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ORIGINAL ARTICLE

Monitoring presence and abundance of two gyrodactylid ectoparasites and their salmonid hosts using environmental DNA

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Abstract

Background: Invasive species represent a major challenge for the conservation of biodiversity. The invasive ectoparasitic fluke *Gyrodactylus salaris* is considered one of the major threats to the Atlantic salmon (*Salmo salar*), and the parasite has so far been detected in 50 rivers in Norway.

Aims: We investigate environmental DNA (eDNA) as a tool for detecting and assessing relative abundance of *G. salaris* and Atlantic salmon, upstream and downstream of a recently constructed artificial migration barrier in the River Driva in Norway. In addition, we also use eDNA to assess abundance of the less pathogenic *G. derjavinoides* and its main host, the brown trout (*S. trutta*).

Material & Methods: We filtered 1 L and 10 L of water through a 0.45 μm cellulose filter and a 2.0 μm glass fiber filter, respectively, at nine different localities along the river. Concentrations of eDNA were assessed using droplet digital PCR (ddPCR) and compared to parasite abundance based on conventional methodology using electrofishing and the counting of individual parasites on juvenile salmon.

Results: All four species could successfully be detected from water samples using two different protocols varying in sample volumes, filter types, and DNA-isolation methods. However, eDNA-occupancy modeling revealed that the probability of detecting the two gyrodactylid species was higher when filtering 10 L water through a 2.0 μm glass fiber filter ($p > .99$) than when filtering 1 L water through a 0.45 μm cellulose filter ($p = .48–.78$). The eDNA concentrations of the two fish species were markedly higher below the migration barrier, reflecting the expected higher biomass of fish. For the two gyrodactylid parasites, eDNA concentrations showed a peak upstream of the migration barrier and decreased below the migration barrier. The observed pattern was consistent with parasite abundance based on conventional methodology.

Discussion: Assessing abundance in rivers using eDNA is challenging and potentially influenced by downstream accumulation and dilution from tributaries, but our results suggest that *G. salaris* eDNA concentrations were indicative of parasite abundance.

Conclusion: We conclude that eDNA is an efficient way of monitoring gyrodactylid parasites and their salmonid hosts, and we suggest that eDNA should be incorporated into future monitoring of *G. salaris*.

KEYWORDS
Atlantic salmon, ddPCR, eDNA, *Gyrodactylus salaris*

1 | INTRODUCTION

Invasive species pose a global challenge and represent an increasing threat to native biodiversity (Pyšek & Richardson, 2010). Invasive pathogens can potentially eradicate local or endemic species, and pathogenic strains of the ectoparasitic fluke *Gyrodactylus salaris* (Platyhelminthes; Monogenea) are considered one of the major threats to the conservation of Atlantic salmon (*Salmo salar*) in Norway (Forseth et al., 2017). Norway has more than 400 Atlantic salmon rivers and represent ca. 25% of the world's wild Atlantic salmon populations. Norwegian authorities have thus taken particular responsibility in protecting the species.

Gyrodactylus salaris is native to watercourses draining into the Baltic Sea, and live fish carrying pathogenic strains of *G. salaris* were translocated from hatcheries in Sweden to Norway on several occasions (Hansen, Bachmann, & Bakke, 2003; Johnsen & Jensen, 1991; Johnsen, Møkkelgjerd, & Jensen, 1999). The first unintentional introductions of the parasite started during the 1970s and stocking of infected Atlantic salmon from hatcheries to several rivers in western and northern Norway caused massive mortality of juvenile fish in the wild (Hansen et al., 2003; Johnsen & Jensen, 1991). From many of these rivers, migrating infected fish spread the parasite to neighboring rivers via brackish fjords (Jansen, Matthews, & Toft, 2007; Soleng & Bakke, 1997). The spread of *G. salaris* was further exacerbated through the escape of non-native rainbow trout (*Oncorhynchus mykiss*) from inland fish farms (Mo, 1991). Later, fish culling effectively eradicated *G. salaris* from all 39 infected Norwegian fish farms (Hytterød et al., 2018).

Pathogenic strains of *G. salaris* have been detected on juvenile Atlantic salmon in 50 Norwegian rivers (Hytterød et al., 2018). As the distribution of the pathogenic strains is restricted to the subsections of the watercourses with anadromous fish, eradication of *G. salaris* is considered possible and the goal of Norwegian authorities is to remove *G. salaris* in all rivers where possible (Miljødirektoratet 2014). The eradication of *G. salaris* in Norwegian rivers started in the early 1980s. By the end of 2017, chemical treatment was completed in 43 rivers. In all but one river, rotenone has been used as a piscicide to kill all the fish and associated parasites (Johnsen, Brabrand, Jansen, Teien, & Bremset, 2008). In the last river, a new method using acidified aluminum was used to selectively kill the *Gyrodactylus* parasites but not the salmonid hosts (Hindar et al., 2015). Thirty-two of the 43 rivers are currently declared free from *G. salaris* while 11 rivers are in a five-year monitoring period post-treatment before they can be declared free of the parasite. In 2018, *G. salaris* remains present in seven Norwegian rivers (Hytterød et al., 2018).

Continued monitoring for the presence of *G. salaris* is an important part of managing Norwegian salmon populations, especially for rivers in the five-year post-treatment period. Traditionally, monitoring of *G. salaris* is done by stereomicroscopic examination of juvenile Atlantic salmon (Solem, Aalbu, & Mo, 2018). Juvenile fish are collected by electrofishing, killed, and preserved in ethanol for later examination. More efficient procedures are desirable because standard sampling methods are based on lethal sampling and are labor-intensive and time-consuming in both the field and laboratory.

