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ORIGINAL ARTICLE

Use of environmental DNA to detect the myxozoan endoparasite *Tetracapsuloides bryosalmonae* in large Norwegian lakes

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Abstract

Environmental DNA (eDNA) is an increasingly used noninvasive and cost-effective method for species detections in surveillance studies. The myxozoan endoparasite *Tetracapsuloides bryosalmonae* is the causative agent of proliferative kidney disease (PKD) in salmonid fish. PKD is a potentially lethal disease of freshwater salmonids when water temperatures exceed 12–14°C for prolonged periods. Periodically, high mortality and decline in farmed and wild salmonid populations in Europe and North America have been reported in the last decades. The aim of this study was to use eDNA as a method to detect *Tetracapsuloides bryosalmonae* from large, deep, dimictic Norwegian lakes. Such habitats are expected to become increasingly important for cold-water salmonids with global warming. Samples were collected from five lakes in southeastern Norway, and parasite DNA was detected by qPCR. eDNA from *T. bryosalmonae* was detected in four of the five lakes surveyed. These findings corresponded with the detection of *T. bryosalmonae* DNA in salmonid kidneys in four of the lakes in a previous survey. The detection of parasites from eDNA varied between years and stations within the same lake, revealing a changing and apparently stochastic spatial distribution of parasite DNA from year to year. Nonetheless, by sampling multiple sites throughout the lakes, we were able to detect *T. bryosalmonae* at the lake level in both survey years. Strategies for eDNA sampling in deep, dimictic lakes are discussed.

KEYWORDS
deep lakes, dimictic, eDNA, sampling strategy, *Tetracapsuloides bryosalmonae*

1 | INTRODUCTION

Environmental DNA (eDNA) is increasingly used as a cost-effective and sensitive supplement to traditional sampling methods in surveys and monitoring programs (Kumar et al., 2020; Taberlet et al., 2012; Valentini et al., 2016). The methods are considered particularly promising when targeting rare or recent invasive species that in general are difficult to detect (Thomsen et al., 2012; Valentini

et al., 2016). eDNA is intra- or extracellular DNA (Pietramellara et al., 2008; Taberlet et al., 2012) collected from a range of environmental samples such as soil, seawater, snow, or air, rather than directly sampled from an individual organism (Ficetola et al., 2008; Taberlet et al., 2012), and can be used for detection of single species or as a substrate for eDNA metabarcoding (where a large number of species are detected simultaneously) (Taberlet et al., 2012; Taberlet et al., 2018). In lakes, eDNA has been used to detect the occurrence and distributions of aquatic parasites since 2014. (Ficetola et al., 2008; Strand et al., 2014).

All parasites depend on their hosts to live and complete their life cycles. Endoparasites, such as myxozoans - which are normally concealed within their hosts, can be particularly difficult to detect and sample in the environment (Bass et al., 2015; Duval et al., 2021; Okamura et al., 2018). Traditional surveys therefore mostly rely on lethal sampling of the host to detect the parasite (Huver et al., 2015; Rusch et al., 2018). eDNA offers sampling and detection of genetic material released from a parasite, without capturing or killing its host (Bass et al., 2015; Duval et al., 2021; Hallett & Bartholomew, 2006; Huver et al., 2015; Rusch et al., 2018).

The myxozoan endoparasite *Tetracapsuloides bryosalmonae* is the causative agent of proliferative kidney disease (PKD), which is a potentially lethal disease of freshwater salmonids when water temperatures exceed 12–14°C for prolonged periods (Hedrick et al., 1993; Okamura et al., 2011; Waldner et al., 2021). PKD has caused high mortality and decline in both wild and farmed populations of salmonid species in Europe and North America for more than four decades (Ferguson & Needham, 1978; Hedrick et al., 1993; Hutchins et al., 2021; Okamura et al., 2011; Opitz & Rhoten, 2016; Ros et al., 2021; Schmidt-Posthaus et al., 2015; Sterud et al., 2007; Svavarsdottir, 2016; Wahli et al., 2002). PKD is still considered an emerging disease as it manifests in new ways, expands to new areas, and increases in severity with an increasingly warm climate (Okamura et al., 2011; Okamura & Feist, 2011; Rubin et al., 2019; Tops et al., 2009).

T. bryosalmonae has a life cycle alternating between the primary bryozoan and an intermediate salmonid host (Anderson et al., 1999; Canning et al., 1999; Longshaw et al., 2002; Okamura et al., 2011) (Figure 1). The parasite produces two morphologically different spores in its two hosts (Feist et al., 2002; Gay et al., 2001; Grabner & El-Matbouli, 2008; Syrová et al., 2020), and rising water temperature can enhance the proliferation of the parasite in both hosts (Bettge, Segner, et al., 2009; Ros et al., 2021; Rubin et al., 2019; Tops et al., 2006; Wahli et al., 2008). Bryozoans are known to live on surfaces that are sheltered from light, sedimentation deposits, and water movements, e.g., submerged plants, dead trees and roots, rocks, piers, and boat fenders (Økland & Økland, 2006; Wood & Okamura, 2005) and are able to produce resistant spores (statoblasts) that can survive under very harsh conditions (Økland & Økland, 2006; Wood & Okamura, 2005). In Norway, most surveys have sampled bryozoans from sheltered substrates in rivers and in the upper 1.5 meters of the littoral zone of ponds and lakes (Økland & Økland, 2005; Økland & Økland, 2006), but there are also reports of *Fredericella sultana* from Norwegian oligotrophic lakes down to 20 meters (Raddum & Johnsen, 1983) and, in some Swiss lakes, even down to 100- and 214-m depth (p. 356 in Marcus (1940)). In Europe, species within the class Phylactolaemata, specifically the two species *F. sultana* and *Plumatella emerginata*, have been found to be the most abundant hosts of *T. bryosalmonae* (Bendixby & Hals, 2009; Hartikainen et al., 2014; Okamura & Wood, 2002; Ros et al., 2021). The parasite has also been observed in *Cristatella mucedo* and *Pectinella magnifica* in the USA (Hartikainen et al., 2014).

The presence of *T. bryosalmonae* has so far mainly been documented by the lethal sampling of its hosts, by analysis of blood or tissue samples from salmonids, and less frequently, parts of bryozoan colonies. However, the use of eDNA for detecting the parasite is evolving and an increasing number of surveys are using eDNA to detect *T. bryosalmonae*, so far mainly from rivers (Fontes et al., 2017; Hutchins et al., 2017; Sepulveda et al., 2020; Sepulveda et al., 2021), and in controlled experiments (Duval et al., 2021; Sieber et al., 2020). In these surveys, samples are analyzed by conventional polymerase chain reactions (PCR), real-time PCR (qPCR), or droplet digital PCR (ddPCR), using species-specific primers (Carraro et al., 2018; Fontes

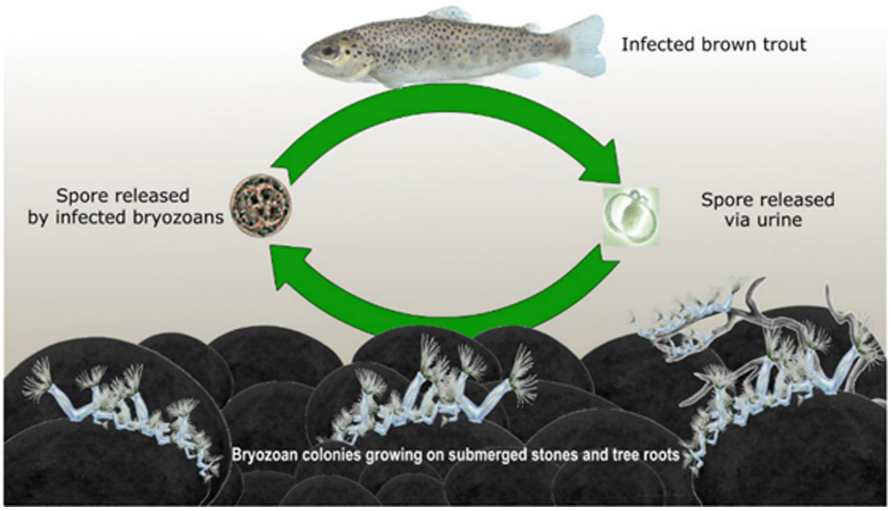

FIGURE 1 The life cycle of *Tetracapsuloides bryosalmonae*, alternating between the primary invertebrate sessile bryozoan host and the intermediate salmonid fish host. Figure from (Sudhagar et al., 2019)

et al., 2017; Hutchins et al., 2017; Sepulveda et al., 2020; Sepulveda et al., 2021).

There are only a few studies on the presence and distribution of *T. bryosalmonae* in deep, large lakes and to the best of our knowledge, no work has been published on *T. bryosalmonae* using water samples and eDNA from large, deep lakes. Deep lakes, with their large cold hypolimnion, are important habitats for many cold-adapted salmonids in the northern hemisphere and their importance might increase in a warmer climate (Gaudard et al., 2018; Oredalen et al., 2022). Two recent surveys have detected parasite DNA in kidney samples from different salmonid species in deep, large lakes (Naldoni et al., 2019; Oredalen et al., 2022), but in general, surveys of wild salmonids have focused on *T. bryosalmonae* and PKD in rivers and shallow lakes (Feist et al., 2002; Hutchins et al., 2021; Kristmundsson et al., 2010; Lewisch et al., 2018; Mo & Jørgensen, 2017; Sterud et al., 2007; Svavarsdottir, 2016; Svavarsdóttir et al., 2021; Wahli et al., 2007).

eDNA studies conducted for species detection in large lakes, focusing primarily on fish (Hänfling et al., 2016; Lawson Handley et al., 2019; Zhang et al., 2020), have highlighted several challenges in terms of detection probability and sampling design due to the "ecology of DNA" (Barnes & Turner, 2016). These challenges include the origin, the state, the transport, and the fate of the DNA in the environment (Barnes & Turner, 2016). Several works have highlighted important factors, including persistence, partly due to different DNA degradation rates that are seen to be higher in marine inshore than in offshore environment (Collins et al., 2018; Dejean et al., 2011; Lawson Handley et al., 2019; Thomsen et al., 2012; Zhang et al., 2020), and temporal and spatial variations in density and distribution of species (Hänfling et al., 2016; Lawson Handley et al., 2019; Zhang et al., 2020). In large, deep, dimictic lakes, detection probability becomes complex and challenging due to the patchy distribution of species and the physical properties of these lakes with large water volumes and periodic mixing and stratification, resulting in elevated summer surface temperatures (Hänfling et al., 2016; Lawson Handley et al., 2019; Zhang et al., 2020). The patchy distribution often observed in lentic waters is due not only to the patchy distribution of the source species but also to variations in degradation and persistence of eDNA (Dejean et al., 2011; Eichmiller et al., 2014; Thomsen et al., 2012), and that eDNA remains closer to its source of production than in lotic habitats (Bedwell & Goldberg, 2020; Dunker et al., 2016). This can lead to the absence or very low concentrations of target DNA in samples taken outside of "hotspots" (Dunker et al., 2016; Eichmiller et al., 2014; C. S. Goldberg et al., 2016), and where present, concentrations below or close to the limit of detection (LOD), the lowest concentration of target analyte that can be detected with confidence, as described in Klymus et al. (2020) and references within. However, the authors point out that even if positive PCR detections from eDNA are sub-LOD, they may be true positives and meaningful, albeit with a reproducibility less than desired (Klymus et al., 2020).

For selecting optimal sampling time and locations in large, deep lakes, the physical, chemical, and ecological processes, and the ecology of target organisms in each lake should be carefully considered (Dejean et al., 2011; Dunker et al., 2016; Eichmiller et al., 2014). Sampling and analysis replicates should always be included to increase sensitivity and reliability when working with eDNA (Dejean et al., 2011; Goldberg et al., 2016).

This survey aimed to use eDNA, extracted from water samples from representative stations, to detect the myxozoan parasite *T. bryosalmonae* in five Norwegian deep, large, dimictic clearwater lakes using real-time PCR. Our hypothesis is that this method can detect parasite presence in large lakes despite the challenges in sampling design and analytical constraints in lentic systems.

2 | MATERIALS AND METHODS

2.1 | Study area

Water samples were collected during late summer in 2018 and 2019 from five deep, clearwater, dimictic lakes in South-eastern Norway: Lake Fyresvatn (6 and 5 stations, respectively), Lake Totak (6 and 5 stations), Lake Møsvatn (3 stations both years), Lake Tinnsjå (6 and 7 stations), and Lake Norsjø (6 and 5 stations) (Figure 2). Sampling was conducted after a warm summer period with elevated water temperatures when we expected the highest probability of encountering parasite spores. All five lakes are nutrient-poor and have been part of watercourses regulated for hydropower purposes for more than 50 years (Appendix 0). The regulation zone, between the highest regulated water level (HRV) and lowest regulated water level (LRV) of four of the lakes, varies between 4 and 18.5 meters (Appendix 0). Lake Norsjø (with a regulation zone of only 0.3 meters) was sampled at HRV both years, while the other lakes were sampled between HRV and LRV (Appendix 0). The surface water temperatures above the deepest area of the lakes were in all cases above 12°C at the time of sampling (Appendix 0).

2.2 | eDNA sampling

Samples were collected by hand (using disposable gloves) in 1 L polypropylene bottles just beneath the water surface at all stations. The upper meter of lake shoreline, mainly by the mouths of inlet rivers and the outlet river, were chosen as sampling stations in each lake. Most of the stations were dominated by large rocks and shingle with little noticeable benthic organisms present. A few stations deviated from this picture, such as Lake Norsjø, the inlet of Lake Fyresvatn (station 1.1), and the outlet of Lake Tinnsjå (station 2.5) where the stations showed a pronounced presence of periphyton, macrophytes, and benthos, and some detritus and sand. In addition, one sample was collected from surface waters above the deepest area of each lake (selected from depth maps), a commonly used site for limnological sampling.

At every station, two sampling replicates (treated separately) were collected during the survey in August 2018 (54 samples collected from 27 stations) and three sampling replicates in September

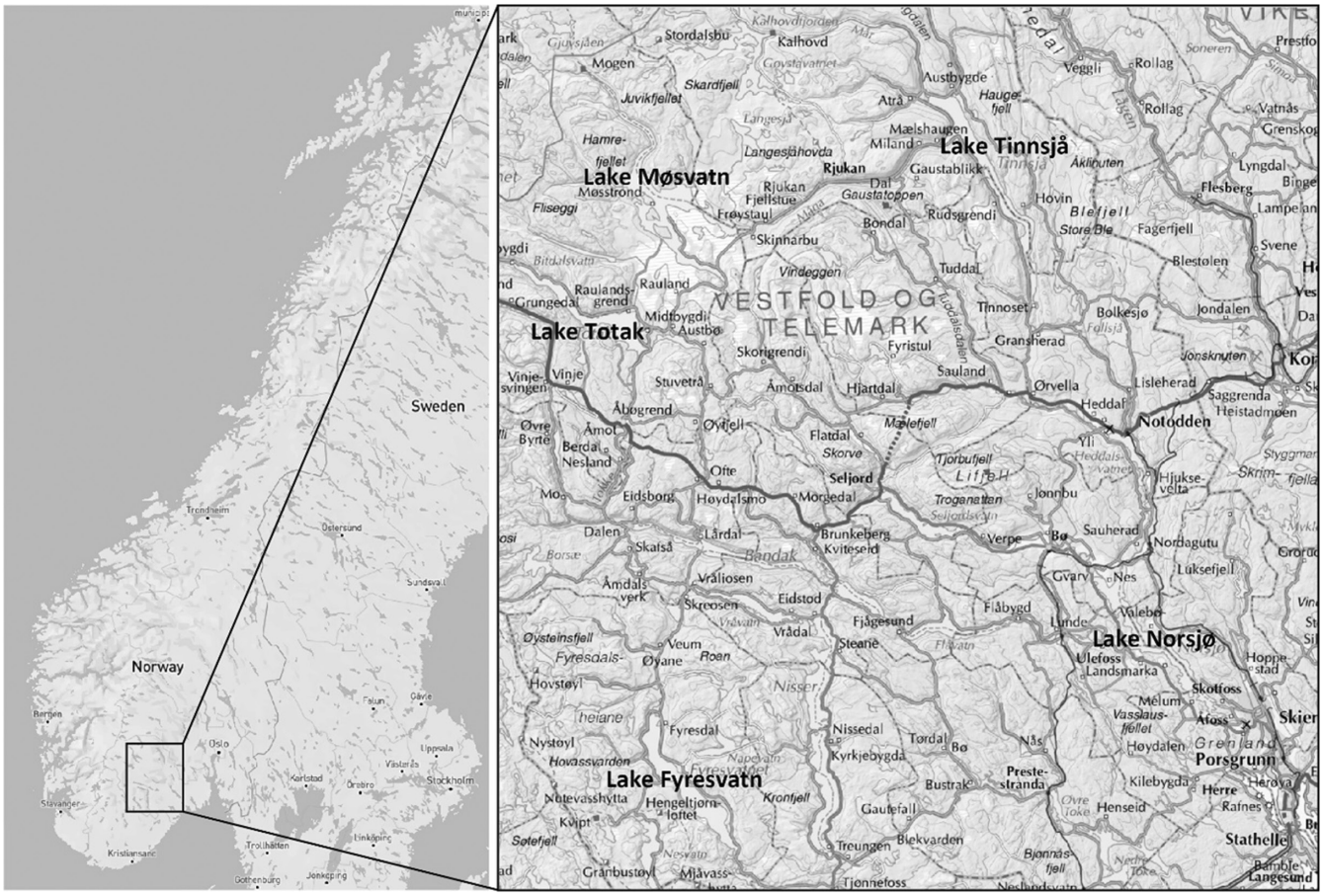

FIGURE 2 Lake localizations in southeastern part of Norway of the five deep, dimictic lakes included in the survey: Lake Fyresvatn, Lake Tinnsjå, Lake Totak, Lake Møsvatn, and Lake Norsjø

TABLE 1 Number of biological replicates with different filtration volumes in 2018 and 2019, and total numbers of biological replicates filtrated both years

	No. of biological replicates analyzed		
	Both years	2018	2019
Total no. of b. replicates:	128	54	74
Filtration volumes:			
B.replicate = 1000 ml	101 (78.9%)	53 (98.1%)	48 (64.9%)
B.replicate <1000 ml	27 (21.1%)	1 (1.9%)	26 (35.1%)
B.replicate =650 ml	3 (2.3%)	0	3 (4.1%)
B.replicate = 850 ml	1 (0.8%)	0	1 (1.4%)
B.replicate 900 ml	5 (3.9%)	0	5 (6.8%)
B.replicate = 910 ml	1 (0.8%)	1 (1.9%)	0
B.replicate = 950 ml	17 (13.3%)	0	17 (23.0%)

2019 (75 samples collected from 25 stations). Three field blanks were included in 2018 and followed the same analytic procedures as the other samples. No field blanks were included in 2019.

