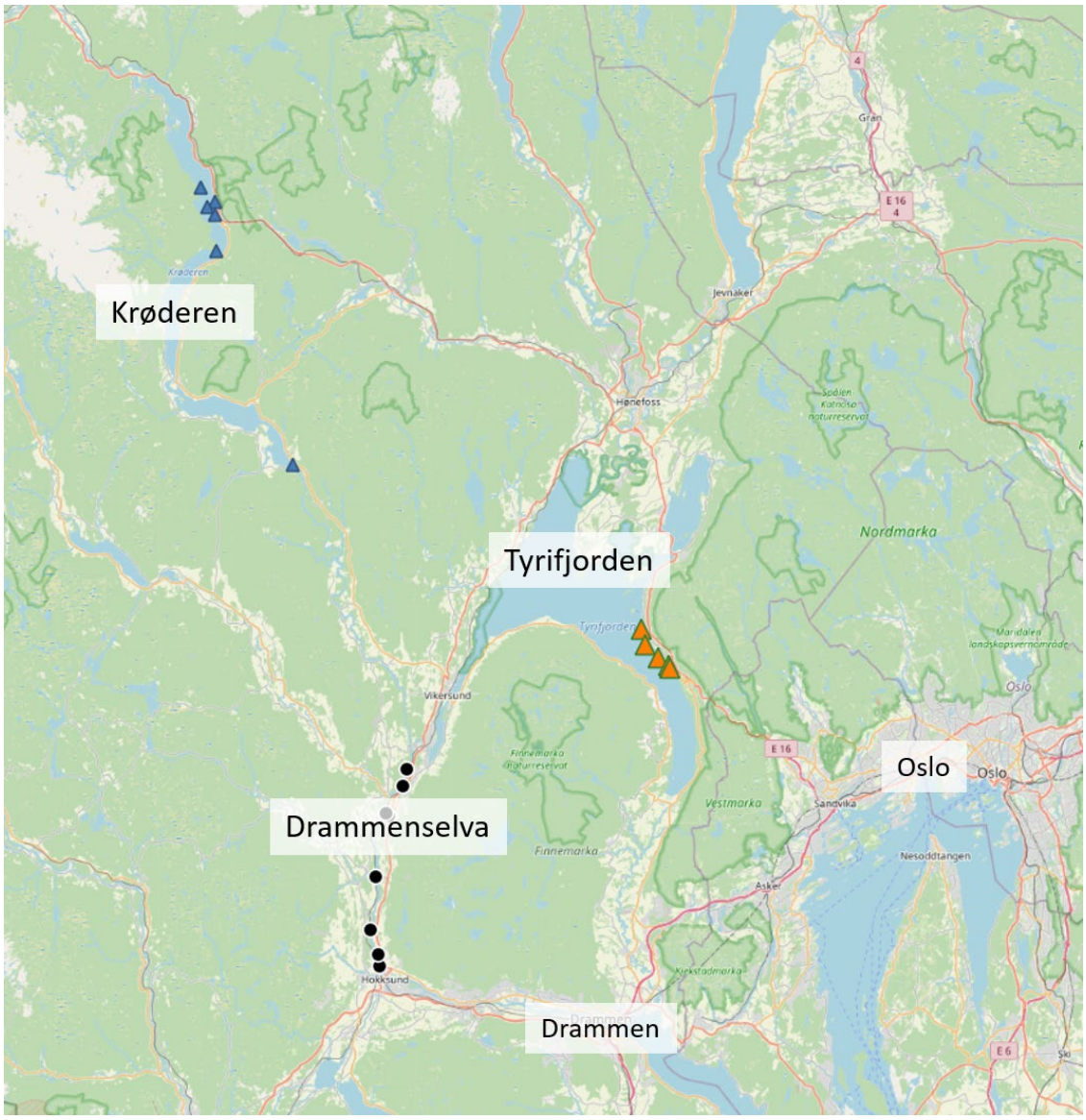

Figure 1. Map showing the location of River Drammenselva and the lakes Krøderen and Tyrifjorden with the sampling stations indicated. More details on the sampling stations can be found in figures 2, 3 and 4.