Environmental DNA (eDNA) represents a new era of noninvasive monitoring, where filtration of water alone can detect minute remains of intra- and extracellular DNA in the freshwater environment (Rees, Maddison, Middleditch, Patmore, & Gough, 2014; Taberlet, Coissac, Hajibabaei, & Rieseberg, 2012; Thomsen & Willerslev, 2015; Valentini et al., 2016). eDNA is a promising tool for detecting and monitoring invasive species as well as rare or threatened species and has been successfully utilized in field studies on a range of different aquatic taxa including mollusks, fish, and amphibians (Ficetola, Miaud, Pompanon, & Taberlet, 2008; Goldberg, Pilliod, Arkle, & Waits, 2011; Jerde, Mahon, Chadderton, & Lodge, 2011; Thomsen et al., 2012; Valentini et al., 2016) and their pathogens and parasites such as the fungi *Batrachochytrium dendrobatidis* and trematode *Ribeiroia ondatrae* (Bass et al., 2015; Dorazio & Erickson, 2017; Huver, Koprivnikar, Johnson, & Whyard, 2015; Taugbøl et al., 2017). Screening of environmental samples for eDNA of target species is a noninvasive method, less labor-intensive, and can be more sensitive for detecting rare species than traditional sampling methods (Valentini et al., 2016). A recent study demonstrated that eDNA of *G. salaris* and its hosts can successfully be detected in water samples (Rusch et al., 2018).

Rivers present an interesting environment for detecting occurrence and estimating abundance of aquatic organisms using eDNA. The constant unidirectional flow could potentially transport eDNA downstream from where the organism actually is present. Invertebrate DNA has been documented as far as 12 km downstream from known locations (Deiner & Altermatt, 2014). Flowing waters in rivers may also lead to transport and accumulation of eDNA at downstream locations. Field studies of a river crayfish have reported increased eDNA concentrations and overestimation of abundance of the target species in lower parts of a river (Rice, Larson, & Taylor, 2018). On the other hand, eDNA concentrations may degrade quickly in turbulent water and field studies of fish suggest that eDNA can also reflect local abundance at fine spatial scales (Doi et al., 2017; Tillotson et al., 2018).

In the River Driva, anadromous fish could until recently migrate about 100 km upstream of the river, including a remote canyon with

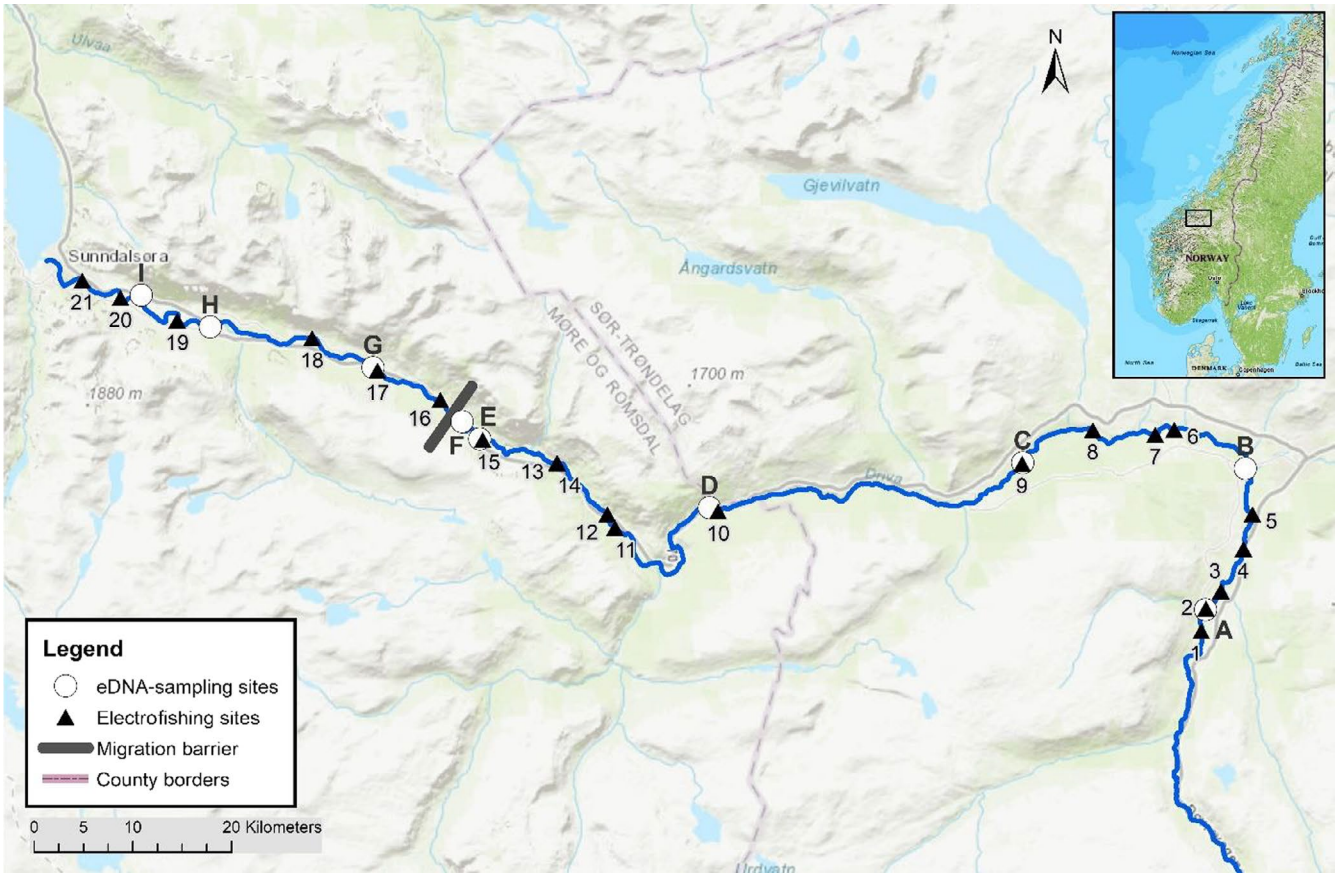

FIGURE 1 Map showing the eDNA sampling sites (A-I) and electrofishing sites (1–21) in the River Driva in central Norway. Locality 1 in the east is near the upper natural limit for anadromous salmonids, and the river outlet is found at Sunndalsøra in the west

rocks, waterfalls, and rapids, up to a natural migration barrier at 580 meters above sea level (m.a.s.l.). Based on this complexity, chemical eradication of *G. salaris* was considered challenging and risky, both for the success and for the personnel involved (Miljødirektoratet 2014). Therefore, an artificial fish migration barrier was built 25 km from the river outlet, at 110 m.a.s.l. The barrier became functional in June 2017 and prevents migration and spawning by Atlantic salmon upstream of this point. Hence, only juvenile salmon from spawning in 2016 or earlier are currently found above the barrier and no anadromous adult salmon. Within 5–6 years, all juvenile salmon upstream of the barrier will have migrated to the sea as smolts or died from the epizootic or other causes. When no hosts or parasites are found above the barrier, a restricted chemical treatment of *G. salaris* can be carried out downstream of the barrier to completely remove the parasite from the river.

