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Patterns of *Tetracapsuloides bryosalmonae* infection of three salmonid species in large, deep Norwegian lakes

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Abstract

Proliferative kidney disease (PKD), caused by the myxozoan endoparasite *Tetracapsuloides bryosalmonae*, is of serious ecological and economical concern to wild and farmed salmonids. Wild salmonid populations have declined due to PKD, primarily in rivers, in Europe and North America. Deep lakes are also important habitats for salmonids, and this work aimed to investigate parasite presence in five deep Norwegian lakes. Kidney samples from three salmonid species from deep lakes were collected and tested using real-time PCR to detect PKD parasite presence. We present the first detection of *T. bryosalmonae* in European whitefish in Norway for the first time, as well as the first published documentation of the parasite in kidneys of Arctic charr, brown trout and whitefish in four lakes. The observed prevalence of the parasite was higher in populations of brown trout than of Arctic charr and whitefish. The parasite was detected in farmed, but not in wild, charr in one lake. This suggests a possible link with a depth of fish habitat and fewer *T. bryosalmonae*-infected and PKD-affected fish. Towards a warmer climate, cold hypolimnion in deep lakes may act as a refuge for wild salmonids, while cold deep water may be used to control PKD in farmed salmonids.

KEYWORDS
climate change, hypolimnion refuge, proliferative kidney disease

1 | INTRODUCTION

Wild and cultured salmonids, including Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*), have high ecological, recreational and economical value in Europe and North America (Abd-Elfattah, Kumar, et al., 2014; Bailey et al., 2020; Forseth, Barlaup, et al., 2017; Gorgoglione et al., 2020; Syrová et al., 2020).

Proliferative kidney disease (PKD) is an emerging disease, which periodically causes high mortality in both farmed and wild freshwater salmonids in these continents (Bruneaux et al., 2017; Ferguson & Needham, 1978; Forseth, Jørgensen, et al., 2017; Hedrick et al., 1993; Mo & Jørgensen, 2017; Okamura & Feist, 2011; Okamura et al., 2011; Schmidt-Posthaus et al., 2015; Smith et al., 1984; Wahli et al., 2002). The disease is caused by the myxozoan endoparasite *Tetracapsuloides bryosalmonae* that alternates between a bryozoan definitive host and a salmonid intermediate host (Anderson et al., 1999; Longshaw et al., 2002; Morris et al., 2000; Okamura et al., 2011). *T. bryosalmonae* spores are released from the bryozoans, enter the fish mainly through the skin and gills and infect primarily the kidney (Bruneaux et al., 2017; Burkhardt-Holm et al., 2005; Kent & Hedrick, 1985; Mo & Jørgensen, 2017; Okamura et al., 2011;

Sterud et al., 2007). Young-of-the-year fish and fish that are not previously exposed to the parasite are particularly vulnerable to infection and PKD-induced mortality (Burkhardt-Holm et al., 2005; Mo et al., 2011). PKD can be lethal alone (Bettge, Segner, et al., 2009; Bettge, Wahli, et al., 2009), but also in combination with secondary infections, the mortality of infected fish in total can be very high—almost 100%, in both farmed and wild salmonids (Feist & Bucke, 1993; Wahli et al., 2007). Severe outbreaks of PKD with subsequent high mortalities have been observed in wild salmonids. In Switzerland, outbreaks of PKD in wild and farmed rainbow trout, brown trout and grayling have been documented almost every year since the first diagnosis in 1979 (Wahli et al., 2002). *T. bryosalmonae* is now considered to be one of the major factors for decline in wild populations of brown trout and rainbow trout in the alpine rivers (Burkhardt-Holm et al., 2005; Gorgoglione et al., 2016; Lewisch et al., 2018; Ros et al., 2021; Sudhagar et al., 2019). Also in other countries in Europe and North America, the parasite and mortality due to PKD are observed in Atlantic salmon, Arctic charr, European whitefish (*Coregonus lavaretus*) and mountain whitefish (*Prosopium williamsoni*) (Feist et al., 2002; Forseth, Jørgensen, et al., 2017; Gorgoglione et al., 2016; Hutchins et al., 2021; Kristmundsson et al., 2010; Mo, 2007; Mo & Jørgensen, 2017; Mo et al., 2011; Naldoni et al., 2019; Opitz & Rhoten, 2016; Skovgaard & Buchmann, 2012; Sterud et al., 2007; Sudhagar et al., 2019; Svavarsdottir, 2016; Vasemagi et al., 2017; Wahli et al., 2002).

Several factors have been suggested to influence the risk of infection and spread of *T. bryosalmonae* in lakes and rivers, often linked with increased water temperatures (Hendrick et al., 1986; Okamura et al., 2011; Rubin et al., 2019; Tops et al., 2006, 2009; Waldner et al., 2021), manipulations of watercourses, fish stocking, increased water pollution, differences in susceptibility and immune responses to the parasite (Bailey et al., 2019; Grabner & El-Matbouli, 2009; Kumar et al., 2013; Ros et al., 2021; Schmidt-Posthaus et al., 2017). Outbreak of PKD is observed when the water temperature exceeds 12–15°C for more than 14 days (Bettge, Segner, et al., 2009; Bettge, Wahli, et al., 2009; Brown et al., 1991; Ferguson, 1981; Hedrick et al., 1993; Okamura et al., 2011; Wahli et al., 2002), and high morbidity and mortality of wild salmonids associated with PKD are mainly observed during summer and early autumn in the Northern Hemisphere (Mo & Jørgensen, 2017; Okamura et al., 2011; Sterud et al., 2007; Wahli et al., 2008). It is expected that the spread of the parasite *T. bryosalmonae* and outbreaks of PKD will constitute an increased threat to salmonids in the future, due to climate change with expected increasing water temperatures, as well as human activities and water pollution (Mo & Jørgensen, 2017; Okamura et al., 2011; Ros et al., 2021; Strepparava et al., 2017; Sudhagar et al., 2019; Tops et al., 2006).

Several bryozoan species belonging to the Class Phylactolaemata are shown to host *T. bryosalmonae*, including *Fredericella sultana*, *Plumatella rugosa*, *P. fruticosa*, *P. emerginata*, *Plumatella* sp., *Cristatella mucedo*, and *Pectinatella magnifica* (Anderson et al., 1999; Longshaw et al., 1999; Okamura et al., 2001; Ros et al., 2021; Sudhagar et al., 2019); *F. sultana* considered to be the main primary host (Abd-Elfattah, Fontes, et al., 2014; Ros et al., 2021). In Norway, bryozoan species belonging to the Class Phylactolaemata are widespread throughout the country (Økland and Økland, 2000, 2001, 2002; 2005; Økland et al., 2003). The bryozoan main habitats are considered to be sheltered bottom substrates in rivers and the littoral zone in lakes (Økland & Økland, 2005; Wood & Okamura, 2005), even if occurrence in deeper areas is also reported (Raddum & Johnsen, 1983). Fish species feeding on benthic prey in epilimnion of lakes during summer season will presumably have a higher risk of being infected by the short-lived parasite spores released from bryozoans than fish living and feeding in the pelagic or in the cold hypolimnion of lakes.

Different fish and parasite strains with various history of host–parasite coevolution might influence salmonid susceptibility to *T. bryosalmonae* (Bailey et al., 2018; Grabner & El-Matbouli, 2008; Okamura et al., 2011). Two main clades of *T. bryosalmonae* have so far been reported, one North American and one European (Henderson & Okamura, 2004). Native European brown trout and brook trout (*Salvelinus fontinalis*) are shown to be active hosts for the European strain of *T. bryosalmonae*, being able to produce spores that can infect new bryozoan colonies (Grabner & El-Matbouli, 2008; Morris & Adams, 2006; Syrová et al., 2020). Rainbow trout and grayling (*Thymallus thymallus*) most probably lack viable spore production of the European parasite clade (Grabner & El-Matbouli, 2008; Syrová et al., 2020), while rainbow trout are shown to produce spores of *T. bryosalmonae* in an American trial (Hedrick et al., 2004). A recent study, from one lake and river in Iceland, suggests that native populations of Arctic charr, brown trout and Atlantic salmon all are active hosts for the European strain of *T. bryosalmonae*. However, there are still remaining questions regarding which salmonids are a 'dead-end' host and which are active host for the two known main strains of *T. bryosalmonae* (Grabner & El-Matbouli, 2008; Naldoni et al., 2019; Tops et al., 2009).

