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Abstract .

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During the last decade, water molds, especially *Saprolegnia parasitica*, have been associated with salmon deaths in rivers around the Northern Baltic Sea. At present, knowledge of the distribution of *S. parasitica* and its abundance in these rivers and in different salmonid species is limited. In this project, we developed a sampling and detection method to monitor the presence of *S. parasitica* in river water using environmental DNA (eDNA) analysis. Salmonid fish and water samples were collected along the River Tornionjoki from summer to late fall in 2022–2023 and, as a reference to validate the eDNA methods, from the River Oulujoki in the year 2022. For the eDNA analysis of *S. parasitica* from water samples, we used two different detection methods simultaneously, i.e. real-time quantitative PCR (qPCR) assay and digital PCR (dPCR) assay.

We found that the dPCR based detection is more sensitive than the qPCR, and thus more applicable for *S. parasitica* detection from natural river waters. During the study years 2022–2023, *S. parasitica* DNA was detected in water samples from the River Tornionjoki, although we had no evidence on *S. parasitica* infection in salmonids. However, we detected other aquatic fungi than *S. parasitica* that were responsible for skin lesions in some of the studied salmonids. On the contrary, both salmon and brown trout with a *S. parasitica* infection were confirmed from the nearby River Oulujoki, where also the variation in *S. parasitica* DNA concentrations in water samples aligned well with the observed numbers of symptomatic fish. Based on current results, the eDNA is a promising method for monitoring the presence and concentrations of *S. parasitica* in river environments. However, further research is still needed to apply eDNA results for predicting the health consequences for wild salmonid populations.

Keywords: Atlantic salmon, Brown trout, water mold, *Saprolegnia parasitica*, environmental DNA, River Tornionjoki, River Oulujoki

Tiivistelmä

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Viimeisen vuosikymmenen aikana lohikalojen voimakkaat vesihometartunnat ovat lisääntyneet pohjoisen Itämeren alueella. Vesihometauti on ollut näkyvästi esillä Tornionjoen lohikuolemien yhteydessä, mutta sitä esiintyy myös muihin pohjoisen Itämeren jokiin pyrkivissä lohikaloissa. Useita eri vesihomelajeja esiintyy luontaisesti makeassa vedessä, mutta vesihometauti kehittyy tavallisimmin *Saprolegnia parasitica* -vesihomelajin tarttuessa kalan vaurioituneeseen ihoon. Tällä hetkellä tiedot *S. parasitica* -vesihomeen esiintymisestä ja sen yleisyydestä jokivesissä sekä eri lohikaloissa ovat rajalliset, eikä muita luonnonlohissa esiintyviä vesihomelajeja ole juurikaan aikaisemmin selvitetty.

Tässä kaksivuotisessa tutkimushankkeessa kehitimme ja testasimme ympäristö-DNA:han (eDNA) perustuvia näytteenotto- ja tunnistusmenetelmiä *S. parasitica* -vesihomeen esiintymisen seurantaan jokivedessä. eDNA-menetelmä perustuu virran mukana kulkeutuvien vesihomeitiöiden ja niiden DNA:n havaitsemiseen vesinäytteistä. Tutkimusta varten keräsimme vesinäytteitä noin kahden viikon välein alkukesästä myöhäissyksyyn Tornionjoesta vuosina 2022–2023 ja lisäksi Oulujoesta vuonna 2022. *S. parasitica* -vesihomeen havaitsemiseksi vesinäytteitä analysoitiin kahden eri PCR-teknologian avulla, eli kvantitatiivisella PCR:llä (qPCR) ja digitaalisella PCR:llä (dPCR). Lisäksi eri vesihomeiden esiintymistä lohikaloilla seurattiin Tornionjoella järjestettävien kalastuskilpailujen yhteydessä sekä Oulujoella käyttäen Merikosken kalatien videotallenteita.

Tulostemme perusteella dPCR-pohjainen menetelmä on herkempi tunnistamaan *S. parasitica* -vesihomeen DNA:n vesinäytteistä kuin qPCR, ja siten soveltuvampi vesihomeen havaitsemiseen luonnonvesistä. *S. parasitica* -vesihomeen ei havaittu aiheuttavan vakavia tautiongelmia Tornionjokeen vaeltaville lohille kumpanakaan seurantavuonna. Vuosina 2022–2023, jolloin lohien määrät Tornionjoessa olivat keskimääräistä alhaisempia, emme havainneet lainkaan *S. parasitica* -tartuntoja lohikaloissa. eDNA-tulokset kuitenkin osoittivat, että taudinaiheuttajaa esiintyi kohtalaisesti jokivedessä. Vuonna 2023 Tornionjoen 20 % tutkituilla lohilla ja harjuksilla esiintyi kuitenkin vesihomemaisia ihomuutoksia, mutta niiden aiheuttajaksi tunnistettiin muita vedessä eläviä home- ja sienilajeja. Vuonna 2022 Oulujoella *S. parasitica* -vesihomeen DNA:ta havaittiin vesinäytteissä jokaisella näytteenottokerralla vaihtelevia määriä. Lisäksi Oulujoella sairastuneista lohesta ja taimenesta todennettiin *S. parasitica* vesihome. Vesinäytteiden DNA-pitoisuuksien perusteella *S. parasitica* -vesihomeen runsaus mukaili jokeen nousseiden vesihomeoireisten lohien ja taimenten määrää.

Tutkimuksemme perusteella eDNA on lupaava menetelmä tautia aiheuttavan *S. parasitica* -vesihomelajin esiintymisen seurantaan jokialueilla. Vesihometauti syntyy kuitenkin usean kaloihin ja niiden elinympäristöön liittyvän tekijän yhteisvaikutuksesta, joten taudin torjunta on hankalaa. Tämän tutkimuksen perusteella ei vielä voida sanoa, mikä merkitys luonnonvesissä löydettävillä *S. parasitica* DNA-määrillä on lohikalojen terveyteen, vaan ennustusmallien

laatimiseksi tarvittaisiin lisätutkimusta sekä useampivuotista seurantaa. Taudin ennaltaehkäisyyn voidaan luonnonkalojen kohdalla vaikuttaa muun muassa välttämällä kaloja vaurioittavia kalastustapoja.

Avainsanat: vesihome, *Saprolegnia parasitica*, lohi, taimen, ympäristö-DNA, Tornionjoki, Oulujoki

Contents

1. Introduction

Severe water mold infections have been reported in connection with the mass deaths in wild salmonid populations in recent years (Rocchi et al. 2017). Water mold is a generic term for fungal-like microorganisms, typically belonging to the class Oomycetes. Certain naturally occurring species of oomycetes, particularly those from the *Saprolegnia* genus, can be pathogenic to salmonids (Elameen et al. 2021). *Saprolegnia* infections, commonly referred to as saprolegniosis, are caused by pathogenic species of this genus and are known to result in significant fish mortality. Saprolegniosis is a particularly serious problem for aquaculture (reviewed by Lindholm-Lehto & Pylkkö 2024), but it has also raised concerns about the viability of endangered wild salmon stocks. So far, investigation of the water molds infecting wild salmonids is limited (but see Neitzel et al. 2004, Tedesco et al. 2021, Engblom et al. 2022).

The *Saprolegnia parasitica* is the most prevalently isolated oomycete from diseased salmonids in aquaculture environments (Tedesco et al. 2021, Korkea-aho et al. 2022), and it occurs naturally across habitats inhabited by freshwater stages of various salmonid species. The fish typically get infected through their fins, gills or damaged skin. The migratory salmonids that are damaged or weakened by fishing, predators, migration, and reproductive stress are particularly vulnerable to get infected with *S. parasitica* when they return to freshwater to spawn. Once attached on a host fish, hyphal growth of *S. parasitica* can be rapid, and it can produce substantial amounts of spores in the surrounding water, which can efficiently disperse with flow and colonize new hosts (Wood & Willoughby 1986, Van der Berg et al. 2013). Information on the occurrence and distribution of *S. parasitica* in natural river environments are scarce (but see Rocchi et al. 2017, Pavić et al. 2022).

