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Comparison of the parasite communities of brown trout (*Salmo trutta)* from two coastal lakes in central Norway

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Astrid Lægran

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Front page: View from the popular bird watching tower by the shore of Litlvatnet, Fremstadvassdraget, Norway (Photo: Norwegian Institute for Nature Research).

Abstract

There are several studies concerning parasite communities in brown trout (*Salmo trutta)* and other salmonids in Norwegian lakes. These are mainly conducted in deeper oligotrophic inland lakes with a few coastal exceptions. Coastal lakes with eutrophic characteristics have received less attention. Therefore, this study aims to investigate similarities and differences in parasite diversity and community composition, within a costal system containing both eutrophic and oligotrophic characteristics. A total of 60 brown trout were examined for metazoan parasites in June 2021 from two coastal lakes in Fremstadvassdraget, central Norway. Litlvatnet - a shallow lake with eutrophic characteristics, and Storvatnet - a deep, oligotrophic lake. The study revealed that parasite diversity, evenness and total abundance of allogenic and autogenic parasites were similar between the lakes. However, differences were revealed in terms of infracommunity parasite composition between the lakes. Larval nematode *Eustrongylides* sp. and the metacercaria stage eyefluke *Diplostomum* sp. were more abundant and/or prevalent in Litlvatnet trout, whereas adult stage kidney digenean *Phyllodistomum umblae*, larval tapeworm *Dibothriocephalus* sp. and an unknown nematode (sp1) had higher abundance and/or prevalence in Storvatn trout. Adult stage intestinal digenean *Crepidostomum brinkmanni* also differed between the lakes, being more aggregated in Litlvatnet. Comparison with oligotrophic inland systems indicates that *P. umblae* may favor oligotrophic systems, whilst *Eustrongylides* sp. thrive in systems with eutrophic characteristics. The other parasite taxa found may be more adaptable to different lake characteristics. Stable carbon and nitrogen isotope- and diet content analysis were also conducted to see if the prey the trout had consumed in the last period reflected the patterns in parasite community. Litlvatnet trout had higher signatures of both nitrogen and carbon isotopes than Storvatnet trout. This may be caused by more marine and agricultural input in Litlvatnet, being the downstream located lake. The diet content also differed between the lakes, with Storvatnet trout consuming more on zooplankton. Fish prey were not abundant, but the high nitrogen isotope values and infection of many parasites possibly transmitted through piscivory, indicates that some trout may have eaten fish prey. This study suggests that differences in lake characteristics like size, depth and trophic status has little effect on parasite diversity. The parasite community composition, however, may be affected, and can partly be explained by the diet and trophic niche differences observed between the lakes

1 Introduction

Parasites are a diverse group of fascinating creatures, having complex and variable life history strategies. They depend on one or more organisms as hosts to survive and reproduce on which the parasites impose a cost. Parasites often utilize the ecosystems' food web to fulfil their life cycle (Kennedy, 1975; Knudsen et al., 2008). Thus, the parasite community composition can reveal the state of an ecosystem and provide information about the host community (Kennedy, 1975; Marcogliese, 2005; Palm, 2011). Metazoan parasites play a central, ecological role in most aquatic ecosystems, as drivers of important ecological processes such as mediating coexistence of species, making prey more susceptible to predators and possibly altering migratory behaviour (Freeland, 1983; Lafferty, 2008a; Lafferty et al., 2008b; Poulin et al., 2012). Unfortunately, metazoan parasites are often ignored in freshwater fish ecological and behavioural studies (Timi & Poulin, 2020). As parasitism is one of the most common life history strategies on earth, investigating the parasite communities should therefore also play an important part in understanding ecological patterns in aquatic ecosystems (Poulin et al., 2007; Timi & Poulin, 2020).

Although parasite ecology is a complex field, where diversity and community composition can be difficult to predict, there still exists other general patterns for further investigation (Johnson et al., 2016; Kamiya et al., 2014; Poulin et al., 2007). Parasite diversity can be altered by host diversity (Hechinger & Lafferty, 2005), and in turn, host diversity can be altered by factors such as the habitat's productivity and heterogeneity (Cardinale et al., 2009; MacArthur & MacArthur, 1961; Rosenzweig, 1995). However, if two ecosystems are equally diverse, it does not mean that the parasite community are similar. When it comes to freshwater lakes, several characteristics have been reported to shape the parasite community. One characteristic that is much investigated is eutrophication (nutrient over-enrichment, Bonsdorff, 2021), with some lakes becoming eutrophic due to anthropogenic stressors such as agriculture (Valtonen et al., 1997; Bennet et al., 2001; Bonsdorff, 2021), and climate change (Nazari-Sharabian et al., 2018; Meerhoff et al., 2022). Eutrophication can both result in an increase or decrease in parasite species richness, depending on the tolerance of multiple parasites and their hosts to the eutrophic environment as well as other factors like pollution and fragmentation (Valtonen et al., 1997; Marcogliese & Cone, 1991; Suthar et al., 2022). Eutrophic lakes are often shown to have more allogenic parasite species (birds or mammals as final hosts), and less autogenic parasite species (fish as final hosts) when compared to less productive lakes (Esch, 1971; Wisniewski, 1958). Eutrophication can also lead to increased anoxic zones or altered degree

of interaction between the lake community and terrestrial mammals and birds, which also are shown to have possible effects on the parasite community (Spalding & Exner, 1993; Coyner et al., 2002; Esch, 1971; Esch et al., 1986).

Predicting parasite diversity and community composition in lakes based on a limited number of factors is challenging, since no single factor explains parasite communities alone (Dogiel, 1961). Studies even suggest that there is no general relationship between lake characteristics and parasite diversity (Kennedy, 1978). Several other lake characteristics in addition to eutrophication can often help to explain the parasite community and be used to predict this to other similar systems. Biotic factors like geographical range of the host (Esch et al., 1990), ratio of migrating fish individuals in a partially migratory population (Rochat et al., 2021), availability of intermediate and final hosts (Dogiel, 1961), availability for marine or terrestrial organisms (Siwertson et al., 2016; Esch et al., 1986) and similarities in fish assemblages (Fernandez et al., 2010) have been previously recognised. In addition, environmental and habitat factors like lake size and depth (Kennedy, 1990; Kortelainen et al., 2006; Marcogliese & Cone, 1991; Xenopoulos et al., 2003), geographical location and distance between the lakes (Dogiel, 1961; Poulin & Morand, 1999; Siwertson et al., 2016), as well as whether the lakes belong to the same drainage or not (rivers and lakes that drains into a common body of water) (Barger et al., 2006) have also been investigated.