The presence of *T. bryosalmonae* and outbreaks of PKD have mostly been studied in rivers and shallow lakes in Europe and North America (Feist et al., 2002; Kristmundsson et al., 2010; Lewisch et al., 2018; Mo & Jørgensen, 2017; Okamura et al., 2001; Opitz & Rhoten, 2016; Sterud et al., 2007; Svavarsdottir, 2016; Wahli et al., 2007), and there is little knowledge about the presence of the parasite in large and deep lakes. In Norway, deep dimictic lakes are important habitats for wild salmonids, as well as interesting locations for existing and potential future freshwater fish farming facilities. The dynamics of these deep lakes differ substantial from rivers and shallow lakes, as they often are clear water lakes low in nutrients and with a stable cold hypolimnion during summer season. This cold, deep layer might represent a potential refugium—and 'rescue'—to cold water-adapted species during summers with elevated lake surface temperatures (Gaudard et al., 2018). One screening of *T. bryosalmonae* in kidneys from Arctic charr in 10 deep lakes of Telemark County (southern Norway) performed by the University of South-Eastern Norway (USN) in 2015 detected the parasite in three lakes (personal observations). As far as we know, there have only been a few other registrations of *T. bryosalmonae* from other deep

European lakes, such as Lake Lucerne in Switzerland where the parasite was detected in 6 out of 7 analysed European whitefish individuals (Naldoni et al., 2019).

The aim of our research was to study the presence of *Tetracapsuloides bryosalmonae* in salmonid species from five selected large, deep, dimictic lakes in southern part of Norway, and to compare the parasite prevalence in different salmonid species in the same lake, and the prevalence in the same species in different lakes.

2 | MATERIALS AND METHODS

2.1 | Study area

Five large and deep, regulated, oligotrophic-to-mesotrophic lakes located in southern Norway (in Vestfold and Telemark County) were selected for this survey (Figure 1). All lakes are nutrient-poor, regarding the two main nutritional variables total nitrogen and total phosphorous (vann-nett.no). Four of the lakes (Fyresvatn, Totak, Møsvatn and Tinnsjå) are regulated for hydropower purposes, with regulation zone ranging from 4.0 to 18.5 m. The regulation zone is the range between the permitted highest (HRV) and the lowest (LRV) water level, defined in the regulation admission from the Norwegian Water Resources and Energy Directorate (NVE). All lakes have been regulated for many years, with the first dam installation spanning from 1907 to 1958. For all lakes, except Lake Norsjø, the regulation has resulted in a littoral zone sparsely populated by macrophytes and benthic invertebrates, as often is the case in lakes with annual winter reduction in water levels (Carmignani & Roy, 2017).

Morphometric, main nutrient and fish data of the lakes are given in Table 1. All five lakes have native populations of Arctic charr and brown trout, while two lakes also have populations of whitefish. Lake Norsjø is the most species-rich lake regarding fish, with 12 registered species (Table 1). Two of the lakes host fish farms: Lake Fyresvatn produces indigenous Arctic charr, and Lake Totak produces indigenous Arctic charr and brown trout.

Vertical temperature profiles were taken in epilimnion above the deepest area (according to bathymetric charts) of each lake in mid-August 2018, from surface down to 30 m.

2.2 | Fish collection

Wild, native salmonid fish from the lakes Fyresvatn, Totak, Møsvatn and Tinnsjå were purchased from local fishermen with detailed knowledge about the best-fishing spots. In Lake Fyresvatn, fish were sampled at three stations in northern part of the lake: Whitefish and brown trout from Kjeøyni (FyKje), Arctic charr and brown trout from Nesland (FyNes) and all three species from Kalsholmane (FyKal). Fishing in Lake Fyresvatn and Lake Tinnsjå was performed during October/November in 2018, Lake Totak in September/October 2018 and Lake Møsvatn in October 2016. Standard bottom-set gillnets were used overnight (fishing approximately for 12–20 h), standing from shore and angular towards the deeper area of the lake

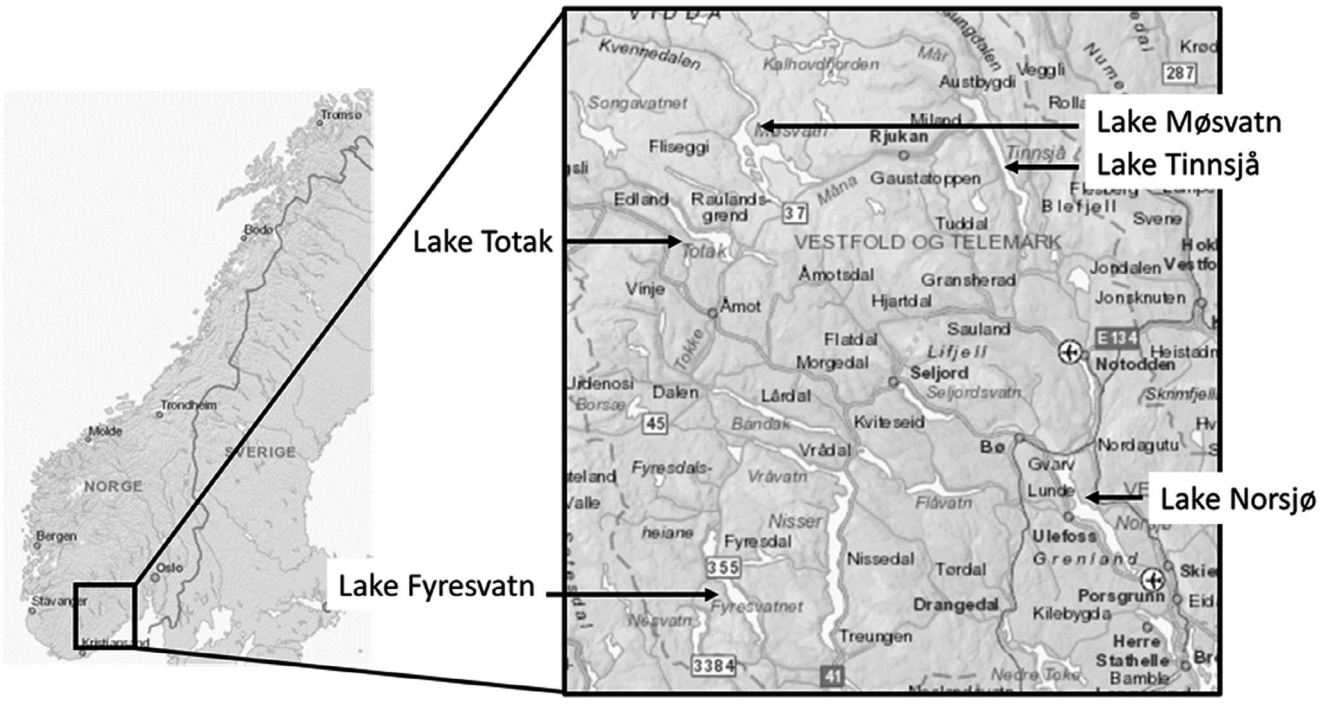

FIGURE 1 Location of the five lakes in this survey, all within the County of Vestfold and Telemark. (Map from Kartverket, Geovekst, kommuner og OSM—Geodata AS, https://vann-nett.no)

TABLE 1 Morphometric, nutrient and fish data of the five lakes included in the survey