Cleaning of equipment and sampling procedure followed C. Goldberg and Strickler (2017), with the exception of using 10% hypochlorite instead of 50% for cleaning of bottles. All sampling bottles (new, 1 Liter, polypropylene) were cleaned in the laboratory before going into the field as follows: first, a standard machine wash for laboratory equipment, then soaking in 10% chlorine for 1 min, and subsequent rinsing three times with distilled water. Before sampling, the bottles were rinsed three times with lake water at each site. All samples were collected by filling the bottles with lake water just beneath the surface, using fresh disposable gloves (VWR Latex, powder free) during sampling at each station. Sampling bottles filled with distilled water served as blanks and followed the same procedures as the samples in subsequent filtering and analyses.

Samples were stored in cooling boxes with cooler bricks during transport from the field to the laboratory and each replicate was

filtered within 24 h. One liter was filtered through sterile disposable 0.45 µm cellulose nitrate filter funnels (47 mm) (Pall corporation, Ann Arbor, MI, USA). Occasionally filters clogged, in which case filtration was stopped after approximately 15 min - and the filtrate volume was measured (Appendix 1). Less than 1000 ml was filtered in 1 biological replicate (1.9%) in 2018 and in 26 replicates (35.1%) in 2019 (Table 1). Of the 26 biological replicates with reduced filtration volume in 2019, 950 ml was filtered in 17 (65.4%) of them. Forceps used for filter handling were cleaned in 50% chlorine for 1 min and rinsed in distilled water between each sample.

Filters from samples collected in 2018 were folded and placed in cryo-vials (Thermo scientific Nalgene Cryoware Cryogenic Vials, catalog number 5000–0020) with 1440 µl ATL buffer (Fossøy et al., 2020; Majaneva et al., 2018) and kept at room temperature approximate 1 year until DNA extraction with Blood and Tissue Kit (Qiagen). Filters from samples collected in 2019 were gently rolled up and placed into tubes from Power Water Kit (Qiagen), and stored for approximately 1 month at −20°C before extraction.

2.3 | Molecular analysis

All DNA extractions were performed in a dedicated pre-PCR laboratory, physically separated from any equipment or products from the post-PCR laboratory. Extraction of filters from 2018 followed the protocol of Blood and Tissue Kit with some modifications, due to a larger volume of ATL buffer than described in the protocol: each filter with corresponding ATL buffer was transferred to a 2.5 ml tube, with 160 µl proteinase-K and incubated for 16 h on a Thermomixer Comfort (Eppendorf AG) at 56°C and 500 rpm. After incubation, the filters were thoroughly manually squeezed using two pairs of forceps to express trapped fluid and then discarded. Subsequently, the buffer-proteinase solution was centrifuged in three steps (due to the large sample volume) through the DNeasy Mini spin columns provided in the extraction kit. Volumes used were 600 µl (×2) and 400 µl, with the addition of the corresponding proportion of ethanol/AL-buffer prescribed in the protocol. Further processing followed the standard procedure of the kit except that, since we expected small amounts of *T. bryosalmonae* DNA in the water, the elution volume was reduced from 200 µl to 100 µl to increase the concentration.

Samples from 2019, stored in tubes from Power Water Kit, followed the kit protocol with one adjustment: an additional heating step was added between steps 5 and 6 for increased lysis.

DNA concentration and purity of undiluted samples were measured by NanoDrop™ Lite Spectrophotometer (Thermo Fisher Scientific) after extraction in order to verify DNA content and the comparability of the two extraction methods (Appendix 2).

DNA extractions were analyzed by real-time PCR (qPCR) (Applied Biosystems StepOne™ and StepOne Plus™ Real-Time PCR systems) on MicroAmp Fast 96-Well Reaction Plates (0.1 ml). Primers specific for 18S rDNA of *T. bryosalmonae* (Bettge, Wahli, et al., 2009) were used: Forward primer PKDtaqf1: 5′-GCG AGA TTT GTT GCA TTT AAA AAG-3′ and reverse primer PKDtaqr1: 5′-GCA CAT GCA GTG TCC AAT CG-3′, giving a target sequence of 73 base pair amplicons (Bettge, Segner, et al., 2009; Bettge, Wahli, et al., 2009). As a positive control and for quantification purposes, a synthetic plasmid, pTbr, which is the cloning vector pUC57 with an insertion of the *T. bryosalmonae* target sequence, was obtained from GenScript (Piscataway, NJ, USA).

qPCR amplification was performed using a 25 µl reaction volume, containing 12.5 µl Taqman Environmental Mastermix 2×, 1 µl each of forward and reverse primers (10 µM), 0.5 µl TaqMan MGB probe (10 µM, 5′- AGT CGG ACG GTT CCA-3′, produced at Thermofisher), 5 µl sterile water (Fresenius Kabi Norge A/S) and 5 µl undiluted sample DNA. The qPCR program consisted of an initial 10-min heating step at 95°C, followed by 45 cycles of 15 s at 95°C and 1 min at 60°C. In each qPCR-run a standard curve (dilution series of plasmid from 10^6 to 1 copy number was included) (Figure 3), and two or three replicates of negative control (RNase-Free Water, Qiagen GmbH) and two replicates of positive control (fish-kidneys positive to *T. bryosalmonae* DNA). Each sampling replicate was analyzed in qPCR triplicates.[1]

2.4 | Sensitivity of qPCR analysis

The limit of detection, LOD, was calculated according to the procedure described by Klymus et al. (2020) using an R-script provided by Merkes et al. (2019). Calculations were based on qPCR analyses of dilution series of the control plasmid pTbr with 10 replicates of each concentration ranging from 10^6 to 1 DNA copies/reaction, and further 10 replicates of each concentration of 100, 80, 60, 40, 20 DNA copies/reaction. Effective LOD for 95% probability of detection in at least one of three replicates was calculated using the curve fitting method (Merkes et al., 2019) as to 10.33 DNA copies/reaction (standard error of 0.55) and rounded down to 10 DNA copies in further discussion (Figure 4).

Positive results were stratified relative to LOD and classified as above LOD and sub-LOD. A biological replicate was considered positive if at least one of the three PCR replicates was positive (with a quantification cycle [C_q] below 40). A station was considered positive if at least one of the biological replicates were positive.

Even when below the LOD, positive signals are still considered meaningful, since truly negative samples will give no signal response (Ellison et al., 2006; Hunter et al., 2017). However, to be considered as a "true" positive, the samples should parallel the controls in the qPCR multicomponent plot. Thus, in this work, additional analysis of the indicative presence of *T. bryosalmonae* in the five lakes was performed, based on positive detections sub-LOD. However, unless otherwise stated, results always refer to values above LOD.

2.5 | Inhibition

In testing for possible inhibition of qPCR by the samples, we assumed similar water quality and inhibition agents in all replicates

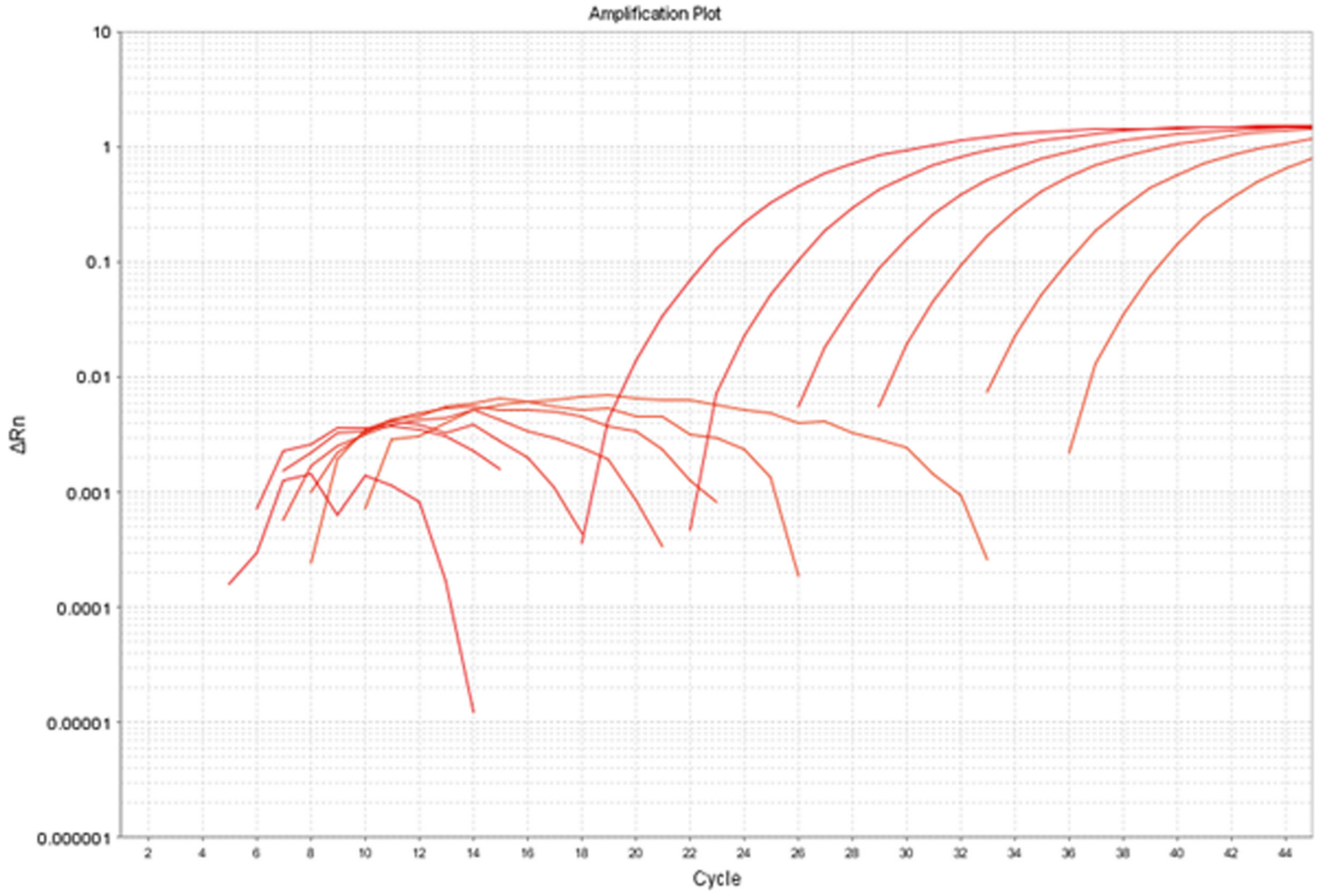

FIGURE 3 Example of standard curve from qPCR, ranging from 10^6 to 10 DNA copies

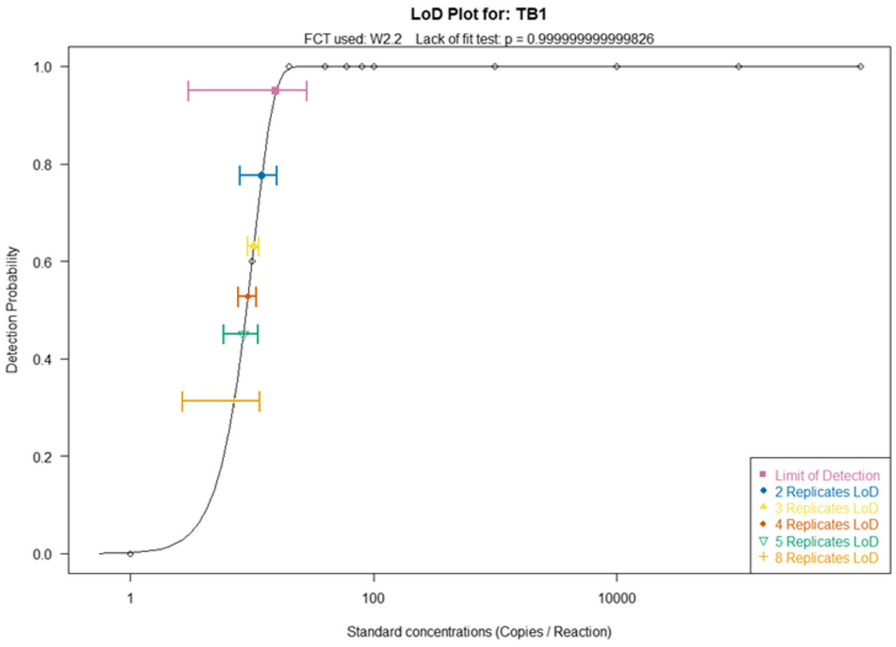

FIGURE 4 LOD for different numbers of biological replicates. By the use of 3 replicates, the LOD is estimated to 10 DNA copies per PCR run

taken at each station. One sampling replicate from each station and year (51 samples) was spiked with 1 µl of positive control, containing 2000 DNA copies of the plasmid. The spiked samples were processed through the standard qPCR protocol used for all samples. It was considered as evidence of inhibition if a qPCR sample had a shift > = 3 in C_T-value, or a reduction in endpoint fluorescence by > = 50%, as advised by Hartman et al. (2005) and Goldberg et al. (2016).

2.6 | Data analysis

Data from qPCR were stored and extracted from StepOne Software v2.3. and further processed in Excel for Microsoft 365 MSO with statistical summaries shown in the tables. All raw data are attached in Appendix 1. The data include 3 to 7 stations per lake over 2 years (2018–2019), 2–3 biological replicates per station, and 3 PCR replicates per biological replicate. The dataset, with a relatively small number of samples, does not allow for more elaborated statistical tests.

The calculation of LOD was performed in a modified R-script from Merkes et al. (2019), which is available at USN figshare; doi.org/10.23642/usn.19387472.v1and doi.org/10.23642/usn.19387469.v1.

3 | RESULTS

All positive detections of *T. bryosalmonae* in all five lakes are shown in Table 2. The highest number of DNA copies from the parasite was observed in the samples from 2019, and the highest number of parasite DNA was detected at the shoreline/boat jetty (1.3) in Lake Fyresvatn, by an inlet river (1.1) in Lake Fyresvatn, by an inlet river in Lake Norsjø (5.1) and by the outlet river in Lake Tinnsjå (Table 2).

T. bryosalmonae was detected above LOD in four of the five lakes in both years (Table 3a). In these four lakes, the naïve occupancy (the frequency of stations with positive detections of *T. bryosalmonae*) varied from 0.2 in Lake Norsjø and Lake Tinnsjå in 2018 to 0.7 in Lake Møsvatn in 2019 (Table 3a).