Since parasites are often transmitted through the food web (Knudsen et al., 2008; Siwertson et al., 2016), their trophic niche can also be an indicator of the parasite community of the host. To investigate this, stable isotope analysis (SIA) and stomach contents are often used as ecological "markers" of the hosts trophic behaviour and resulting parasite community (Knudsen et al., 2008; Knudsen et al., 2014; Locke et al., 2012; Timi & Poulin, 2020). Trophic transmitted parasites can reveal long-term niche patterns depending on the duration of the parasite's life cycle stage in a particular host, which can last up to several years (Abdussalam et al., 1995; Bjelic-Cabrilo et al., 2013). Stomach content shows the more short-term pattern of what the host has been consuming the last days, whereas SIA shows the trophic position and niche habitat of the host over the last months (Knudsen et al., 2014; Post, 2002).

Several studies have investigated the metazoan parasite communities in brown trout (*Salmo trutta)* in Europe (Paterson et al., 2019b; Knudsen et al., 2008; Molloy et al., 1995; Byrne et al., 1999). However, most of these studies were performed in oligotrophic inland-systems and/or rivers. In contrast, this study focuses on eutrophication and other lake characteristics in

a coastal watercourse. Smaller lakes also dominate the global distribution of lakes (Woolway et al., 2020), and these lakes are often extra exposed to eutrophication, which is a global problem (Downing, 2014). Therefore, this study can be helpful to investigate the impact eutrophication has on the parasite community in brown trout in this part of the world.

The aims of this study were to investigate how parasite diversity and community composition in brown trout differ between two coastal lakes in Fremstadvassdraget, Trønderlag, Norway. To reveal trophic niche patterns, stable isotopes and diet were also investigated between the lakes to support the trophic transmitted parasite data.

The hypothesises were the following:

1. The parasite diversity will be higher in the downstream located lake with eutrophic characteristics.
2. Parasite species with birds or mammals as final host (allogenic parasites) will be more abundant and/or prevalent in the downstream located lake with eutrophic characteristics. Parasites with brown trout as final host (autogenic parasites) will be more abundant in the upstream located, oligo/mesotrophic lake.

2 Material and methods

2.1 Study lakes

The study sites Litlvatnet and Storvatnet are two connected, coastal lakes located in the Fremstad catchment, Orkland, Trønderlag, Norway at the outer region of Trondheimsfjorden (63º370‘N, 9º380‘E). The river Fremstadelva (0.8 km) connects the two lakes, whilst Heggaelva (1 km) connects Litlvatnet to Trondheimsfjorden (Figure 1). Litlvatnet has a surface area of 0.47 km^2, max. depth of 3 m and is situated 5 m above sea level (Table 1). This lake has a prolonged history of modification, with water levels reduced several times to increase agricultural land. Phosphorous and nitrogen from fertilizers have drained into the lake (Holtan, 1988). In 1983, Litlvatnet became a nature reserve to prevent the lake from drying out and to protect one of few eutrophic lakes in southern Trønderlag (Winnem, 2010; Ulsund, 2013). In August 1987 the phosphorous concentration from the upper two meters of the lake were 27.1 µg/L (Holtan, 1988). Monthly water sampling between April and November 2021 detected a mean phosphorus concentration of 17.0 µg/L (range 12.2 (August) - 27.5 (September) µg/L (Table 1). The mean temperature was 10.7 ± 0.9 $^{\circ}C$, percent dissolved oxygen was 97.6 ± 1.4 % and secchi depth was 1.9 ± 0.1 m during this sampling period (Table 1).

Storvatnet has a surface area of 2.92 km^2, max. depth of 16 m and is situated 6 m above sea level. The impact from agriculture here is more limited, because less of the catchment are croplands and the water volume is higher (Winnem, 2010; Ulsund, 2013). Storvatnet has a lower phosphorus level (9.2 ± 0.5 µg/L), higher secchi depth (2.6 ± 0.1 m) and a slightly lower percentage of dissolved oxygen (93.1 ± 0.9) than Litlvatnet (Table 1). Based on NVE Atlas, the catchment of Litlvatnet is approximately 27 km^2, which also includes the Storvatnet catchment (16 km^2). The agricultural land for the entire catchment makes up about 20%. The rest is mostly coastal forest, crags and delicious forest (estimated from Norges vassdrags og energidirektorat, 2022). The hard ground consists mostly of gneiss, but also some calcareous rocks. The ground under the croplands is old seabed, with a lot of calcareous shells and algae residues (Baadsvik & Suul, 1977).

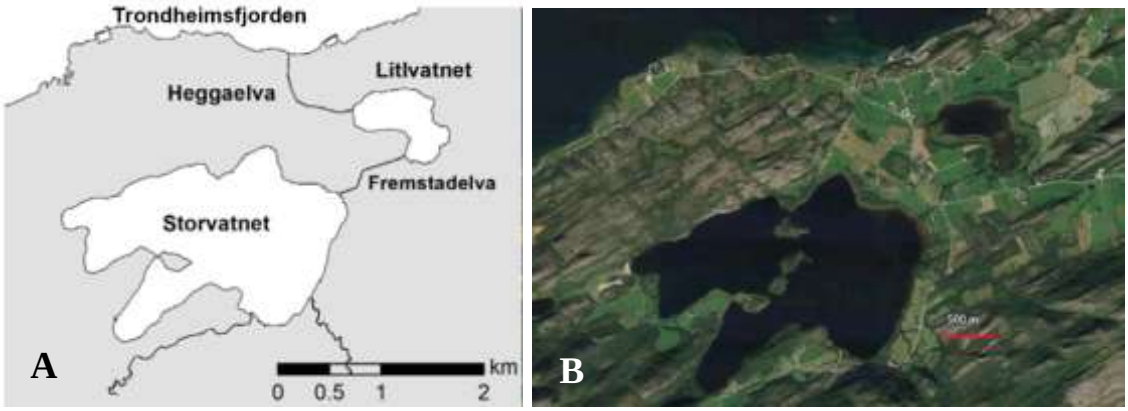


Figure 1 – A - map showing the study location consisting of the lakes Storvatnet and Litlvatnet and the rivers Heggaelva and Fremstadelva (modified from Paterson et al., 2021). B - satellite photo from the catchment (from Google Maps).