The use of environmental DNA (eDNA) has become more common in monitoring aquatic ecosystems by detecting DNA shed by *e.g.* different fish species (Goldberg et al. 2016, Yates et al. 2021). Yet rarely have they been applied for fish pathogen monitoring in their natural environments (but see *e.g.* Rusch et al. 2018). Environmental DNA techniques have been employed to monitor the presence of *Saprolegnia parasitica* in both fish farms and natural aquatic environments (Rocchi et al. 2017, Korkea-aho et al. 2022, Pavic et al. 2022). However, the detection of *S. parasitica* load from water samples is affected by the volumes of the spores in space and time, while the conditions for spore production and dispersion differ greatly between fish farms and natural waters. In the river environment, higher water flow and lower densities of host fish are likely to reduce the *S. parasitica* load in the water. Therefore, the eDNA sampling and analytical protocols should be developed further to increase the accuracy of *S. parasitica* detections under natural conditions.

This study was carried out in two Northern Baltic rivers to estimate the prevalence of water mold infections in wild salmonids, and to investigate the applicability of eDNA methods for monitoring the *S. parasitica* load in these rivers. The River Tornionjoki – the border river between Finland and Sweden – is the largest river around the Baltic Sea where Atlantic salmon (*Salmo salar*) can migrate freely for hundreds of kilometers. Since 2014, occasional deaths of salmon with water mold infections have been reported along the river (Axen & Koski 2017), and the emergence of fish deaths has given rise to concern about the health of the stock. It is not yet known what proportion of the salmon mortality is due to *S. parasitica,* or other water

molds, as the health monitoring has previously focused mainly on investigating other pathogens and causes for the poor condition of salmon than water mold as such (Axen & Koski 2017). We also studied the nearby River Oulujoki as a Saprolegnia positive reference river. The River Oulujoki is a heavily regulated river by seven consecutive hydropower plants. In addition to the negative effects of damming on natural river dynamics, the Atlantic salmon and sea trout (*Salmo trutta*) populations are sustained through stocking efforts.

In both study rivers, salmon with severe water mold infections have been previously observed mainly from late summer to late fall. Here, we aimed to assess the presence and concentration of *S. parasitica* DNA in river water before, during and after the expected occurrence of salmon with saprolegniosis. We also inspected salmon caught in fishing competitions along the River Tornionjoki during the summer months and took skin samples from potential water mold infections. In the River Oulujoki, we used video material from a fish ladder located at the lowest dam to assess the prevalence of symptomatic salmon and trout entering the river.

The methodological testing for eDNA was conducted in two phases. In 2022, we collected water samples from both rivers every two weeks between July and November and tested the applicability of eDNA analytical methods previously optimized for fish farms (Korkea-aho et al. 2022) to detect *S. parasitica* DNA in the river environment. After initial testing and adjusting the eDNA methods, the water sampling and analyses were repeated in 2023 in two locations along the River Tornionjoki (Figure 1).

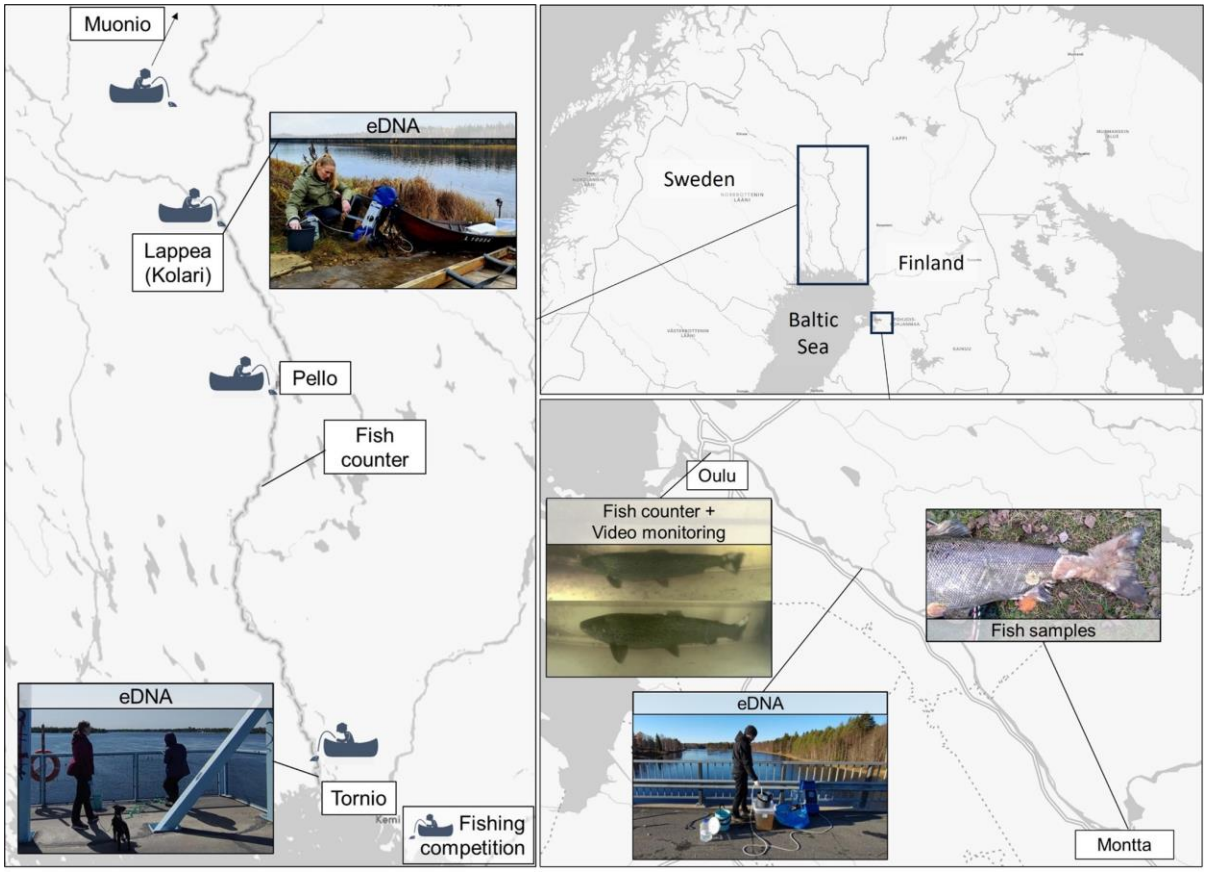

Figure 1. Environmental DNA sampling sites and the locations where the fish health assessments were made along both the River Tornionjoki (left) and the reference River Oulujoki (bottom right).

2. Materials and methods

2.1. Study rivers

The locations for fish and eDNA sampling along the River Tornionjoki in 2022–2023 and the River Oulujoki in 2022 are illustrated in Figure 1.

2.1.1. River Tornionjoki

In the first year of the study in 2022, eDNA sampling was performed in Lappea village (in Kolari), locating in the mid-parts of the River Tornionjoki (ca. 170 km from the river mouth), where the River Tornionjoki combines with the River Muonionjoki. The eDNA sampling site is about 1 km below the merge of these rivers, where the river is ca. 300 meters wide. A boat was used to collect a total of 50 liters of surface water (10 × 5 liters; 5 l every 30 meters along the river cross section) for eDNA filtering (see 2.2. for detailed protocol). This site was chosen because several infected salmon were observed in the area during the years preceding this study (Juha Pieskä, pers. comm.). A year before this study (in 2021), we conducted a survey to evaluate the salmon condition and water mold presence in salmon in Lappea area. These findings are reported in Appendix 1.

In 2023, eDNA water samples were taken from two locations along the River Tornionjoki. The first sampling site was the same location in Lappea as in year 2022. The pooled water sample (45 L) for eDNA filtering comprised of 15 liters of surface water taken from ca. 50, 150 and 250 meters from the shore using a boat. The second sampling site was chosen from the river estuary. The site was in the City of Tornio, located at the mouth of the river. The water samples were collected from a pedestrian bridge crossing the river (ca. 310 meters), and 15 liters of surface water for was taken again from 50, 150 and 250 meters from the shore. eDNA sampling was conducted at the same time both in Lappea and Tornio for the first three times (June, July, and August, see Table 2). From late August until late October, eDNA sampling was conducted only in Tornio every second week (six times in total).

To monitor prevalence of water molds in the salmonids in 2022–2023, we inspected the fish at major fishing competitions organized along the River Tornionjoki (in Muonio, Lappea/ Kolari, Pello and Tornio, see Figure 1, Appendix 3). The fish caught during the competitions were measured for total length and weight, sex determined, and inspected for skin lesions. The observed lesions and their sites on fish epithelia were recorded. When water mold was suspected, the skin was incised, and the skin pieces were cultivated on agar and preserved in RNAlater™ Stabilization Solution (Invitrogen™) for PCR analyses. In laboratory, the sampled water molds were identified to species or genus level with morphological, PCR and sequencing analyses (see below).