	Fyresvatn	Totak	Møsvatn	Tinnsjå (e.g. 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)	377/120[a]	306/62	68.5/20	460/190	171/87
Altitude (m.a.s.l.)[a]	280	687	919	190	15
Tot P (µg/l)[a]	3.9 (2015–2018)	5.0 (2019)	3.6 (2016–2018)	7.4 (2015)	5.5 (2015–2019)
Tot N (µg/l)[a]	321 (2015–2018)	158 (2019)	90.4 (2016–2018)	196 (2015)	256 (2015–2019)
Highly modified waterbodies (HMWB)	Yes	Yes	Yes	No	No
HRV/LRV (m)[a]	279.75/275.15	687.3/680	918.5/900	191.2/187.2	15.3/15.0
Water area[a]	Nidelva	Tokke-Vinje	East Telemark	East Telemark	Mid Telemark
Most common fish species[b]	**whitefish** (*Coregonus lavaretus*) **brown trout** (*Salmo trutta*) **Arctic charr** (*Salvelinus alpinus*)	***Arctic charr*** (*Salvelinus alpinus*) **brown trout** (*Salmo trutta*) **minnow** (*Phoxinus phoxinus*) **three-spined stickleback** (*Gasterosteus aculeatus*)	***Arctic charr*** (*Salvelinus alpinus*) **brown trout** (*Salmo trutta*) **minnow** (*Phoxinus phoxinus*)	***Arctic charr*** (*Salvelinus alpinus*), probably two morphs; one living deep littoral and one living pelagial **brown trout** (*Salmo trutta*) **perch** (*Perca fluviatilis*) **minnow** (*Phoxinus phoxinus*) possible: **fourhorn sculpin** (*Myoxocephalus quadricornis*), living in deep waters	**whitefish** (*Coregonus lavaretus*), different morphs **brown trout** (*Salmo trutta*) **Arctic charr** (*Salvelinus alpinus*), different morphs **perch** (*Perca fluviatilis*) **smelt** (*Osmerus eperlanus*) **pike** (*Esox Lucius*) **salmon** (*Salmo salar*) **minnow** (*Phoxinus phoxinus*) **three-spined stickleback** (*Gasterosteus aculeatus*) **crucian carp** (*Carassius carassius*) **tench** (*Tinca tinca*) **river lamprey** (*Lampreta fluviatilis*)

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://www.vannportalen.no/kunnskapsgrunnlaget/klassifisering/), [b]Lydersen (2015).

covering the littoral zone in epilimnion and parts of the hypolimnion in Tinnsjå, Totak, Møsvatn and Norsjø. In Fyresvatn, fishing was performed in shallow areas (<10 m) at all stations. We aimed at collecting minimum 30 fish from each species in these lakes (Table 2), but we received fewer from Lake Tinnsjå.

In Norsjø, a separate comprehensive fishing from three stations (north, middle and south in the lake) at three different seasons was performed in late May (spring), late July (summer) and mid-September (autumn) 2018. Brown trout was caught in the middle (only three individuals) and the northern (two individuals) station, and Arctic charr and whitefish were caught in all three stations, both species with highest numbers in the northern station (Table 3). Stations, schematic depth profiles, maximum depths, regulation heights and maximum depth of fishing are shown in Figure 2.

Two series of standard bottom-set gillnets (1.5 m × 25 m) were deployed at each station for each sampling season. In total, 251 charr, 102 whitefish and five brown trout were caught in Norsjø. In accordance with Norwegian Animal Research Authority, it requires no ethical permission for collection with gill nets and the associated killing of fish (FOR-2015-06-18-761 with changes FOR-2017-04-05-451, the Norwegian Ministry of Agriculture and Food).

All sampled fish from all lakes were wrapped separately in plastic bags and immediately frozen for subsequent storage and transport to the laboratory, where they were kept frozen until examination.

In total, 601 wild and 29 farmed fish were analysed from all lakes. The 29 farmed fish from Fyresvatn were collected by staff at the fish farm facility, located close to station Nesland (FyNes), in October 2019 (Table 2). Farmed fish was produced from local fry produced in indoor facilities utilizing water from municipal waterwork in 2015 and moved to lake pens in February 2017.

In the laboratory, the fish were thawed, the total length from the snout to the end of the tailfin measured to the closest mm, and weight measured to the closest gram. Each fish was opened with a pair of scissors, and a small subsample (approx. 2 mm^3) was taken from the 1/3 hind part of the kidney and transferred to a vial (Thermo Scientific Nalgene Cryoware Cryogenic Vials, catalog number 5000-0020) with 1.5 ml 96% ethanol. To avoid contamination of possible DNA from *T. bryosalmonae* between samples, all equipment was disinfected with 50% chlorine and rinsed in distilled water before and between every subsampling. The vials were stored in fridge (4°C) until DNA extraction.

2.3 | Molecular analysis

DNA from the kidney samples was extracted following the protocol of Blood and Tissue Kit from Qiagen (DNeasy Blood & Tissue Handbook 07/2006), and total DNA content and purity were subsequently measured (Thermo Scientific NanoDrop Lite Spectrophotometer). Samples for further PCR analyses at USN were standardized with TE buffer to 50 ng DNA/25 µl sample volume.

To detect *Tetracapsuloides bryosalmonae* DNA in the fish kidney extracts, 18S rDNA was used (PKDtaqf1 (GCGAGATTTGTT GCATTTAAAAAG) and PKDtaqr1 (GCACATGCAGTGTCCAATCG), giving a PCR product of 73 base pairs (bp) (GCGAGATTTGTTG CATTTAAAAAGCTCGTAGTCGGACGGTTCCACAATTTTGTC GATTGGACACTGCATGTGC) (Bettge, Segner, et al., 2009; Bettge, Wahli, et al., 2009)).

From four of the investigated lakes (Fyresvatn, Totak, Møsvatn and Tinnsjå), 250 kidney samples were analysed at NINA with digital droplet PCR (ddPCR) (QX200 Droplet Digital PCR System with AutoDG™, Bio-Rad Laboratories). The total volume of each well was 22 µl, including 10 µl ddPCR Supermix, 0.8 µl each of PKDtaqf1 and PKDtaqr1, 0.055 µl Probe PKD FAM (CAAAATTGTGGAACCGTCCGACTACGA) (Bettge, Segner, et al., 2009; Bettge, Wahli, et al., 2009) and 1 µl DNA template. The final concentrations of primers and probes were 0.9 and 0.25 µM, respectively. Analyses included two technical replicates of each sample, as well as one negative and one positive control. The specifications of the programme ddPCR60 were as follows: one cycle in 10 min at 95°C, followed by 40 cycles of 30 s at 95°C, 40 cycles of 1 min at 60°C, one cycle in 10 min of 98°C and finally one cycle of cooling down to 4°C.

Kidney samples from 358 fish collected in Lake Norsjø and 30 farmed fish from Lake Fyresvatn were analysed with real-time PCR (qPCR) (Applied Biosystems StepOne™ and StepOne Plus™ Real-Time PCR systems) on MicroAmp Fast 96-Well Reaction Plates (0.1 ml) at USN laboratory. The total volume of each well was 25 µl, including 12.5 µl Applied Biosystems SYBR Green Mastermix 2x (ROX), 0.75 µl each of PKDtaqf1 and PKDtaqr1 and 5 µl sample. The final concentrations of primers were 0.3 µM. The specifications of the qPCR programme were as follows: one cycle in 10 min at 95°C, followed by 45 cycles of 15 s at 95°C and 1 min at 60°C, and finally a coding step of 10 min down to 4°C. For quantification,

TABLE 2 No. of collected fish from each species (wild and farmed) in the five lakes, mesh sizes and approximate depth of fishing

Lake	Station	Total no. of fish	Arctic charr	Brown trout	Whitefish	mesh size (mm)	Approximate depth (m)
Fyresvatn	Wild	108	40	32	36	22–39	0–10
Fyresvatn	Farmed	29	29			Landing net	0–15
Totak	Wild	60	30	30		22	
Møsvatn	Wild	30	30				
Tinnsjø	Wild	48	22	26		40	0–40
Norsjø	Wild	355	249	5	101	13.5–45	2 to 40–60 m (north, mid), 2–20 m (south)