The fish community of Litlvatnet consists of five species: brown trout (*Salmo trutta* - both migratory and resident individuals), three-spined stickleback (*Gasterosteus aculeatus*), European flounder (*Platichthys flesus*), European eel (*Anguilla anguilla*) and Atlantic salmon (*Salmo salar*) (Ulsund, 2013). The lake supports rich plant and wildlife communities, with great numbers of wetland birds using Litlvatnet for resting and feeding during migration (Winnem, 2010; Baadsvik & Suul, 1977). A benthic fauna investigation from 1975-1976 consisted of high numbers of Oligochaete and *Chironomus* larvae (non-biting midges), which indicates that the lake was eutrophic (Baadsvik & Suul, 1977). The Storvatn fish community contains brown trout, from which some individuals are migrating both between the lakes and to the fjord (Paterson et al., 2021), three-spined stickleback (*pers. obs.*) and Atlantic salmon (Miljødirektoratet, 2022). No systematic plant and wildlife surveys have been undertaken at Storvatnet.

2.2 Fish sampling

In June 2021, brown trout from Storvatnet and Litlvatnet were caught using Nordic benthic gill nets (area: 45 m^2 (length: 30 m height: 1.5 m) mesh sizes: 5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43, 55 mm)) set overnight. Seven gill nets were set in Storvatnet (depth: 0.5-12 m), and 11 gill nets in Litlvatnet (depth: 0.5-2.9 m). A total of 30 fish from each lake (size range 204-311 mm) were selected for parasite examination. The fish were measured (fork length, mm) and weighed (g). Muscle samples from behind the dorsal fin were frozen for stable isotope analysis, and organs were kept for parasite and diet (stomach content) examination.

Table 1 – Lake and brown trout characteristics of Storvatnet and Litlvatnet in Orkland county, Trønderlag, Norway. Water sampling were performed monthly from April to November 2021. The phosphorus level measurement was done at 2x secchi depth for Storvatnet and a mixed sample from 1, 2 and 3 m for Litlvatnet. The other measurements were made at every meter of depth. The trout were sampled in June 2021.

Lake	Litlvatnet	Storvatnet
Area (km^2)	0.47	2.92
Max. depth (m)	3	16
Altitude (m. a. s.)	5	6
Phosphorus ($\bar{x}$ ± SE; µg/L)	17.0 ± 1.7	9.2 ± 0.5
Dissolved oxygen ($\bar{x}$ ± SE; %)	97.6 ± 1.4	93.1 ± 0.9
Secchi depth ($\bar{x}$ ± SE, m)	1.9 ± 0.1	2.6 ± 0.1
Water column temperature ($\bar{x}$ ±SE, ^{o}C)	10.7 ± 0.9	10.3 ± 0.3
Surface water temperature ($\bar{x}$ ±SE, ^{o}C)	11.0 ± 1.9	11.5 ± 1.2
Fork length ($\bar{x}$ ± SE[a], mm)	269.9 ± 4.6	272.3 ± 4.3
Weight ($\bar{x}$ ± SE[b], g)	200.7 ± 9.8	202.8 ± 8.1

a – no significant difference in fork length (mm) between lakes. T-test: t = -0.37987, p-value = 0.705

b- no significant difference in weight (g) between lakes. T-test: t = -0.16697, p-value = 0.868

2.3 Parasite processing and identification

The number of ectoparasites (e.g. copepods *Argulus coregoni* and *Salmincola* sp.) on the fins, skin and operculum were first counted. Then, *Dibothriocephalus* spp. tapeworm larvae (formerly *Diphyllobothrium;* Waeschenbach et al., 2017) encysted in the body cavity were estimated using the CYST-counting method (Kuhn et al., 2017). Both *Dibothriocephalus dendriticum* and *Dibothriocephalus ditremum* were present, but they were only identified to the genus level. The cysts were later opened to confirm the taxa, and the ones that contained other parasites than *Dibothriocephalus* spp. (e.g. nematodes) were subtracted from the total estimated number of *Dibothriocephalus* spp.

The gills, liver, heart, kidney, ureters, gall bladder, spleen and swim bladder of each fish were systematically screened for endoparasites and gill ectoparasites using a dissecting microscope. The gill arches were separated and put on 96 % ethanol to bleach the colour to enhance the

detection of monogenean (e.g. *Discocotyle* sp.). The cnidarian parasite Myxozoa that form cysts full of spores were only noted as absent/present, whilst the other taxa were counted by each observed anterior end. The stomach, intestine and eyes were frozen at -20 ^{o} C and examined later in the lab. Stomachs were processed by firstly examining the outer surface with a disection microscope, and any observed cysts were opened. After removing the stomach contents, the internal stomach surface was rechecked for cysts before the rest of the stomach contents were examined for parasites. Studies show that trematode eye flukes do not have a left-right eye preference (Paterson et al., unpublished), therefore only the left eyes were examined for eye fluke metacercaria (*Diplostomum* sp., *Tylodelphys* sp. and *Apatemon* sp.). The numbers were doubled to estimate the total infection. If the left eye had no infections of these taxa, the right eye was also examined, to confirm whether the fish was infected or not. If present, the fish were considered infected in the prevalence analysis, but the abundance remained based on the left eye only to avoid bias. All parasites were fixed in 96% ethanol for later morphological and/or genetical identification. The parasites were first identified to the lowest possible taxonomic level (morphological) based on keys in Moravec (2004).

For the genetical identification the DNA was extracted by CHELEX extraction. A 5% solution of Chelex® and MQ-water with 0.1 mM Proteinase K was used. The flatworms and acanthocephalans were molecularly identified using a partial fragment of the large ribosomal subunit (28S rDNA) that is normally used for this group (Blasco-Costa et al., 2016). A partial fragment of the small ribosomal subunit (18S rDNA) was used for the nematode molecular identification (Černotíková et al., 2011).

For the trematodes and acanthocephalan 28S rDNA were amplified using the primers U178 (5′-GCA CCC GCT GAA YTT AAG-3′) and L1642R (5′-CCA GCG CCA TCC ATT TTC A-3′) (Lockyer *et al.*, 2003); and LSU5F (5′-TAG GTC GAC CCG CTG AAY TTA AGC-3′). 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) primers were used for the cestodes (Littlewood et al., 2000; Olson et al., 2003). Finally, 18S rDNA from the nematodes were amplified using PhilonemaF (5′-GCC TAT AAT GGT GAA ACC GCG AAC-3′) and PhilPCRr0 -primers (5′-CCG TT CAA GCC ACT GC ATT A-3′) (Černotíková et al., 2011).

The PCR amplification protocol for the 28S marker followed Blasco-Costa et al. (2009). The 18S followed (Černotíková et al., 2011). The amplicons generated by the PCR amplifications were verified for a single band PCR product via electrophoresis before purified with a mix of exonuclease I and Thermosensitive Alkaline Phosphatase enzyme (Werle et al., 1994). After

verification and purification, the amplicons were sent to Macrogen Europe (Amsterdam, Netherlands) for sequencing from both strands with the same PCR primers used for amplification. All genetical analysis until the sequencing part were performed at the Natural History Museum of Geneva (Switzerland).