Local people and fishermen along the river were contacted and informed also through social media to report their observations of infected salmon. Unfortunately, we did not receive any samples of severely infected fish during the study years. In 2023, however, the ice cover on the River Tornionjoki formed early, during the period when deceased fish are typically observed (from October to November, see Appendix 1).

2.1.2. River Oulujoki

We used the River Oulujoki as a reference river where an increasing number of salmon with water mold infections have been observed during recent years (Louhi et al. 2024). In addition, sea trout infected with water mold have been observed in the river. The River Oulujoki is a heavily regulated river. The fish ascending to the river can pass the Merikoski hydropower plant, located at the mouth of the river, through fish ladders. Once a fish passes the ladder, it can only reach as far as the Montta hydropower plant in Muhos, about 40 km upstream from the river mouth. In connection with the Montta hydropower plant, there is a fish trapping device for capturing mature salmon to be transferred to the spawning grounds above the dam(s).

The eDNA water samples from the River Oulujoki were collected in Madekoski area within the City of Oulu (ca. 12 km from the river mouth, Figure 1). The eDNA samples were taken from the River Oulujoki within a few days of the samples from the River Tornionjoki (Lappea) in 2022. We repeated the eDNA sampling in the River Oulujoki for a total of 9 times between early July and early November (Table 2). The water sampling followed the same protocol (i.e., 10 × 5 liters of surface water were collected for eDNA filtering, see 2.2.). The water was lifted from a bridge crossing the river (with an interval of ca. 10 meters along the 120-meter bridge).

In the River Oulujoki, a video monitoring system and a fish counter have been set up (by Oulun Energia Oy and Simsonar Oy) at the end of Merikoski fish ladder. We used the video recordings to estimate the prevalence of fish having skin lesions, likely caused by water mold (Figure 2).

Figure 2. Snapshots of infected salmon and trout ascending in the River Oulujoki, taken from the video recordings from the Merikoski fish ladder.

The samples of infected fish from the River Oulujoki were obtained from Montta. A health inspection is regularly made from salmon and trout captured below the Montta dam before they are transferred to their spawning sites above the dam (see Uusitalo et al. 2022). One Atlantic salmon and one sea trout with visible lesions on their skin were sampled on September 30th, 2022, and water mold species was identified in species level with PCR analyses.

2.2. eDNA sampling protocol

At each sampling occasion (location and time; Figure 1 and Table 2), the same eDNA sampling protocol was used. Surface water was collected from an intersect of the river at each sampling site and pooled resulting in 45–50 liters of water. We measured water temperature from the pooled water contents. Then, three replicate samples were filtered using an eDNA sampler (Smith-Root, US) and self-preserving 5 µm PES filters (Smith-Root, US). Water filtering was done by automatically pumping until a maximum of 3-liter water volume per replicate sample was achieved. A negative control water sample was filtered using 2 liters of sterile milli-Q-water or bottled spring water (from the store) per each sampling occasion (referred to as field control from now on).

The amount of filtered water per sample varied between the sampling occasions. From the mid-parts of the River Tornionjoki (Lappea), full 3.0 liters for each replicate water sample could be filtered every time in 2022–2023. In the estuary (Tornio), 3.0 liters were filtered every time between June and August 2023. In September and October, a mean of 1.6–2.5 liters was successfully filtered. From the River Oulujoki, the average water volume filtered was only 2.1 liters (ranging from 1.1 liters on August 24 to 3.0 liters on November 4).

2.3. Laboratory analyses

2.3.1. DNA extraction

From the water samples (*i.e.*, PES filters) and fish tissue samples collected in 2022, DNA was extracted with Qiagen DNeasy® Blood & Tissue Kit (Qiagen, USA) according to the Animal Tissue protocol with minor modifications in Luke's eDNA laboratory (Figure 3). From the 2023 eDNA samples, DNA extraction followed in main parts the same protocol as for the year 2022 samples. However, minor modifications were made with an aim to improve the release of DNA from the *S. parasitica* spores by using a mechanical (MP Bio FastPrep-24™) homogenizer and a thermal lysis method. In each extraction series, a negative extraction control was included that only had the reagents but no DNA sample. DNA from fish tissue samples preserved in RNAlater was extracted using the year 2023 protocol.

Figure 3. eDNA laboratory dedicated for reagent pipetting in Luke, Jokioinen. As eDNA is easily contaminated, eDNA laboratories (DNA-free) are isolated from other laboratories and contaminations are prevented by wearing fully covering overalls. UV light, ethanol and chlorine are used to sterilize rooms and PCR cabins.

2.3.2. qPCR and dPCR amplification

The extracted eDNA was analyzed with both real-time quantitative PCR method (qPCR) (Rocchi et al. 2017, Korkea-aho et al. 2022) and with digital PCR (dPCR). Positive control strain *S. parasitica* VH28 and negative control strain of an oomycete *Aphanomyces astaci* (*i.e.*, crayfish plague) with an extraction negative control sample and NTC (*i.e.*, No Template Control) were included with samples as positive and negative control for each PCR run. An internal amplification control (IAC) was added to check for qPCR inhibition.

The qPCR was performed with BIO-RAD CFX96™ Real-Time System C1000 Touch Thermal Cycler and analyzed with Bio-Rad CFX Maestro Software Version 2.3. For the eDNA samples and fish tissue samples collected in year 2022, qPCR was performed according to Korkea-aho et al. (2021) method. Because of high PCR inhibition in the eDNA water samples in qPCR, eDNA samples from the year 2023 were run using TaqMan™ Environmental Master Mix 2.0 (Applied Biosystems) according to manufacturer's PCR program:
10 min 95 °C, 40x [15 s 95 °C + 1 min 60 °C].

The dPCR analyses were done using QIAcuity Digital PCR System (Qiagen, Germany). The same method was used for both 2022 and 2023 eDNA samples, as well as the same validated primer pair as for qPCR. The PCR was conducted in a 40µl volume containing 10µl of 4x concentrated QIAcuity Probe Mastermix, 1.1µl of each primer (10 µM), 0.5 µl of probe

(10 µM) and 10.9 µl of eDNA. The reaction was filled up to 40 µl with PCR clean water. The samples were analyzed in QIAcuity Nanoplate 26k 24-well plates where each reaction is divided into approximately 26,000 partitions. To obtain more accurate concentrations of the target DNA, each sample (*i.e.*, the extracted DNA from each PES filter) was analyzed in duplicate (*i.e.*, technical replicates). Each dPCR run included also separate positive control, and NTC samples No IAC was used for dPCR. The PCR conditions for dPCR are shown in Table 1.

Table 1. The PCR conditions for digital PCR (dPCR).

Number of repetitions	Temperature (°C)	Duration
1	95	2 min
40	95	15 s
	60	30s

2.3.3. Fish skin samples

A tissue sample from skin lesions suspected to have a water mold infection were preserved in RNAlater™ Stabilization Solution (Invitrogen™), and later cultivated on PG-1 agar supplemented with antibiotics (ampicillin and oxolinic acid 10 µg/mLT, Dieguez-Uribeondo et al. 1996). PG-1 agar was incubated at +15 °C for 3–7 days and re-cultivated until pure culture was obtained.

The PG-1 cultivation and induced sporulation of cultivated mycelium were performed for morphological identification of the isolated strains (Dieguez-Uribeondo et al. 1996). From each cultivated mycelium and tissue sample preserved in RNAlater, DNA was extracted (see 2.3.2) and their identification tested for *S. parasitica* with qPCR (2.3.2.). If the isolated mycelium was not identified as *S. parastica*, its DNA was sanger sequenced from ITS region, and the genus or species of mycelium was confirmed by comparing the ITS sequence to GeneBank sequence data (NCBI) (Engblom et al. 2022).

2.4. eDNA data analyses

To first obtain a general overview of presence/absence of *S. parasitica* DNA among all replicated water samples and field control samples, we produced a heatmap of qPCR and dPCR results for all sampling occasions (*i.e.*, time and location). The sampling occasion was considered negative for *S. parasitica*, if all three water samples and their two technical replicates were negative for *S. parasitica* DNA. Depending on the number of positive technical replicates, we considered the sampling occasion to be from weakly to strongly positive. For example, if only one or a few of the replicates were positive, the sampling occasion was considered weakly positive.