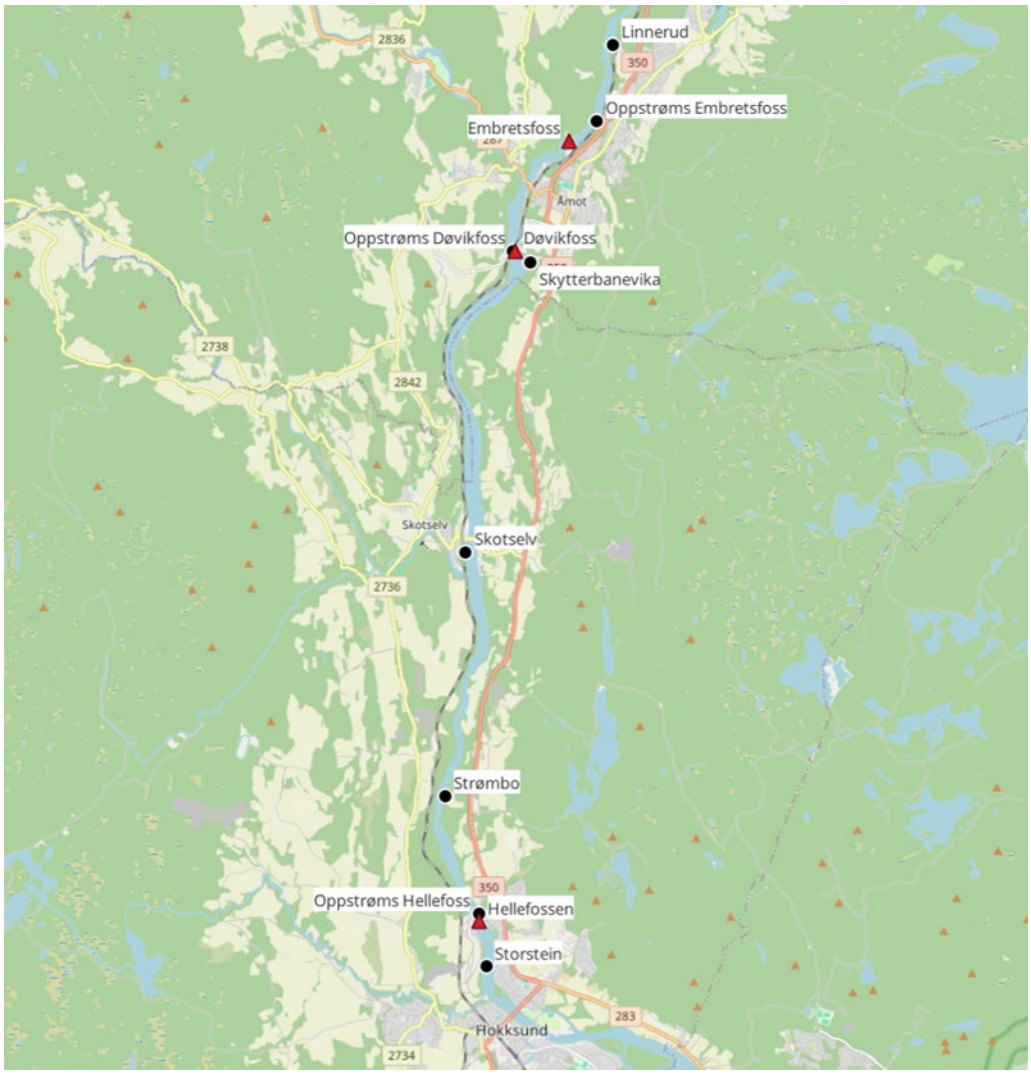

Figure 2. Sampling stations (black dots) for eDNA and electrofishing in the River Drammenselva in 2023 and 2024. The barriers for upstream migration of salmon, Hellefoss, Døvikfoss and Embretsfoss, are shown by red triangles. See also details in Table 1.

Lake Tyrifjorden was included in the surveillance program for 2023. Lake Tyrifjorden is a large lake covering an area of 122.8 km2 and with a maximum depth of 288 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*), Tench (*Tinca tinca*), bream (*Abramis brama*), 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*) [17].

In Lake Tyrifjorden, Arctic char were caught from five different stations from the eastern part of the lake using gillnets (see Figure 3) during autumn 2023. Sampling in the autumn is considered the best period for catching Arctic char in gillnets as these fish spawn in the autumn and thus gather at the spawning grounds, making it easier to catch sufficient numbers of fish. It is also likely that parasite transmission occurs during the spawning period and that the intensity of parasites is higher in this period, increasing the chances of detecting an infection [18]. Environmental DNA samples were collected on the 7th October 2023, close to the stations used for gillnetting, covering the entire fishing area (see Figure 3).