The River Driva also holds a large population of anadromous brown trout (*Salmo trutta*), which have a high economic value for conservation and sport fishing. To maintain local stocks of brown trout, fish are caught below the barrier and released upstream. Upstream populations of brown trout will also provide a source population for recolonization after eradication treatments have been completed at downstream locations. Controlled trials have shown that *G. salaris* cannot survive on brown trout for long periods (Jansen & Bakke, 1995; Paladini et al., 2014), and translocations of (genetically verified) trout upstream are not considered problematic for control or eradication of *G. salaris*.

In this study, we assess eDNA as a tool for estimating local abundance of the pathogenic *G. salaris* and Atlantic salmon in the River Driva. In addition, we also use eDNA for detection of the less pathogenic *G. derjavinoides* and its main host, the brown trout. *Gyrodactylus derjavinoides* can also infect Atlantic salmon but it is not causing massive mortality in either species (Mo, 1997). The large-scale conservation effort of introducing an artificial barrier presents a unique opportunity for investigating eDNA as a monitoring tool in rivers. eDNA concentrations of Atlantic salmon and *G. salaris* are expected to decrease gradually at upstream locations while the barrier is in place, whereas eDNA concentrations of brown trout and *G. derjavinoides* are expected to remain relatively stable. Here, we present the results from the first year of eDNA monitoring, where we test two different filter types and use eDNA-occupancy models (Dorazio & Erickson, 2017) to assess the efficiency of our sampling protocols and to investigate whether eDNA concentrations reflect local abundance of fish and parasites under natural conditions in the River Driva.

2 | MATERIAL AND METHODS

2.1 | eDNA sampling

Samples were collected at nine localities on 8 November 2017 in the River Driva (Figure 1). Three localities were sampled below the

barrier, and six were sampled above the barrier. The uppermost locality was close to the natural migration barrier at 580 m.a.s.l.

For each locality, we filtered two replicate samples of 1 L water through a 0.45 µm cellulose filter (Pall MicroFunnel 300 ST; Pall Corporation), and two replicate samples of 10 L water on a 2.0 µm glass fiber filter (Merck Millipore). The water samples were filtered using a vacuum pump (Microsart e.jet; Sartorius GmbH) connected to a 3-place filter funnel manifold (Pall Corporation). Filter holders and all collecting equipment were bleached in 10% chlorine between each sample to avoid contamination among stations. Negative field control samples were unfortunately not included in this study. However, several negative results suggest that contamination between samples was not a systematic problem, and the distinct pattern of eDNA abundance of *G. salaris* conformed to results from conventional methodology and is unlikely to stem from contamination in the field. The 0.45 µm cellulose filters were immediately placed in 2-ml plastic tubes with 1,440 µl ATL buffer (Qiagen), whereas the 2.0 µm glass fiber filters were placed in 5-ml plastic tubes with 4,050 µl ATL buffer. All samples were stored at room temperature until further processing in the genetics laboratory at the Norwegian Institute for Nature Research in Trondheim.

2.2 | Laboratory analysis

In the laboratory, 160 or 450 µl (2 mg/ml) Proteinase K (Qiagen), respectively, was added to the sampling tubes collected in the field and incubated overnight at 56°C. DNA was isolated using DNeasy DNA blood & tissue kit (Qiagen) following the modified protocol of Spens et al. (2017) for the 0.45 µm cellulose filters, and using a FastDNA 50 ml SPIN Kit for Soil (MP Biomedicals) for the 2.0 µm glass fiber filters. The FastDNA 50 ml SPIN Kit requires a high elution volume (2 ml), which results in low final DNA concentrations. Preliminary tests revealed low detection rates of the two gyrodactylid species, and we modified our initial protocol to use a centrifugal filter unit (Microcon DNA Fast Flow Filter, Merck Millipore) to concentrate the DNA eluate. We added 500 µl eluate to the Fast Flow Filter and gained on average 64 µl (range: 40–90 µl) of concentrated DNA. The measured volume of concentrated DNA was used in the calculations of DNA concentrations. All results relating to the 2.0 µm glass fiber filter are based on the concentrated DNA eluate.

Concentration of target DNA was assessed using droplet digital PCR (QX200 Droplet Digital PCR system with AutoDG™, Bio-Rad Laboratories). All samples were analyzed using species-specific primers developed for *G. salaris and G. derjavinoides* (Collins et al., 2010), brown trout (Gustavson et al., 2015), and Atlantic salmon (this study, Table 1). For salmon, we developed a new species-specific assay based on a part of the mitochondrial D-loop. This part of the D-loop is regularly used at the Norwegian Institute for Nature Research (NINA) for assessing species identity and levels of hybridization between Atlantic salmon and brown trout (Karlsson et al., 2013). We used Primer Express 3.0.1 (Applied Biosystems) to design primers and TaqMan MGB probes (Table 1). Species specificity was assessed by testing cross-amplification in other local fish species (Table S1), and

TABLE 1 Details of primers used for detection of the two gyrodactylid parasites and their two salmonid hosts

Species	Primer name	Gene	Forward primer sequence	Reverse primer sequence	Probe sequence	Source
G. derjavinoides	Gder2	*ITS*	TTGGTGCCCCATGATGGT	CGCACCTATTCAGCTAGTCTTAATACA	CTGCTCAGAACTCATCTC	Collins et al. (2010)
G. salaris	Gsal2	*ITS*	CGATCGTCACTCGGAATCG	GGTGGCGCACCTATTCTACA	TCTTATTAACCAGTTCTGC	Collins et al. (2010)
S. trutta	Str	*COI*	TTTTGTTTGGGCCGTGTTAGT	TGCTAAAACAGGGAGGGAGAGT	ACCGCCGTCCTCT	Gustavson et al. (2015)
S. salar	NINAsalar	*CTRL*	GCATTCGGCACCGACTACA	CGCCAACTAGGCCTTTATATATTTAAC	TCATTGGTACCACTTTTA	This study

the results showed no amplification in any of the tested species. We also tested cross-amplification for the brown trout assay and found some positive results (Table S2). In particular, five out of seven Arctic char (*Salvelinus alpinus*) samples showed low levels of amplification, in addition to one sample each from Atlantic salmon and European perch (*Perca fluviatilis*). However, none of these samples were collected for this specific purpose, and it is possible that several species have been caught in the same gillnet, kept in the same transport container or dissected with the same knife. Hence, we cannot exclude the possibility of cross-contamination in the field. The low levels of amplification using tissue samples suggest that cross-amplification is unlikely to be a problem when analyzing water samples. For the two Gyrodactylid assays, we always included DNA isolated from both species as a positive control in addition to a regular PCR-negative control sample based on RNase-free water (Qiagen).