The resulting sequences were assembled and inspected for errors in Geneious ver. 8.1.9 (Kearse et al., 2012). Each chromatogram was examined automatically and by eye. Consensus sequences were submitted to BLAST to verify that the closest match was a sequence belonging to the same genus, and species, or at least closely related to the preliminary identification.

2.4 Stable isotope analyses (SIA)

The stable isotope samples were freeze-dried for approximately 48 hours and grounded into a fine powder. Approximately 0.5-0.6 mg were used for final SIA. The samples were run through a continuous flow isotope mass spectrometer (EA+CF-SIRSM) and analysed with an elemental analyser for carbon and nitrogen isotope ratios (δ13C, δ15N) to investigate the trophic position and niche habitat of the host (Eloranta et al., 2013). All stable isotope analyses were conducted at the University of Jyväskylä, Finland.

2.5 Diet

The diet content was examined simultaneously with the stomach parasite examination. The stomach fullness was estimated in percentage fullness from 0-100. The stomach content was then scraped out and washed before being examined for parasites using the dissecting microscope. Next the stomach content was identified and estimated as percentage of the total volume (from here on referred to as prey abundance (%) (Amundsen, 1995; Amundsen et al., 1996).

2.6 Calculation of indices

The mean abundance (total number of parasite individuals of a particular taxa in a sample of a particular host divided by the total number of hosts examined, both infected and uninfected), prevalence (the percentage of hosts infected with one or more individuals of a particular parasite taxa) (Bush et al., 1997). The mean intensity was also calculated but is only reported in Table S 4, Appendix A.

$$J(A,B) = \frac{A \cap B}{A \cup B}$$ *Equation 1 – Jaccard index*

The Jaccard index (J) of similarity was used to investigate the similarity in parasite taxa between the two lakes (Equation 1) where *A* and *B* are the number of parasite taxa per lake, the intercept ($\cap$) is the number of taxa that the two lakes have in common, and union ($\cup$) is the total number of parasite taxa in both lakes, both shared and unshared.

Taxon richness (S; total number of taxa), total parasite abundance (N), diversity (DMg, Equation 2) and evenness (BP, Equation 3) were calculated at an infracommunity level (defined from Bush et al., 1997). This can be conveyed as the community for each fish.

$$DMg = \frac{S-1}{lnN}$$ *Equation 2 – Margalef's diversity index*

The Margalef's diversity index (Margalef, 1958; Equation 2) was used to investigate differences in infracommunity parasite diversity between lakes. Where S is the number of species or taxa, and N is the total number of parasites. Whilst indices like the Shannon index have more widespread use in parasitology, is not always the best choice due to its sensitivity to sample size (Magurran, 2003). The advantage with Margalef's index is that it attempts to compensate for the effect sampling size can have on the index by dividing it by the natural logarithm of the total number of organisms collected (Gamito, 2010). A high number tells us that the parasite community is diverse, because there are many taxa relative to the logarithm of total abundance.

$$BP = \frac{N_{max}}{N}$$ *Equation 3 – Berger-Parker evenness index*

To investigate the relationship between the most dominant taxa and the total number of parasites, the Berger-Parker evenness index (Berger & Parker, 1970; Equation 3) was used. The number of individuals from the most abundant parasite taxa per fish (N_{max}) were divided by the total number of individuals from all parasite taxa (total parasite abundance per fish), and the means for each lake were calculated. A high number tells us that the highest abundant taxa contribute a lot to the total number of parasites, and therefore the parasite community probably is less homogenous.

$R_0 = 1 - \frac{1}{2}\sum|pij - pik|\ x\ 100\ \%$ *Equation 4 – Schoner's overlap index*

To calculate diet, overlap the Schoner index (Schoner, 1970) were used. P_{xj} and P_{yj} refers to the relative abundance of the diet for fish lake x and y. To simplify this is what is done: the lowest abundance (%) for each group were summarized (lowest taxonomic group, total 25 diet groups).

2.7 Statistical analyses

All statistical analysis were conducted using R-studio version 4.1.2. Generalized linear models (GLM) were used to test whether parasite taxon richness, total abundance, diversity, and evenness differed between the lakes. All measures were first plotted to see which distribution was most likely. A Shapiro Wiilk test (Shapiro & Wilk, 1965) was then used to check whether a Gaussian family was most appropriate for normally distributed data, whereas AER::dispersiontest (Kleiber, 2020) was used to determine if the distribution was over-dispersed to determine if poisson or quasipoisson family were the preferable choice for dispersed data. If the dispersion value were >0 the data was over-dispersed and quasipoisson distribution was fitted to the model.

Fish length and age usually show a positive relationship, as old fish are usually more exposed to more parasites than young fish (Kamiya, 2014, Poulin & Morand, 2000). Therefore, fish length was included as a fixed factor in the GLMs. Both additive (simple) and interactive (complex) GLMs were performed. The interactive models differ from the additive in the way that it checks if there are a significant relationship between lakes and fish length. The one with the best fit (complex if p-value from ANOVA comparing GLM's were less than 0.05, simple if p-value were above 0.05) were chosen.

This same procedure was also used to analyse differences in the abundance of each the parasite taxa between lakes, with exception of the rare taxa (*Tylodelphys* sp., *Proteocephalus* sp., Acanthocephala, *Apatemon* sp., *Argulus coregoni* and *Salmincola* sp.). Myxozoa, which were only noted as present/absent were not included in the abundance analysis. For the prevalence analysis, the count data were converted to binary data (0 for absent and 1 for present), and GLMs were refitted with a binomial family.

Non-metric multidimensional scaling (NMDS) was used to compare the parasite communities between the lakes using the package vegan (Oksanen et al., 2019). A permutation test (vegan::permutest) was performed to confirm if a Permutated Analysis of Variance (PERMANOVA) was an appropriate choice. If not appropriate ($p<0.05$), the data was log-transformed before conducting the PERMANOVA. The PERMANOVA was conducted using the vegan::adionis2 function. Similarity percentages, also known as Simper (Clarke, 1993) were also conducted to see which parasite taxa contributed most to the community differences.

For the stable isotope analysis (SIA) the distribution assumptions for a parametric test were not met. Since there were two groups to compare, the non-parametric Mann-Whitney U test were performed using Stats::wilcox.test (Bolar et al., 2019). This test is the non-parametric counterpart for the t-test, and compares the mean rank sum instead of the sample mean (Whitlock et al., 2015). The relationship between fish length and carbon and nitrogen isotope signature relationship (δ13C and δ15N) were also tested using Mann-Whitney U. If the p-value were above 0.05 the hypothesis of that fish length sum of squares mean did not differ between the lakes and would not affect the SIA results. A plot with 95 % CI ellipses were made to visualize the relationship between δ13C and δ15N in the different lakes were made using GGplot and GGForce packages (Wickham et al., 2016). Ellipse overlaps were calculated to show isotopic niche overlap using the SIBER package (Jackson et al., 2011).