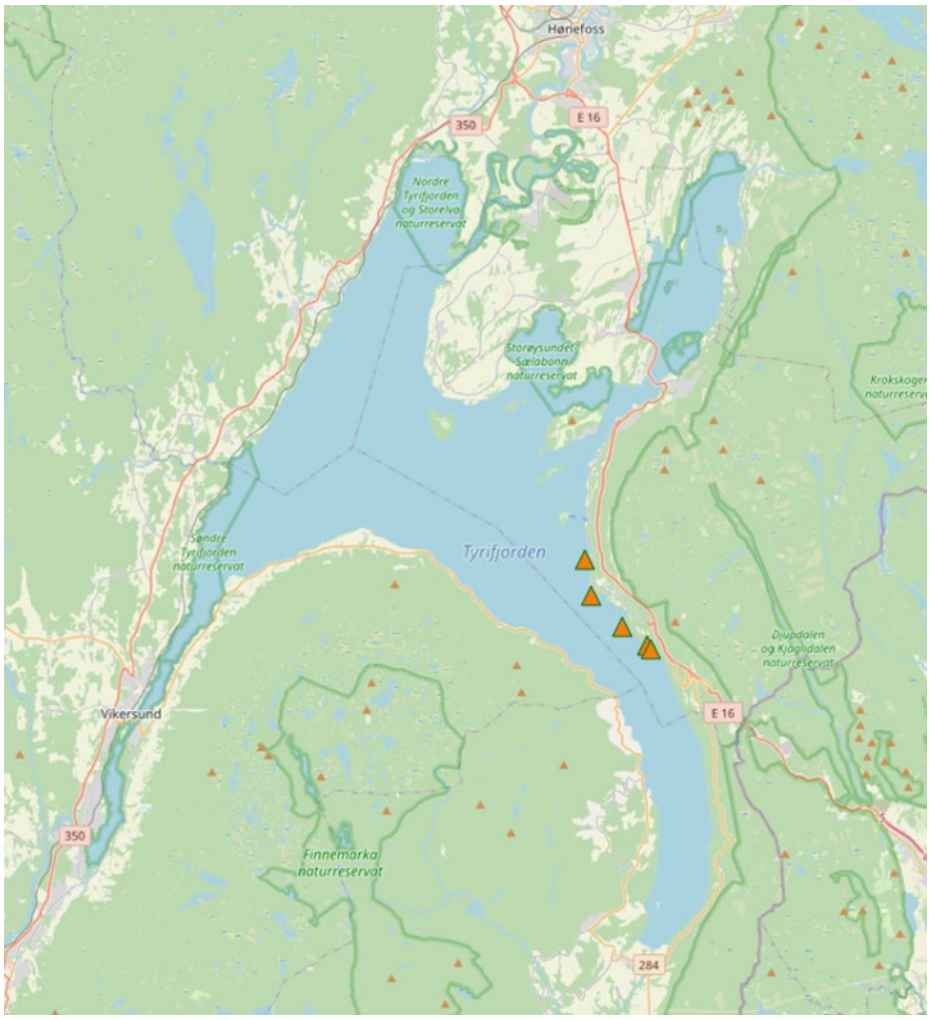

Figure 3. Map of Lake Tyrifjorden with the sampling stations used for gillnetting for Arctic char (*Salvelinus alpinus*) in 2023 shown as orange triangles.

In 2024, the lake Krøderen was included in the surveillance program. Lake Krøderen is a lake covering an area of 44 km2 and with a maximum depth of 130 m. In addition to Arctic char, the fish fauna in the lake consists of brown trout, whitefish (*Coregonus lavaretus*), minnows, crucian carp, pike, and perch [19].

In Lake Krøderen, Arctic char were caught in gill nets from a number of different stations in the lake during a period in autumn. Due to low catch (see results), a considerable effort (14 nights fishing) was put into gill-netting in this lake. Environmental DNA samples were collected on the 18th November 2024, close to five of the stations used for gillnetting. In addition, one extra station close to the water outlet was included (see Figure 4).