The ddPCRs consisted of 0.9 µM forward and reverse primers, 0.25 µM of the probes, ddPCR™ Supermix for Probes (No dUTP) (Bio-Rad Laboratories), dH_2O, and 5 µl template-DNA. We initially tested both 1 and 5 µl template-DNA volume and found that the higher volume increased the concentration and detectability of the two Gyrodactylid species and with no signs of inhibition. For the test of cross-amplification using tissue samples of various fish species, we used 1 µl template-DNA. To generate droplets, an AutoDG™ Instrument (Bio-Rad Laboratories) was used, with subsequent PCR amplification in a Veriti™ 96-Well Thermal Cycler (Applied Biosystems). The following thermal cycling conditions were used: an initial denaturation step at 95°C for 10 min, 40 cycles of denaturation at 95°C for 30 s, annealing and extension at 60°C for 1 min, a final step of denaturation at 98°C for 10 min, and a final hold at 4°C. PCR plates were transferred to a QX200™ Droplet Reader (Bio-Rad Laboratories) to automatically detect the fluorescent signal in the droplets. QuantaSoft software v.1.7.4 (Bio-Rad Laboratories) was used to separate positive from negative droplets, according to manufacturer's instructions. PCR-negative control samples revealed that one or two positive droplets sometimes occurred without the presence of DNA template. A low level of false positives is consistent with results using other assays for other species in our laboratory, and we have conservatively set a limit of minimum three positive droplets for assessing a sample as positive (Dobnik, Spilsberg, Bogožalec Košir, Holst-Jensen, & Žel, 2015).

2.3 | Conventional monitoring

Conventional monitoring was conducted during the period from the end of August to start of October in 2017 and involved more localities (N = 21) than were included in the eDNA study (N = 9). Juvenile salmon were sampled with a backpack electroshocker (Bohlin et al., 1989). Electrofishing was repeated three times for seven localities and one time only for the remaining 15 localities. Juvenile densities were estimated by the removal method of Zippin (1958) for the seven locations fished three times. Estimated catchability for those localities was then used to calculate mean density of juvenile salmon for all 21 localities (Solem et al., 2018). All juvenile salmon were killed with an overdose of anesthesia (Benzocaine) and stored in separate bottles with ethanol before being brought to the laboratory where the number of *G. salaris* on each fish was counted. An estimated total number of *G. salaris* per locality was calculated as the product of mean density of juvenile salmon and mean number of *G. salaris* per fish, controlling both for fish density and infection rate at each locality (Table S3).

2.4 | Statistical analysis

Concentrations of target DNA were recalculated to a standardized measure of number of DNA copies per liter water, to control for different sample volumes and DNA-isolation protocols in the laboratory. The DNA concentration based on the ddCPR analysis was calculated as:

$$\mathrm{DNA_{conc}} = \frac{-\log_{10}(\text{number of negative droplets/total number of droplets})}{\text{drop volume}} \quad (1)$$

using a drop volume of 0.00085 µl. A standardized measure of DNA copies per liter of water was then calculated as:

$$\mathrm{DNA_{copies/L}} = \frac{(\mathrm{DNA_{conc}}/\mathrm{PCR\ volume_{ddPCR}}) * \text{Eluate volume}}{\text{Water volume}} \quad (2)$$

where PCR volume was 22 µl, Template volume was 5 µl and Eluate volume was 100 µl or 2 ml. Water volumes were either 1 or 10 L, depending on the type of filter used. Statistical modeling and graphics were conducted in R (R Core Team 2018). All plots were drawn using ggplot2 (Wickham, 2009), and the map was designed using ArcMap v10.6.

We modeled occupancy rates for *G. salaris and G. derjavinoides* eDNA using multilevel occupancy models in the eDNAoccupancy package in R (Dorazio & Erickson, 2017). Multilevel models estimated three probabilities: the probability of occupancy at a sampling site (psi or ψ), the probability of occupancy in a replicate water sample (theta or θ), and the probability of detection in a replicate PCR (p). Models were fit using MCMC methods and run for a total of 11,000 iterations. We assessed model convergence for estimated parameters with the plotTrace function of the eDNAoccupancy package. For most analyses, we used 11,000 iterations, including an initial burn-in period of 1,000 iterations that was discarded, and 10,000 iterations that were used for parameter estimation. For the analysis of *G. salaris* with glass fiber filters, we ran the model for 15,000 iterations, discarded a burn-in period of 5,000 iterations, and again used 10,000 iterations for parameter estimation. Data files and R scripts documenting our analysis are available as electronic supplements.

3 | RESULTS

We detected eDNA of *G. salaris*, *G. derjavinoides*, Atlantic salmon, and brown trout both downstream and upstream of the migration barrier (Figure 2). The concentration of eDNA had large variation