The dietary overlaps of trout between the lakes were calculated using the Schoener's index (Equation 4). The prey items were then grouped into the following categories: benthos, zooplankton, surface insects and fish. Prey abundance (%) for each group was analysed for difference in mean rank sum between the lakes using the Mann Whitney U test. Canonical Correlation Analysis (CCorA) were performed to visualize the relationship between trophic transmitted parasites and prey abundance using the vegan package (Oksanen et al., 2019). The diet data were chord transformed, and the parasite data were log-transformed using vegan::decostand to meet the assumptions for a CCorA. To interpret this analysis the Canonical Correlation values for each axis (in this case the first two axis since there were only two variables) were read to see how much this axis explains the diet – parasite relationship. If the values are low, the parasite community in the fishes could not be explained by the stomach content. The relationship between prey group and trophic transmitted parasite taxa were also visually interpreted in a biplot.

3 Results

3.1 Parasite community

The trout in Storvatnet were infected with a total of 14 metazoan parasite taxa, whilst Litlvatnet trout had 17 parasite taxa (Table 4). Four taxa were only present in Litlvatnet trout; the acanthocephalan *Neoechinorhynchus* sp., copepod *Argulus coregoni* and adult cestode *Proteocephalus longicollis*, as well as one individual of an unknown intestinal nematode (sp 3; Appendix B). An unknown adult stomach nematode (sp 2; Appendix B) was only present in Storvatnet. The rest of the taxa were present in both lakes (see Table 4 for an overview). Among the total of 18 taxa, 10 were autogenic (AU) – with fish as final host, whilst five were allogenic (AL) taxa using birds or mammals as final hosts. The remaining taxa were unidentifiable nematodes, without any clear adult or larval features, and are therefore not classified as AU or AG. The total abundance of both autogenic (N_{AU}) and allogenic (N_{AL}) taxa did not differ between the lakes (all $p > 0.05$; Table 2, Table S1 Appendix A) The Jaccard coefficient showed a high similarity (0.72) in community structure of parasites between overlapping species among the lakes. Neither the mean infracommunity taxon richness (S), total abundance (N), diversity (DMg) or evenness (BP) differed between the lakes (Table 2, Table S1, Appendix A). Taxon richness, total abundance, and total abundance of allogenic (N_{AL}) parasite taxa did all show a positive correlation with fish length (Table 2, Table S1 Appendix A).

The parasite infracommunity composition differed between the lakes (PERMANOVA on log transformed data, $p < 0.05$; Figure 3). The taxa that contributed most to these differences were *Eustrongylides* sp. (79 %), *Phyllodistomum* sp. (67 %), *Diplostomum* sp. (55 %), *Crepidostomum* sp. (40 %) and *Dibothriocephalus* spp. (22 %) (Similarity percentages, SIMPER).

Table 2 The mean (± standard error) of the total abundance (N), taxon richness (S), Margalef's diversity index (DMg) and Berger Parker evenness index (BP) at an intracommunity level per lake. Total abundance for autogenic (N_{AU}) and allogenic (N_{AL}) taxa are also included.

	Litlvatnet	Storvatnet	Contrasts [a]
Taxon richness (S)	5.47 ± 0.29	5.33 ± 0.32	Lake: NS **Length: S increase with length**
Total abundance (N)	56.4 ± 6.92	78.2 ± 21.7 (60.5 ± 13.0)	Lake: NS **Length: N increase with length**
Diversity (DMg)	1.18 ± 0.06	1.20 ± 0.07 (1.20 ± 0.073)	NS
Evenness (BP)	0.58 ± 0.03	0.59 ± 0.03 (0.58 ± 0.033)	NS
Total abundance autogenic (N_{AU}) parasites	14.4 ± 3.2	14.3 ± 2.7 (12.9 ± 2.4)	NS
Total abundance allogenic (N_{AL}) parasites	41.8 ± 6.7	61.4 ± 19.7 (45.6 ± 12.1)	Lake: NS **Fork length: N_{AL} increase with increasing length**

Values in parentheses refer to calculation with one highly infected trout in Storvatnet removed.
a - summary of contrast analysis NS=not significant =p-value > 0,05
For supplementary information see table S1 in appendix A.

3.2 Prevalence

Prevalence of the adult cestode *Eubothrium crassum* (LT:63.3%, ST: 73.3%) and trematode eye fluke metacercaria *Diplostomum* sp. (LT:100%, ST: 96.67%) were generally high in trout from both lakes (Table 4). The prevalence of the kidney fluke *Phyllodistomum* sp. (LT: 6.7 %, ST: 60%), and the unknown stomach and intestinal nematodes (sp1; LT: 13.3 %, ST: 26.7 %) was higher in Storvatnet, whereas the larval nematode *Eustrongylides* sp. was the only taxa with higher prevalence in Litlvatnet (LT: 70%, ST: 33.3%) (Table 4). The taxa with less than 15 % prevalence in both lakes were excluded from further analysis due to low sample size. For these taxa, the trematodes *Apatemon* sp. and *Tylodelphys* sp. had the exact same prevalence (10%) in both lakes, whilst Acanthocephala, the adult cestode *Proteocephalus* sp. and fish louse *A. coregoni* were present in a few trout from Litlvatnet only. The gill louse *Salmincola* sp. was present in low numbers in both lakes (Table 4).

The taxa that showed a positive correlation between prevalence and fish length were the larval tapeworm *Dibothriocephalus* spp., the adult stage nematode *Pseudocapillaria* sp., *Eustrongylides* sp. and Myxozoa (Table 4, Table S 2 Appendix A). The prevalence of the unknown nematode sp. 1, and *Pseudocapillaria* sp. showed a significant interaction between fish length and prevalence between the lakes (Table 4, Table S 2 Appendix A).