A few qPCR samples gave positive detections solely with values sub-LOD, in Lake Fyresvatn and Lake Totak, indicating a possibly higher naïve occupancy relative to calculations that only included values above LOD (Table 3b).

Within lakes, the number of stations with detection of *T. bryosalmonae* was quite similar from year to year. The frequency of detection varied slightly (Table 3), but the number of samples is too limited to give a meaningful statistical power.

At station level, there was less consistency in parasite detection between years than at lake level (Table 4,). Even where the same number of stations showed parasite detections in a lake in both years, the actual positive stations were to a large extent different (Figure 5). Within each lake, the number of stations consistently positive to parasite presence varied between 0% and 33%, the number of consistently negative varied between 0 and 100%, and the number with variable results varied between 0 and 80%. (Table 4a). Stations with detected DNA concentrations including values sub-LOD are shown in Table 4b.

In total 28 stations were sampled in this survey, 27 in 2018 and 25 in 2019. Four habitat types were included; 13 stations close to inlet rivers, 5 stations by shorelines with no inlets/outlets, 5 stations in the middle of the lake, and 5 stations by outlet rivers. Positive detections were registered in all types of stations (Table 5). When comparing station types across the different lakes, there is a slight tendency for higher detection of the parasite at the shoreline/jetty and mid-lake stations, compared with inlet river and outlet river stations (Table 6).

Spiking with IPC showed that only one of 52 samples gave a shift in C_T -values of >1 cycle (C_T: 28.6–29.4) and none showed a reduction in endpoint fluorescence. One spiked sample failed to amplify, but the sample was repeatedly positive for *T. bryosalmonae* in 3/3 replicates, so the spiking result was regarded as an anomaly. The three field blanks from 2018 were all negative when tested by qPCR.

Extraction with Blood and Tissue Kit gave a higher total DNA yield than extraction with Power Water Kit. Samples extracted with Blood and Tissue Kit in 2018 had an average/median DNA content of 40.9/37.2 ng DNA/µl, respectively, while det corresponding average/median for samples extracted with Power Water Kit in 2019 was 10.0/9.1 ng DNA/µl (Appendix 2).

4 | DISCUSSION

Tetracapsuloides bryosalmonae was detected by eDNA above the LOD level in four of the five lakes in 2018 and 2019. When DNA concentrations below LOD level were included, the parasite was detected in all five lakes in both years.

4.1 | Variation in detection of parasite DNA between stations and station types

Although the number of stations with parasite detection in each lake was the same in both years of the eDNA survey, the result for individual stations varied from year to year (Table 4, Figure 5). In only two cases, in Lake Fyresvatn at the inlet and in Lake Møsvatn at the shore/jetty, *T. bryosalmonae* was detected in both years. On the other hand, in all four lakes where *T. bryosalmonae* was detected in both 2018 and 2019, several stations were negative both years: Lake Møsvatn (1 station), Lake Norsjø (3 stations), Lake Tinnsjå (3 stations) and Lake Totak (5 stations) (Table 4).

No detection of the parasite at a station may indicate that the parasite is absent, or that the levels are too low and/or the distribution is too patchy to allow consistent detection. It may also result from inhibition of the PCR reaction, but since no evidence of inhibition was found at any station, the latter explanation is less likely. We thus conclude that *T. bryosalmonae* is thinly and/or patchily distributed in the lakes sampled. The challenges in the sampling of eDNA from lentic waters, including patchier distribution of the organisms than in lotic systems, are demonstrated by many former investigations (Dejean et al., 2011; Dunker et al., 2016; Eichmiller et al., 2014; Lawson Handley et al., 2019). These challenges and finding the "hotspot" to detect the species of interest are especially important when sampling in large, deep lakes (Bedwell & Goldberg, 2020; Eichmiller et al., 2014), since DNA normally is not transported far away from the source in these waterbodies (Bedwell & Goldberg, 2020; Dunker et al., 2016; Eichmiller et al., 2014).

TABLE 2 Summary of all positive detections of *Tetracapsuloides bryosalmonae* at the different station types

		Quantity1	Quantity2	Quantity3	Quantity average				
Sample name (year)	**Station type**	**DNA copies pr. PCR-run**				**Ct1**	**Ct2**	**Ct3**	**C_T average**
Fyr 1.1.1 (18)	by inlet river, Stykkjevika	14.1		14.3	14.2	36,714	Undetermined	36,698	36.706
Fyr 1.2.2 (18)	by inlet river, Fardal			11.6	11.6	Undetermined	Undetermined	37,002	37.002
Fyr 1.3.1 (18)	Shoreline/boat jetty			7.4	7.4	Undetermined	Undetermined	37,652	37.652
Fyr midt1 (18)	deep lake			16.4	16.4	Undetermined	Undetermined	36,420	36.420
Fyr midt2 (18)	deep lake	11.2			11.2	36,973	Undetermined	Undetermined	36.973
M 4.1.1 (18)	Shoreline/boat jetty	5.6			5.6	37,980	Undetermined	Undetermined	37.980
M 4.1.2 (18)	Shoreline/boat jetty			26.3	26.3	Undetermined	Undetermined	35,742	35.742
N midt1 (18)	deep lake		4.4		4.4	Undetermined	38,050	Undetermined	38.050
N midt2 (18)	deep lake		15.4		15.4	Undetermined	36,263	Undetermined	36.263
Ti 2.2.1 (18)	by inlet river, Måroset		11.1		11.1	Undetermined	36,983	Undetermined	36.983
Tot 3.2.1 (18)	by inlet river, Arabygdi		5.0		5.0	Undetermined	37,869	Undetermined	37.869
Tot 3.3.1 (18)	by inlet river, Bitåi			4.9	4.9	Undetermined	Undetermined	37,894	37.894
Tot 3.4.2 (18)	by inlet river, Longvik			0.1	0.1	Undetermined	Undetermined	44,113	44.113
Fyr 1.1.2 (19)	by inlet river, Stykkjevika	1568.9	787.4	554.3	970.2	29,623	30,663	31,193	30.493
Fyr 1.3.1 (19)	Shoreline/boat jetty	228.0	161.9	221.1	203.6	32,534	33,050	32,580	32.721
Fyr 1.3.2 (19)	Shoreline/boat jetty	170.3	404.9	113.7	229.6	32,974	31,667	33,583	32.741
Fyr 1.3.3 (19)	Shoreline/boat jetty	1600.6	1711.2	2112.3	1808.0	29,592	29,492	29,174	29.419
Fyr 1.5.1 (19)	by outlet river			4.5	4.5	Undetermined	Undetermined	38,722	38.722
Fyr 1.5.2 (19)	by outlet river		15.0		15.0	Undetermined	36,945	Undetermined	36.945
Fyr midt-2 (19)	deep lake			5.4	5.4	Undetermined	Undetermined	38,428	38.428
M 4.1.2 (19)	Shoreline/boat jetty	4.9		39.6	22.3	37,178	Undetermined	34,446	35.812
M midt-1 (19)	deep lake			30.4	30.4	Undetermined	Undetermined	34,794	34.794
N 5.1.1 (19)	by inlet river, Aslaksborg	653.2	626.7	610.3	630.0	31,099	31,160	31,199	31.153
Ti 2.4.2 (19)	by inlet river/jetty			10.6	10.6	Undetermined	Undetermined	37,449	37.449
Ti 2.5.1 (19)	by outlet river		33.2	5.9	19.6	Undetermined	35,781	38,309	37.045
Ti 2.5.2 (19)	by outlet river	144.1	125.7	150.7	140.1	32,754	32,933	32,695	32.794
Tot midt-3 (19)	deep lake			5.1	5.1	Undetermined	Undetermined	37,124	37.124

Note: Single quantity values (DNA copies per PCR run) for each PCR replicate, and average values (in gray color) of quantity and C_T.

TABLE 3 (a) Number of stations where *Tetracapsuloides bryosalmonae* were detected above LOD over the total number of stations in each lake; (b) Detections of *T. bryosalmonae* including values both above and below LOD, as number of stations with detection over the total number of stations in each lake

Year	Lake Fyresvatn	Lake Møsvatn	Lake Norsjø	Lake Tinnsjå	Lake Totak
a		**Detections above LOD**			
2018	3/6 (0.5)	1/3 (0.3)	1/6 (0.2)	1/6 (0.2)	0/6 (0.0)
2019	3/5 (0.6)	2/3 (0.7)	1/5 (0.2)	2/7 (0.3)	0/5 (0.0)
b		**Detections both above and below LOD**			
2018	4/6 (0.7)	1/3 (0.3)	1/6 (0.2)	1/6 (0.2)	2/6 (0.3)
2019	4/5 (0.8)	2/3 (0.7)	1/5 (0.2)	2/7 (0.3)	1/5 (0.2)

Note: Detection frequency per lake in brackets. Lake Fyresvatn and Lake Totak (gray color) showed a possible higher detection frequency if positive detections below LOD were included of *T. bryosalmonae*.

TABLE 4 Number of stations in each lake with (1) positive detections of *Tetracapsuloides bryosalmonae* both years or (2) no detections both year, and (3) stations with different detection in 2018/2019

	(a) Detections above LOD			(b) All detections		
Lake	Detection both years	No detection both years	Different detection each year	Detection both years	No detection both years	Different detection each year
Fyresvatn	**1 (0.20)**	0	**4 (0.80)**	**3 (0.60)**	0	**2 (0.40)**
Møsvatn	1 (0.33)	1 (0.33)	1 (0.33)	1 (0.33)	1 (0.33)	1 (0.33)
Norsjø	0	3 (0.60)	2 (0.40)	0	3 (0.60)	2 (0.40)
Tinnsjå	0	3 (0.50)	3 (0.50)	0	3 (0.50)	3 (0.50)
Totak	0	**5 (1.00)**	**0**	0	**2 (0.40)**	**3 (0.60)**

Note: (a) Refers to detection values above LOD; (b) refers to detections including both above and sub-LOD. Frequency of the respective stations in brackets. Numbers in bold (Lake Fyrsvatn and Lake Totak) are the detections that differ when values sub-LOD are included in the material, compared with only detections above LOD. When values sub-LOD are included, two more stations in Lake Fyresvatn have positive detections of *T. bryosalmonae* both years, and Lake Totak have three stations with positive parasite detections in one of the 2 years.

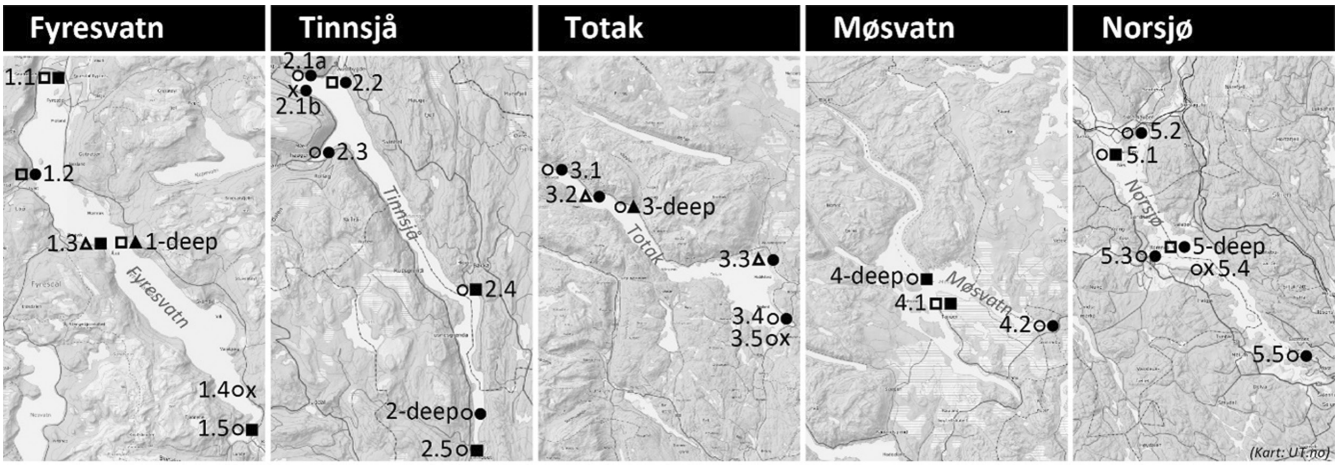

FIGURE 5 Detections of *Tetracapsuloides bryosalmonae* in eDNA sampled from the five lakes in 2018 and 2019. First number is Lake number; second number is station number. Square symbols represent detections above LOD (open symbol □ 2018, filled ■ 2019); triangles represent detections sub-LOD (open symbol △2018, filled ▲2019). Open circles represent stations with no detections (open symbol ○2018, filled ●2019). X represents lack of sampling at the station either year (first position 2018 or second position 2019)

This survey included four different station types: inlet river, shoreline/jetty, outlet river, and open water over the deep area of the lake. Although the parasite was detected at all station types, the results showed a higher percentage of parasite detection at shoreline/jetty- (63%) and mid-lake stations (50%) than by inlet- (28%) and outlet rivers (22%) over the 2 years (Table 6).

Somewhat surprisingly, the parasite was detected in open water above the deepest area in four of the lakes, Lake Fyresvatn (both

TABLE 5 Number of stations with positive detections of *Tetracapsuloides bryosalmonae* (in bold) over total number of the different station type in each lake in 2018 and 2019

Lake	2018						2019					
	No. stations	No. Samples	Station types				No. stations	No. Samples	Station types			
			Inlet	Outlet	Shoreline pier/jetty	Deep-lake			Inlet	Outlet	Shoreline pier/jetty	Deep-lake
Fyresvatn	**6**	12	**2**/2	0/1	**1***/2	**1**/1	**5**	15	**1**/2	**1**/1	**1**/1	**1***/1
Møsvatn	**3**	6		0/1	**1**/1	0/1	**3**	9		0/1	**1**/1	**1**/1
Norsjø	**6**	12	0/3	0/1	0/1	**1**/1	**5**	15	**1**/3	0/1		0/1
Tinnsjå	**6**	12	**1**/4	0/1		0/1	**7**	21	**1**/5	**1**/1		0/1
Totak	**6**	12	**1***/3	0/1	**1***/1	0/1	**5**	12	0/3		0/1	**1***/1

Note: Gray color mark differences (in number of stations and positive detections) for the station types within each lake in 2018 and 2019.

years), Lake Totak (sub-LOD, 2019), Lake Møsvatn (2019), and Lake Norsjø (2018). Above these deepest areas, often used for sampling in limnological surveys, we expected no or very few parasite spore detections due to high degree of dilution, and no presence of benthic bryozoans. Furthermore, statoblasts of *F. sultana* and *P. emerginata* (the most common bryozoan hosts for *T. bryosalmonae*) that can produce *T. bryosalmonae* spores, are sessile (Wood & Okamura, 2005). However, spores and DNA released from the free-roaming fish host might be a possible explanation for the parasite detection in open water, as shown in a recent experimental study that used eDNA to detect *T. bryosalmonae* released in urine (named "uDNA") from brown trout (Duval et al., 2021). A previous fish survey from the large Lake Windermere in the UK also showed that a majority of the fish species present in the lake were detected by eDNA from shoreline sampling during summer (Lawson Handley et al., 2019; Zhang et al., 2020). The exception outlined by Lawson Handley et al. (2019) was that eDNA from Arctic charr was only detected offshore in midwater and bottom samples in the lake during summer stratification. Interestingly, both benthic and littoral species were detected throughout the water column during winter (Lawson Handley et al., 2019).

Possible implications from these findings to this present study are that *T. bryosalmonae* spores released from salmonids presumably could be traced both in shoreline and in open water areas during late summer before autumn lake circulation. It would probably be less meaningful to sample eDNA for *T. bryosalmonae* detection during the more homogenous water column in winter as suggested by Lawson Handley et al. (2019) for fish eDNA sampling, given that the soft-valved parasite spores released from salmonids have a lifetime of less than 24 h (Hartikainen & Okamura, 2015b).