Figure 4. Map of Lake Krøderen showing sampling stations for eDNA monitoring (blue triangles) in 2024.

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. [16]. Sampling in the river was conducted from the river bank, while samples in the lakes were taken from a boat using a water sampler (Ruttner Water sampler, 5 L, KC Denmark). 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 4 ml ATL buffer. DNA was isolated in the laboratory using a Nucleospin Plant II midi kit and Qiagen buffer according to Fossøy et al. [15].

The eDNA samples from the River Drammenselva was analysed with qPCR assays designed to detect the following four targets; G. *salaris* [14], G. *derjavinoides* [20], Atlantic salmon [21], and brown trout (*Salmo trutta*) [22]. The assays for brown trout and G. *derjavinoides* were included as positive controls; i.e. brown trout is common in the river, 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 or most localities.

From the Lake Tyrifjorden and Lake Krøderen, the eDNA samples were analysed with qPCR assays designed to detect three targets; G. *salaris* [14], G. *derjavinoides* [20], and brown trout [22]. The assay for brown trout was included as a positive control as this fish species is found in both Lake Tyrifjorden and Lake Krøderen.

The qPCR analyses on the eDNA samples obtained on 7th November 2023, 24th April 2024 and 16th of May 2024 were run without technical replicates, i.e. only one PCR per sample (i.e. per filter). For these samples, the Cq cut-off value was set to 40, and all results below this value were considered positive. For the eDNA samples obtained on 31th October 2024 however, the qPCR analyses were run with three technical replicates (See table 1). The Cq cut-off value was set to 40, and each sample were considered positive if two out of the three replicates had a Cq value below 40.

Table 1. Overview of sampling stations for eDNA monitoring and electrofishing in the River Drammenselva for 2023 and 2024. eDNA = eDNA samples were taken; EL = electrofishing was conducted. Grey colouring of cells indicate the stations between Hellefossen and Embretsfoss (see text) and corresponds to the area marked in grey in figure 5 and 6. *qPCR run with three technical replicates.

Station	07.11.2023	24.04.2024	16.05.2024	31.10.2024
Linnerud	eDNA	eDNA		eDNA*/EL
Oppstrøms Embretsfoss		eDNA	eDNA	
Oppstrøms Døviksfoss	eDNA			eDNA*/EL
Skytterbanevika	eDNA			eDNA*/EL
Skotselv	eDNA			
Strømbo				eDNA*/EL
Oppstrøms Hellefoss	eDNA	eDNA	eDNA	eDNA*/EL
Storstein	eDNA		eDNA	eDNA*/EL

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 Tyrifjorden and Lake Krøderen, Arctic char was caught using gillnets (mesh size 35 to 45 mm) by a local fisherman in the autumn 2023 and 2024, respectively. 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.

Parasite species identification

DNA was extracted from a selection of 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 (i.e. which were not G. *salaris*), was subjected to PCR and DNA sequencing of the ribosomal internal transcribed spacer 2 (ITS2) following standard protocols [23, 24]. The ITS2 fragment is a common species barcode used to identify and differentiate between the different *Gyrodactylus* species, and used here to confirm the qPCR results.

Results and discussion

River Drammenselva

Electrofishing

In 2023, electrofishing was not conducted due to severe flooding. However, in 2024, a total of 25 Atlantic salmon were caught by electrofishing at station Storstein, located below Hellefossen. The sizes of the Atlantic salmon ranged from 45 to 125 mm. No Atlantic salmon were caught by electrofishing at the five stations upstream Hellefoss in 2024, and none have been caught in that stretch of river since 2020, the year following the closure of the fish ladder. Other fish species caught during electrofishing in the River Drammenselva were brown trout (*Salmo trutta*), minnows (*Phoxinus phoxinus*), ruffe (*Acerina cernua*) and pike (*Esox lucius*).

Parasitological examination

The prevalence of *Gyrodactylus* spp. infection in fish sampled downstream of Hellefossen (station Storstein) was 100% in 2024, consistent with previous years. The intensity of infection was generally high and varied from 100 to more than a thousand. As G. *salaris* has been confirmed present at this station several times [see e.g 25], no *Gyrodactylus* specimens were thus identified to species.