TABLE 3 Prevalence of *T. bryosalmonae* in populations of the three salmonid species in five lakes (a) and adherent stations (b). Positive samples were defined as samples with number of parasite DNA copies above LOD (three copies of parasite DNA) within a confidence interval of 95%. Prevalence is the percentage of positive samples of the total number of fish of the respective species. Numbers in bold are the prevalence, and numbers within brackets are number of fish infected above total number of the fish species sampled in the lake (a) or station (b)

(a)	Prevalence		
Lake	Arctic_charr	Brown_trout	Whitefish
Fyresvatn (wild)	**0** (0/40)	**84** (27/32)	**0** (0/36)
Fyresvatn (farmed)	**24** (7/29)		
Møsvatn	**0** (0/30)		
Norsjø	**11** (27/249)	**80** (4/5)	**18** (18/101)
Tinnsjå	**73** (16/22)	**100** (26/26)	
Totak	**0** (0/30)	**37** (11/30)	

(b)		Prevalence		
Lake	Station	Arctic_charr	Brown_trout	Whitefish
Fyresvatn (wild fish)	Kalsholmen (FyKar)	**0** (0/10)	**88** (7/8)	**0** (0/4)
Fyresvatn (wild fish)	Kjeholmen (FyKje)	**0** (0/0)	**80** (4/5)	**0** (0/32)
Fyresvatn (wild fish)	Nesland (FyNes)	**0** (0/30)	**84** (16/19)	
Fyresvatn (farmed fish)	Pen 1 (FYRFarm1)	**29** (7/24)		
Fyresvatn (farmed fish)	Pen 8 (FYRFarm8)	**0** (0/5)		
Møsvatn	Møs	**0** (0/30)		
Norsjø	NOR-mid	**10** (8/83)	**100** (3/3)	**17** (4/24)
Norsjø	NOR-north	**8** (8/96)	**50** (1/2)	**19** (13/67)
Norsjø	NOR-south	**16** (11/70)		**10** (1/10)
Tinnsjå	TIN-Lug	**73** (16/22)	**100** (26/26)	
Totak	TOT-south	**0** (0/30)	**37** (11/30)	

we included a dilution series of plasmid (pUC57) from GenScript, with the insertion of the 73-bp PCR product of our selected primers, in each of our 15 qPCR runs. The dilution series ranged from 1×10^6 to 1×10^0 DNA copies/25 µl. Every sample of fish kidney was analysed with three technical replicates. Two to three positive controls and two blanks (negative controls) were included in every PCR run. To ensure compatibility between the PCR methods at NINA and USN, 12 samples with different DNA contents analysed at NINA were reanalysed in duplicates and at several dilutions at USN. Positive results regarding *T. bryosalmonae* in NINA analyses showed positive results at USN laboratory, and negative results at NINA laboratory gave negative results in USN laboratory (Table A1). Selected positive NINA samples were used as positive controls in every PCR run at USN.

2.4 | Sensitivity of qPCR analyses

The sensitivity of the method at NINA was assessed by determining the absolute limit of detection (aLOD) according to Dobnik et al. (2015), with a minimum of three positive droplets of target DNA to assess a sample as positive (Dobnik et al., 2015).

Level of detection (LOD) at USN analyses was estimated by analysing 10 replicates of each concentration of standard curves ranging from 1×10^6 to 1×10^{-1} and subsequently calculating the effective LOD value with 95% confidence interval (Merkes et al., 2019) using the curve-fitting method approach for the use of three replicates, as explained by Klymus et al. (2020) Effective LOD in our samples was calculated to 3.3 DNA copies in at least one replicate. In addition, the peak of the melting curve of the sample had to be within ±1 unit of the melting peak of the positive controls and standard curves, and cycle numbers below 40. From this, a positive sample is defined as a minimum of three DNA copies in one of three replicates, in further analyses.

2.5 | Statistics

Statistics were performed in RStudio, version 1.4.1103, 'Wax Begonia' (458706c3, 2021-01-07) for Windows, and Excel for Microsoft 365 MSO (16.0.13530.20626) 32-bit. Statistical differences in fish lengths were compared through one-way ANOVA with Tukey's HSD post hoc tests, and differences in prevalence between fish species and lakes respectively were compared with the chi-squared test with modified R-script from Statistical Tools for High-Throughput Data

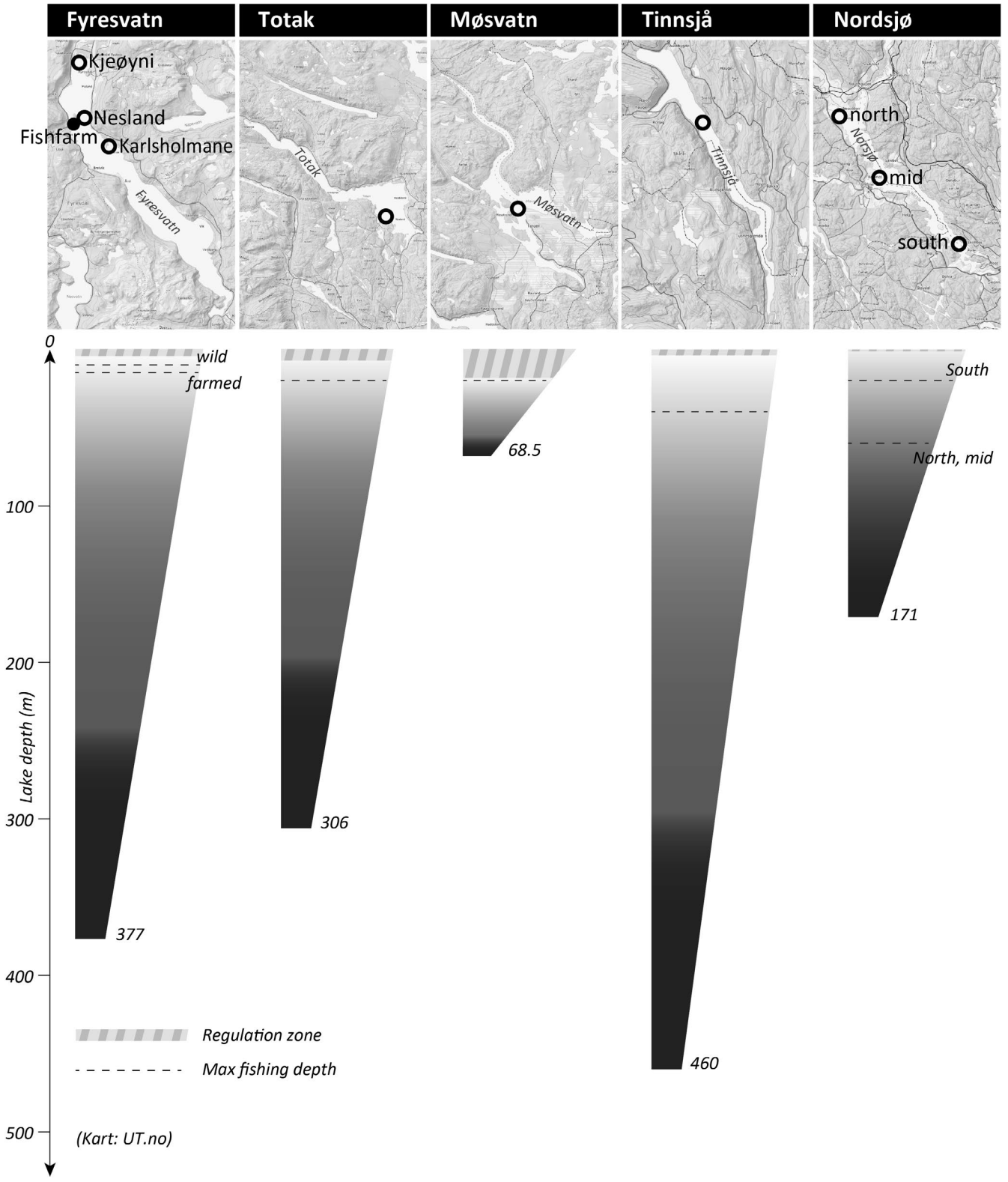

FIGURE 2 Fishing sites, schematic depth profile, maximum lake depth (m), regulation zone (m) and maximum fishing depth (m) of each lake

Analysis (STHDA) (http://www.sthda.com/english/wiki/chi-square-test-of-independence-in-r). When input numbers were <5, Fisher's exact test for count data was used.