4.2 | Detection of parasite DNA in eDNA survey versus fish survey

The detection of *T. bryosalmonae* by eDNA was consistent with parasite detection in fish in three of the five lakes (Lake Fyresvatn, Lake Tinnsjå, and Lake Norsjø) sampled in 2018 (Oredalen et al., 2022). In fish, *T. bryosalmonae* was recently reported in wild Arctic charr Lake Tinnsjå and Lake Norsjø and in farmed Arctic charr in Lake Fyresvatn. The parasite was detected in brown trout in Lake Fyresvatn, Lake Tinnsjå, Lake Norsjø, and Lake Totak. European whitefish was infected with *T. bryosalmonae* in Lake Norsjø (Oredalen et al., 2022). If detections of parasite DNA sub-LOD from Lake Totak were included in the eDNA survey, the results corresponded with the previous fish survey from the same lake. The results from Lake Møsvatn differed in the two surveys; *T. bryosalmonae* was not detected in Arctic charr in 2016 but was detected in eDNA in both 2018 and 2019. A plausible explanation of this difference would be parasite presence in brown trout, which are present, but were not sampled in 2016. As previously shown in both Lake Fyresvatn and Lake Totak, brown trout are more susceptible to *T. bryosalmonae* infection than wild Arctic charr (Oredalen et al., 2022). Other possible reasons for

TABLE 6 Number and percentage of all detections of *Tetracapsuloides bryosalmonae* at each station type in 2018, 2019, and both years merged

	2018				
Station type:	**Number of stations**	**Number of samples (replicates merged)**	**Detection**	**No detection**	**% detection**
Inlet rivers	12	12	4	8	33
Outlet rivers	5	5	0	5	0
Shoreline/jetty	5	5	3	2	60
Mid-lake	5	5	2	3	40
Total	27	27	9	18	33
	2019				
Inlet rivers	13	13	3	10	23
Outlet rivers	4	4	2	2	50
Shoreline/jetty	3	3	2	1	67
Mid-lake	5	5	3	2	60
Total	25	25	10	15	40
	Both years				
Inlet rivers	13	25	7	18	28
Outlet rivers	5	9	2	7	22
Shoreline/jetty	5	8	5	3	63
Mid-lake	5	10	5	5	50
Total	28	52	19	33	37

Note: Detections both above and below LOD both years.

the divergence between datasets in Lake Møsvatn could be that 1) the charr sampled in 2016 were quite large (255 mm, STDEV ± 13.1) and *T. bryosalmonae* could have been present in smaller and younger charr (not sampled) 2) despite no evidence of infection of charr in 2016 - they could have been infected in 2018 or 3) the sampling sites for fish and eDNA were different.

4.3 | Variations in DNA concentrations levels

In Lake Totak concentrations of *T. bryosalmonae* DNA sub-LOD were detected in both years, at two stations in 2018 (5.0 and 4.9 DNA copies/reaction) and the third station in 2019 (5.1 DNA copies/reaction) (Appendix 1). Despite these low values, these results indicated parasite presence in the lake, and the presence was consistent with the detection of *T. bryosalmonae* in brown trout in the 2018 fish survey (Oredalen et al., 2022). Different sampling locations may be a possible explanation for the low detection of the parasite in eDNA samples compared with detection in fish samples from Lake Totak, if spores released from fish were the main source of eDNA detection. Infected trout were sampled in the south-western part of the lake, while water for eDNA analyses was sampled in the north, eastern and southern parts. The distance between sampling sites in western or eastern parts is substantial, with expected minor water exchange. Sampling of eDNA was performed at water levels close to HRV in both years (Appendix 2) as was the sampling of fish in 2018. This means that the hydrological conditions were comparable during all samplings, with most of the littoral zone submerged and the possibility of parasite spores being present in the surface water. However, we cannot exclude the possibility that some of the positive results come from the dead remnants of *T. bryosalmonae* populations no longer present in the lake (Cristescu, 2019).

Concentrations of parasite DNA below LOD were observed in all the investigated lakes and in both years. However, a general pattern in the analyses was that the highest concentrations (and lower C_T) were measured in 2019, at four stations in three lakes (Table 2), but there was no apparent correlation between concentrations at the same stations in the 2 years.

As the samples were stored differently and extracted by two different extraction kits in the 2 years, this may be a possible factor influencing the different concentration levels. The measurements of total DNA content in the samples (measured by nanodrop) were ten times higher in the samples extracted with Blood and Tissue Kit in 2018 than in the samples extracted with Power Water Kit in 2019 (Appendix 2). Even so, the highest concentration of DNA from *T. bryosalmonae* was measured in the Power Water Kit. Despite these differences, both methods allowed for the detection of the parasite. The two extraction methods are also compared in other studies that concluded an equally well performance (Djurhuus et al., 2017; Kumar et al., 2020).

4.4 | Sampling strategy and challenges of eDNA studies

Tetracapsuloides bryosalmonae depend on an invertebrate bryozoan host and a vertebrate salmonid host to complete its life cycle and maintain its presence in a habitat. Transmission from fish to fish seems to be precluded (D'Silva et al., 1984; Ferguson & Ball, 1979). From this, we conclude that when *T. bryosalmonae* is detected in a lake, both salmonids and bryozoans most probably must be present. The littoral zone, which is thought to be the main habitat for bryozoans (Økland & Økland, 2005; Økland & Økland, 2006), was apparently unpopulated by benthic organisms in four of the lakes in this study. These lakes (Lake Møsvatn, Lake Totak, Lake Fyresvatn, and Lake Tinnsjå) are regulated for hydropower purposes with regulation zones between 4 and 18.5 meters (Appendix 0). Repeated water level drawdowns during each winter and early spring lead to subsequent wash-out of organic matter of the affected upper shoreline (Borgstrøm & Hansen, 2000; Hellsten & Riihimäki, 1996; Hirsch et al., 2017). The overall regulation scheme in these lakes is to reduce water levels in winter and spring, and filling of the reservoirs with snowmelt from the high mountain areas in early summer and precipitation during summer and autumn. Although the littoral zones of most of these lakes are sparsely populated by benthic organisms, the spores of *T. bryosalmonae* may potentially be protected and survive within the dormant and resistant bryozoan statoblasts. The statoblasts usually have a production peak in autumn, and can survive prolonged winter conditions of freezing and drying of the littoral zone (Hartikainen & Okamura, 2015a; Raddum & Johnsen, 1983; Wood & Okamura, 2005), germinate in spring (Raddum & Johnsen, 1983) and subsequently release "hiking" parasite spores. Bryozoan colonies and spores may also survive in deeper layers of the lake, below the regulation zone, as seen in other oligotrophic lakes (Marcus, 1940; Raddum & Johnsen, 1983) or in the inlet streams (Økland & Økland, 2005; Økland & Økland, 2006).

At the end of the summer season, when water temperatures in the epilimnion are usually highest the highest abundance and proliferation of infectious *T. bryosalmonae* spores is expected, both in bryozoan and salmonid hosts (Bettge, Segner, et al., 2009; Bettge, Wahli, et al., 2009; Okamura et al., 2011; Strepparava et al., 2017; Tops et al., 2006; Tops et al., 2009). This was the rationale for the sampling strategy of this survey: Sampling surface water at the time when we expected the highest density of parasite spores, at the most likely parasite-host habitats in the littoral zone and by river inlets and outlets. Bryozoans have been reported from such habitats ((Økland & Økland, 2006) and salmonids use these habitats for feeding and spawning (Borgstrøm et al., 1995; Klemetsen et al., 2003). A possible disadvantage of stations close to river inlets is the potential dilution of spores and DNA that may be present in the water.

The eDNA survey mostly detected rather low concentrations of parasite DNA. One reason for this may be that we have missed "hotspots"—the sampling sites with the highest abundance of parasite DNA (Bedwell & Goldberg, 2020; Eichmiller et al., 2014; Goldberg et al., 2016) from bryozoans or fish. Another reason may be that spore production from one or both hosts was low overall, or that locally produced spores were diluted or had decayed (Bedwell & Goldberg, 2020; Dejean et al., 2011; Goldberg et al., 2016). This highlights the importance of finding the right place and time when using eDNA methods, and planning for representative and sufficient samples (Bedwell & Goldberg, 2020; Dunker et al., 2016; Eichmiller et al., 2014).

Our results showed that the parasite presence could be detected by eDNA at the lake level. Despite indications of the higher percentage of parasite DNA detection in shoreline/jetty and deep lake stations, this survey cannot conclude on any clear and systematic differences in detection between the 2 years or the four station types: river inlets and outlets, free water above the deepest area of the lakes and shoreline with pier or jetty. That said, all station types in this survey (except above the deepest area) were chosen with an assumption of them being suitable habitats for both hosts - with a consequent higher a priori probability of parasite presence. Our findings indicate that number of samples could be more important than the specific station types. A recent study on crayfish and its parasite *Aphanomyces astaci* in different types of water bodies found that seven water samples per site were needed to achieve a 95% success rate (Natalie Sieber et al., 2021). The same survey concluded that if results sub-LOD were included, three samples were sufficient to reach the same detection success.

eDNA methods have limitations when it comes to revealing dynamics in spatial and temporal infection by *T. bryosalmonae*, including the origin of the DNA and infection status of the host (Duval et al., 2021). This shortcoming of molecular methods compared with traditional sampling surveys, which can reveal for instance different development stages of the species, sex, age, etc., has also been raised in other studies (Hallett & Bartholomew, 2006; Valentini et al., 2016). Additionally, sampling of eDNA must be considered a snapshot of the status in the water at a specific time point, as DNA has been shown to become undetectable within a few days or weeks, depending on sedimentation and degradation rates (Barnes et al., 2014; Bedwell & Goldberg, 2020; Dejean et al., 2011; Thomsen et al., 2012). In contrary, infected fish can "sample" the parasite present in the water over a longer exposure period (Hallett & Bartholomew, 2006).

Questions remain about the number and placing of stations needed to achieve representative samples of eDNA from ponds and lakes (Harper et al., 2019). These questions are highly relevant to the detection of *T. bryosalmonae* if the method is to be used to monitor the parasite and support control measures in large and deep lakes. However, it is possible to detect *T. bryosalmonae* throughout the year from salmonid tissues as they migrate from the littoral to the pelagic zone and between hypo- and epilimnion, in particular in brown trout where the parasite can persist for at least 5 years after exposure (Soliman et al., 2018). The more stationary bryozoans release fragments, spores, and statoblasts with peaks in abundance mostly in autumn. Particularly in large, regulated lakes with limited bryozoan abundance and cold water most of the year, the period of elevated chance for detection of *T. bryosalmonae* from eDNA in late

summer/early fall is shorter compared with detection in salmonids, which are always present. Nevertheless, this survey showed that the parasite could be detected using eDNA in large, deep lakes in late summer. However, more extensive sampling in such large, deep lakes should be carried out to gain more knowledge on the optimal sampling sites and time for the detection of *T. bryosalmonae*. This could preferably be done by continuous (automated) sampling over larger areas of a lake at different times (Sassoubre et al., 2016) or by pooling several subsamples into one merged sample for screening analyses, as suggested in Bruce et al. (2021). Another option worth investigating is to use water temperature measurements to determine the time point of the highest infection probability *T. bryosalmonae* in salmonids. This appears to peak after 30–55 days of exposure to water temperatures above 15°C (Rubin et al., 2022; Strepparava et al., 2017). This publication concluded that the probability of highest parasite detection (assessed by histology) in brown trout was after ~1500 degree days or 30 days with an average temperature ≥15°C, in two Swizz rivers. It would be interesting to collect eDNA from the water after such a temperature-induced period of "maximum infection," when infectious spores may be released from brown trout urine. Experiments have shown that the release of infectious parasite spores from brown trout was temperature-dependent, and started after 55 days postexposure of the fish in water temperature of 15°C (Strepparava et al., 2017). The detection of *T. bryosalmonae* and the infection status of the salmonid host using eDNA (or uDNA) released from the urine of brown trout has been successfully achieved (Duval et al., 2021), leading to further promising possibilities for future eDNA detection and monitoring of *T. bryosalmonae*.

5 | CONCLUSIONS

Tetracapsuloides bryosalmonae was detected in all five lakes surveyed. In Lake Totak, all detections were sub-LOD, but the presence of the parasite was in agreement with results from brown trout in the same lake in 2018. In three of the lakes, Lake Fyresvatn, Lake Tinnsjå, and Lake Norsjø, lake level detections of eDNA were consistent with detections in salmonids from the previous fish survey. *T. bryosalmonae* was detected by eDNA in Lake Møsvatn in 2018, but not in Arctic charr, which was sampled in 2016. Our results confirm that eDNA can detect DNA of *T. bryosalmonae* even in large lakes with presumably patchy and dilute occurrence. However, parasite detection from eDNA varied between the 2 years and between stations within the same lake, revealing a shifting and apparently stochastic spatial distribution of parasite DNA between years. The results highlight not only the importance of sampling strategy in eDNA surveys in large, deep lakes but also the possibility of using a noninvasive method for monitoring the presence and spread of this emerging parasite.

AUTHOR CONTRIBUTIONS

The concept and design of the study were carried out by Tone Jøran Oredalen, Tor Atle Mo, and Mona Sæbø. Material preparation and data collection were performed by Tone Jøran Oredalen and Natalie Haugan. Laboratory design, analysis, and fine-tuning were performed by Tone Jøran Oredalen and Natalie Haugan, and advised by Mona Sæbø and Andrew Jenkins. The first draft of the manuscript was written by Tone Jøran Oredalen, and all authors contributed to revisions of drafts and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study is openly available in "USN figshare" with doi.org/10.23642/usn.19387472.v1 and doi.org/10.23642/usn.19387469.v1.

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ENDNOTES

[1] Exceptions: 3 qPCR replicates missing at station Totak 3.2 and 2 qPCR replicates missing at station Møsvatn 4.1, due to loss during laboratory processing

REFERENCES

Anderson, C. L., Canning, E. U., & Okamura, B. (1999). Molecular data implicate bryozoans as hosts for PKX (phylum myxozoa) and identify a clade of bryozoan parasites within the myxozoa. *Parasitology*, *119*, 555–561. <Go to ISI>://WOS:000084223500003

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, *17*(1), 1–17. https://doi.org/10.1007/s10592-015-0775-4

Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., & Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science & Technology*, *48*(3), 1819–1827. https://doi.org/10.1021/es404734p

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

Bedwell, M. E., & Goldberg, C. S. (2020). Spatial and temporal patterns of environmental DNA detection to inform sampling protocols in lentic and lotic systems. *Ecology and Evolution*, *10*(3), 1602–1612. https://doi.org/10.1002/ece3.6014

Bendixby, L., & Hals, P. I. (2009). *Distribution and abundance of bryozoan species in river Åelva and the prevalence of the PKD parasite Tetracapsuloides bryosalmonae* (master master thesis-60 credits). Norwegian University of Life Science, Norwegian University of Life Science.

Bettge, K., Segner, H., Burki, R., Schmidt-Posthaus, H., & Wahli, T. (2009). Proliferative kidney disease (PKD) of rainbow trout: Temperature- and time-related changes of Tetracapsuloides bryosalmonae

DNA in the kidney. *Parasitology*, *136*(6), 615–625. https://doi.org/10.1017/s0031182009005800

Bettge, K., Wahli, T., Segner, H., & Schmidt-Posthaus, H. (2009). Proliferative kidney disease in rainbow trout: Time- and temperature-related renal pathology and parasite distribution. *Diseases of Aquatic Organisms*, *83*, 67–76. https://doi.org/10.3354/dao01989

Borgstrøm, R., & Hansen, L. P. (2000). *Fisk i ferskvann: et samspill mellom bestander, miljø og forvaltning* (2. utg. ed.). Landbruksforlaget.

Borgstrøm, R., Jonsson, B., & L'Abèe-Lund, J. H. (1995). *Ferskvannsfisk-Økologi, kultivering og utnytting*. Norges Forskningsråd (NFR).

Bruce, K., Blackman, R., Bourlat, S. J., Hellström, A. M., Bakker, J., Bista, I., Kristine Bohmann, Agnès Bouchez, Rein Brys, Katie Clark, Vasco Elbrecht, Stefano Fazi, Vera Fonseca, Bernd Hänfling, Florian Leese, Elvira Mächler, Andrew R. Mahon, Kristian Meissner, Kristel Panksep, Jan Pawlowski, Paul Schmidt Yáñez, Mathew Seymour, Bettina Thalinger, Alice Valentini, Paul Woodcock, Michael Traugott, Valentin Vasselon. Deiner, K. (2021). *A practical guide to DNA-based methods for biodiversity assessment*. *Advanced books*, *1*. Advanced Books. https://doi.org/10.3897/ab.e68634

Canning, E. U., Curry, A., Feist, S. W., Longshaw, M., & Okamura, B. (1999). Tetracapsula bryosalmonae n.sp. for PKX organism, the cause of PKD in salmonid fish. *Bulletin of the European Association of Fish Pathologists*, *19*, 203–206.