Environmental DNA monitoring in river Drammenselva

In 2023, a total of 18 eDNA samples were collected from six stations (Figure 2, three replicate samples per station) and analysed. The results are summarised in figures 5 and 6. As for the the previous years, *Gyrodactylus salaris* eDNA was only detected in the sample from below Hellefossen (station Storstein). In 2023, eDNA from Atlantic salmon was detected at Linnerud, the negative control site above Embretsfoss. As per today, there is not reasonable explanation for this detection. Drammenselva experienced a massive flooding in August due to extreme weather, but even with the increased water level during that flooding, Embretsfoss is still considered an absolute migration barrier for Atlantic salmon. We can only speculate as to why we detect DNA from Atlantic salmon in samples for a station that was supposed to be negative. All samples taken from this site until 2023 have all been negative. Whether the extreme weather resulted in overflow of the sewage system, carrying eDNA from Atlantic salmon originating from private households in to the river, or whether there are other explanations, is uncertain. However, there is no indication that the detection is a result of contamination in the laboratory, as all negative control samples were negative.

To further document the presence or absence of eDNA from Atlantic salmon upstream of Embretsfoss, extra samples were taken on the 24th April 2024 and 13th May 2024. The results from these samples were negative for Atlantic salmon for the stations upstream of Embretsfoss. However, in April 2024, amplification of Atlantic salmon were detected at station Oppstrøms Hellefoss with cq values over the set cut-off. Due to the high cq values, these results are not considered reliable evidence and are therefore considered negative. Atlantic salmon qPCR for water samples obtained in October 2024 were run in technical triplicate for each filter, where one of three technical replicate amplified for some of the samples (Station Oppstrøms Døviksfoss, Oppstrøms Hellefoss,

and Skytterbanevika) with Cq values under 40. These results were nevertheless considered negative since only one of three replicates was positive.