Results *Tetracapsuloides bryosalmonae* was detected in kidneys of native fish from four (Fyresvatn, Totak, Tinnsjå and Norsjø) of the five investigated lakes (Table 3). In all four lakes, the parasite was

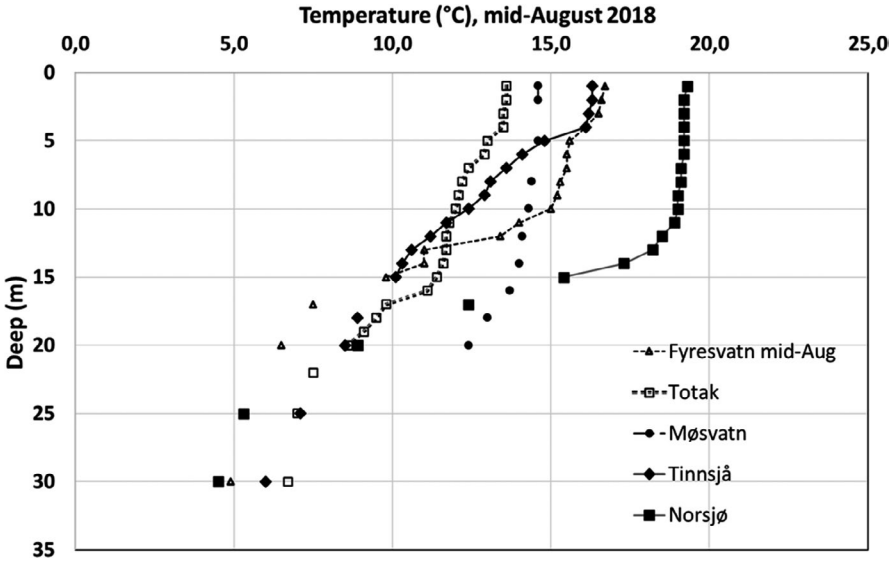

FIGURE 3 Temperature vertical profiles 0–30 m in the five investigated lakes: Fyresvatn, Totak, Møsvatn, Tinnsjå and Norsjø, in mid-August 2018

detected in brown trout—with 100% prevalence in Tinnsjå, 80%–88% in Fyresvatn, 80% in Norsjø and 37% in Totak.

Wild Arctic charr were sampled in all five lakes, but the parasite was only detected in charr from Tinnsjå and Norsjø, with the prevalence of 73% and 11%, respectively. *T. bryosalmonae* was detected in 7 (24%) out of 29 farmed Arctic charr in Fyresvatn. The parasite was detected in 18 (18%) out of 101 whitefish from Norsjø, while whitefish from Fyresvatn ($n = 36$) were negative.

Pearson's chi-squared test revealed that 'infection' and 'fish species' were statistically associated ($n = 630$, $\chi^2 = 171.18$, $df = 2$, p-value < 2.2e-16), and infection by *T. bryosalmonae* was higher in brown trout than in the two other salmonid species. Infection in brown trout contributed by 66.8% to these chi-square statistics. Likewise, Pearson's chi-squared test showed a significant association between 'Lake' and 'infection' ($\chi^2 = 145.39$, $df = 5$, p-value < 2.2e-16), where the cell that contributed most to the analyses was infection and Tinnsjå (66.4%) (input data in Table A2).

In Lake Norsjø, fish were sampled at three stations during spring, summer and fall in 2018. Pearson's chi-squared test showed no significant differences between 'infection' and 'lake stations' when all seasons were merged ($\chi^2 = 0.13$, $df = 2$, p-value = .94). There were neither any significant differences in infection between stations during spring ($\chi^2 = 0.64$, $df = 2$, p-value = 0.73), summer (Fisher's exact test for count data, p-value = .93) or autumn (Fisher's exact test for count data, p-value = .10) (input data in Table A2).

Water temperatures in three of the lakes (Norsjø, Fyresvatn and Tinnsjå) were warmer than 15°C down to 5 m. Norsjø was the warmest of all the lakes, with 19°C recorded at 10 m (Figure 2).

2.6 | Fish lengths and weights

One-way ANOVA with Tukey's HSD post hoc tests showed that Arctic charr from Tinnsjå were significantly longer than wild charr from Lake Fyresvatn ($p < .01$) and Lake Norsjø ($p < .00$). Charr from Totak was significantly longer than charr from Norsjø ($p < .01$) (Figure 3). Within Fyresvatn, there were no significant differences between fish lengths of wild charr in the three stations, but significant differences between farmed (longer) and wild charr: FyrFarm1 and FyKar ($p < .00$), FyrFarm1 and FyNes ($p = .00$), FyrFarm8 and FyKar ($p < .00$) and between FyrFarm8 and FyNes ($p < .00$) (details given in Table A3).

In Lake Norsjø, Arctic charr from the southern part were significantly shorter than charr from the middle and northern areas, with a p-value of .00 for all. For brown trout, fish lengths were significantly different between all four lakes where these species were sampled (NB: only five individuals of brown trout sampled in Norsjø). Between stations within the same lake (Fyresvatn and Norsjø), there were no significant differences in trout lengths ($p = .00$). Whitefish was sampled only in Norsjø and Fyresvatn. Fish lengths were significant lower in Norsjø than in Fyresvatn ($p = .00$), but there were no significant differences between stations within the same lake. (Data and R-script available in figshare, length distribution of fish species in the different lakes and stations in Appendix Figure A1).

3 | DISCUSSION

Here, we present the first detection of *T. bryosalmonae* DNA in European whitefish in Norway, as well as the first published documentation of the parasite presence in native Arctic charr, whitefish and brown trout in deep, dimictic Norwegian lakes.

Tetracapsuloides bryosalmonae was detected in fish kidneys in four of the five investigated deep lakes: Fyresvatn, Totak, Tinnsjå and Norsjø. In all four lakes, the parasite was detected in brown trout populations in high prevalence, ranging from 100% in Lake Tinnsjå to 84% in Lake Fyresvatn and 37% in Lake Totak. Although wild Arctic charr were detected in all five lakes, infected charr only occurred in Lake Tinnsjå (73%) and Lake Norsjø (11%). Interestingly, the parasite

prevalence was 24% in farmed Arctic charr from Lake Fyresvatn (2019), in contrast to the negative results from wild charr sampled in the same lake in 2018. Whitefish were present in Lake Fyresvatn and Lake Norsjø, in accordance with earlier findings (Lydersen, 2015), but *T. bryosalmonae* was only detected in whitefish in Lake Norsjø (18%).

The observations include some low prevalence of infection and measurements close to LOD, which illustrates some of the methodological challenges when it comes to field sampling, subsampling and molecular analyses. Further, sampling site, sampling size and small subsamples of tissue and unevenly distributed parasite cells can result in analytical limitations, especially at low infection intensities (Skovgaard & Buchmann, 2012), which we observed in some of the whitefish and Arctic charr. Due to the absence of mass mortality, dead fish can easily be overlooked (Okamura et al., 2011; Skovgaard & Buchmann, 2012; Sudhagar et al., 2019; Wootten & McVicar, 1982), or more likely, they are eaten by a predator in the early stage of disease development. Even with such possible limitations, we trust that our results give a realistic picture of the presence of infection of *T. bryosalmonae* in the five selected lakes. However, we cannot state that the parasite is not present in species and lakes we found to be negative.