To compare variation in *S. parasitica* load in the river water between years and locations, we used the average gain of DNA (copies/µl), obtained from dPCR, for each sampling occasion. As each replicate water sample was analyzed in duplicate, we first calculated the average of DNA copies for each water sample. There were also substantial differences in the filtered water volume per water sample (see 2.2.). Therefore, we adjusted the DNA gain (number of

copies/µl) to the filtered water volume (liters) for each replicate sample, and the obtained value will be referred to as DNA concentration from now on. To better compare the eDNA results between rivers and across years with varying flow conditions, we also calculated the flow-corrected *S. parasitica* DNA rate following Levi et al. (2019). This was done by multiplying the DNA concentration from dPCR (DNA copies/µl, relative to filtered water volume) for each sampling occasion by the river flow (m^3/s) on that day.

For the River Tornionjoki, we considered the daily count of salmon and sea trout migrating upstream as an estimate of temporal variation in abundance of potential hosts. To assess the numbers of salmon(ids) returning to the River Tornionjoki to spawn, we used the day-specific data that the Natural Resources Institute Finland (Luke) gathers in their annual monitoring of the salmon population in Pello (Figure 1) (Isometsä ym. 2021). We used environmental monitoring data that is available from Hertta database maintained by Finnish Environment Institute (SYKE). Water temperature was obtained from Tornio (Kukkolankoski). Water flow data was available from Pello, near to the location of the fish counter (see Figure 1). We acknowledge that these data points do not match with the exact conditions as present at the eDNA sampling locations, but they do offer robust patterns for temporal variations in environmental factors for the River Tornionjoki.

For the River Oulujoki, the fish numbers were obtained from the fish counter located in Merikoski fish ladder (Figure 1). Daily data of the water flow and temperature were obtained from Merikoski and Montta hydropower plants. In addition, the video monitoring system at the end of fish ladder allowed to evaluate the prevalence of symptomatic individuals in contrast to the eDNA results in the River Oulujoki. The prevalence of infected salmonids was evaluated against the *S. parasitica* DNA concentration using a two-week running count of symptomatic fish. These fish likely congregated in the reservoir between the first two dams but were also expected to die within a few weeks (Misk et al. 2022).

Due to the nature of the of our data, accurate statistical analyses of environmental effects on *S. parasitica* concentrations could not be performed. Anyhow, we used SPSS 29.0 for simple correlation tests between *S. parasitica* DNA measures and day-specific water temperature, flow, or numbers of migrating or infected fish. The results are primarily descriptive, and the high number of positive field controls makes the findings from those dates indicative.

3. Results

3.1. Identification of water mold species in sampled fish

The condition of salmon caught and inspected along the River Tornionjoki during the eDNA sampling years are described in Appendices 2 and 3.

In 2022, no visibly infected salmon with water mold were caught in the fishing competitions along the River Tornionjoki (n = 57, Appendix 2). The same year, one salmon and one sea trout were caught from the River Oulujoki in the fall and sampled for water mold diagnostics. These fish were confirmed to have *Saprolegnia parasitica* infection (Figure 4).

In 2023, altogether 25 fish were inspected from several places along the River Tornionjoki in July and August (Appendix 3). 20 percent of all studied fish showed very minor changes in their skin. The isolated water molds differed in their morphology when grown on agar, and based on their colony morphology, two of them were fungal-like rather than water molds (oomycetes). None of the isolated water molds were identified as *S. parasitica* with qPCR from the skin nor from isolated hyphan. The suspected water molds were identified by ITS sequence as *Botryitinia* sp., *Didymella* sp., *Mucor* sp., *Pithomyces* sp., and *Cladosporium* sp.

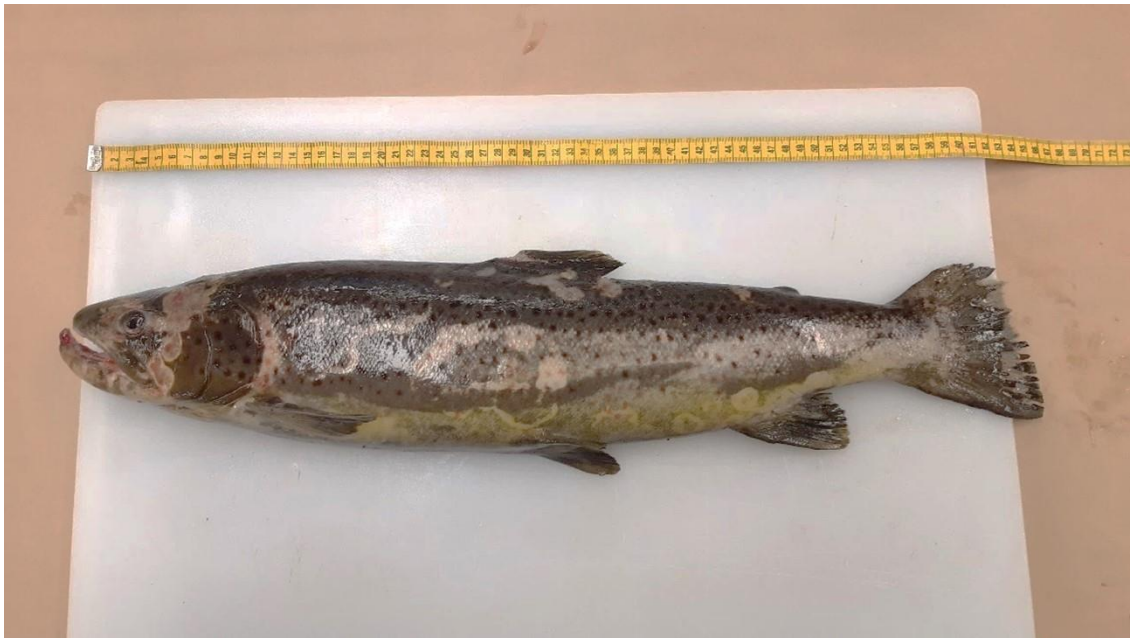

Figure 4. A sea trout from the River Oulujoki with hyphal growth on its skin. The trout was sampled in October 2022 and confirmed to have *Saprolegnia parasitica* infection.

3.2. Presence of *Saprolegnia parasitica* DNA in water samples

The dPCR assays outperformed qPCR assays in detection for *S. parasitica* from the eDNA samples collected from natural rivers. All qPCR results for the samples collected in 2022 were found negative for *S. parasitica* DNA. The dPCR results were weakly positive for four sampling occasions from the River Tornionjoki, while all sampling occasions for the River Oulujoki were positive, being weaker in mid-summer and stronger towards late fall (Table 2a).

After modifying the analytical protocol for 2023 samples, the qPCR revealed two positive DNA yield for Lappea samples collected in June and August but not for any samples collected from Tornio (Table 2b). Again, the dPCR produced more positive samples. It should be noted that also the field controls were positive in several sampling occasions in both rivers.

Table 2. A heatmap summarizing the results from dPCR and qPCR for eDNA water samples collected A) 2022 in mid-parts of the River Tornionjoki and the reference River Oulujoki, and B) 2023 at two locations along the River Tornionjoki. Positive results for *S. parasitica* DNA are presented along a green gradient (very light green = very weak positive, etc.). Sampling occasions with only negative water samples are presented with orange color. The plus (+) sign indicates a positive field control for that sampling occasion.

A) Year 2022	River Tornionjoki - Lappea		River Oulujoki	
	dPCR	qPCR	dPCR	qPCR
1.-3.7.2022	+			+
15.7.2022	n/a	n/a		
3.-7.8.2022			+	
23.-24.8.2022			+	
8.-9.8.2022	+		+	
22.-23.9.2022				
4.-7.10.2022	+		+	
21.10.2022	n/a	n/a	+	
4.11.2022	n/a	n/a		

B) Year 2023	River Tornionjoki - Lappea		River Tornionjoki - Tornio	
	dPCR	qPCR	dPCR	qPCR
16.6.2023	+			
25.7.2023	+		+	
4.-5.8.2023				
12.8.2023	n/a	n/a	+	
25.8.2023	n/a	n/a	+	
8.9.2023	n/a	n/a	+	
23.9.2023	n/a	n/a		
7.10.2023	n/a	n/a		
27.10.2023	n/a	n/a	+	

3.3. Variation in *Saprolegnia parasitica* DNA concentrations

The *Saprolegnia parasitica* DNA presence, concentrations and flow-corrected DNA rates varied non-linearly along the sampling periods within each sampling location (Figure 5), *i.e.* they did not increase as more salmonids accumulated in the rivers towards the fall.