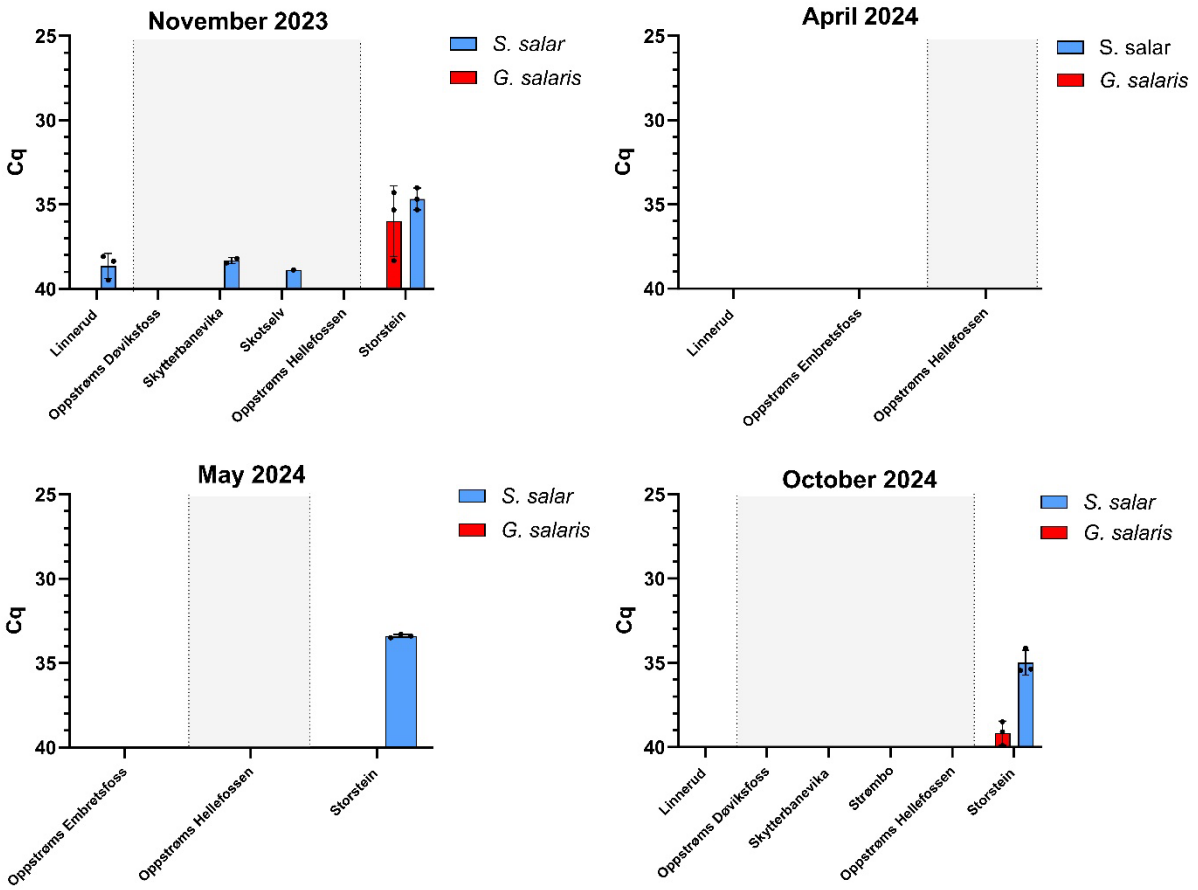

Figure 5. Bar plot showing the average Cq-values (±SD) for *Gyrodactylus salaris* (red) and Atlantic salmon, Salmo salar (blue), for each station included in the eDNA monitoring. The Cq-value reflects the level of target DNA in a sample, where lower Cq-value indicates higher DNA content. Each black dot represents a positive sample with correlating Cq-value. The area marked in grey represents the area between Embretsfossen and Hellefossen.

As for the analyses of the targets included as field positive controls, brown trout eDNA was detected at all stations in 2023 and 2024, while G. *derjavinoides* eDNA was only detected at few stations (See Figure 6).

The combined results from the eDNA survey and electrofishing indicate that the closure of the fishing ladder in Hellefoss has had the desired effect, as only a few Atlantic salmon were caught by electrofishing above Hellefoss in 2020 [26] and none in 2021 [25], 2022[27], and 2024 (this report). However, the environmental DNA analyses cannot rule out a continued presence of Atlantic salmon upstream of Hellefoss, however at a very low density. The closure of the migration barrier at Hellefoss 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 4+ in 2023 and 5+ in 2024. 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 positive samples from 2023 from the stations between Hellefossen and Embretsfoss culd theoretically be caused by the presence of 4+ smolts. However, this would not explain the positive result from station Linnerud. We therefore consider it most likely that the eDNA detected in samples upstream of Hellefossen originated from another source than live Atlantic salmon in the river.