Carraro, L., Hartikainen, H., Jokela, J., Bertuzzo, E., & Rinaldo, A. (2018). Estimating species distribution and abundance in river networks using environmental DNA. *Proceedings of the National Academy of Sciences*, *115*, 11724–11729. https://doi.org/10.1073/pnas.1813843115

Collins, R. A., Wangensteen, O. S., O'Gorman, E. J., Mariani, S., Sims, D. W., & Genner, M. J. (2018). Persistence of environmental DNA in marine systems. *Communications Biology*, *1*(1), 185. https://doi.org/10.1038/s42003-018-0192-6

Cristescu, M. E. (2019). Can environmental RNA revolutionize biodiversity science? *Trends in Ecology & Evolution*, *34*(8), 694–697. https://doi.org/10.1016/j.tree.2019.05.003

D'Silva, J., Mulcahy, M. F., & de Kinkelin, P. (1984). Experimental transmission of proliferative kidney disease in rainbow trout, Salmo gairdneri Richardson. *Journal of Fish Diseases*, *7*(3), 235–239. https://doi.org/10.1111/j.1365-2761.1984.tb00928.x

Dejean, T., Valentini, A., Duparc, A., Pellier-Cuit, S., Pompanon, F., Taberlet, P., & Miaud, C. (2011). Persistence of environmental DNA in freshwater ecosystems. *PLoS One*, *6*(8), e23398. 10.1371/journal.pone.0023398

Djurhuus, A., Port, J., Closek, C. J., Yamahara, K. M., Romero-Maraccini, O., Walz, K. R., Goldsmith, D. B., Michisaki, R., Breitbart, M., Boehm, A. B., & Chavez, F. P. (2017). Evaluation of filtration and DNA extraction methods for environmental DNA biodiversity assessments across multiple trophic levels. *Frontiers in Marine Science*, *4*, 1–11. https://doi.org/10.3389/fmars.2017.00314

Dunker, K. J., Sepulveda, A. J., Massengill, R. L., Olsen, J. B., Russ, O. L., Wenburg, J. K., & Antonovich, A. (2016). Potential of environmental DNA to evaluate northern pike (Esox lucius) eradication efforts: An experimental test and case study. *PLoS One*, *11*(9), e0162277. https://doi.org/10.1371/journal.pone.0162277

Duval, E., Blanchet, S., Quéméré, E., Jacquin, L., Veyssière, C., Lautraite, A., Garmendia, L., Yotte, A., Parthuisot, N., Côte, J., & Loot, G. (2021). Urine DNA (uDNA) as a non-lethal method for endoparasite biomonitoring: Development and validation. *Environmental DNA*, *3*(5), 1035–1045. https://doi.org/10.1002/edn3.228

Eichmiller, J. J., Bajer, P. G., & Sorensen, P. W. (2014). The relationship between the distribution of common carp and their environmental DNA in a small lake. *PLoS One*, *9*(11), e112611. https://doi.org/10.1371/journal.pone.0112611

Ellison, S. L. R., English, C. A., Burns, M. J., & Keer, J. T. (2006). Routes to improving the reliability of low level DNA analysis using real-time PCR. *BMC Biotechnology*, *6*, 1–11. Artn 33 10.1186/1472-6750-6-33

Feist, S. W., Peeler, E. J., Gardiner, R., Smith, E., & Longshaw, M. (2002). Proliferative kidney disease and renal myxosporidiosis in juvenile salmonids from rivers in England and Wales. *Journal of Fish Diseases*, *25*(8), 451–458. https://doi.org/10.1046/j.1365-2761.2002.00361.x

Ferguson, H. W., & Ball, H. J. (1979). Epidemiological aspects of proliferative kidney disease amongst rainbow trout Salmo gairdneri Richardson in Northern Ireland. *Journal of Fish Diseases*, *2*(3), 219–225. https://doi.org/10.1111/j.1365-2761.1979.tb00161.x

Ferguson, H. W., & Needham, E. A. (1978). Proliferative kidney-disease in rainbow-trout Salmo-Gairdneri Richardson. *Journal of Fish Diseases*, *1*(1), 91–108. https://doi.org/10.1111/j.1365-2761.1978.tb00008.x

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

Fontes, I., Hartikainen, H., Holland, J. W., Secombes, C. J., & Okamura, B. (2017). *Tetracapsuloides bryosalmonae* abundance in river water. *Diseases of Aquatic Organisms*, *124*(2), 145–157. https://doi.org/10.3354/dao03116

Fossøy, F., Brandsegg, H., Sivertsgård, R., Pettersen, O., Sandercock, B. K., Solem, Ø., Hindar, K., & Mo, T. A. (2020). Monitoring presence and abundance of two gyrodactylid ectoparasites and their salmonid hosts using environmental DNA. *Environmental DNA*, *2*(1), 53–62. https://doi.org/10.1002/edn3.45

Gaudard, A., Weber, C., Alexander, T. J., Hunziker, S., & Schmid, M. (2018). Impacts of using lakes and rivers for extraction and disposal of heat. *WIREs Water*, *5*(5), 1–18. https://doi.org/10.1002/wat2.1295

Gay, M., Okamura, B., & de Kinkelin, P. (2001). Evidence that infectious stages of Tetracapsula bryosalmonae for rainbow trout Oncorhynchus mykiss are present throughout the year. *Diseases of Aquatic Organisms*, *46*(1), 31–40. https://doi.org/10.3354/dao046031

Goldberg, C., & Strickler, K. (2017). eDNA protocol sample collection [protocol]. https://s3.wp.wsu.edu/uploads/sites/686/2017/01/WSU-eDNA-sampling-protocol-Jan2017.pdf

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., Spear, S. F., McKee, A., Oyler-McCance, S. J., Cornman, R. S., Laramie, M. B., Mahon, A. R., Lance, R. F., Pilliod, D. S., Strickler, K. M., Waits, L. P., Fremier, A. K., Takahara, T., Herder, J. E., & Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, *7*(11), 1299–1307. https://doi.org/10.1111/2041-210x.12595

Grabner, D. S., & El-Matbouli, M. (2008). Transmission of *Tetracapsuloides bryosalmonae* (myxozoa: Malacosporea) to *Fredericella sultana* (Bryozoa: Phylactolaemata) by various fish species. *Diseases of Aquatic Organisms*, *79*(2), 133–139. http://www.int-res.com/articles/dao2007/79/d079p133.pdf

Hallett, S. L., & Bartholomew, J. L. (2006). Application of a real-time PCR assay to detect and quantify the myxozoan parasite Ceratomyxa shasta in river water samples. *Diseases of Aquatic Organisms*, *71*(2), 109–118. https://www.int-res.com/abstracts/dao/v71/n2/p109-118/

Harper, L. R., Buxton, A. S., Rees, H. C., Bruce, K., Brys, R., Halfmaerten, D., Read, D. S., Watson, H. V., Sayer, C. D., Jones, E. P., Priestley, V., Mächler, E., Múrria, C., Garcés-Pastor, S., Medupin, C., Burgess, K., Benson, G., Boonham, N., Griffiths, R. A., … Hänfling, B. (2019). Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds. *Hydrobiologia*, *826*(1), 25–41. https://doi.org/10.1007/s10750-018-3750-5

Hartikainen, H., Gruhl, A., & Okamura, B. (2014). Diversification and repeated morphological transitions in endoparasitic cnidarians (myxozoa: Malacosporea). *Molecular Phylogenetics and Evolution*, *76*(Supplement C), 261–269. https://doi.org/10.1016/j.ympev.2014.03.010

Hartikainen, H., & Okamura, B. (2015a). Ecology and evolution of Malacosporean-bryozoan interactions. In B. Okamura, A. Gruhl, & J. L. Bartholomew (Eds.), *Myxozoan evolution, ecology and development* (pp. 201–216). Springer International Publishing.

Hartikainen, H., & Okamura, B. (2015b). Host-parasite interactions: Ecology and evolution of malacosporean-bryozoan interactions. In *Myxozoan evolution, ecology and development* (pp. 199–216). SpringerLink.

Hartman, L. J., Coyne, S. R., & Norwood, D. A. (2005). Development of a novel internal positive control for Taqman® based assays. *Molecular and Cellular Probes*, *19*(1), 51–59. https://doi.org/10.1016/j.mcp.2004.07.006

Hedrick, R. P., MacConnell, E., & de Kinkelin, P. (1993). Proliferative kidney disease of salmonid fish. *Annual Review of Fish Diseases*, *3*(Supplement C), 277–290. https://doi.org/10.1016/0959-8030(93)90039-E

Hellsten, S., & Riihimäki, J. (1996). Effects of lake water level regulation on the dynamics of littoral vegetation in northern Finland. *Hydrobiologia*, *340*(1), 85–92. https://doi.org/10.1007/BF00012738

Hirsch, P. E., Eloranta, A. P., Amundsen, P.-A., Brabrand, Å., Charmasson, J., Helland, I. P., Power, M., Sánchez-Hernández, J., Sandlund, O. T., Sauterleute, J. F., Skoglund, S., Ugedal, O., & Yang, H. (2017). Effects of water level regulation in alpine hydropower reservoirs: An ecosystem perspective with a special emphasis on fish. *Hydrobiologia*, *794*(1), 287–301. https://doi.org/10.1007/s10750-017-3105-7

Hunter, M. E., Dorazio, R. M., Butterfield, J. S., Meigs-Friend, G., Nico, L. G., & Ferrante, J. A. (2017). Detection limits of quantitative and digital PCR assays and their influence in presence-absence surveys of environmental DNA. *Molecular Ecology Resources*, *17*(2), 221–229. https://doi.org/10.1111/1755-0998.12619

Hutchins, P. R., Sepulveda, A. J., Hartikainen, H., Staigmiller, K. D., Opitz, S. T., Yamamoto, R. M., Huttinger, A., Cordes, R. J., Weiss, T., Hopper, L. R., Purcell, M. K., & Okamura, B. (2021). Exploration of the 2016 Yellowstone River fish kill and proliferative kidney disease in wild fish populations. *Ecosphere*, *12*(3), e03436. https://doi.org/10.1002/ecs2.3436

Hutchins, P. R., Sepulveda, A. J., Martin, R. M., & Hopper, L. R. (2017). A probe-based quantitative PCR assay for detecting *Tetracapsuloides bryosalmonae* in fish tissue and environmental DNA water samples. *Conservation Genetics Resources*, Technical note, *10*, 317–319. https://doi.org/10.1007/s12686-017-0812-3

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: A Publication of the Ecological Society of America*, *25*(4), 991–1002. https://doi.org/10.1890/14-1530.1

Hänfling, B., Lawson Handley, L., Read, D. S., Hahn, C., Li, J., Nichols, P., & Blackman, R. C. (2016). Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. *Molecular Ecology*, *25*(13), 3101–3119. https://doi.org/10.1111/mec.13660

Klemetsen, A., Amundsen, P.-A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F., & Mortensen, E. (2003). Atlantic salmon Salmo salar L., brown trout Salmo trutta L. and Arctic charr Salvelinus alpinus (L.): A review of aspects of their life histories. *Ecology of Freshwater Fish*, *12*(1), 1–59. https://doi.org/10.1034/j.1600-0633.2003.00010.x

Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., Jackson, C. A., Lance, R. F., Mangan, A. M., Monroe, E. M., Piaggio, A. J., Stokdyk, J. P., Wilson, C. C., & Richter, C. A. (2020). Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA*, *2*(3), 271–282. https://doi.org/10.1002/edn3.29

Kristmundsson, A., Antonsson, T., & Arnason, E. (2010). First record of proliferative kidney disease in Iceland. *Bulletin of the European Association of Fish Pathologists*, *30*(1), 35–40. Retrieved from <Go to ISI>://WOS:000274748300006

Kumar, G., Eble, J. E., & Gaither, M. R. (2020). A practical guide to sample preservation and pre-PCR processing of aquatic environmental DNA. *Molecular Ecology Resources*, *20*(1), 29–39. https://doi.org/10.1111/1755-0998.13107

Lawson Handley, L., Read, D. S., Winfield, I. J., Kimbell, H., Johnson, H., Li, J., Hahn, C., Blackman, R., Wilcox, R., Donnelly, R., Szitenberg, A., & Hänfling, B. (2019). Temporal and spatial variation in distribution of fish environmental DNA in England's largest lake. *Environmental DNA*, *1*(1), 26–39. https://doi.org/10.1002/edn3.5

Lewisch, E., Unfer, G., Pinter, K., Bechter, T., & El-Matbouli, M. (2018). Distribution and prevalence of T. bryosalmonae in Austria: A first survey of trout from rivers with a shrinking population. *Journal of Fish Diseases*, *41*(10), 1549–1557. https://doi.org/10.1111/jfd.12863

Longshaw, M., Le Deuff, R. M., Harris, A. F., & Feist, S. W. (2002). Development of proliferative kidney disease in rainbow trout, Oncorhynchus mykiss (Walbaum), following short-term exposure to Tetracapsula bryosalmonae infected bryozoans. *Journal of Fish Diseases*, *25*(8), 443–449. https://doi.org/10.1046/j.1365-2761.2002.00353.x

Majaneva, M., Diserud, O. H., Eagle, S. H. C., Boström, E., Hajibabaei, M., & Ekrem, T. (2018). Environmental DNA filtration techniques affect recovered biodiversity. *Scientific Reports*, *8*(1), 4682. https://doi.org/10.1038/s41598-018-23052-8

Marcus, E. (1940). *Mosdyr (Bryozoa eller Polyzoa)* (Vol. *46*). G. E. C Gads forlag.

Merkes, C. M., Klymus, K. E., Allison, M., Goldberg, C., Helbing, C. C., Hunter, M. E., Craig A Jackson, Richard Lance, Anna Mangan, Emy Monroe, Antoineete Piagio, Joel P Stokdyk, Chris Wilson, Cathy A Richter, & Richter, C. (2019). Generic qPCR Limit of Detection (LOD)/Limit of Quantification (LOQ) calculator. R Script. https://doi.org/10.5066/P9GT00GB. https://github.com/cmerkes/qPCR_LOD_Calc

Mo, T. A., & Jørgensen, A. (2017). A survey of the distribution of the PKD-parasite *Tetracapsuloides bryosalmonae* (cnidaria: Myxozoa: Malacosporea) in salmonids in Norwegian rivers – Additional information gleaned from formerly collected fish. *Journal of Fish Diseases*, *40*, 621–627. https://doi.org/10.1111/jfd.12542

Naldoni, J., Adriano, E. A., Hartigan, A., Sayer, C., & Okamura, B. (2019). Malacosporean myxozoans exploit a diversity of fish hosts. *Parasitology*, *146*(7), 968–978. https://doi.org/10.1017/S0031182019000246

Okamura, B., & Feist, S. W. (2011). Emerging diseases in freshwater systems. *Freshwater Biology*, *56*(4), 627–637. https://onlinelibrary.wiley.com/toc/13652427/56/4

Okamura, B., Hartigan, A., & Naldoni, J. (2018). Extensive uncharted biodiversity: The parasite dimension. *Integrative and Comparative Biology*, *58*(6), 1132–1145. https://doi.org/10.1093/icb/icy039

Okamura, B., Hartikainen, H., Schmidt-Posthaus, H., & Wahli, T. (2011). Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. *Freshwater Biology*, *56*(4), 735–753. https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2427.2010.02465.x

Okamura, B., & Wood, T. S. (2002). Bryozoans as hosts for *Tetracapsula bryosalmonae*, the PKX organism. *Journal of Fish Diseases*, *25*(8), 469–475. https://onlinelibrary.wiley.com/doi/full/10.1046/j.1365-2761.2002.00376.x

Opitz, S., & Rhoten, J. (2016). 2016 mountain whitefish kill on the Yellowstone River. Bozeman, MT. http://mtflyfishmag.com/wp-content/uploads/2018/03/2016-Mountain-Whitefish-Kill-on-the-Yellowstone-River-Finalx.pdf

Oredalen, T. J., Sæbø, M., & Mo, T. A. (2022). Patterns of Tetracapsuloides bryosalmonae infection of three salmonid species in large, deep

Norwegian lakes. *Journal of Fish Diseases*, *45*(8), 1–18. https://doi.org/10.1111/jfd.13548

Pietramellara, G., Ascher-Jenull, J., Borgogni, F., Ceccherini, M. T., Guerri, G., & Nannipieri, P. (2008). Extracellular DNA in soil and sediment: Fate and ecological relevance. *Biology and Fertility of Soils*, *45*, 219–235. https://doi.org/10.1007/s00374-008-0345-8

Raddum, G. G., & Johnsen, T. M. (1983). Growth and feeding of Fredericella sultana (bryozoa) in the outlet of a humic acid lake. *Hydrobiologia*, *101*(1), 115–120. https://doi.org/10.1007/bf00008663

Ros, A., Baer, J., Basen, T., Chucholl, C., Schneider, E., Teschner, R., & Brinker, A. (2021). Current and projected impacts of the parasite Tetracapsuloides bryosalmonae (causative to proliferative kidney disease) on central European salmonid populations under predicted climate change. *Freshwater Biology*, *66*(6), 1182–1199. https://doi.org/10.1111/fwb.13709