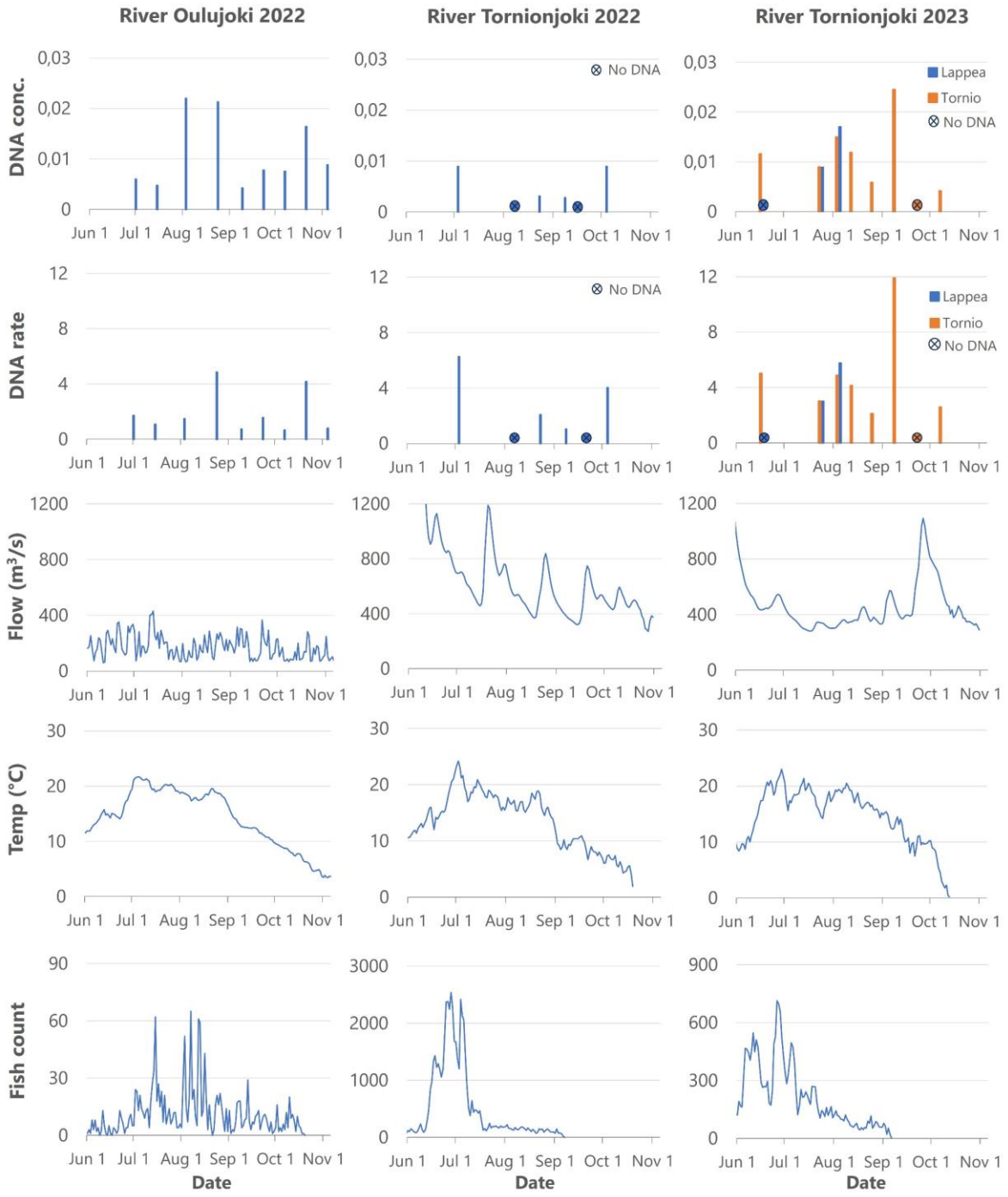

Figure 5. The dPCR results show variation in *Saprolegnia parasitica* DNA concentration (DNA copies/µl, adjusted for filtered water volume) and flow-corrected DNA rates (DNA concentration × water flow) in water samples from the River Tornionjoki and Oulujoki. In addition, monitoring is included for water flow (m^3/s), water temperature (°C), and fish count, which tracks the daily number of salmon and trout swimming upstream.

In the River Tornionjoki, *S. parasitica* DNA concentration (adjusted for filtered water volume), remained low throughout 2022 compared to 2023 (Figure 5). In both years, there were also sampling occasions where no DNA was detected (Table 2, Figure 5). In 2023, DNA concentrations fluctuated more across sampling times but differed significantly between locations (Tornio and Lappea) only in early summer (mid-June), when *S. parasitica* DNA was detected only in the estuary. In the River Oulujoki, the *S. parasitica* DNA was more consistently present and its concentration in the water samples was on average higher compared to the River Tornionjoki in the same year (2022). The flow-corrected *S. parasitica* DNA rates followed a similar pattern to the uncorrected DNA concentrations across all sampling locations. However, the flow-corrected DNA concentrations in the River Oulujoki showed less variation than the uncorrected ones, and more comparable to the concentrations measured from the River Tornionjoki during the same year.

The high flow rates coincided with sampling occasions in the River Tornionjoki when no *S. parasitica* DNA was detected (Figure 5). In the free-flowing River Tornionjoki, the water flow rates varied greatly over time, and the flow patterns differed also between the study years (Figure 5). In 2022, there were noticeable flow fluctuations with several peaks reaching between 800 and 1200 m^3/s. In 2023, fewer distinct flow fluctuations occurred during the summer months, but there was one significant flow peak up to 1100 m^3/s in late September. *S. parasitica* DNA was present in the River Tornionjoki on most of the sampling occasions, but the negative water samples in both years were collected during or soon after high flow periods (see Figure 5). In the River Oulujoki, the water flow is regulated, and it remained mostly between 100-200 m^3/s with relatively minor fluctuations, being thus more consistent than in the River Tornionjoki (Figure 5). Accordingly, *S. parasitica* DNA was present in the water samples throughout the sampling period (Table 2), but its concentration varied (Figure 5).

There were also noticeable peaks of *S. parasitica* DNA concentrations during the sampling periods in both rivers. In 2022, *S. parasitica* DNA concentrations in the River Tornionjoki were low, without any noticeable peaks. In 2023, the DNA concentrations were higher, with one significant peak in September, before the highest flow event (Figure 5). In the River Oulujoki, *S. parasitica* DNA concentrations fluctuated throughout the sampling period in 2022, with more pronounced peaks in August and October. Neither water temperature variations nor the daily numbers of migrating salmonids correlated with the fluctuations or observed peaks in *S. parasitica* DNA concentrations in either river (Figure 5).

For the River Oulujoki, we were also able to assess the prevalence of infected salmon and trout on *S. parasitica*. The video monitoring data between May 7 and October 19, 2022, revealed that 7% of the salmon (N=1,021) and 3% of trout (N=565) passing the fish ladder had a varying degree of skin lesions indicative to water mold infection (see Figure 2). The first symptomatic salmonids, primarily trout, were observed at the fish ladder in mid-June. The numbers of infected salmon began to increase in mid-July, peaking in early August (Figure 6). The concentration of *S. parasitica* DNA, rather than the flow-corrected DNA rate, strongly aligned with the number of symptomatic salmonids congregated in the area (Figure 6). Notably, the highest and lowest peaks in *S. parasitica* DNA concentrations coincided with the largest and lowest numbers of symptomatic salmonids, respectively.