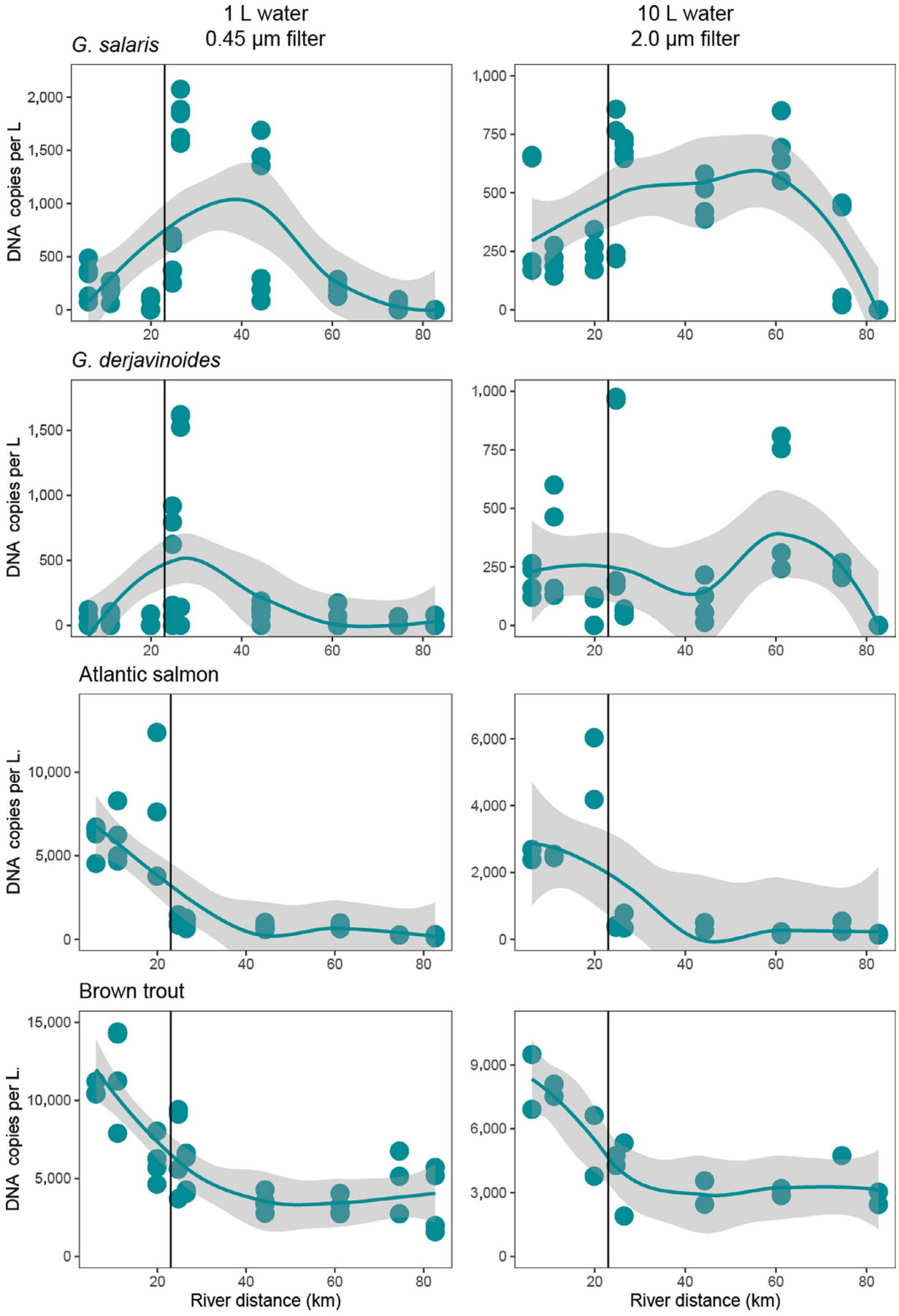
1 L water
0.45 µm filter
10 L water
2.0 µm filter
G. salaris
G. derjavinoides
Atlantic salmon
Brown trout
DNA copies per L
DNA copies per L.
River distance (km)

FIGURE 2 Number of DNA copies per liter of water for two gyrodactylid ectoparasites and their two salmonid hosts along the River Driva, Norway, 2017. Sampling protocols were based on analyses of 1 L water using a 0.45 µm cellulose filter (left panels) or 10 L water using a 2.0 glass fiber filter (right panels). The vertical line at ca. 25 km river distance indicates the position of the migration barrier separating the downstream (0–25 km) and upstream reaches (25–85 km). The green lines depict the smoothed conditional means with 95% confidence intervals in gray

among species and localities, with the two salmonid species having five to ten times higher eDNA concentrations than their parasites, and trout having higher concentrations than salmon. Both fish species showed an increasing eDNA-concentration downstream, with salmon also showing a distinct difference in eDNA-concentration downstream relative to upstream of the barrier (Figure 2). *G. salaris* showed the highest eDNA concentration in the middle part of the river, above the barrier, whereas *G. derjavinoides* appeared to have a more even eDNA concentration along the river (Figure 2).

The spatial pattern of eDNA concentrations of *G. salaris* did not match the eDNA concentrations of salmon (Figure 2), nor the estimated abundance of juvenile salmon (Figure 3) but appeared to be related to the estimated abundance of *G. salaris* based on electrofishing along the river (Figure 3).

Results of eDNA-occupancy modeling revealed a strong effect of the two different protocols on the likelihood of detecting the two parasite species (Table 2). The probability *p* of detecting the parasite in the PCR increased from .78 to .95 for *G. salaris* and from .49 to .94 for *G. derjavinoides* (Table 2) when analyzing 10 L of water on the 2.0 µm glass fiber filters compared with analyzing 1 L of water on the 0.45 µm cellulose filters. A small overlap in the 95% Bayesian Credible Interval (BCI) for *G. salaris* suggests that this increase was not statistically significant, whereas the likelihood of detecting *G. derjavinoides* was significantly different between the two protocols (Table 2). Estimates of the probability of the two species occurring at a sampling locality (*psi* = 0.84 and 0.84) or the probability of species-specific eDNA from the two species being present in a water sample (*theta* = 0.93 and 1.0) did not differ significantly between the two protocols (Table 2).

4 | DISCUSSION

In our study, we demonstrate the suitability of eDNA as an efficient method for the simultaneous assessment of Atlantic salmon, brown trout, and their two gyrodactylid ectoparasites. All four species could successfully be detected in water samples filtered on site and eDNA concentrations of *G. salaris* appeared to reflect parasite abundance along the river. Thus, eDNA monitoring represents a noninvasive methodology for assessing infection of *G. salaris* in Atlantic salmon, and our results may have important implications for monitoring ectoparasites in aquatic environments.