The relatively high prevalence of *T. bryosalmonae* observed in brown trout populations could indicate a possible future infection potential in the investigated lakes where brown trout was present. Brown trout is known to be an active host (Grabner & El-Matbouli, 2008; Hedrick et al., 2004) capable of releasing viable spores of *T. bryosalmonae* that infected the bryozoan *Fredericella sultana* after 5 years post-exposure (Abd-Elfattah, Kumar, et al., 2014; Soliman et al., 2018). These findings indicate that infected brown trout might sustain and spread infection of *T. bryosalmonae* over time in wild habitats (Burkhardt-Holm et al., 2005; Kristmundsson et al., 2010; Lahnsteiner et al., 2011; Mo & Jørgensen, 2017; Wahli et al., 2002, 2008; Waldner et al., 2019). In addition, a recent Icelandic survey detected viable spores of the European strain of *T.bryosalmonae* not only in native brown trout, but also in populations of Arctic charr and Atlantic salmon, strongly suggesting that all the three native species are active hosts for the European strain of the parasite (Svavarsdóttir et al., 2021). It is reasonable to assume that *T. bryosalmonae* present in Norway also belong to the European strain, as is the case in Iceland (Svavarsdóttir et al., 2021), but this remains to be examined further. This is an interesting perspective regarding the three native salmonid species inhabiting Telemark lakes in the present study. By being able to infect new bryozoan colonies with viable *T. bryosalmonae* spores, it is possible that both infected brown trout and Arctic charr can contribute to the exposure to other salmonids present in the investigated deep lakes.

We detected *T. bryosalmonae* in whitefish kidneys for the first time in Norway, in Lake Norsjø (18%). No infection was detected in whitefish from Fyresvatn. *T. bryosalmonae* is earlier reported in European whitefish from Finland (Sobociński et al., 2018) with prevalence levels from 28% to 53% and from Lake Lucerne in Switzerland (Naldoni et al., 2019) with a prevalence of 86% ($n = 7$). Naldoni et al. (2019) found indications of parasite spore production in whitefish, but due to co-infection of a sphaerosporid species, they could not confirm that European whitefish is a true host for *T. bryosalmonae* . The parasite was also detected in mountain whitefish (*Prosopium williamsoni*) in a severe outbreak with high mortalities in Yellowstone, Montana, USA, in 2016 (Hutchins et al., 2021; Opitz, 2017; Opitz & Rhoten, 2016). Hutchins et al. (2021) detected *T. bryosalmonae* presence in a broad host range in a wide distribution area of Montana, but mountain whitefish have the highest prevalence (50%) among the species. Molecular analyses of new and old samples revealed that the *T. bryosalmonae* had a long-term history in Yellowstone, belonging to the American parasite strain (Hutchins et al., 2021). Despite indications of spore production in whitefish (Naldoni et al., 2019) it is not proven whether whitefish is an active or 'dead-end' host for the PKD parasite.

The observed prevalence of the parasite in kidneys of wild Arctic charr was generally lower than in brown trout in the present survey, and prevalence varied between infected charr populations in the investigated lakes. In three of the lakes, we did not observe *T. bryosalmonae* in charr kidneys, while we found prevalence of 11% and 73% in Lake Norsjø and Tinnsjå, respectively.

Specific age of the fish was not determined, but the lengths indicate that the majority were adults. This was also supported by the observation of mature gonads in many of the fish included in the survey. In general, young fish that has not previously been exposed to the parasite are expected to be more susceptibility to the parasite and PKD (Burkhardt-Holm et al., 2005; Ferguson & Ball, 1979; Okamura et al., 2011), but there are also observations showing that earlier infected wild salmons might be reinfected or sustain established infection (Mo et al., 2011; Morris et al., 2000). Sustained infection is also observed in other species, including brown trout and grayling (Morris, Adams, Feist, et al., 2000) and rainbow trout (Foott & Hedrick, 1987). Experiments have shown that fish surviving the first infection can develop resistance to reinfection or develop a coevolution of host–parasite (resistance enabled tolerance) (Bailey et al., 2019), and previous exposure might be more important than age (Ferguson & Ball, 1979).

A key factor for infection of fish by *T. bryosalmonae* is the presence of the main bryozoan host, since there is no evidence that the parasite can transmit directly from fish to fish—despite experiments conducted to investigate this possibility (D'silva et al., 1984; Ferguson & Ball, 1979). Since the parasite is confirmed to be present in the fish in four of the investigated lakes, it is reasonable to conclude that the bryozoans have been present. In general, benthic fauna will be substantially reduced under repeatedly fluctuating water levels in hydropower reservoirs (Borgstrøm & Hansen, 2000). The regulation heights vary between the reservoirs in this survey, from 18.5 m in Lake Møsvatn to 0.3 m in Lake Norsjø (Table 1). One possibility of bryozoan survival in such systems could be to produce statoblasts, dormant and highly resistant stages that are able to persist under very harsh conditions (Hartikainen & Okamura, 2015; Wood & Okamura, 2005), such as freezing and drying conditions under

reduced water levels during cold season. Experiments have shown that covert infection of statoblasts by *T. bryosalmonae* can carry infection for 5–17 months (Hill & Okamura, 2007). Another possible explanation could be that the colonies live below the lowest regulated water level in the reservoirs. Even if most observations of bryozoans in Norway have been from the upper 1.5 m of the littoral zone (Økland & Økland, 2005), observations of *F. sultana* (Blumenbach) have been made down to 20 m in one Norwegian oligotrophic lake (Raddum & Johnsen, 1983). Raddum and Johnsen (1983) also refer to findings of *F. sultana* back to 1938 and 1940, down to depths of 65 and 214 m in freshwater localities outside Norway. A third possibility could be that the bryozoans live higher upstream in the tributaries and that colony fragments and infected spores are fed into the lake from inlet rivers. One of the inlet rivers to Lake Tinnsjå, River Måna, come from Lake Møsvatn. This lake has the highest altitude of the five lakes included in this survey (919 m.a.s.l.) and a regulation height of 18.5 m. We did not observe any infection of *T. bryosalmonae* in samples from Lake Møsvatn.

Lake Totak is located 687 m above sea level and was the coldest of the investigated lakes, with an average temperature of 12.9°C in 0- to 10-m depth. Brown trout in the lake was infected with *T. bryosalmonae* (37%), but we found no infection in wild Arctic charr.

Arctic charr and brown trout populations in Lake Tinnsjå both had high prevalence of the parasite, 73% and 100%, respectively. Lake Tinnsjå has low nutrient concentrations, clear water and a quite steep and very scarcely populated littoral zone (due to regulation) with no conspicuous colonies of bryozoans. Temperature in the upper 5 m of the water column was above 15°C during measurements in August 2018. Data from the Norwegian Metrological Institute show that the air temperatures in Norway in May were the warmest measured since 1900, and the air temperature in summer season from June to August 2018 was 3–4°C over the normal period (1961–1990) in east of Norway (https://www.met.no/publikasjoner/met-info/met-info-2018). Based on our measurements of water temperatures and the documented warm weather during summer 2018, we are confident that temperatures in upper layers of all the investigated lakes have been sufficient for both proliferation of *T. bryosalmonae* and disease development as required temperature for PKD development in fish hosts is 12–15°C over a period of 14 days (Bettge, Segner, et al., 2009; Bettge, Wahli, et al., 2009; Brown et al., 1991; Ferguson, 1981; Wahli et al., 2002) .