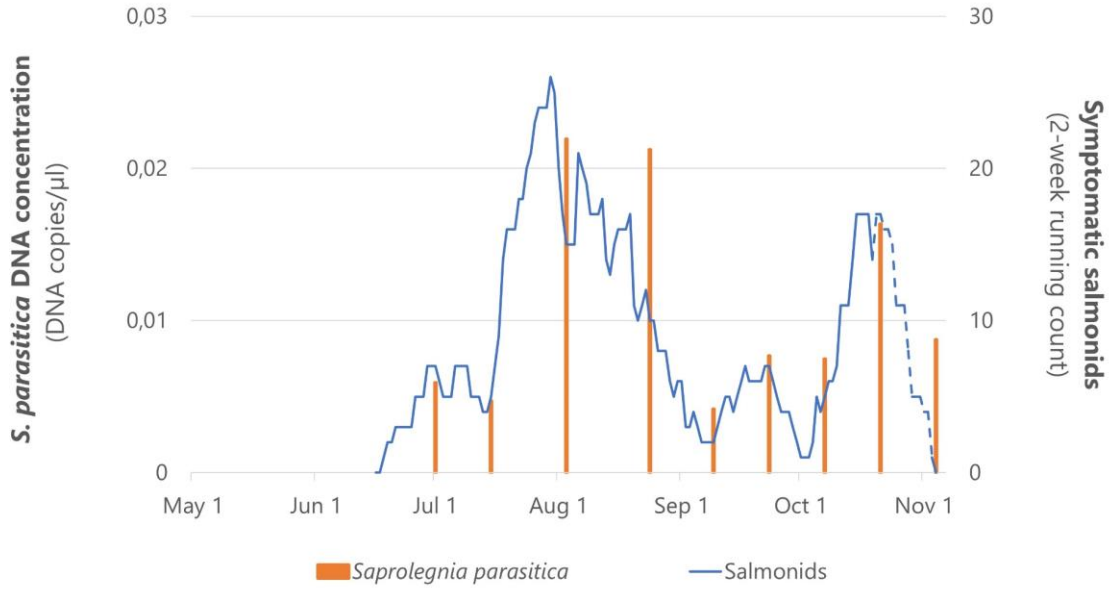

Figure 6. Variation in *Saprolegnia parasitica* DNA concentration (DNA copies/µl, adjusted to filtered water volume) in the water of the River Oulujoki, in relation to the number of symptomatic salmonids (total of salmon and trout) that had passed the fish ladder during a 2-week period prior to eDNA sampling. Numbers of symptomatic salmon (n=73) and trout (n=19) were tracked from video recordings available from May 7th to October 19th, 2022.

4. Discussion

During the study years 2022-2023, we did not observe water mold related disease outbreaks in the River Tornionjoki salmonids. Instead, we detected other aquatic fungi than *S. parasitica* that were responsible for skin lesions in the studied wild salmonids. However, *S. parasitica* DNA was detected infrequently in water samples from the River Tornionjoki in both years. For comparison, both salmon and brown trout with a *S. parasitica* infection were confirmed from the nearby River Oulujoki, where also *S. parasitica* DNA was detected on each sampling occasion.

4.1. Water molds in salmonids

The prevalence of water mold (*i.e.*, the number of symptomatic fish) in the River Tornionjoki has varied among years (Axen & Koski 2017, see also Appendices 1–3). In 2022, our health survey on salmon from the River Tornionjoki did not reveal any fish that would have shown indications of *S. parasitica* (Appendix 2). In 2023, 20% of the studied fish had distinct skin lesions (four salmon and one grayling, Appendix 3), which could have been early symptoms of a water mold growth. However, none of the isolated water molds were identified as *S. parasitica*. For comparison, 38 deceased salmon were studied in 2014–2016 from the River Tornionjoki, 21 of which had symptoms of saprolegniosis. 17 of the isolated water mold strains were found to belong to genus *Saprolegnia*, of which 7 were *S. parasitica* (Axen & Koski 2017).

Overall information of oomycete and fungi isolated from wild fish has been scarce. The growth in skin lesions of fish samples collected in 2023 from the River Tornionjoki were identified as *Botryitinia* sp., *Didymella* sp., *Mucor* sp., *Pithomyces* sp. and *Cladosporium* sp., which are mainly environmentally occurring fungi. *Cladosporium herbarum*, *Mucor hiemalis* and *Mucor circinelloides* have been documented to be pathogenic or opportunistically pathogenic for fish (Ke et al. 2010, Magray et al. 2020, Meyers et al. 2019), but the *Mucor* sp. and *Cladosporium* sp. isolated in this study were not identified as any of those species. Therefore, we may conclude that there are several other aquatic fungi besides *S. parasitica* that may be responsible for skin lesions in wild salmonids.

Contrary to earlier findings on deceased salmon in the River Tornionjoki (c.f. Axen & Koski 2017), one of our goals was to estimate the development of water mold symptoms in salmonids in relation to *S. parasitica* presence in river water. Thus, we aimed to monitor the fish also with early phases of infection. The fins or any mechanical skin damages (due to *e.g.* fishing gears or predators) typically serve as a typical starting point for infection (Pavić et al., 2022), including saprolegniosis (Axen and Koski 2017). Accordingly, the skin lesions in salmon were mainly observed in the fins or at the root of the fins, but interestingly, no signs of other damages or underlying diseases were found. The fish with severe saprolegniosis behave abnormally and mostly remain passive, and therefore they cannot be caught by angling gear used in fishing competitions. Unfortunately, we did not receive any additional samples of severely infected fish during this study to confirm *S. parasitica* in the River Tornionjoki salmon.

In this study, we confirmed *Saprolegnia parasitica* infections in both salmon and trout from the River Oulujoki. In addition, the evaluation of the salmon and trout conditions from video

footage revealed that ca. 6% of those entering the river through the fish ladder had a varying degree of skin lesions indicative to a water mold infection. Compared to the wild, naturally reproducing population in the River Tornionjoki, the vast majority of salmonids migrating to the River Oulujoki are of hatchery origin (Louhi et al. 2024). Tedesco et al. (2021) found that *S. parasitica* is more prevalent in farmed than in wild salmonids. It should be studied further whether the salmonid populations of our study rivers differ in susceptibility to saprolegniosis.

4.2. Detection of *Saprolegnia parasitica* in water samples

Based on our results, the dPCR was a more promising method to detect the presence of *S. parasitica* DNA in the river water samples than qPCR. The qPCR is more pronounced for inhibitors than the dPCR. Inhibitors, such as humus and algae, are typical in natural waters. Accordingly, the humus content was suspected to be the main cause for high PCR inhibition in the eDNA samples collected in 2022. The amount of humus dissolved in the water is also substantially higher in the River Oulujoki compared to the River Tornionjoki. Moreover, *Saprolegnia* spores may attach to organic material flowing in the river water, while high organic load has also been associated with *Saprolegnia* infections (Pavić et al., 2022).

S. parasitica was observed in water samples from the River Tornionjoki through the sampling periods and without any observations of infected fish. This confirms that *S. parasitica* is a widespread opportunistic pathogen and naturally present in natural fresh waters as shown also in previous studies (Pavić et al. 2022). The dPCR system generally has an advantage to distinguish smaller amounts of DNA from water samples than the qPCR, especially here as the concentrations of *S. parasitica* DNA were expected to be very low in the river water samples compared to those taken from fish farms. Accordingly, we found that the DNA concentrations measured with dPCR in the study rivers were very low (< 0.03 DNA copies/µl) compared to the fish farms (~10 DNA copies/µl using filter pore size 1.2 µm, O. Turunen et al. unpublished data). These results encourage applying the dPCR method in further eDNA research done in natural waters. However, it is also noteworthy that many of the field controls turned out to be positive for *S. parasitica*, especially in dPCR analyses, and even when the river samples were negative (Table 2). Thus, the results from the sampling occasions having a positive field control should be considered with care.

4.3. Sources of variation in *Saprolegnia parasitica* eDNA

Distribution and abundance of *S. parasitica* eDNA in the river environment may be affected by various environmental factors (Pavic et al. 2022, Rocchi et al. 2017). First, the measurable concentration of DNA from the river environment results from both the amount of DNA spread by the target organisms and the flow of water (Levi et al. 2019). Second, the number of host fish infected by *S. parasitica* affects the rate of spore production (*i.e.* released DNA) into its surrounding environment (Wood & Willoughby 1986, Van der Berg et al. 2013). Third, also other factors, especially water temperature, may contribute *S. parasitica* abundance by affecting on host immunity but also on *S. parasitica* mycelial growth and spore production rate (Matthews 2019, Pavić et al. 2021).

Water flow does not directly contribute to the *S. parasitica* abundance, but it does impact the spore distribution in the river environment. In this study, fluctuations in water flow were associated with the detection of *S. parasitica* DNA in water samples, although the impact of

flow on the concentration of DNA in these samples was less pronounced. In the River Tornionjoki, which experiences high natural flow variability, the absence of *S. parasitica* DNA was preceded by a high flow period. In 2022, multiple high-flow peaks and relatively lower *S. parasitica* DNA concentrations were observed in the River Tornionjoki, whereas higher DNA concentrations under steadier flow conditions were detected during summer months in 2023 (see Figure 5). However, the eDNA results from 2022 and 2023 are not fully comparable due to methodological improvements between the study years. Meanwhile in the regulated River Oulujoki with a steady and relatively low flow, *S. parasitica* DNA was detected on every sampling occasion.