Rubin, A., Bailey, C., Strepparava, N., Wahli, T., Segner, H., & Rubin, J.-F. (2022). Reliable field assessment of proliferative kidney disease in wild Brown trout, Salmo trutta, populations: When is the optimal sampling period? *Pathogens*, *11*, 1–11. https://doi.org/10.3390/pathogens11060681

Rubin, A., de Coulon, P., Bailey, C., Segner, H., Wahli, T., & Rubin, J.-F. (2019). Keeping an eye on wild Brown trout (Salmo trutta) populations: Correlation between temperature, environmental parameters, and proliferative kidney disease. *Frontiers in Veterinary Science*, *6*(281), 1–16. https://doi.org/10.3389/fvets.2019.00281

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 & Vectors*, *11*(1), 333. https://doi.org/10.1186/s13071-018-2916-3

Sassoubre, L. M., Yamahara, K. M., Gardner, L. D., Block, B. A., & Boehm, A. B. (2016). Quantification of environmental DNA (eDNA) shedding and decay rates for three marine fish. *Environmental Science & Technology*, *50*(19), 10456–10464. https://doi.org/10.1021/acs.est.6b03114

Schmidt-Posthaus, H., Hirschi, R., & Schneider, E. (2015). Proliferative kidney disease in brown trout: Infection level, pathology and mortality under field conditions. *Diseases of Aquatic Organisms*, *114*(2), 139–146. https://doi.org/10.3354/dao02855

Sepulveda, A. J., Birch, J. M., Barnhart, E. P., Merkes, C. M., Yamahara, K. M., Marin, R., Kinsey, S. M., Wright, P. R., & Schmidt, C. (2020). Robotic environmental DNA bio-surveillance of freshwater health. *Scientific Reports*, *10*(1), 14389. https://doi.org/10.1038/s41598-020-71304-3

Sepulveda, A. J., Hoegh, A., Gage, J. A., Caldwell Eldridge, S. L., Birch, J. M., Stratton, C., Hutchins, P. R., & Barnhart, E. P. (2021). Integrating environmental DNA results with diverse data sets to improve bio-surveillance of river health. *Frontiers in Ecology and Evolution*, *9*(132), 1–13. https://doi.org/10.3389/fevo.2021.620715

Sieber, N., Hartikainen, H., Krieg, R., Zenker, A., & Vorburger, C. (2021). Parasite DNA detection in water samples enhances crayfish plague monitoring in asymptomatic invasive populations. *Biological Invasions*, *24*, 281–297. https://doi.org/10.1007/s10530-021-02644-y

Sieber, N., Hartikainen, H., & Vorburger, C. (2020). Validation of an eDNA-based method for the detection of wildlife pathogens in water. *Diseases of Aquatic Organisms*, *141*, 171–184. https://doi.org/10.3354/dao03524

Soliman, H., Kumar, G., & El-Matbouli, M. (2018). Tetracapsuloides bryosalmonae persists in brown trout Salmo trutta for five years post exposure. *Diseases of Aquatic Organisms*, *127*(2), 151–156. https://doi.org/10.3354/dao03200

Sterud, E., Forseth, T., Ugedal, O., Poppe, T. T., Jorgensen, A., Bruheim, T., Fjeldstad, H. P., & Mo, T. A. (2007). Severe mortality in wild Atlantic salmon *Salmo salar* due to proliferative kidney disease (PKD) caused by *Tetracapsuloides bryosalmonae* (myxozoa). *Diseases of Aquatic Organisms*, *77*(3), 191–198. https://doi.org/10.3354/dao01846

Strand, D. A., Jussila, J., Johnsen, S. I., Viljamaa-Dirks, S., Edsman, L., Wiik-Nielsen, J., Viljugrein, H., Engdahl, F., & Vrålstad, T. (2014). Detection of crayfish plague spores in large freshwater systems. *Journal of Applied Ecology*, *51*(2), 544–553. https://doi.org/10.1111/1365-2664.12218

Strepparava, N., Segner, H., Ros, A., Hartikainen, H., Schmidt-Posthaus, H., & Wahli, T. (2017). Temperature-related parasite infection dynamics: The case of proliferative kidney disease of brown trout. *Parasitology*, *145*(3), 281–291. https://doi.org/10.1017/S0031182017001482

Sudhagar, A., Kumar, G., & El-Matbouli, M. (2019). The Malacosporean Myxozoan parasite Tetracapsuloides bryosalmonae: A threat to wild salmonids. *Pathogens*, *9*(16), 1–16. https://www.mdpi.com/2076-0817/9/1/16

Svavarsdottir, F. R. (2016). *Proliferative kidney disease (PKD) in Icelandic fresh water. Distribution and prevalence of Tetracapsuloides bryosalmonae and its effect on salmonid populations in Iceland* (master master thesis). Háskóli Íslands, Samskipti ehf. Reykjavik, Ísland. https://skemman.is/bitstream/1946/24816/1/Lokautgafa_FjolaRut_Mastersritgerd.pdf

Svavarsdóttir, R. F., Freeman, A. M., Antonsson, Þ., Árnason, F., & Kristmundsson, Á. (2021). The presence of sporogonic stages of Tetracapsuloides bryosalmonae in Icelandic salmonids detected using in situ hybridisation. *Folia Parasitologica*, *68*(1), 1–8. https://doi.org/10.14411/fp.2021.020

Syrová, E., Palíková, M., Mendel, J., Seidlová, V., Papežíková, I., Schmidt-Posthaus, H., Somerlíková, K., Minářová, H., Mareš, L., Mikulíková, I., Pikula, J., & Mareš, J. (2020). Field study indicating susceptibility differences between salmonid species and their lineages to proliferative kidney disease. *Journal of Fish Diseases*, *43*(10), 1201–1211. https://doi.org/10.1111/jfd.13221

Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). *Environmental DNA: For biodiversity research and monitoring*.

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

Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., Orlando, L., & 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

Tops, S., Hartikainen, H. L., & Okamura, B. (2009). The effects of infection by *Tetracapsuloides bryosalmonae* (myxozoa) and temperature on *Fredericella sultana* (Bryozoa). *International Journal for Parasitology*, *39*(9), 1003–1010. https://www.sciencedirect.com/science/article/pii/S0020751909000927

Tops, S., Lockwood, W., & Okamura, B. (2006). Temperature-driven proliferation of *Tetracapsuloides bryosalmonae* in bryozoan hosts portends salmonid declines. *Diseases of Aquatic Organisms*, *70*(3), 227–236. https://doi.org/10.3354/dao070227

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G. H., Geniez, P., Pont, D., Argillier, C., Baudoin, J. M., … 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

Wahli, T., Bernet, D., Segner, H., & Schmidt-Posthaus, H. (2008). Role of altitude and water temperature as regulating factors for the geographical distribution of *Tetracapsuloides bryosalmonae* infected fishes in Switzerland. *Journal of Fish Biology*, *73*(9), 2184–2197. https://doi.org/10.1111/j.1095-8649.2008.02054.x

Wahli, T., Bernet, D., Steiner, P. A., & Schmidt-Posthaus, H. (2007). Geographic distribution of *Tetracapsuloides bryosalmonae* infected fish in swiss rivers: An update. *Aquatic Sciences*, *69*(1), 3–10. https://doi.org/10.1007/s00027-006-0843-4

Wahli, T., Knuesel, R., Bernet, D., Segner, H., Pugovkin, D., Burkhardt-Holm, P., Escher, M., & Schmidt-Posthaus, H. (2002). Proliferative kidney disease in Switzerland: Current state of knowledge. *Journal of Fish Diseases*, *25*(8), 491–500. https://doi.org/10.1046/j.1365-2761.2002.00401.x

Waldner, K., Borkovec, M., Borgwardt, F., Unfer, G., & El-Matbouli, M. (2021). Effect of water temperature on the morbidity of Tetracapsuloides bryosalmonae (myxozoa) to brown trout (Salmo trutta) under laboratory conditions. *Journal of Fish Diseases*, *44*(7), 1005–1013. https://doi.org/10.1111/jfd.13361

Wood, T. S., & Okamura, B. (2005). *A new key to the freshwater bryozoans of Britain, Ireland and continetal Europe, with notes on their ecology* (Vol. *63*). Cumbria.

Zhang, S., Lu, Q., Wang, Y., Wang, X., Zhao, J., & Yao, M. (2020). Assessment of fish communities using environmental DNA: Effect of spatial sampling design in lentic systems of different sizes. *Molecular Ecology Resources*, *20*(1), 242–255. https://doi.org/10.1111/1755-0998.13105

Økland, J., & Økland, K. A. (2005). Freshwater bryozoans (Bryozoa) of Norway V: Review and comparative discussion of the distribution and ecology of the 10 species recorded. *Hydrobiologia*, *534*(1–3), 31–55. <Go to ISI>://WOS:000227070700004

Økland, J., & Økland, K. A. (2006). Mosdyrene (Bryozoa) i ferskvann i Norge. *Fauna*, *59*, 2–14.

How to cite this article: Oredalen, T. J., Mo, T. A., Jenkins, A., Haugan, N., & Sæbø, M. (2022). Use of environmental DNA to detect the myxozoan endoparasite *Tetracapsuloides bryosalmonae* in large Norwegian lakes. *Environmental DNA*, *00*, 1–29. https://doi.org/10.1002/edn3.338

APPENDIX

Morphometric data and salmonid species present in the five lakes included in the survey

	Fyresvatn	Totak	Møsvatn	Tinnsjå (ex. Vestfjorden)	Norsjø
Area (km^2)[a]	49.795	37.264	79.097	49.535	55.116
Volume (m^3)[a]	218	2360	1574	9700	5100
Max/Mid depth (m)[a]	377/120	306/62	68.5/20	460/190	171/87
Altitude (m.a.s.l.)[a]	280	687	919	190	15
HRV/LRV	279.8/275.2	687.3/680	918.5/900	191.2/187.2	15.3/15.0
ΔHRV-LRV (m)[a]	4.6	7.3	18.5	4.0	0.3
Water level (m) [b] 2018/2019	277.9 /279.2	686.9/686.3	915.3 /915.6	188.5 /190.3	15.3 /15.3
Temperature (°C)	16.7/15.3	13.6/12.1	14.6/12.5	16.3/12.5	19.3/NA
First regulation year[c]	1914	1958	1944	1907	1952
Salmonid species present[d]	whitefish[1] brown trout[2] Arctic charr[3]	Arctic charr brown trout	Arctic charr brown trout	Arctic charr brown trout	whitefish brown trout Arctic charr

Note: Data references: [a]https://vannett.no/portal/waterbody/ extracted 07.04.2021., HRV: Highest Regulated water level (m), LRV—lowest regulated water level (m). https://vann-nett.no/portal/#/waterbody/, [b]The Norwegian Water Resources and Energy Directorate (NVE). Temperature measured from surface water above the deepest area of the lake. [c]https://atlas.nve.no/, [d]Lydersen (2015). [1]Coregonus lavaretus, [2]Salmo trutta, [3]Salvelinus alpinus, [4]Salmo salar.

APPENDIX 1

Raw data from PCR

Sample Name	Cт	Quantity in PCR sample	Voume filtrated, ml
Fyr 1.3.1 (19)	32.53	228.0	1000
Fyr 1.3.1 (19)	33.05	161.9	1000
Fyr 1.3.1 (19)	32.58	221.1	1000
Fyr 1.3.2 (19)	32.97	170.3	900
Fyr 1.3.2 (19)	31.67	404.9	900
Fyr 1.3.2 (19)	33.58	113.7	900
Fyr 1.3.3 (19)	29.59	1600.6	1000
Fyr 1.3.3 (19)	29.49	1711.2	1000
Fyr 1.3.3 (19)	29.17	2112.3	1000
Fyr 1.1.2 (19)	29.62	1568.9	1000
Fyr 1.1.2 (19)	30.66	787.4	1000
Fyr 1.1.2 (19)	31.19	554.3	1000
Fyr 1.1.1 (19)	Undetermined	0.0	1000
Fyr 1.1.1 (19)	Undetermined	0.0	1000
Fyr 1.1.1 (19)	Undetermined	0.0	1000
Fyr 1.1.3 (19)	Undetermined	0.0	650
Fyr 1.1.3 (19)	Undetermined	0.0	650
Fyr 1.1.3 (19)	Undetermined	0.0	650
Fyr 1.2.1 (19)	Undetermined	0.0	950
Fyr 1.2.1 (19)	Undetermined	0.0	950
Fyr 1.2.1 (19)	Undetermined	0.0	950
Fyr 1.2.2 (19)	Undetermined	0.0	1000
Fyr 1.2.2 (19)	Undetermined	0.0	1000
Fyr 1.2.2 (19)	Undetermined	0.0	1000
Fyr 1.2.3 (19)	Undetermined	0.0	1000
Fyr 1.2.3 (19)	Undetermined	0.0	1000
Fyr 1.2.3 (19)	Undetermined	0.0	1000
Fyr 1.5.1 (19)	Undetermined	0.0	1000
Fyr 1.5.1 (19)	Undetermined	0.0	1000
Fyr 1.5.1 (19)	38.72	4.5	1000
Fyr 1.5.2 (19)	Undetermined	0.0	900
Fyr 1.5.2 (19)	36.95	15.0	900
Fyr 1.5.2 (19)	Undetermined	0.0	900
Fyr 1.5.3 (19)	Undetermined	0.0	1000
Fyr 1.5.3 (19)	Undetermined	0.0	1000
Fyr 1.5.3 (19)	Undetermined	0.0	1000
Fyr midt-1 (19)	Undetermined	0.0	1000
Fyr midt-1 (19)	Undetermined	0.0	1000
Fyr midt-1 (19)	Undetermined	0.0	1000
Fyr midt-2 (19)	Undetermined	0.0	1000
Fyr midt-2 (19)	Undetermined	0.0	1000
Fyr midt-2 (19)	38.43	5.4	1000
Fyr midt-3 (19)	Undetermined	0.0	1000
Fyr midt-3 (19)	Undetermined	0.0	1000

Sample Name	Ct	Quantity in PCR sample	Voume filtrated, ml
Fyr midt-3 (19)	Undetermined	0.0	1000
Ti 2.1a.1 (19)	Undetermined	0.0	950
Ti 2.1a.1 (19)	Undetermined	0.0	950
Ti 2.1a.1 (19)	Undetermined	0.0	950
Ti 2.1a.2 (19)	Undetermined	0.0	950
Ti 2.1a.2 (19)	Undetermined	0.0	950
Ti 2.1a.2 (19)	Undetermined	0.0	950
Ti 2.1a.3 (19)	Undetermined	0.0	950
Ti 2.1a.3 (19)	Undetermined	0.0	950
Ti 2.1a.3 (19)	Undetermined	0.0	950
Ti 2.1b.1 (19)	Undetermined	0.0	950
Ti 2.1b.1 (19)	Undetermined	0.0	950
Ti 2.1b.1 (19)	Undetermined	0.0	950
Ti 2.1b.2 (19)	Undetermined	0.0	950
Ti 2.1b.2 (19)	Undetermined	0.0	950
Ti 2.1b.2 (19)	Undetermined	0.0	950
Ti 2.1b.3 (19)	Undetermined	0.0	950
Ti 2.1b.3 (19)	Undetermined	0.0	950
Ti 2.1b.3 (19)	Undetermined	0.0	950
Ti 2.2.1 (19)	Undetermined	0.0	1000
Ti 2.2.1 (19)	Undetermined	0.0	1000
Ti 2.2.1 (19)	Undetermined	0.0	1000
Ti 2.2.2 (19)	Undetermined	0.0	1000
Ti 2.2.2 (19)	Undetermined	0.0	1000
Ti 2.2.2 (19)	Undetermined	0.0	1000
Ti 2.2.3 (19)	Undetermined	0.0	1000
Ti 2.2.3 (19)	Undetermined	0.0	1000
Ti 2.2.3 (19)	Undetermined	0.0	1000
Ti 2.3.1 (19)	Undetermined	0.0	1000
Ti 2.3.1 (19)	Undetermined	0.0	1000
Ti 2.3.1 (19)	Undetermined	0.0	1000
Ti 2.3.2 (19)	Undetermined	0.0	1000
Ti 2.3.2 (19)	Undetermined	0.0	1000
Ti 2.3.2 (19)	Undetermined	0.0	1000
Ti 2.3.3 (19)	Undetermined	0.0	1000
Ti 2.3.3 (19)	Undetermined	0.0	1000
Ti 2.3.3 (19)	Undetermined	0.0	1000
Ti 2.4.1 (19)	Undetermined	0.0	950
Ti 2.4.1 (19)	Undetermined	0.0	950
Ti 2.4.1 (19)	Undetermined	0.0	950
Ti 2.4.2 (19)	Undetermined	0.0	900
Ti 2.4.2 (19)	Undetermined	0.0	900
Ti 2.4.2 (19)	37.45	10.6	900
Ti 2.4.3 (19)	Undetermined	0.0	900
Ti 2.4.3 (19)	Undetermined	0.0	900
Ti 2.4.3 (19)	Undetermined	0.0	900
Ti 2.5.1 (19)	Undetermined	0.0	950