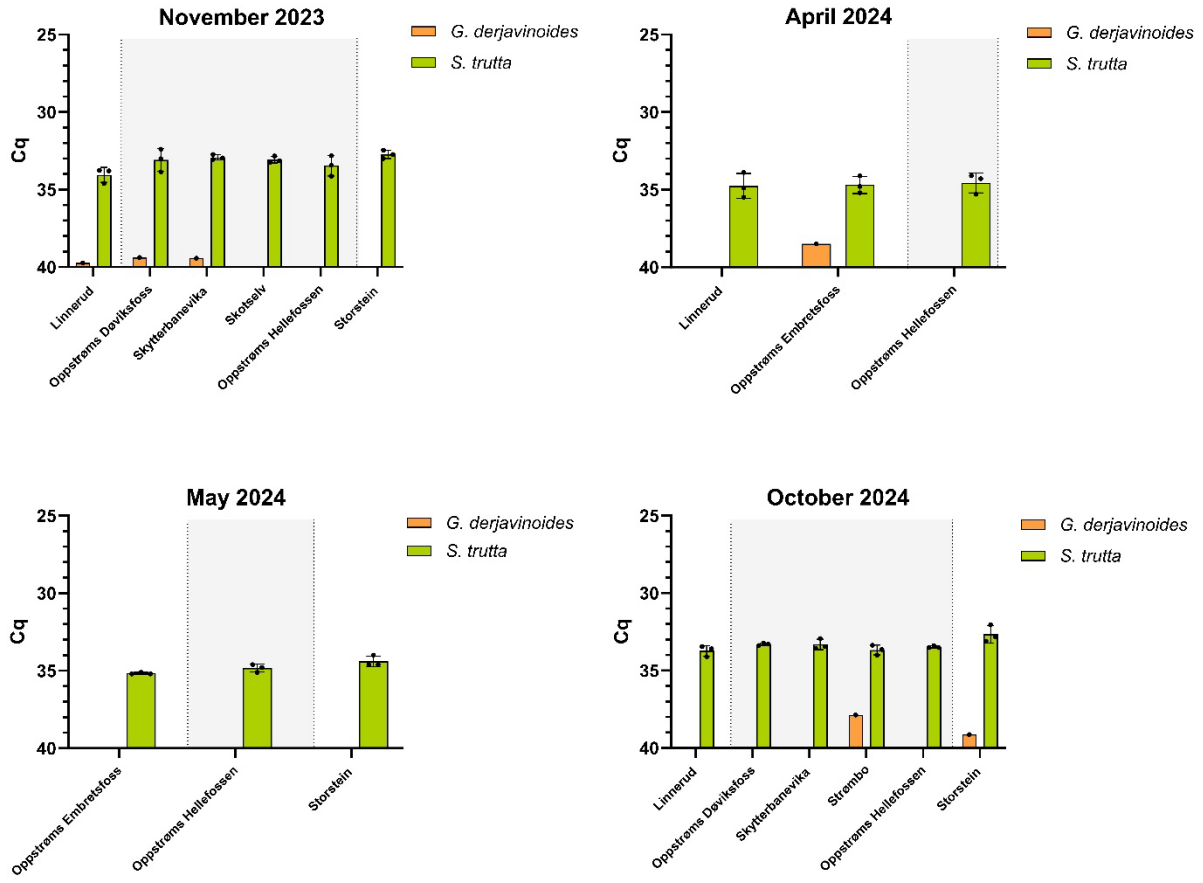

Figure 6. Bar plot showing the average Cq-values (±SD) for *Gyrodactylus derjavinoides* (orange) and brown trout, Salmo trutta (green), for each station included in the eDNA monitoring. The Cq-value reflects the level of target DNA in a sample, where lower Cq-value indicates higher DNA. Each black dot represents a positive sample with correlating Cq-value. The area marked in grey represents the area between Embretsfossen and Hellefossen.

Lake Tyrifjorden, 2023

Species identification of *Gyrodactylus* specimens in Lake Tyrifjorden

Altogether, 201 arctic char from five stations in Lake Tyrifjorden were collected and the fins were examined. The intensity of infection on individual fish varied from 0 to over 200 *Gyrodactylus* spp. per fin.

A total of 376 *Gyrodactylus* specimens were sampled from all infected caudal fins and dorsal fins, and analysed using the qPCR assay specific for G. *salaris*. All of these tests were negative for G. *salaris*. The subsample of 54 specimens that were subjected to PCR and DNA sequencing, also confirmed that *Gyrodactylus salaris* was not present amongst the analysed specimens.

Environmental DNA analyses in Lake Tyrifjorden

qPCR analysis of the water samples yielded no positive results for G. *salaris* in any of the stations in Lake Tyrifjorden. The assays for brown trout and G. *derjavinoides* were included as positive controls. Environmental DNA from G. *derjavinoides* were detected in filters from four of the stations (station 2-5), while all filters from all stations were positive for brown trout.

Lake Krøderen, 2024

Species identification of *Gyrodactylus* specimens in Lake Krøderen

Altogether, 14 arctic char were collected from the Lake Krøderen, and the fins were examined. The sample size was smaller than expected despite a large fishing effort. However, no *Gyrodactylus* specimens were detected.

Environmental DNA analyses in Lake Krøderen

qPCR analysis of the water samples yielded no positive results for G. *salaris* in any of the stations in Lake Krøderen. The assays for brown trout and G. *derjavinoides* were included as positive controls. Environmental DNA from G. *derjavinoides* were detected in filters from two of the stations (station 2 and 4), while environmental DNA from five out of six stations were positive for brown trout. Apart from acting as a positive control in the analyses, the results obtained for the detection of G. *derjavinoides* also demonstrates that eDNA can be used to detect DNA from minute organisms, such as parasites, in large lakes.

Conclusion

All results from eDNA monitoring for G. *salaris* upstream of Hellefossen have been negative since 2020, the year after the closure of the fish ladder. In addition, no Atlantic salmon have been caught upstream of Hellefossen in the same period. The combined results from the eDNA monitoring and electrofishing have since the start of the programme demonstrated that the closure of the fishing ladder in Hellefossen have had the desired effect. Based on environmental DNA monitoring alone, the results are inconclusive. Thus, based on this method alone, we cannot conclude that Atlantic salmon is absent upstream of Hellefossen.

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