Spatial variation in eDNA concentrations indicates that the installation of an artificial migration barrier in the River Driva in 2017 has been a successful management action. For the two salmonid host species, eDNA concentrations increased downstream and were markedly higher below the migration barrier. For the two gyrodactylid species, and for *G. salaris* in particular, eDNA concentrations were highest in the middle part of the river and decreased downstream of the barrier. Rivers may pose a challenge for assessing local species presence and abundance using eDNA, as intra- and extracellular DNA can be transported long distances downstream (Deiner & Altermatt, 2014). However, a recent study on sockeye salmon (*Oncorhynchus nerka*) found that eDNA degrades below detectable levels within 1.5 km downstream and thus appears to reflect local abundance (Tillotson et al., 2018). The increasing fish eDNA-concentration downstream in this study could indicate accumulation of eDNA, but a higher density of fish or the presence of larger fish downstream could also explain this pattern. For salmon, eDNA concentrations reflected the expected difference in biomass upstream of and downstream of the barrier

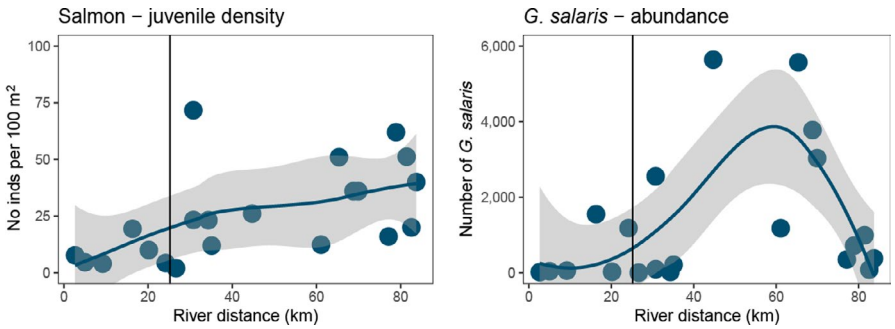

FIGURE 3 Density of juvenile salmon and estimated abundance of *Gyrodactylus salaris* based on conventional sampling using electrofishing at 21 localities in the River Driva, Norway, 2017. The estimated numbers were calculated as the product of mean density of juvenile salmon and mean number of *G. salaris* per fish, controlling both for infection rate and fish density at each locality. Data based on Solem et al. (2018) are listed in Table S3. The vertical line at ca. 25 km river distance indicates the position of the migration barrier that separates the downstream (0–25 km) and upstream reaches (25–85 km). The blue lines depict the smoothed conditional means with 95% confidence intervals in gray

TABLE 2 Parameter estimates from multilevel occupancy models for two species of gyrodactylid parasites in the River Driva, Norway, 2017

	Gyrodactylus salaris			*Gyrodactylus derjavinoides*		
Filter type	***psi***	***theta***	***p***	***psi***	***theta***	***p***
0.45 µm	0.84 (0.56–0.98)	0.94 (0.74–0.99)	.78 (.64–.89)	0.82 (0.50–0.99)	0.82 (0.52–0.99)	.49 (.31–.67)
2.0 µm	0.84 (0.56–0.98)	0.97 (0.78–1.0)	.95 (.84–.99)	0.85 (0.57–0.99)	0.90 (0.70–0.98)	.94 (.82–.99)

Note: Model parameters included occupancy per sampling site (psi), occupancy per water sample (theta), and probability of detection in a PCR (*p*) with 95% Bayesian credible intervals (BCI) in parentheses. Parameter estimates were taken from an intercept only model without covariates.

(Figure 2). No adult salmon, and no salmon-by-trout hybrids, were allowed to migrate past the barrier after 2017, and hence, only juveniles were present upstream of this point. Tributaries may pose another challenge, with supply of water either having higher or lower density of target DNA compared with the main river, thus affecting the final eDNA concentrations. An outlet from a local hydropower station below the barrier increases water flow in the River Driva and could potentially dilute eDNA concentrations in the main river below this point. Tributaries should be sampled in future studies to assess the amount of eDNA being introduced to the main river. Last, water temperatures often increase downstream in rivers and could increase degradation rates of eDNA, as well as activity and eDNA shedding rate of target species (Lacoursière-Roussel, Rosabal, & Bernatchez, 2016). The water temperature increased from 0.3 to 3.4°C from the highest to lowest locality in our study. DNA degradation is expected to be low within this range of temperatures.

Estimation of abundance based on eDNA concentrations can be hampered by catching entire specimens on the filter. Rusch et al. (2018) reported a single filter that had a 1,000-fold higher concentration of *G. salaris* than three other filters sampled at the same locality and presumed that the outlier was caused by catching one or more entire organism. We did not experience any such outliers in our data but making use of prefilters that would exclude entire specimens or larger fragments could be a possible solution in future studies.

The density of juvenile salmon and infection rates with *G. salaris* were investigated using electrofishing one to three months before our eDNA survey. The electrofishing study included more localities (N = 21) and surveyed different stretches of the river. However, despite different timing and localities, eDNA concentrations appeared to reflect levels of infection/abundance of *G. salaris* in juvenile Atlantic salmon (Figure 3) and did not reflect either salmon eDNA concentrations (Figure 2) or juvenile salmon density (Figure 3). Hence, our results suggest that in this 100 km long river stretch, eDNA can be used for both detection and assessing local abundance of *G. salaris*. Future studies should also analyze infection rates of *G. salaris* in different rivers and compare eDNA concentrations with electrofishing to assess whether this methodology also can detect differences in infection rates between rivers. Challenges for between-river comparisons include differences in water flow, temperature, turbidity, and acidity as well as differences in host–parasite relationships between rivers, all factors that could influence the concentration of *G. salaris* eDNA.

The eDNA-occupancy modeling revealed an effect of filter type and water volume on the probability of detecting the two parasite species, significantly so for *G. derjavinoides*. Although the coarser 2.0 µm glass fiber filter appeared to sample less DNA relative to the finer 0.45 µm cellulose filter, the possibility of filtrating much larger water volumes compensated for any potential loss. The number of DNA copies per liter water was lower for the glass fiber filter (Figure 2), but the probability of detection *p* was much higher (Table 2). Interestingly, neither the probability of species presence *psi*, nor the probability of eDNA present on the sample/filter *theta*, differed greatly between the two filter types. Hence, eDNA from the two gyrodactylid species is equally likely to be found in 1 L water as in 10 L water, but the limitation for detection seems to be found in the PCR analysis. The final species-specific DNA concentration of the DNA eluate used in the PCR is likely to be lower for the 0.45 µm than the 2.0 µm filter, and this difference could possibly explain the contrast in detectability. In this study, we used two separate DNA-isolation methods for the two filter types, which also could have influenced the final result. In addition, we also concentrated the eluate from the 2.0 µm glass fiber filter, as the isolation method using large 50-ml tubes requires an unusually large eluate volume (2 ml compared to 100 µl).