Sample Name	Cт	Quantity in PCR sample	Voume filtrated, ml
Ti 2.5.1 (19)	35.78	33.2	950
Ti 2.5.1 (19)	38.31	5.9	950
Ti 2.5.2 (19)	32.75	144.1	1000
Ti 2.5.2 (19)	32.93	125.7	1000
Ti 2.5.2 (19)	32.70	150.7	1000
Ti 2.5.3 (19)	Undetermined	0.0	900
Ti 2.5.3 (19)	Undetermined	0.0	900
Ti 2.5.3 (19)	Undetermined	0.0	900
Ti midt-1 (19)	Undetermined	0.0	950
Ti midt-1 (19)	Undetermined	0.0	950
Ti midt-1 (19)	Undetermined	0.0	950
Ti midt-2 (19)	Undetermined	0.0	1000
Ti midt-2 (19)	Undetermined	0.0	1000
Ti midt-2 (19)	Undetermined	0.0	1000
Ti midt-3 (19)	Undetermined	0.0	1000
Ti midt-3 (19)	Undetermined	0.0	1000
Ti midt-3 (19)	Undetermined	0.0	1000
Tot 3.1.1 (19)	Undetermined	0.0	1000
Tot 3.1.1 (19)	Undetermined	0.0	1000
Tot 3.1.1 (19)	Undetermined	0.0	1000
Tot 3.1.2 (19)	Undetermined	0.0	1000
Tot 3.1.2 (19)	Undetermined	0.0	1000
Tot 3.1.2 (19)	Undetermined	0.0	1000
Tot 3.1.3 (19)	Undetermined	0.0	1000
Tot 3.1.3 (19)	Undetermined	0.0	1000
Tot 3.1.3 (19)	Undetermined	0.0	1000
Tot 3.2.1 (19)	Undetermined	0.0	1000
Tot 3.2.1 (19)	Undetermined	0.0	1000
Tot 3.2.1 (19)	Undetermined	0.0	1000
Tot 3.2.2 (19)	Undetermined	0.0	1000
Tot 3.2.2 (19)	Undetermined	0.0	1000
Tot 3.2.2 (19)	Undetermined	0.0	1000
M midt-1 (19)	Undetermined	0.0	1000
M midt-1 (19)	Undetermined	0.0	1000
M midt-1 (19)	34.79	30.4	1000
Tot 3.3.1 (19)	Undetermined	0.0	850
Tot 3.3.1 (19)	Undetermined	0.0	850
Tot 3.3.1 (19)	Undetermined	0.0	850
Tot 3.3.2 (19)	Undetermined	0.0	650
Tot 3.3.2 (19)	Undetermined	0.0	650
Tot 3.3.2 (19)	Undetermined	0.0	650
Tot 3.3.3 (19)	Undetermined	0.0	650
Tot 3.3.3 (19)	Undetermined	0.0	650
Tot 3.3.3 (19)	Undetermined	0.0	650
Tot 3.4.1 (19)	Undetermined	0.0	1000
Tot 3.4.1 (19)	Undetermined	0.0	1000
Tot 3.4.1 (19)	Undetermined	0.0	1000

Sample Name	Cт	Quantity in PCR sample	Voume filtrated, ml
Tot 3.4.2 (19)	Undetermined	0.0	1000
Tot 3.4.2 (19)	Undetermined	0.0	1000
Tot 3.4.2 (19)	Undetermined	0.0	1000
Tot 3.4.3 (19)	Undetermined	0.0	950
Tot 3.4.3 (19)	Undetermined	0.0	950
Tot 3.4.3 (19)	Undetermined	0.0	950
Tot midt-1 (19)	Undetermined	0.0	1000
Tot midt-1 (19)	Undetermined	0.0	1000
Tot midt-1 (19)	Undetermined	0.0	1000
Tot midt-2 (19)	Undetermined	0.0	1000
Tot midt-2 (19)	Undetermined	0.0	1000
Tot midt-2 (19)	Undetermined	0.0	1000
Tot midt-3 (19)	Undetermined	0.0	950
Tot midt-3 (19)	Undetermined	0.0	950
Tot midt-3 (19)	37.12	5.1	950
M 4.1.1 (19)	Undetermined	0.0	1000
M 4.1.2 (19)	37.18	4.9	1000
M 4.1.2 (19)	Undetermined	0.0	1000
M 4.1.2 (19)	34.45	39.6	1000
M 4.1.3 (19)	Undetermined	0.0	1000
M 4.1.3 (19)	Undetermined	0.0	1000
M 4.1.3 (19)	Undetermined	0.0	1000
M 4.2.1 (19)	Undetermined	0.0	1000
M 4.2.1 (19)	Undetermined	0.0	1000
M 4.2.1 (19)	Undetermined	0.0	1000
M 4.2.2 (19)	Undetermined	0.0	1000
M 4.2.2 (19)	Undetermined	0.0	1000
M 4.2.2 (19)	Undetermined	0.0	1000
M 4.2.3 (19)	Undetermined	0.0	1000
M 4.2.3 (19)	Undetermined	0.0	1000
M 4.2.3 (19)	Undetermined	0.0	1000
M midt-2 (19)	Undetermined	0.0	1000
M midt-2 (19)	Undetermined	0.0	1000
M midt-2 (19)	Undetermined	0.0	1000
M midt-3 (19)	Undetermined	0.0	1000
M midt-3 (19)	Undetermined	0.0	1000
M midt-3 (19)	Undetermined	0.0	1000
N 5.1.1 (19)	31.10	653.2	1000
N 5.1.1 (19)	31.16	626.7	1000
N 5.1.1 (19)	31.20	610.3	1000
N 5.1.2 (19)	Undetermined	0.0	1000
N 5.1.2 (19)	Undetermined	0.0	1000
N 5.1.2 (19)	Undetermined	0.0	1000
N 5.1.3 (19)	Undetermined	0.0	1000
N 5.1.3 (19)	Undetermined	0.0	1000
N 5.1.3 (19)	Undetermined	0.0	1000
N 5.2.1 (19)	Undetermined	0.0	1000

Sample Name	Cт	Quantity in PCR sample	Voume filtrated, ml
N 5.2.1 (19)	Undetermined	0.0	1000
N 5.2.1 (19)	Undetermined	0.0	1000
N 5.2.2 (19)	Undetermined	0.0	1000
N 5.2.2 (19)	Undetermined	0.0	1000
N 5.2.2 (19)	Undetermined	0.0	1000
N 5.2.3 (19)	Undetermined	0.0	1000
N 5.2.3 (19)	Undetermined	0.0	1000
N 5.2.3 (19)	Undetermined	0.0	1000
N 5.3.1 (19)	Undetermined	0.0	1000
N 5.3.1 (19)	Undetermined	0.0	1000
N 5.3.1 (19)	Undetermined	0.0	1000
N 5.3.2 (19)	Undetermined	0.0	950
N 5.3.2 (19)	Undetermined	0.0	950
N 5.3.2 (19)	Undetermined	0.0	950
N 5.3.3 (19)	Undetermined	0.0	950
N 5.3.3 (19)	Undetermined	0.0	950
N 5.3.3 (19)	Undetermined	0.0	950
N 5.5.1 (19)	Undetermined	0.0	1000
N 5.5.1 (19)	Undetermined	0.0	1000
N 5.5.1 (19)	Undetermined	0.0	1000
N 5.5.2 (19)	Undetermined	0.0	1000
N 5.5.2 (19)	Undetermined	0.0	1000
N 5.5.2 (19)	Undetermined	0.0	1000
N 5.5.3 (19)	Undetermined	0.0	1000
N 5.5.3 (19)	Undetermined	0.0	1000
N 5.5.3 (19)	Undetermined	0.0	1000
N midt-1 (19)	Undetermined	0.0	950
N midt-1 (19)	Undetermined	0.0	950
N midt-1 (19)	Undetermined	0.0	950
N midt-2 (19)	Undetermined	0.0	950
N midt-2 (19)	Undetermined	0.0	950
N midt-2 (19)	Undetermined	0.0	950
N midt-3 (19)	Undetermined	0.0	950
N midt-3 (19)	Undetermined	0.0	950
N midt-3 (19)	Undetermined	0.0	950
Fyr 1.1.1 (18)	36.71	14.1	910
Fyr 1.1.1 (18)	Undetermined	0.0	910
Fyr 1.1.1 (18)	36.70	14.3	910
Fyr 1.1.2 (18)	Undetermined	0.0	1000
Fyr 1.1.2 (18)	Undetermined	0.0	1000
Fyr 1.1.2 (18)	Undetermined	0.0	1000
Fyr 1.2.1 (18)	Undetermined	0.0	1000
Fyr 1.2.1 (18)	Undetermined	0.0	1000
Fyr 1.2.1 (18)	Undetermined	0.0	1000
Fyr 1.2.2 (18)	Undetermined	0.0	1000
Fyr 1.2.2 (18)	Undetermined	0.0	1000
Fyr 1.2.2 (18)	37.00	11.6	1000

Sample Name	Cт	Quantity in PCR sample	Voume filtrated, ml
Fyr 1.3.1 (18)	Undetermined	0.0	1000
Fyr 1.3.1 (18)	Undetermined	0.0	1000
Fyr 1.3.1 (18)	37.65	7.4	1000
Fyr 1.3.2 (18)	Undetermined	0.0	1000
Fyr 1.3.2 (18)	Undetermined	0.0	1000
Fyr 1.3.2 (18)	Undetermined	0.0	1000
Fyr 1.4.1 (18)	Undetermined	0.0	1000
Fyr 1.4.1 (18)	Undetermined	0.0	1000
Fyr 1.4.1 (18)	Undetermined	0.0	1000
Fyr 1.4.2 (18)	Undetermined	0.0	1000
Fyr 1.4.2 (18)	Undetermined	0.0	1000
Fyr 1.4.2 (18)	Undetermined	0.0	1000
Fyr 1.5.1 (18)	Undetermined	0.0	1000
Fyr 1.5.1 (18)	Undetermined	0.0	1000
Fyr 1.5.1 (18)	Undetermined	0.0	1000
Fyr 1.5.2 (18)	Undetermined	0.0	1000
Fyr 1.5.2 (18)	Undetermined	0.0	1000
Fyr 1.5.2 (18)	Undetermined	0.0	1000
Fyr midt1 (18)	Undetermined	0.0	1000
Fyr midt1 (18)	Undetermined	0.0	1000
Fyr midt1 (18)	36.42	16.4	1000
Fyr midt2 (18)	36.97	11.2	1000
Fyr midt2 (18)	Undetermined	0.0	1000
Fyr midt2 (18)	Undetermined	0.0	1000
Fyr Blank1 (18)	Undetermined	0.0	1000
Fyr Blank1 (18)	Undetermined	0.0	1000
Fyr Blank1 (18)	Undetermined	0.0	1000
M 4.1.1 (18)	37.98	5.6	1000
M 4.1.1 (18)	Undetermined	0.0	1000
M 4.1.1 (18)	Undetermined	0.0	1000
M 4.1.2 (18)	Undetermined	0.0	1000
M 4.1.2 (18)	Undetermined	0.0	1000
M 4.1.2 (18)	35.74	26.3	1000
M midt1 (18)	Undetermined	0.0	1000
M midt1 (18)	Undetermined	0.0	1000
M midt1 (18)	Undetermined	0.0	1000
M midt2 (18)	Undetermined	0.0	1000
M midt2 (18)	Undetermined	0.0	1000
M midt2 (18)	Undetermined	0.0	1000
M 4.2.1 (18)	Undetermined	0.0	1000
M 4.2.1 (18)	Undetermined	0.0	1000
M 4.2.1 (18)	Undetermined	0.0	1000
M 4.2.2 (18)	Undetermined	0.0	1000
M 4.2.2 (18)	Undetermined	0.0	1000
M 4.2.2 (18)	Undetermined	0.0	1000
Ti 2.1.1 (18)	Undetermined	0.0	1000
Ti 2.1.1 (18)	Undetermined	0.0	1000

Sample Name	Cт	Quantity in PCR sample	Voume filtrated, ml
Ti 2.1.1 (18)	Undetermined	0.0	1000
Ti 2.1.2 (18)	Undetermined	0.0	1000
Ti 2.1.2 (18)	Undetermined	0.0	1000
Ti 2.1.2 (18)	Undetermined	0.0	1000
Ti 2.2.1 (18)	Undetermined	0.0	1000
Ti 2.2.1 (18)	36.98	11.1	1000
Ti 2.2.1 (18)	Undetermined	0.0	1000
Ti 2.2.2 (18)	Undetermined	0.0	1000
Ti 2.2.2 (18)	Undetermined	0.0	1000
Ti 2.2.2 (18)	Undetermined	0.0	1000
Ti 2.3.1 (18)	Undetermined	0.0	1000
Ti 2.3.1 (18)	Undetermined	0.0	1000
Ti 2.3.1 (18)	Undetermined	0.0	1000
Ti 2.3.2 (18)	Undetermined	0.0	1000
Ti 2.3.2 (18)	Undetermined	0.0	1000
Ti 2.3.2 (18)	Undetermined	0.0	1000
Ti 2.4.1 (18)	Undetermined	0.0	1000
Ti 2.4.1 (18)	Undetermined	0.0	1000
Ti 2.4.1 (18)	Undetermined	0.0	1000
Ti 2.4.2 (18)	Undetermined	0.0	1000
Ti 2.4.2 (18)	Undetermined	0.0	1000
Ti 2.4.2 (18)	Undetermined	0.0	1000
Ti 2.5.1 (18)	Undetermined	0.0	1000
Ti 2.5.1 (18)	Undetermined	0.0	1000
Ti 2.5.1 (18)	Undetermined	0.0	1000
Ti 2.5.2 (18)	Undetermined	0.0	1000
Ti 2.5.2 (18)	Undetermined	0.0	1000
Ti 2.5.2 (18)	Undetermined	0.0	1000
Ti midt1 (18)	Undetermined	0.0	1000
Ti midt1 (18)	Undetermined	0.0	1000
Ti midt1 (18)	Undetermined	0.0	1000
Ti midt2 (18)	Undetermined	0.0	1000
Ti midt2 (18)	Undetermined	0.0	1000
Ti midt2 (18)	Undetermined	0.0	1000
To 3.1.1 (18)	Undetermined	0.0	1000
To 3.1.1 (18)	Undetermined	0.0	1000
To 3.1.1 (18)	Undetermined	0.0	1000
To 3.1.2 (18)	Undetermined	0.0	1000
To 3.1.2 (18)	Undetermined	0.0	1000
To 3.1.2 (18)	Undetermined	0.0	1000
To 3.2.1 (18)	Undetermined	0.0	1000
To 3.2.1 (18)	37.87	5.0	1000
To 3.2.1 (18)	Undetermined	0.0	1000
To 3.2.2 (18)	Undetermined	0.0	1000
To 3.2.2 (18)	Undetermined	0.0	1000
To 3.2.2 (18)	Undetermined	0.0	1000
To 3.3.1 (18)	Undetermined	0.0	1000