Lake Norsjø is located downstream in the same watercourse as Lake Møsvatn and Lake Tinnsjå, and in Lake Norsjø, we found high prevalence (80%) of *T. bryosalmonae* with DNA concentrations well above LOD in brown trout, but we only managed to sample five individuals. We also detected the parasite in Arctic charr and whitefish, but in low prevalence, 11 and 18%, respectively. Lake Norsjø was the warmest of the surveyed lakes, with almost 20°C at the surface, and more than 15°C down to the thermocline at approximately 15 m, a temperature in the range where *T. bryosalmonae* can proliferate and develop. The lake is oligotrophic to mesotrophic and has 12 registered fish species (Lydersen, 2015). Results from Olk et al. (2020) indicated that the Arctic charr mainly fed in the littoral and profundal zone, the whitefish in the littoral and pelagic zone and the perch primarily in the littoral zone in Lake Norsjø. Generally, brown trout will normally live and feed in the littoral zone of epilimnion, and often use the tributary as spawning area (Klemetsen et al., 2003, and references therein). These habitats will presumably make brown trout more exposed to spores of *T. bryosalmonae* if infected bryozoans are present in these habitats, than charr feeding more in the pelagic and cold profundal zone, and whitefish in the littoral and pelagic zone.

Water temperature is by itself important for the development of *T. bryosalmonae*, but the temperature can also influence the competition and habitat preferences among fish species. Arctic charr can utilize the profundal zone of very deep lakes like few other northern freshwater species, and a warm epilimnion can be the reason why that Arctic charr move to hypolimnion during summer (Klemetsen et al., 2003). Several studies have shown that whitefish and Arctic charr prefer the same trophic niches regarding habitat and main diet, with whitefish often being the most competitive species of the two (Jensen et al., 2017). There are also several examples of stable coexistence, for instance, if the lake has an extensive profundal zone with a spatial refugee niche for the charr or there is a third competitor present, like in Lake Fyresvatn (Jensen et al., 2017; Sandlund et al., 2010) and Lake Norsjø (Olk et al., 2020). An investigation on resource partitioning between Arctic charr, whitefish and brown trout in Lake Fyresvatn in 2005/2006, based on analyses of stomach content and stable isotopes, found that both the charr and whitefish shifted the habitat utilization seasonally (Jensen et al., 2017). The charr and whitefish overlapped significantly in habitat in the littoral or pelagic zone in June and October, but the charr dominated in the profundal zone throughout the season and access to this habitat was an essential contribution for charr population resource utilization during hot summer periods in Lake Fyresvatn. Jensen et al. (2017) found that when brown trout was present in Lake Fyresvatn, as a third competitor, the coexistence of Arctic charr and whitefish was facilitated. This emphasizes the importance of habitat preferences and competition between species as possible underlying factors for potential exposure, infection and disease development, also regarding *T. bryosalmonae* and PKD. While the charr live in the cold profundal zone in Lake Fyresvatn during summer (Jensen et al., 2017), it is expected to be less exposed to infection from parasite spores during the warm season. While living in the littoral and pelagic zone in June and October, the charr might be exposed to infected spores, but water is also colder at that time in large Norwegian lakes, and the risk of disease development is smaller. Brown trout, preferring to live and feed in the littoral zone throughout the summer season, will presumably have a higher risk of infection and disease development than both Arctic charr and Whitefish preferring the pelagic or profundal zone during the warmest season. Differences in susceptibility to infection between the species might also be a contributing factor, but it is impossible to distinguish between exposure and susceptibility in such an ecosystem.

No infection of *T. bryosalmonae* was detected in wild Arctic charr in Lake Fyresvatn in 2018, but a prevalence of 24% was detected in farmed charr in the same lake in 2019. All fish were caught in

October/November from the same depth range (0–15 m) in both years, but we expect the wild fish to have a wider depth range over time, as it can move freely in the deep lake for feeding and shelter. In our study, the wild charr was sampled in the littoral zone in early November 2018, most of the charr sampled close to the fish farm (Nesland) (Figure 2). It is reasonable to assume that these wild charrs have been living in the cold profundal zone during summer, as reported from the survey of Jensen et al. (2017). The farmed charr in Lake Fyresvatn was sampled in late October, but these fish had no opportunity of migrating down to the profundal zone during the warm season, as max depth of the fish-pen was 15 m. Farmed fish were hatched and lived indoor in municipal water for 2 years, before being moved to the lake where they stayed in the same pens, without stressful relocations, for 2½ years. Since the wild and farmed charr are caught in different years, and spatial and temporal variations in infection are important, we cannot conclude on differences in prevalence One possible explanation for these differences that should be explored further could be the different habitats of farmed and wild fish, the latter with access to the cold profundal zone as a possible counteracting mechanism against infection of *T. bryosalmonae* and development of PKD to cold-adapted fish. If so, these deep, dimictic lakes might represent a 'rescue' habitat to wild salmonids facing the parasite *T. bryosalmonae* , due to their two distinct temperature zones during summer stratification that differ them from rivers and shallow lakes. Deep dimictic lakes could represent future avenues for fish farms, by making their cages go deeper into the cold hypolimnion during summer stratification, or pumping cold water into the fish tanks, and thereby prevent development of PKD. However, the possible impact of cold hypolimnion in the battle against *T. bryosalmonae* infection should be tested further by controlled experiments.

Several studies have aimed to reveal and understand the mechanisms of different susceptibilities to PKD among salmonid species (Syrová et al., 2020). So far, rainbow trout has been found to be the most susceptible salmonid species to PKD—especially under farming conditions (Bucke et al., 1991)—and more susceptible than brown trout (Bailey et al., 2018; Grabner & El-Matbouli, 2009) and brook trout (Grabner & El-Matbouli, 2008). Syrova et al. (2020) found differences in susceptibility, not only between species but also among different lineages of charr and rainbow trout.

In our survey, we observed a lower prevalence of *T. bryosalmonae* in Arctic charr and whitefish than in brown trout. One possible explanation for this could be less susceptibility of charr and whitefish than brown trout, but it could also be caused by less exposure to parasite spores due to different habitat preferences and competition between the different salmonids. *T. bryosalmonae* was detected in farmed Arctic charr in Lake Fyresvatn, but not in wild charr. Our suggestion is that these deep, clear water lakes might represent *both* a risk and a rescue for the salmonids towards a warmer climate. A risk because of *T. bryosalmonae* , even in this environment, can proliferate and establish in brown trout, which is an active host for the parasite. A rescue because of the access to cold hypolimnion water can prevent severe development of PKD, especially in the cold water-adapted Arctic charr. We also ask the question, whether it is possible that fish also could recover from symptomatic PKD by accessing the cold hypolimnion water.

4 | FUTURE INVESTIGATIONS AND CONCLUSION

There might be different life histories and different strains of both fish and parasite in these lakes that have not been investigated in this survey. This could be an interesting task to investigate further—not only within these lakes but also in a wider perspective including strains from different countries, if possible. It could also be interesting to sample and analyse *T. bryosalmonae* presence in brown trout in Møsvatn, where we found no parasites in the 30 sampled Arctic charr. Controlled experiments should be performed, aimed at revealing the effect of temporal and spatial variation in fish habitats that can possibly lead to different prevalence in farmed and wild Arctic charr. Such experiments should preferably be conducted both in Lake Fyresvatn and in Lake Totak, which also inhabit a fish farm of indigenous charr and brown trout.

The present work is the first observation of *T. bryosalmonae* in European whitefish in Norway, as well as the first published documentation of the parasite present in Arctic charr, whitefish and brown trout in deep, dimictic Norwegian lakes. We detected *T. bryosalmonae* DNA in fish kidneys in four of the five investigated deep lakes. In all four lakes, the parasite was present in brown trout in relatively high prevalence, ranging between 37% and 100%. Lower prevalence of *T. bryosalmonae* was observed in both Arctic charr and whitefish compared with brown trout. Wild Arctic charr were present in all five lakes, with only positive *T.bryosalmonae* detections in Lake Tinnsjå (73%) and Lake Norsjø (11%). Interestingly, we found a parasite prevalence of 24% in farmed Arctic charr from Lake Fyresvatn ($n = 29$), despite no findings in wild charr in the same lake ($n = 40$). Whitefish is present in Lake Fyresvatn ($n = 36$) and Lake Norsjø ($n = 101$), but *T. bryosalmonae* infections were only detected in Lake Norsjø (18%).