The genetic assay we used for *G. salaris* shows a low level of unwanted amplification of *G. derjavinoides* (Collins et al., 2010). Rusch et al. (2018) developed a new *G. salaris* eDNA assay, which also amplifies *G. derjavinoides* at low levels. However, they discovered that cross-amplification was not present when using ddPCR, and thus the issue should not be a problem for our analysis. Both assays also amplify *G. thymalli*, a parasite of grayling (*Thymallus thymallus*). Hence, detection of *G. salaris* may be problematic in river systems where Atlantic salmon co-occur with grayling infected by *G. thymalli*. Grayling was discovered in a tributary lake to the River Driva in 2015 after illegal release, but have not been detected in recent years. Positive amplification is thus indicative for *G. salaris* in our samples.

The artificial migration barrier in the River Driva presents an interesting case study for applied eDNA monitoring. We expect a gradual decrease over years in the eDNA concentrations of both Atlantic salmon and *G. salaris* in the upstream reaches as juvenile salmon die or those that survive migrate as smolts downstream of the barrier. We aim to document the reduction and eventual disappearance of the two species using eDNA in this river, and we plan to compare eDNA with conventional surveys using electrofishing to see how infection rate of *G. salaris* affects eDNA detectability.

Analyses of eDNA reduces both time and cost compared with traditional monitoring and does not require lethal sampling. We conclude that eDNA is an efficient way of monitoring gyrodactylid parasites and their hosts, and we suggest that eDNA analyses should be incorporated as part of the future monitoring for this invasive parasite species.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The study was designed and planned by FF and RS; RS and OP conducted the fieldwork; HB performed the laboratory work; BKS completed the occupancy modeling; ØS collected the conventional data; FF and TAM drafted the first version of the manuscript, and all authors contributed substantially to the final version.

DATA AVAILABILITY STATEMENT

Data relating to this article and R scripts for the occupancy modeling can be found as supplementary material.

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REFERENCES

Bass, D., Stentiford, G. D., Littlewood, D. T. J., & Hartikainen, H. (2015). Diverse applications of environmental DNA methods in parasitology. *Trends in Parasitology*, *31*(10), 499–513. https://doi.org/10.1016/j.pt.2015.06.013

Bohlin, T., Hamrin, S., Heggberget, T. G., Rasmussen, G., & Saltveit, S. J. (1989). Electrofishing - theory and practice with special emphasis on salmonids. *Hydrobiologia*, *173*, 9–43. https://doi.org/10.1007/BF00008596

Collins, C. M., Kerr, R., McIntosh, R., & Snow, M. (2010). Development of a real-time PCR assay for the identification of *Gyrodactylus* parasites infecting salmonids in northern Europe. *Diseases of Aquatic Organisms*, *90*(2), 135–142. https://doi.org/10.3354/dao02201

Deiner, K., & Altermatt, F. (2014). Transport distance of invertebrate environmental DNA in a natural river. *PLoS One*, *9*(2), e88786. https://doi.org/10.1371/journal.pone.0088786

Dobnik, D., Spilsberg, B., Bogožalec Košir, A., Holst-Jensen, A., & Žel, J. (2015). Multiplex quantification of 12 European Union authorized genetically modified maize lines with droplet digital polymerase chain reaction. *Analytical Chemistry*, *87*(16), 8218–8226. https://doi.org/10.1021/acs.analchem.5b01208

Doi, H., Inui, R., Akamatsu, Y., Kanno, K., Yamanaka, H., Takahara, T., & Minamoto, T. (2017). Environmental DNA analysis for estimating the abundance and biomass of stream fish. *Freshwater Biology*, *62*(1), 30–39. https://doi.org/10.1111/fwb.12846

Dorazio, R. M., & Erickson, R. A. (2017). eDNAoccupancy: An R package for multiscale occupancy modelling of environmental DNA data. *Molecular Ecology Resources*, *18*, 368–380. https://doi.org/10.1111/1755-0998.12735

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, *4*(4), 423–425. https://doi.org/10.1098/rsbl.2008.0118

Forseth, T., Barlaup, B. T., Finstad, B., Fiske, P., Gjøsæter, H., Falkegård, M., … Wennevik, V. (2017). The major threats to Atlantic salmon in Norway. *Ices Journal of Marine Science*, *74*(6), 1496–1513. https://doi.org/10.1093/icesjms/fsx020

Goldberg, C. S., Pilliod, D. S., Arkle, R. S., & Waits, L. P. (2011). Molecular detection of vertebrates in stream water: A demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PLoS ONE*, *6*(7), e22746. https://doi.org/10.1371/journal.pone.0022746

Gustavson, M. S., Collins, P. C., Finarelli, J. A., Egan, D., Conchúir, R. Ó., Wightman, G. D., … Carlsson, J. (2015). An eDNA assay for Irish *Petromyzon marinus* and *Salmo trutta* and field validation in running water. *Journal of Fish Biology*, *87*(5), 1254–1262. https://doi.org/10.1111/jfb.12781

Hansen, H., Bachmann, L., & Bakke, T. A. (2003). Mitochondrial DNA variation of Gyrodactylus spp. (Monogenea, Gyrodactylidae) populations infecting Atlantic salmon, grayling, and rainbow trout in Norway and Sweden. *International Journal for Parasitology*, *33*(13), 1471–1478.

Hindar, A., Garmo, Ø., Hagen, A. G., Hytterød, S., Høgberget, R., Moen, A., & Olstad, K. (2015). *Treatment with AIS to eradicate the salmon parasite Gyrodactylus salaris in River Lærdalselva in 2011 and 2012*. Norwegian Institute for Water Research, Report, 6701–2014.

Huver, J. R., Koprivnikar, J., Johnson, P. T. J., & Whyard, S. (2015). Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. *Ecological Applications*, *25*(4), 991–1002. https://doi.org/10.1890/14-1530.1.sm

Hytterød, S., Darrud, M., Johansen, K., Larsen, S., Mohammad, S. N., & Hansen, H. (2018). *The surveillance programme for Gyrodactylus salaris in Atlantic salmon and rainbow trout in Norway 2017 - annual report*. Norwegian Veterinary Institute, Annual Report.