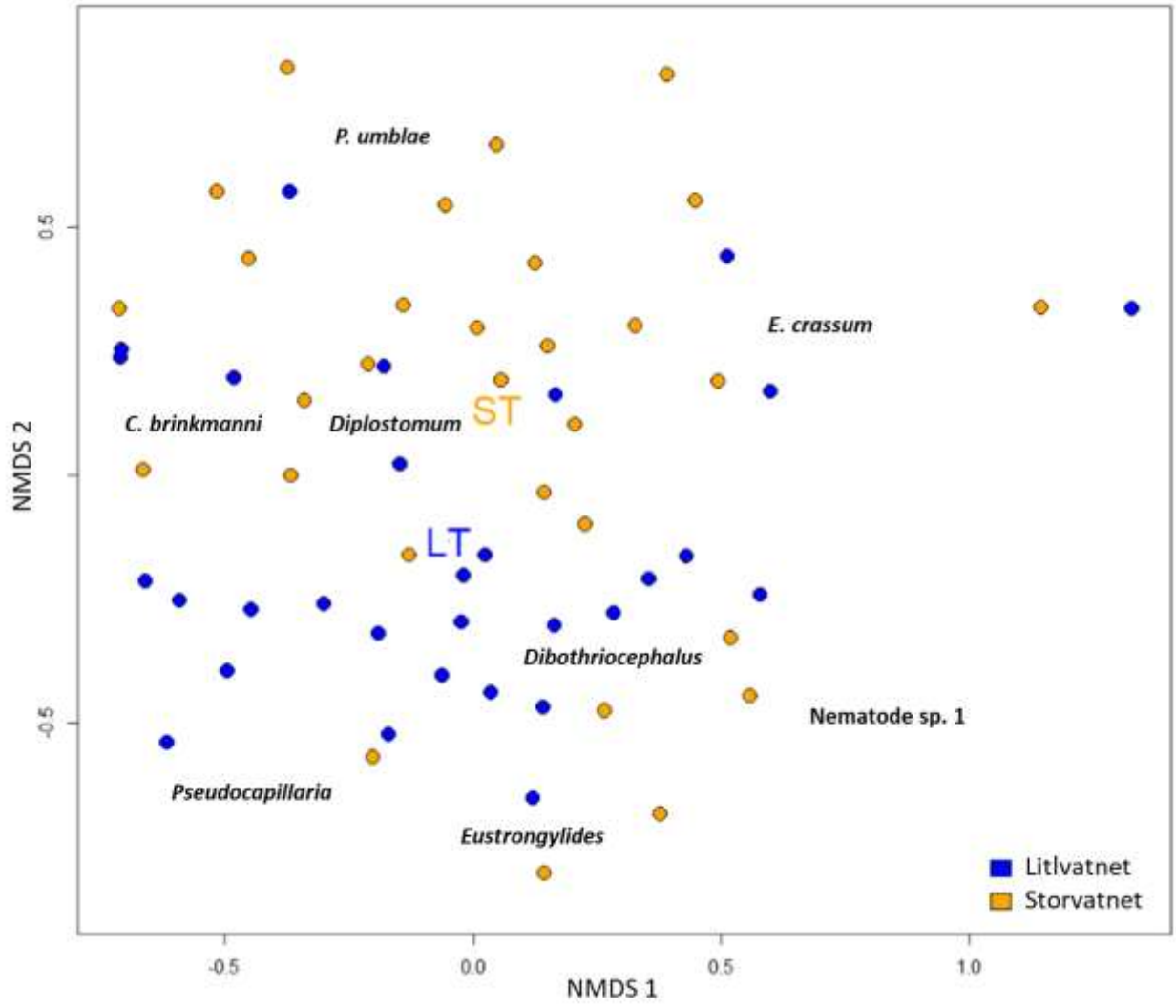


Figure 2 - Parasite community composition of lakes Litlvatnet (blue) and Storvatnet (orange) in Fremstadvassdraget, Trønderlag, Norway. Visualized with Non-Metric multidimensional scaling (NMDS) which is based on Bray-Curtis dissimilarities of parasite infra-communities. The coloured letters show the mean for each lake, and each coloured dot represent each individual trout. The text for each taxa are centred.

3.3 Abundance

The most abundant taxa for each lake were *Diplostomum* sp. (23.2 ± 3.4), and *Dibothriocephalus* spp. (44.4 ± 17.7 (without outlier:30.1 ± 10.7)) in Litlvatnet and Storvatnet, respectively. *Diplostomum* sp. and *Eustrongylides* sp. had a higher abundance in Litlvatnet, whilst *Phyllodistomum* sp., unknown nematode sp. 1 and *Dibothriocephalus* spp. were most abundant in Storvatnet (Table 4, Table S 3, Appendix A). *Dibothriocephalus* spp. abundance no longer differed between the lakes when one highly infected individual was removed.

The parasite taxa with a positive relationship between abundance and fish length were *Diplostomum* sp, *Dibothriocephalus* spp., and unknown nematode sp1. *Dibothriocephalus* spp. also showed a significant interaction between the slopes of abundance and fish length between the lakes (Table 4, Table S 3, Appendix A).

3.4 Stable isotopes

Litlvatnet had higher $\delta^{13}C$ and $\delta^{15}N$ values than Storvatnet (Figure 3; W=808, p<0.001; W=826, p<0.001). The relationship between isotope signatures and fish length were not significant (Kruskall-Wallis test, p>0.05), so fish length was not included in the analysis. The relative overlap between the ellipses of $\delta^{13}C$ and $\delta^{15}N$ values for each lake were 17% between the two lakes (Figure 3).

3.5 Diet

The diet overlap was intermediate (44 %) for the lowest taxonomic grouping level (Total of 25 groups, see Figure 4. Contrast analyses showed that surface insects and benthos did not differ significantly between lakes (W=470, p=0.73; W=582 p=0.053). Zooplankton were more abundant in Storvatnet (W=148, p<0.001). Fish were relatively rare in the diet and were therefore not analysed. The correlation between diet and trophically transmitted parasites was strong (80%) with axis 1 explaining 49% and axis 2 explaining 31% of the correlation (Figure 5).