Sample Name	Ct	Quantity in PCR sample	Voume filtrated, ml
To 3.3.1 (18)	Undetermined	0.0	1000
To 3.3.1 (18)	37.89	4.9	1000
To 3.3.2 (18)	Undetermined	0.0	1000
To 3.3.2 (18)	Undetermined	0.0	1000
To 3.3.2 (18)	Undetermined	0.0	1000
To 3.4.1 (18)	Undetermined	0.0	1000
To 3.4.1 (18)	Undetermined	0.0	1000
To 3.4.1 (18)	Undetermined	0.0	1000
To 3.4.2 (18)	Undetermined	0.0	1000
To 3.4.2 (18)	Undetermined	0.0	1000
To 3.4.2 (18)	44.11	0.1	1000
To 3.5.1 (18)	Undetermined	0.0	1000
To 3.5.1 (18)	Undetermined	0.0	1000
To 3.5.1 (18)	Undetermined	0.0	1000
To 3.5.2 (18)	Undetermined	0.0	1000
To 3.5.2 (18)	Undetermined	0.0	1000
To 3.5.2 (18)	Undetermined	0.0	1000
To midt1 (18)	Undetermined	0.0	1000
To midt1 (18)	Undetermined	0.0	1000
To midt1 (18)	Undetermined	0.0	1000
To midt2 (18)	Undetermined	0.0	1000
To midt2 (18)	Undetermined	0.0	1000
To midt2 (18)	Undetermined	0.0	1000
N Blank1 (18)	Undetermined	0.0	1000
N Blank1 (18)	Undetermined	0.0	1000
N Blank1 (18)	Undetermined	0.0	1000
N 5.1.1 (18)	Undetermined	0.0	1000
N 5.1.1 (18)	Undetermined	0.0	1000
N 5.1.1 (18)	Undetermined	0.0	1000
N 5.1.2 (18)	Undetermined	0.0	1000
N 5.1.2 (18)	Undetermined	0.0	1000
N 5.1.2 (18)	Undetermined	0.0	1000
N 5.2.1 (18)	Undetermined	0.0	1000
N 5.2.1 (18)	Undetermined	0.0	1000
N 5.2.1 (18)	Undetermined	0.0	1000
N 5.2.2 (18)	Undetermined	0.0	1000
N 5.2.2 (18)	Undetermined	0.0	1000
N 5.2.2 (18)	Undetermined	0.0	1000
N 5.3.1 (18)	Undetermined	0.0	1000
N 5.3.1 (18)	Undetermined	0.0	1000
N 5.3.1 (18)	Undetermined	0.0	1000
N 5.3.2 (18)	Undetermined	0.0	1000
N 5.3.2 (18)	Undetermined	0.0	1000
N 5.3.2 (18)	Undetermined	0.0	1000
N 5.4.1 (18)	Undetermined	0.0	1000
N 5.4.1 (18)	Undetermined	0.0	1000
N 5.4.1 (18)	Undetermined	0.0	1000

Sample Name	Cт	Quantity in PCR sample	Voume filtrated, ml
N 5.4.2 (18)	Undetermined	0.0	1000
N 5.4.2 (18)	Undetermined	0.0	1000
N 5.4.2 (18)	Undetermined	0.0	1000
N 5.5.1 (18)	Undetermined	0.0	1000
N 5.5.1 (18)	Undetermined	0.0	1000
N 5.5.1 (18)	Undetermined	0.0	1000
N 5.5.2 (18)	Undetermined	0.0	1000
N 5.5.2 (18)	Undetermined	0.0	1000
N 5.5.2 (18)	Undetermined	0.0	1000
N midt1 (18)	Undetermined	0.0	1000
N midt1 (18)	38.05	4.4	1000
N midt1 (18)	Undetermined	0.0	1000
N midt2 (18)	Undetermined	0.0	1000
N midt2 (18)	36.26	15.4	1000
N midt2 (18)	Undetermined	0.0	1000
N Blank2 (18)	Undetermined	0.0	1000
N Blank2 (18)	Undetermined	0.0	1000
N Blank2 (18)	Undetermined	0.0	1000

Note: C_T-values, which are marked in red, are not included in the results.

APPENDIX 2

Measured ng DNA/µL in eDNA samples after extraction with Qiagen Blood and Tissue Kit and Power Water Kit, respectively

	2018							2019						
Station	sample date	Filter date	Extraction method	Filter volum (ml)	Extraction date	Nanodrop ngDNA/µLA	260/A280 renhet	Sample date	Filter date	Extraction method	Filter volume (ml)	Extraction date	Nanodrop ngDNA/µl	A260/A280 renhet
Fyr 1.1-1	14.08.2018	14.08.2018	B&T	910	18.09.2019	96.9	1.46	10.09.2019	10.09.2019	PW	1000	10.10.2019	12.2	1.76
Fyr 1.1-2	14.08.2018	14.08.2018	B&T	1000	18.09.2019	86.3	1.54	10.09.2019	12.09.2019	PW	1000	10.10.2019	17.4	1.65
Fyr 1.1-3								10.09.2019	12.09.2019	PW	650	10.10.2019	9.8	1.54
Fyr 1.2-1	14.08.2018	14.08.2018	B&T	1000	18.09.2019	26.9	1.73	10.09.2019	10.09.2019	PW	950	10.10.2019	9.6	1.47
Fyr 1.2-2	14.08.2018	14.08.2018	B&T	1000	18.09.2019	32.5	1.67	10.09.2019	10.09.2019	PW	1000	10.10.2019	9.7	1.46
Fyr 1.2-3								10.09.2019	10.09.2019	PW	1000	10.10.2019	9.2	1.39
Fyr 1.3-1	14.08.2018	14.08.2018	B&T	1000	19.09.2019	65.9	1.9	10.09.2019	10.09.2019	PW	1000	10.10.2019	14.2	1.55
Fyr 1.3-2	14.08.2018	14.08.2018	B&T	1000	19.09.2019	56.6	1.54	10.09.2019	10.09.2019	PW	900	10.10.2019	9.8	1.62
Fyr 1.3-3								10.09.2019	10.09.2019	PW	1000	10.10.2019	8.3	1.53
	14.08.2018	14.08.2018	B&T	1000	19.09.2019	31.8	1.74							
	14.08.2018	14.08.2018	B&T	1000	19.09.2019	32.7	1.78							
Fyr 1.5-1	14.08.2018	14.08.2018	B&T	1000	19.09.2019	34.3	1.68	10.09.2019	10.09.2019	PW	1000	10.10.2019	15.3	1.76
Fyr 1.5-2	14.08.2018	14.08.2018	B&T	1000	19.09.2019	25.7	1.77	10.09.2019	10.09.2019	PW	900	10.10.2019	7.8	1.67
Fyr 1.5-3								10.09.2019	10.09.2019	PW	1000	11.10.2019	8.3	1.45
Fyr Midt-1	14.08.2018	14.08.2018	B&T	1000	19.09.2019	32.2	1.78	10.09.2019	10.09.2019	PW	1000	11.10.2019	6.7	1.5
Fyr Midt-2	14.08.2018	14.08.2018	B&T	1000	19.09.2019	26.3	1.79	10.09.2019	10.09.2019	PW	1000	11.10.2019	7.1	1.48
Fyr Midt-3								10.09.2019	10.09.2019	PW	1000	11.10.2019	6.9	1.5
Ti 2.1a-1	15.08.2018	16.08.2018	B&T	1000	19.09.2019	29.1	1.67	12.09.2019	12.09.2019	PW	950	11.10.2019	4.9	1.41
Ti 2.1a-2	15.08.2018	16.08.2018	B&T	1000	19.09.2019	20.1	1.7	12.09.2019	12.09.2019	PW	950	11.10.2019	4.4	1.43
Ti 2.1a-3								12.09.2019	12.09.2019	PW	950	11.10.2019	4.5	1.44
Ti 2.1b-1								12.09.2019	12.09.2019	PW	950	11.10.2019	9.7	1.64
Ti 2.1b-2								12.09.2019	12.09.2019	PW	950	11.10.2019	9.9	1.56
Ti 2.1b-3								12.09.2019	12.09.2019	PW	1000	11.10.2019	9.5	1.58
Ti 2.2-1	15.08.2018	16.08.2018	B&T	1000	19.09.2019	42.3	1.58	12.09.2019	12.09.2019	PW	1000	11.10.2019	6.2	1.45
Ti 2.2-2	15.08.2018	16.08.2018	B&T	1000	20.09.2019	38.5	1.63	12.09.2019	12.09.2019	PW	1000	11.10.2019	7.4	1.41
Ti 2.2-3								12.09.2019	12.09.2019	PW	1000	11.10.2019	6.1	1.47
Ti 2.3-1	15.08.2018	16.08.2018	B&T	1000	20.09.2019	43.8	1.64	12.09.2019	12.09.2019	PW	1000	11.10.2019	7.7	1.62
Ti 2.3-2	15.08.2018	16.08.2018	B&T	1000	20.09.2019	53	1.66	12.09.2019	12.09.2019	PW	1000	11.10.2019	8.8	1.67
Ti 2.3-3								12.09.2019	12.09.2019	PW	1000	11.10.2019	7.5	1.54
Ti 2.4-1	15.08.2018	16.08.2018	B&T	1000	20.09.2019	31.4	1.81	12.09.2019	12.09.2019	PW	950	12.10.2019	6.1	1.42

	2018							2019						
Station	sample date	Filter date	Extraction method	Filter volum (ml)	Extraction date	Nanodrop ngDNA/µLA	260/A280 renhet	Sample date	Filter date	Extraction method	Filter volume (ml)	Extraction date	Nanodrop ngDNA/µl	A260/A280 renhet
Ti 2.4-2	15.08.2018	16.08.2018	B&T	1000	20.09.2019	33.9	1.89	12.09.2019	12.09.2019	PW	900	12.10.2019	7.5	1.5
Ti 2.4-3								12.09.2019	12.09.2019	PW	900	12.10.2019	7.5	1.58
Ti 2.5-1	15.08.2018	16.08.2018	B&T	1000	20.09.2019	25.2	1.76	12.09.2019	12.09.2019	PW	950	12.10.2019	7.4	1.6
Ti 2.5-2	15.08.2018	16.08.2018	B&T	1000	20.09.2019	24.9	1.76	12.09.2019	12.09.2019	PW	1000	12.10.2019	6.5	1.46
Ti 2.5-3								12.09.2019	12.09.2019	PW	900	12.10.2019	7.1	1.66
Ti Midt-1	15.08.2018	16.08.2018	B&T	1000	20.09.2019	27	1.76	12.09.2019	12.09.2019	PW	950	12.10.2019	7.2	1.53
Ti Midt-2	15.08.2018	16.08.2018	B&T	1000	20.09.2019	32.5	1.81	12.09.2019	12.09.2019	PW	1000	12.10.2019	8.1	1.5
Ti Midt-3								12.09.2019	12.09.2019	PW	1000	14.10.2019	7.3	1.53
Tot 3.1-1	15.08.2018	16.08.2018	B&T	1000	20.09.2019	33.3	1.78	12.09.2019	12.09.2019	PW	1000	14.10.2019	8.6	1.65
Tot 3.1-2	15.08.2018	16.08.2018	B&T	1000	20.09.2019	29.3	1.93	12.09.2019	12.09.2019	PW	1000	14.10.2019	9.1	1.48
Tot 3.1-3								12.09.2019	12.09.2019	PW	1000	14.10.2019	6.4	1.71
Tot 3.2-1	15.08.2018	16.08.2018	B&T	1000	20.09.2019	33.6	1.82	12.09.2019	12.09.2019	PW	1000	14.10.2019	7.9	1.65
Tot 3.2-2	15.08.2018	16.08.2018	B&T	1000	20.09.2019	37	1.79	12.09.2019	12.09.2019	PW	1000	14.10.2019	9	1.63
Tot 3.2-3								12.09.2019	12.09.2019	PW	1000	14.10.2019	8	1.64
Tot 3.3-1	15.08.2018	16.08.2018	B&T	1000	20.09.2019	39.1	1.83	12.09.2019	12.09.2019	PW	850	21.10.2019	16	1.68
Tot 3.3-2	15.08.2018	16.08.2018	B&T	1000	20.09.2019	44.1	1.86	12.09.2019	12.09.2019	PW	650	21.10.2019	13	1.68
Tot 3.3-3								12.09.2019	12.09.2019	PW	650	21.10.2019	11.8	1.62
Tot 3.4-1	15.08.2018	16.08.2018	B&T	1000	24.09.2019	64.5	1.47	12.09.2019	12.09.2019	PW	1000	21.10.2019	11.6	1.53
Tot 3.4-2	15.08.2018	16.08.2018	B&T	1000	24.09.2019	75.3	1.68	12.09.2019	12.09.2019	PW	1000	21.10.2019	8.6	1.46
Tot 3.4-3								12.09.2019	12.09.2019	PW	950	21.10.2019	10.6	1.37
	15.08.2018	16.08.2018	B&T	1000	24.09.2019	42.5	1.75							
	15.08.2018	16.08.2018	B&T	1000	24.09.2019	34.7	1.24							
Tot Midt-1	15.08.2018	16.08.2018	B&T	1000	24.09.2019	27.2	1.79	12.09.2019	12.09.2019	PW	1000	21.10.2019	6.4	1.41
Tot Midt-2	15.08.2018	16.08.2018	B&T	1000	24.09.2019	34.1	1.85	12.09.2019	12.09.2019	PW	1000	21.10.2019	7.5	1.62
Tot Midt-3								12.09.2019	12.09.2019	PW	950	21.10.2019	8	1.43
N 5.1-1	17.08.2018	17.08.2018	B&T	1000	24.09.2019	48.5	1.8	06.09.2019	06.09.2019	PW	1000	22.10.2019	20.6	1.69
N 5.1-2	17.08.2018	17.08.2018	B&T	1000	24.09.2019	42	1.79	06.09.2019	06.09.2019	PW	1000	22.10.2019	17.2	1.68
N 5.1-3								06.09.2019	06.09.2019	PW	1000	22.10.2019	18.3	1.8
N 5.2-1	17.08.2018	17.08.2018	B&T	1000	24.09.2019	42.8	1.68	06.09.2019	06.09.2019	PW	1000	22.10.2019	12.7	1.63
N 5.2-2	17.08.2018	17.08.2018	B&T	1000	24.09.2019	38.9	1.66	06.09.2019	06.09.2019	PW	1000	22.10.2019	11.9	1.61
N 5.2-3								06.09.2019	06.09.2019	PW	1000	22.10.2019	17.2	1.66

	2018							2019						
Station	sample date	Filter date	Extraction method	Filter volum (ml)	Extraction date	Nanodrop ngDNA/µLA	260/A280 renhet	Sample date	Filter date	Extraction method	Filter volume (ml)	Extraction date	Nanodrop ngDNA/µl	A260/A280 renhet
N 5.3-1	17.08.2018	17.08.2018	B&T	1000	24.09.2019	32	1.76	17.09.2019	17.09.2019	PW	1000	22.10.2019	12.4	1.61
N 5.3-2	17.08.2018	17.08.2018	B&T	1000	24.09.2019	35.6	1.81	17.09.2019	17.09.2019	PW	950	22.10.2019	12.8	1.63
N 5.3-3								17.09.2019	17.09.2019	PW	950	22.10.2019	12.1	1.6
N 5.4-1	17.08.2018	17.08.2018	B&T	1000	24.09.2019	44.1	1.67	17.09.2019	17.09.2019	PW	1000	22.10.2019	13.1	1.58
N 5.4-2	17.08.2018	17.08.2018	B&T	1000	24.09.2019	50.8	1.67	17.09.2019	17.09.2019	PW	1000	22.10.2019	12.4	1.61
N 5.4-3								17.09.2019	17.09.2019	PW	1000	22.10.2019	10.1	1.66
	17.08.2018	17.08.2018	B&T	1000	24.09.2019	54.7	1.55							
	17.08.2018	17.08.2018	B&T	1000	24.09.2019	59.3	1.8							
N Midt-1	17.08.2018	17.08.2018	B&T	1000	24.09.2019	41.1	1.79	17.09.2019	17.09.2019	PW	950	22.10.2019	10.2	1.7
N Midt-2	17.08.2018	17.08.2018	B&T	1000	24.09.2019	57.6	1.62	17.09.2019	17.09.2019	PW	950	22.10.2019	12	1.66
N Midt-3								17.09.2019	17.09.2019	PW	950	22.10.2019	10.8	1.6
M 4.1-1	15.08.2018	16.08.2018	B&T	1000	19.09.2019	52.1	1.73	12.09.2019	12.09.2019	PW	1000	21.10.2019	9.1	1.51
M 4.1-2	15.08.2018	16.08.2018	B&T	1000	19.09.2019	42.7	1.76	12.09.2019	12.09.2019	PW	1000	21.10.2019	9.6	1.56
M 4.1-3								12.09.2019	12.09.2019	PW	1000	21.10.2019	12.5	1.63
M 4.2-1	15.08.2018	16.08.2018	B&T	1000	19.09.2019	37.4	1.88	12.09.2019	12.09.2019	PW	1000	21.10.2019	8.9	1.56
M 4.2-2	15.08.2018	16.08.2018	B&T	1000	19.09.2019	18.9	1.75	12.09.2019	12.09.2019	PW	1000	21.10.2019	8.1	1.42
M 4.2-3								12.09.2019	12.09.2019	PW	1000	21.10.2019	9	1.5
M Midt-1	15.08.2018	16.08.2018	B&T	1000	19.09.2019	49.6	1.83	12.09.2019	12.09.2019	PW	1000	22.10.2019	14.1	1.53
M Midt-2	15.08.2018	16.08.2018	B&T	1000	19.09.2019	16.1	1.9	12.09.2019	12.09.2019	PW	1000	22.10.2019	9.7	1.46
M Midt-3								12.09.2019	12.09.2019	PW	1000	22.10.2019	7.9	1.51
					max	96.9						max	20.6	
					min	16.1						min	4.4	
					average	40.9						average	9.9	
					median	37.2						median	9.1	