Jansen, P. A., & Bakke, T. A. (1995). Susceptibility of brown trout to *Gyrodactylus salaris* (Monogenea) under experimental conditions. *Journal of Fish Biology*, *46*(3), 415–422. https://doi.org/10.1111/j.1095-8649.1995.tb05981.x

Jansen, P. A., Matthews, L., & Toft, N. (2007). Geographic risk factors for inter-river dispersal of *Gyrodactylus salaris* in fjord systems in Norway. *Diseases of Aquatic Organisms*, *74*(2), 139–149. https://doi.org/10.3354/dao074139

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, *4*(2), 150–157. https://doi.org/10.1111/j.1755-263X.2010.00158.x

Johnsen, B. O., Brabrand, Å., Jansen, P. A., Teien, H.-C., & Bremset, G. (2008). Evaluering av bekjempelsesmetoder for *Gyrodactylus salaris*. *Rapport Fra Ekspertgruppe. Utredning for DN. 7*, 1–140.

Johnsen, B. O., & Jensen, A. J. (1991). The Gyrodactylus story in Norway. *Aquaculture*, *98*(1), 289–302. https://doi.org/10.1016/0044-8486(91)90393-L

Johnsen, B. O., Møkkelgjerd, P. I., & Jensen, A. J. (1999). The parasite *Gyrodactylus salaris* on salmon parr in Norwegian rivers, status report at the beginning of year 2000. *NINA Oppdragsmelding*, *617*, 1–129.

Karlsson, S., Hagen, M., Eriksen, L., Hindar, K., Jensen, A. J., de Leaniz, C. G., … Ryman, N. (2013). A genetic marker for the

maternal identification of Atlantic salmon x brown trout hybrids. *Conservation Genetics Resources*, *5*(1), 47–49. https://doi.org/10.1007/s12686-012-9730-6

Lacoursière-Roussel, A., Rosabal, M., & Bernatchez, L. (2016). Estimating fish abundance and biomass from eDNA concentrations: Variability among capture methods and environmental conditions. *Molecular Ecology Resources*, *16*(6), 1401–1414. https://doi.org/10.1111/1755-0998.12522

Miljødirektoratet. (2014). *Handlingsplan mot lakseparasitten Gyrodactylus salaris for perioden 2014–2016*.

Mo, T. A. (1991). Variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) in a fish farm, with comments on the spreading of the parasite in south-eastern Norway. *Systematic Parasitology*, *20*(1), 1–9. https://doi.org/10.1007/bf00009706

Mo, T. A. (1997). Seasonal occurrence of *Gyrodactylus derjavini* (Monogenea) on brown trout, *Salmo trutta*, and Atlantic salmon, *S. salar*, in the Sandvikselva river, Norway. *The Journal of Parasitology*, *83*(6), 1025–1029. https://doi.org/10.2307/3284356

Paladini, G., Hansen, H., Williams, C. F., Taylor, N. G., Rubio-Mejía, O. L., Denholm, S. J., … Shinn, A. P. (2014). Reservoir hosts for *Gyrodactylus salaris* may play a more significant role in epidemics than previously thought. *Parasites and Vectors*, *7*(1), 576. https://doi.org/10.1186/s13071-014-0576-5

Pyšek, P., & Richardson, D. M. (2010). Invasive species, environmental change and management, and health. *Annual Review of Environment and Resources*, *35*(1), 25–55. https://doi.org/10.1146/annurev-environ-033009-095548

R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, *51*(5), 1450–1459. https://doi.org/10.1111/1365-2664.12306

Rice, C. J., Larson, E. R., & Taylor, C. A. (2018). Environmental DNA detects a rare large river crayfish but with little relation to local abundance. *Freshwater Biology*, *63*(5), 443–455. https://doi.org/10.1111/fwb.13081

Rusch, J. C., Hansen, H., Strand, D. A., Markussen, T., Hytterød, S., & Vrålstad, T. (2018). Catching the fish with the worm: A case study on eDNA detection of the monogenean parasite *Gyrodactylus salaris* and two of its hosts, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). *Parasites and Vectors*, *11*(1), 333. https://doi.org/10.1186/s13071-018-2916-3

Solem, Ø., Aalbu, F., & Mo, T. (2018). *Ungfiskundersøkelser i Drivavassdraget. Årsrapport 2017*. Norwegian Institute for Nature Research, NINA Rapport 1417.

Soleng, A., & Bakke, T. A. (1997). Salinity tolerance of *Gyrodactylus salaris* (Platyhelminthes, Monogenea): Laboratory studies. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(8), 1837–1864. https://doi.org/10.1139/f97-089

Spens, J., Evans, A. R., Halfmaerten, D., Knudsen, S. W., Sengupta, M. E., Mak, S. S. T., … Hellström, M. (2017). Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: Advantage of enclosed filter. *Methods in Ecology and Evolution*, *8*(5), 635–645. https://doi.org/10.1111/2041-210x.12683

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012). Environmental DNA. *Molecular Ecology*, *21*(8), 1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Taugbøl, A., Dervo, B. K., Bærum, K. M., Brandsegg, H., Sivertsgård, R., Ytrehus, B., … Fossøy, F. (2017). Første påvisning av den patogene soppen *Batrachochytrium dendrobatidis* (Bd) i Norge - Bruk av miljø-DNA for påvisning av fremmede arter. *Norwegian Institute for Nature Research*, *NINA Rapport*, *1399*, 25.

Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., … Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, *21*(11), 2565–2573. https://doi.org/10.1111/j.1365-294X.2011.05418.x

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, *183*, 4–18. https://doi.org/10.1016/j.biocon.2014.11.019

Tillotson, M. D., Kelly, R. P., Duda, J. J., Hoy, M., Kralj, J., & Quinn, T. P. (2018). Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. *Biological Conservation*, *220*, 1–11. https://doi.org/10.1016/j.biocon.2018.01.030

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., … Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, *25*(4), 929–942. https://doi.org/10.1111/mec.13428

Wickham, H. (2009). *ggplot2: Elegant graphics for data analysis*. New York, NY: Springer-Verlag.

Zippin, C. (1958). The removal method of population estimation. *The Journal of Wildlife Management*, *22*(1), 82–90. https://doi.org/10.2307/3797301

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