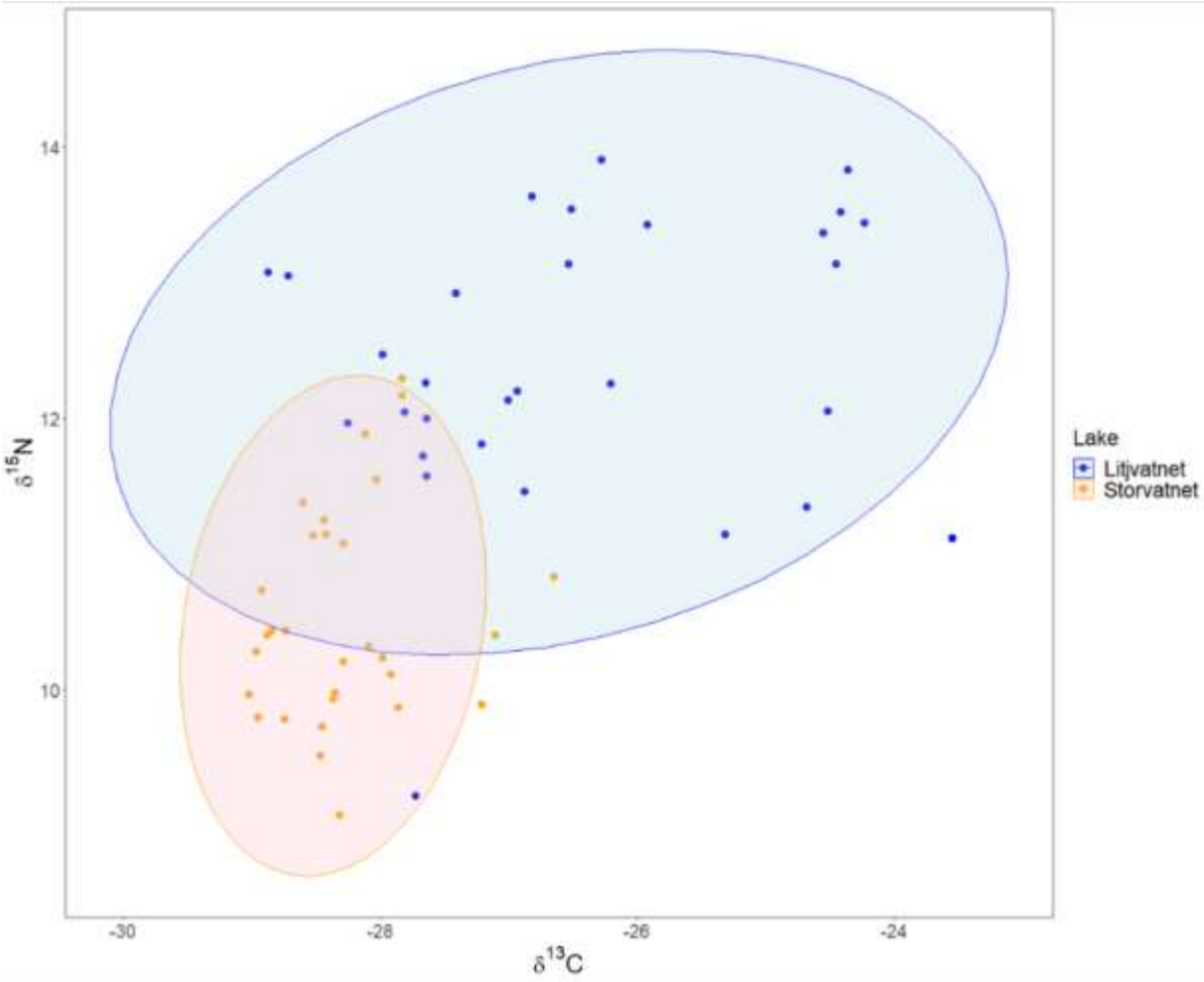


Figure 3 – Elliptical projection for carbon and nitrogen isotope ratios (δ13C, δ15N) between Litlvatnet (blue) and Storvatnet (orange) in Fremstadvassdraget, Trønderlag, Norway. The ellipses are drawn at a 95% confidence interval around each population.

Axis 1 also separated those trout that had recently preyed upon zooplankton from those who had eaten fish (four individuals). Axis 2 separated trout which had consumed benthos from those who recently preyed upon one of the tree other prey groups. Correlation analysis also found a linear relationship with *Crepidostomum brinkmanni* and *Pseudocapillaria* sp. to benthos, *Phyllodistomum umblae* to zooplankton, and the two remaining nematodes and *Dibothriocephalus* spp. to fish. Surface insects are most correlated to *E. crassum.* Fish that are infected with *Eustrongylides* sp. and unknown nematode (sp. 1) have similar diet content based on the CCorA plot (Figure 5).

Table 3 - Mean prey abundance (%) ± standard error for the main prey groups for Litlvatnet and Storvatnet, Fremstadvassdraget, Trønderlag, Norway. Values based on estimations of the percentage of the total volume of the stomach content.

	Litlvatnet	Storvatnet	Contrasts diet [a]
Zooplankton	3.6 ± 2.5	35.4 ± 8.2	**ST > LT**
Benthos	65.6 ± 7.1	46.4 ± 7.8	NS
Surface insects	16.3 ± 5.1	13.0 ± 5.0	NS
Fish	1.3 ± 1.0	5.2 ± 3.6	-

a - contrast analysis performed using non-parametric Mann Whitney U test. NS=not significant (P>0.05). ST=Storvatnet, LT=Litlvatnet.
See figure 4 for a more detailed overview.