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CONFLICT OF INTEREST

No conflict of interest has been declared by the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study will be made openly available in 'figshare' at https://doi.org/10.23642/usn.c.5656243.v1.

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APPENDIX

TABLE A1 Results of the presence (above LOD) of *T.bryosalmonae* DNA in fish kidneys, analysed at two different laboratories: NINA (ddPCR) and USN (qPCR). Description of criteria for positive samples in the text

Sample name	Species	Result NINA (ddPCR)	Result USN (qPCR)	Comment
FyNes046	brown trout	pos	pos	
FyNes053	brown trout	pos	pos	
FyNes103	Arctic charr	neg	neg	
FyKar 062	brown trout	pos	pos	
FyKje039	brown trout	pos	pos	
TiLu029	Arctic charr	pos	pos	
TiLu031	Arctic charr	pos	pos	used as positive sample in PCR runs
TiLu037	Arctic charr	pos	pos	
Tilu043	Arctic charr	pos	pos	
TiLu045	Arctic charr	pos	pos	
Totak021	brown trout	neg	neg	
Møs001	Arctic charr	neg	neg	

TABLE A2 Infected and uninfected fish at all stations in Lake Norsjø, for all three seasons merged, and spring, summer and autumn shown separately

	All seasons			Spring			Summer			Autumn		
Stations	NOR-North	NOR-Mid	NOR-South	NOR-North	NOR-Mid	NOR-South	NOR-North	NOR-Mid	NOR-South	NOR-North	NOR-Mid	NOR-South
Uninfected	143	95	68	48	26	25	50	38	24	45	31	19
Infected	22	15	12	12	4	6	8	4	3	2	7	3

TABLE A3 Significant differences in fish lengths between fish species in different lakes

Significant differences in fish weight					
Species	**Lakes**	**Average difference**	**lwr, 95% conf.int.**	**upr, 95% conf.int.**	***p*-Value**
Brown trout	Norsjø-Fyresvatn	−67.08125	−121.789403	−12.37310	.0097995
	Tinnsjå-Fyresvatn	97.10337	67.065884	127.14085	.0000000
	Totak-Fyresvatn	36.75208	7.840531	65.66364	.0068527
	Tinnsjå-Norsjø[a]	164.18462	108.629974	219.73926	.0000000
	Totak-Norsjø[a]	103.83333	48.879300	158.78737	.0000208
	Totak-Tinnsjå	−60.35128	−90.834295	−29.86827	.0000080
Whitefish	Norsjø-Fyresvatn	−127.1865	−170.6495	−83.72346	0

Significant differences in fish weight						
Species	**Lake**	**Station within lake**	**Average difference**	**lwr, 95% conf.int.**	**upr, 95% conf.int.**	***p*-Value**
Arctic charr	Fyresvatn	FYRFarm1-FyKar	442.116667	278.655931	605.5774027	.0000000
		FYRFarm8-FyKar	566.900000	329.029689	804.7703107	.0000000
		FYRFarm1-FyNes	411.816667	292.881511	530.7518220	.0000000
		FYRFarm8-FyNes	536.600000	326.818104	746.3818955	.0000000
	Norsjø	NOR-south-NOR-mid	−157.711704	−228.187057	−87.2363506	.0000000
		NOR-south-NOR-north	−197.376488	−265.633775	−129.1192015	.0000000

[a]Only five fish.

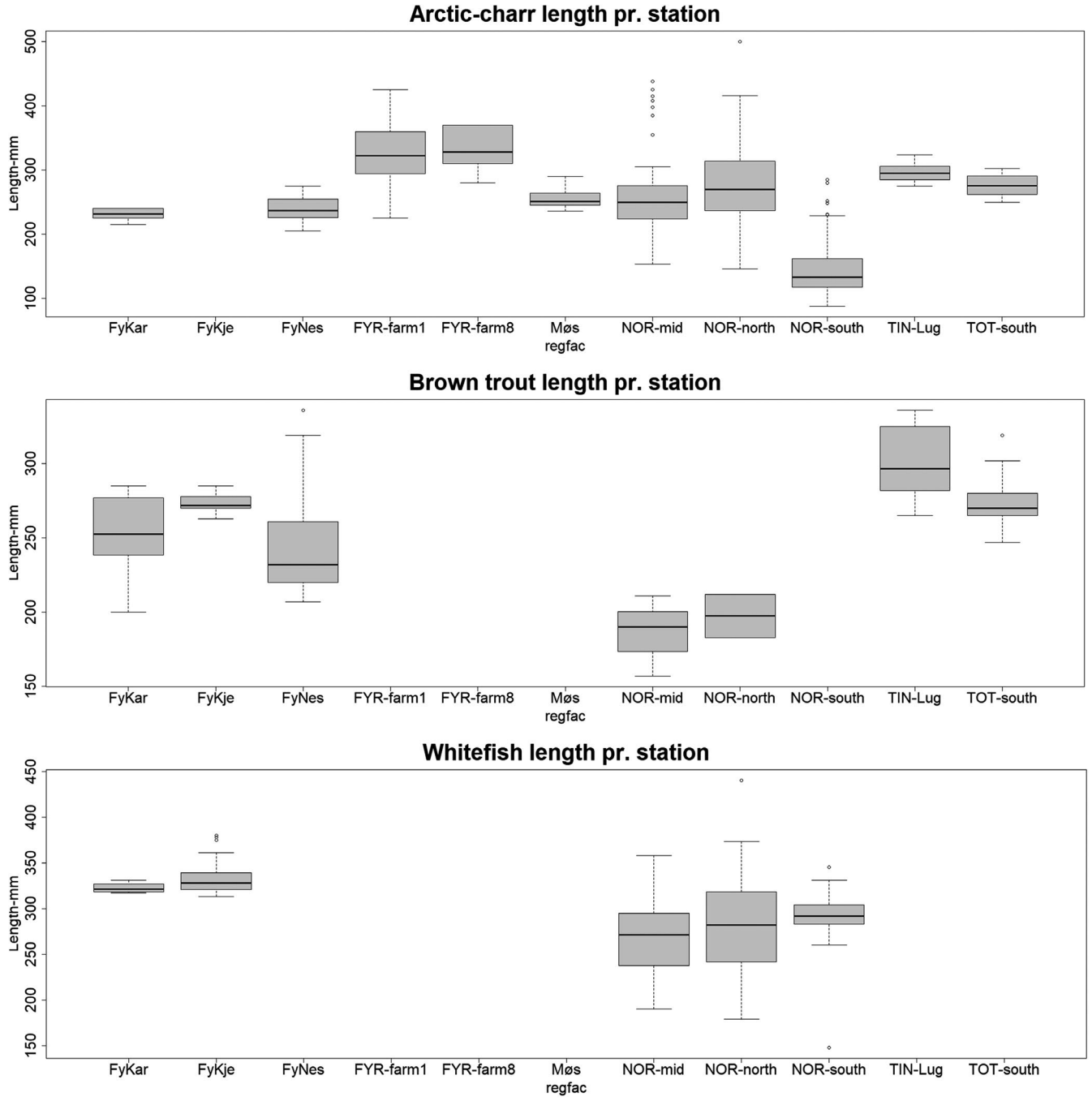

FIGURE A1 Length distribution of sampled Arctic charr, brown trout and European whitefish in four deep, dimictic lakes (2018): Fyresvatn (Fyr), Norsjø (Nor), Tinnsjå (Tin) and Totak (Tot) and Lake Møsvatn (Møs) in 2016. In Lake Fyresvatn, fish were sampled from 3 areas: Kalsholmane (FyKar), Kjeøyni (FyKje) and Nesland (FyNes); and two enclosures in a local fish farm: FyrFarm1 and FYRFarm8. In Lake Norsjø, fish were sampled from three areas: north, mid and south in the lake, three times during the year: spring, summer and autumn