To compare the relative abundances in the *S. parasitica* across rivers and study years, we applied flow-corrected DNA rates. The obtained *S. parasitica* DNA rates mostly followed the same temporal pattern than the DNA concentrations in the water samples, but the flow-corrected DNA rates suggested more similar abundance of *S. parasitica* in both study rivers in 2022. It should, however, be noted that the applied flow-correction is a very simplified method to account for the flow effects when estimating species abundance based on the eDNA concentration. It relies on the assumption that an individual sheds target DNA at a constant rate into the environment, as it has been validated for estimating adult salmon numbers in a watershed (Levi et al. 2019). In the case of *S. parasitica* (and other similar fish pathogens), the release of DNA into the river environment is also strongly affected by prevalence of infected host fish (see discussion below, and Figure 6). For example, the number of symptomatic fish in the River Oulujoki appeared to correlate stronger with concentrations of *S. parasitica* DNA than the flow-corrected DNA rates. Additional research would be necessary to determine if combining eDNA concentrations with the flow measurements can produce relevant indices of *S. parasitica* abundance and/or infection pressure for wild salmonids (see also Yates 2021).

High fish density increases the probability of diseases, including *S. parasitica* (Wood et al. 2010), spreading in a fish population. The prevalence of infected fish also correlates with the *S. parasitica* load in the water of fish farms (Korkea-aho et al. 2022). In 2022, a total of 51,123 salmonids migrated to the River Tornionjoki, while in 2023 only 21,614 of salmonids were counted. Although we had no direct evidence of *S. parasitica* infected salmonids, the eDNA results showed that this disease agent was present in the River Tornionjoki during both study years. However, due to the low numbers of migrating salmon and the large size of the River Tornionjoki watershed, it is likely that potentially infected fish remain undetected. For comparison, only a total of 1,586 salmon and trout passed through Merikoski fish ladder in 2022, and 7% of salmon and 3% of trout showed indications of water mold. However, these fish congregate within a relatively small reservoir area between the first two dams. Most of the mortality in juvenile salmon due to *Saprolegnia* occurs within two weeks post-infection (Misk et al. 2022). Less is known about disease progression in adult salmonids, or eDNA degradation of *S. parasitica* in natural waters. However, the prevalence of symptomatic fish in the River Oulujoki, observed at the fish ladder during the two weeks prior to eDNA measurements, aligned well with the variation in *S. parasitica* DNA concentration (Figure 6). The observations particularly from the River Oulujoki validate the eDNA technique for detecting *S. parasitica* in river environment: when *S. parasitica* was found abundant in fish, its DNA was detected with high concentration also in water samples.

Changes in water temperature influence the prevalence of saprolegniosis in fish farms, with symptomatic fish and high mortality rates occurring from fall to spring (reviewed by

Lindholm-Lehto & Pylkkö 2024). The rapid change and/or decreasing temperatures may lead to stress reactions and immunosuppression in fish, making them more susceptible to saprolegniosis (Duan et al. 2018, Korkea-aho et al. 2022). Meanwhile, mycelial growth of *S. parasitica* on fish skin is highest at temperatures between 15-25°C (Matthews 2019). However, a rapid temperature drop and lower water temperatures in general stimulate *S. parasitica* spore production (Bly et al. 1992, Pavić et al. 2021). We found no correlation between *S. parasitica* DNA concentration and water temperature. More frequent eDNA sampling would be required to observe the impact of temperature changes on *S. parasitica* load in the river environments.

Taken together, several physico-chemical parameters of water can influence the *S. parasitica* load in natural environments (Pavić et al. 2022, Rocchi et al. 2017). Due to the intermittent eDNA sampling at ~2-week intervals, our observations remain only indicative, though they encourage further research – particularly on the effects of water flow regimes on *S. parasitica* load in free-flowing versus hydropower-regulated rivers. Ultimately, automated and more frequent eDNA sampling may offer an optimal solution, as sampling from surface water at limited time points can be affected by environmental factors such as water flow rates and temperature as well as rainfall or wind. Also, other potential environmental sources for variation in *S. parasitica* load in river environment, such as water quality (including nutrients and minerals), organic load (humus) and pH (see Pavić et al. 2022), should be considered in future studies.

4.4. Applicability and further development of eDNA methods

Based on current results, the eDNA technique might be applied to monitor the *Saprolegnia parasitica* presence and load in river environments. *S. parasitica* diagnostic has been previously performed on fish after an observed disease outbreak, so the eDNA diagnostics using water samples would be less invasive for fish and may help to detect the *S. parasitica* earlier than with the usual diagnostic tools. Thus, the eDNA can complement conventional fish health monitoring programs. However, *S. parasitica* also exists freely in natural waters, and less is known about the impact on fish if the *S. parasitica* load fluctuates. So far more is known from aquaculture environments, where high *S. parasitica* amount is detected in water when its prevalence in fish is high (Korkea-aho et al. 2022, Pavić et al. 2022). However, monitoring the prevalence of infected fish in large natural watersheds, such as the River Tornionjoki, is challenging. Before the eDNA technique can be applied in e.g. risk-management, further studies are needed to determine how well the concentrations of *S. parasitica* DNA in water samples correlate with the number of infected fish across river environments.

Although our results on the use of eDNA methods in river environments were somewhat contradictory, we strongly recommend further development of eDNA sampling and analytical methods in natural waters. While eDNA concentrations can provide rough quantitative estimates of abundance in natural environments (Yates et al. 2021), it is important to consider variation in river-specific conditions and fish-pathogen interactions to enhance the accuracy of these estimates for *S. parasitica*. Moving forward, we recognize the need to establish and refine sampling and analytical protocols to better address river-specific conditions. First, it is noteworthy that the incidence of positive field controls was rather high in this study, which could not be directly explained by sampling date, location, control water used (milliQ vs.

spring water) or by persons who performed the sampling or further analyses. This may indicate the existence of *S. parasitica* spores in the air as negative field controls are filtered at the sampling locations. If so, more precise methods when and while filtering the field controls are needed. For example, sampling could be done directly from the water surface instead of first transferring water to buckets. Second, different filter types should be tested. Here, we used filters with a pore size of 5 µm but there is recent evidence from fish farm conditions that smaller pore sizes (1.2 µm or even 0.45 µm) could capture *S. parasitica* DNA more efficiently. The downside of using a smaller pore size is the potential reduction in the volume of water that can be pumped through the filter. In the River Oulujoki, this limit was frequently reached during sampling due to the high humus content. In contrast, the water quality in the River Tornionjoki is different and may have allowed for higher volumes to be filtered. The filtering protocol might also benefit from adjustments to local conditions, and unlimited water volume pumping should be tested.

4.5. Conclusions

The eDNA has great potential to be used as a detection and monitoring tool for *S. parasitica* load in natural waters. Due to the low prevalence of symptomatic fish in the River Tornionjoki during this study, our eDNA results cannot yet be applied to predict increased health risks for this salmon population. Monitoring symptomatic fish along a large, free-flowing river like the River Tornionjoki poses significant challenges, and fish monitoring methods should be developed in conjunction with eDNA techniques. The results from the River Oulujoki were more encouraging, suggesting a strong correlation between *S. parasitica* DNA concentrations and the prevalence of symptomatic fish in the area, thereby validating the eDNA technique for monitoring *S. parasitica* abundance in river environments. These findings also imply that the use of eDNA technique requires careful analyses of river-specific conditions before it can be implemented for *e.g.*, risk management.

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Appendices

Appendix 1. Condition of salmon caught in the mid-parts of the River Tornionjoki (Lappea) in 2021

Appendix 2. Water mold monitoring along the River Tornionjoki in 2022

Appendix 3. Salmon health monitoring along the River Tornionjoki in 2023

Appendix 1. Condition of salmon caught in the mid-parts of the River Tornionjoki (Lappea) in 2021

In late summer and fall 2021, we performed general monitoring for symptoms of water mold infection in salmon caught from the River Tornionjoki, and particularly in the village of Lappea (Figure 1). We measured the fish for total length and weight and took photographs as well as scale samples for age determination.

During August 2021 we surveyed a total of 81 caught salmon for their general condition (30 of them during a 2-day fishing competition on the 7–8th of August). Seven of those fish showed early indications of water mold infection starting from their fins. However, the suspected water mold was not confirmed by any further analyses. In addition, a local fisherman contacted us about five severely infected salmon once the salmon fishing season had ended (Aug 31) (Figure 7). The first salmon with detrimental water mold infection was observed in early August (August 6th). The majority of severely infected salmon were observed in October and November.

Figure 7. Individual characteristics of salmon with a water mold infection found dead or dying in Lappea village in late summer and fall of year 2021. SW refers to number of sea winters, i.e., years spent at sea before returning to the River Tornionjoki. Photos: Oona Herzog and Juha Pieskä.

13.10.2021
Female
86 cm, 4.2 kg
age 6+ (2SW)

15.10.2021
Male
102 cm, 8.4 kg
age 5+ (2SW)

4.11.2021
Male
60 cm, 1.5 kg
age 4+ (1SW)

5.11.2021
Male
57 cm, 1.4 kg
age 4+ (1SW)

Figure 7. Continued.

Appendix 2. Water mold monitoring along the River Tornionjoki in 2022

In 2022, three salmon were inspected during broodfish capture in the river mouth in June in collaboration with the general health survey in the River Tornionjoki conducted by Finnish Food Authority. Between June–August 2022, we inspected a total of 54 salmon during four different fishing competitions (one in Tornio and Pello, and two in Lappea/Kolari). All fish that showed any external symptoms or indications of any potential diseases are shown in Table 3. No indications of water mold were observed in these salmon, but several fish had showed hemorrhage in their ventral skin. In addition, a local fisherman continued monitoring the salmon after the fishing season closure, but no severely infected fish were caught during fall 2022.

Table 3. Details of salmon studied in fishing competitions organized along the River Tornionjoki in 2022. For locations on the map, see Figure 1. ND= Not detected.

Date and location Sampled fish species	Length (cm)	Weight (kg)	Sex	Macroscopic changes	Water mold species
13.6.2022, Tornio					
Salmon	114	16.5	Male	No changes	ND
Salmon	110	13.5	Female	Slight hemorrhage on ventral skin	ND
Salmon	116	16.2	Female	Slight hemorrhage on ventral skin	ND
18.6.2022, Pello					
Salmon	117	14.8	Male	Slight hemorrhage on ventral skin, incised wounds around skin	ND
Salmon	96	8.1	Male	Very slight hemorrhage on ventral skin	ND
Salmon	96	8.3	Female	No changes	ND
Salmon	110	12.3	Male	No changes	ND
Salmon	113	14.5	Male	Slight hemorrhage on ventral skin	ND
Salmon	98.5	8.8	Female	No changes	ND
Salmon	82	5.1	Male	No changes	ND
Salmon	100	8.2	Female	No changes	ND
Salmon	109	12.9	Female	No changes	ND
Salmon	86.5	6.4	Female	No changes	ND
Salmon	86	8.0	Male	Slight hemorrhage on ventral skin	ND
Salmon	98	8.2	Female	Slight hemorrhage on ventral fin	ND
Salmon	101.5	9.2	Female	No changes	ND
Salmon	84	5.8	Male	No changes	ND
Salmon	91	7.4	Male	No changes	ND

Table 3. Continued.

Date and location Sampled fish species	Length (cm)	Weight (kg)	Sex	Macroscopic changes	Water mold species
18.6.2022, Pello					
Salmon	79.5	4.6	Female	No changes	ND
Salmon	105	11.7	Female	No changes	ND
Salmon	82	4.6	Female	No changes	ND
Salmon	81	4.6	Female	No changes	ND
3.7.2022, Kolari/Lappea					
Salmon	86	6.3	Female	No changes	ND
Salmon	105	9.1	Male	No changes	ND
7.8.2022, Lappea					
Salmon	79	4.4	Female	No changes	ND
Salmon	93	8.2	Female	Slight hemorrhage on ventral fin	ND
Salmon	83	5.4	Female	No changes	ND
Salmon	78	4.1	Female	No changes	ND
Salmon	87	5.8	Female	No changes	ND
Salmon	89	6.3	Female	No changes	ND
Salmon	91	6.9	Female	No changes	ND
Salmon	119.5	16.3	Male	No changes	ND
Salmon	91	7.2	Female	No changes	ND
Salmon	86	6.6	Female	No changes	ND
Salmon	86	5.3	Female	No changes	ND
Salmon	81	4.8	Female	No changes	ND
Salmon	106	12.3	Female	No changes	ND
Salmon	85	5.9	Female	Lamprey bite under the neck	ND
Salmon	79	5.1	Female	No changes	ND
Salmon	102	9.5	Male	No changes	ND
Salmon	96	8.2	Female	No changes	ND
Salmon	81	4.4	Female	A seal bite on the side	ND
Salmon	91	6.7	Male	Slight hemorrhage on ventral skin and pelvic fin	ND
Salmon	93	8.2	Female	No changes	ND
Salmon	86	5.9	Female	No changes	ND
Salmon	90	7.4	Female	No changes	ND
Salmon	93	7.3	Female	No changes	ND
Salmon	107	11.5	Female	No changes	ND

Table 3. Continued.

Date and location Sampled fish species	Length (cm)	Weight (kg)	Sex	Macroscopic changes	Water mold species
7.8.2022, Lappea					
Salmon	90	7.2	Female	A healed wound on the left side	ND
Salmon	84	5.0	Female	No changes	ND
Salmon	85	5.7	Female	No changes	ND
Salmon	101	9.4	Male	No changes	ND
Salmon	105	10.1	Female	A healed wound on the left side	ND
Salmon	112,5	13.1	Female	No changes	ND
13.8.2022, Tornio					
Salmon	89	7.0	Male	Slight hemorrhage on ventral skin	ND
Salmon	101	11.8	Female	No changes	ND
Salmon	72	70	Male	Slight hemorrhage on pelvic fin	ND

Appendix 3. Salmon health monitoring along the River Tornionjoki in 2023

In 2023, the salmon numbers in the River Tornionjoki were extremely low, and we were able to inspect only a total of 25 salmon and four graylings in four different fishing competitions (organized in Tornio, Pello, Kolari and Muonio). 20 percent of all the fish studied showed very minor changes in their skin. The health monitoring was done in collaboration with the general health survey in the River Tornionjoki conducted by Finnish Food Authority. Also, we did not receive any observations of infected salmon from local fishermen or citizens during or after the fishing season. All fish sampled in 2023 are shown in Table 4.

Table 4. Details of salmonid fish studied from the River Tornionjoki in 2023. ND= Not detected. For locations on the map, see Figure 1.

Date and location Sampled fish species	Length (cm)	Weight (kg)	Sex	Macroscopic changes	Water mold species
16.6.2023, Lappea					
Salmon	n/a	ca. 6.0	Female	No changes	ND
17.6.2023, Pello					
Salmon	100	9.8	Female	No changes	ND
25.7.2023, Muonio					
Salmon	81	5.3	Female	No changes	ND
Salmon	89	7.1	Female	No changes	ND
Salmon	82	6.0	Female	Lesion on skin behind dorsal and adipose fins	*Didymella* sp.
Grayling	38.5	0.5	n/a	Several fish lice (*Argulus* sp.) on skin	ND
5.-6.8.2023, Lappea					
Salmon	59.5	1.8	Male	No changes	ND
Salmon	83.5	5.2	Female	No changes	ND
Salmon	91	6.7	Male	Lesion behind dorsal fin and around tail fin	*Mucor* sp.
Salmon	57.5	1.5	Male	Bite mark on skin	ND
Salmon	53	1.4	Male	No changes	ND
Salmon	61	1.9	Male	No changes	ND
Salmon	109	11.9	Female	No changes	ND
Salmon	106	12.5	Female	No changes	ND
Salmon	62	2.0	Male	No changes	ND
Salmon	92.5	7.7	Female	No changes	ND
Salmon	60.5	1.9	Male	No changes	ND
Salmon	85	5.1	Male	Lesion on anal fin	*Pithomyces* sp.
Salmon	94	7.7	Male	No changes	ND
Salmon	86	5.7	Male	No changes	ND

Table 4. Continued.

Date and location Sampled fish species	Length (cm)	Weight (kg)	Sex	Macroscopic changes	Water mold species
12.8.2023, Tornio					
Salmon	100	10.3	Female	Lesions in skin near dorsal and tail fin	*Cladosporium* sp.
Salmon	80	5.2	Female	No changes	ND
Grayling	n/a	n/a	n/a	No changes	ND
Grayling	n/a	n/a	n/a	No changes	ND
Grayling	n/a	n/a	n/a	Bite mark with greyish hyphal growth	*Botryotinia* sp.

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