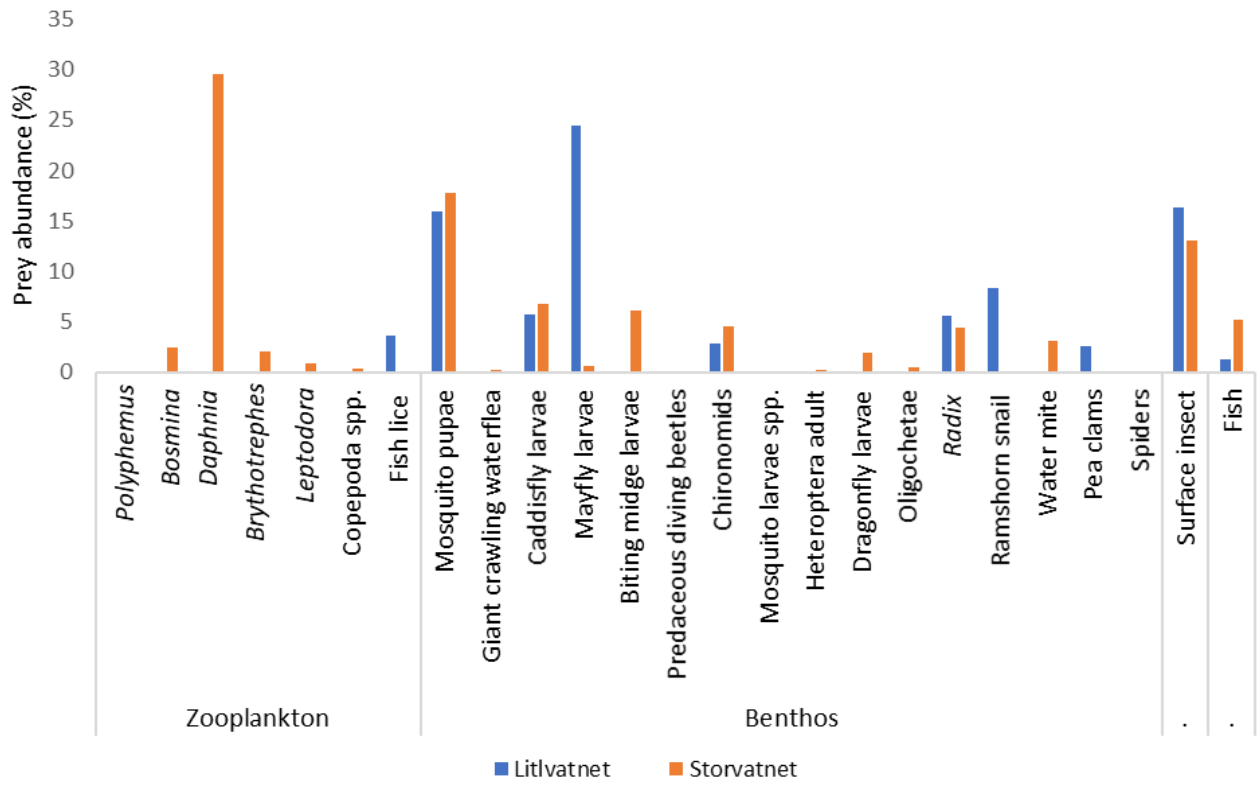


Figure 4 – Diet (prey abundance, %) of brown trout (Salmo trutta) in Litlvatnet (blue) and Storvatnet (orange) in Fremstadvassdraget, Trønderlag, Norway. The main prey groups are also indicated.

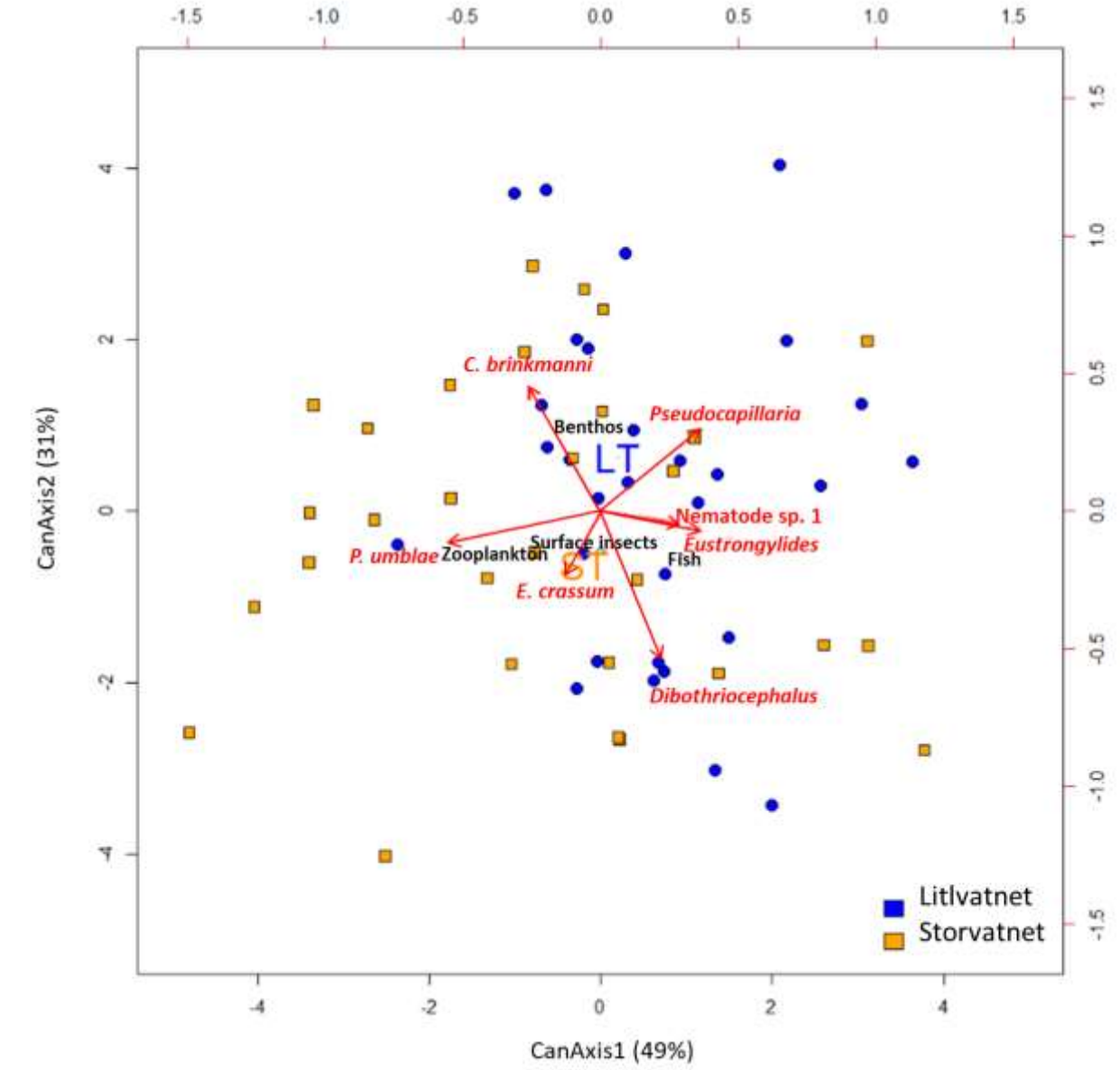


Figure 5 - The relationship between stomach content (black text) and parasite community composition (red text and arrows) of brown trout (Salmo trutta) in Litlvatnet (blue, n=30) and Storvatnet (orange, n=30) in Fremstadvassdraget, Trønderlag, Norway. Derived from canonical correlation analysis (CCorrA). Large, coloured letters show the mean for each lake, and each dot represent one trout. The text for each prey group and parasite taxa are centred.

Table 4 - Overview of life cycles, site of infection, prevalence (PR, %) mean abundance ± standard error (A ± SE) and summary of contrast analysis for parasites harbouring brown trout (Salmo trutta) in Fremstadvassdraget, Trønderlag, Norway.

Taxa			Site of infection	T.T	1st IMH	2ndIMH	Final host	PR (%)	A ±SE	PR (%)	A±SE	Contrasts prevalence [a]	Contrasts abundance [a]
Lake								**Litlvatnet (LT)**		**Storvatnet (ST)**			
Trematoda	*Apatemon* sp.	AL	Cavity	No	Gastropod	Fish	Bird	10	0.2 ± 0.1	10	0.3 ± 0.2	-	-
	Crepidostomum brinkmanni	AU	Intestine	Yes	Bivalve	Arthropod	Fish	63.3	10.8 ± 3.2	83.3	5.8 ± 1.5	NS	NS
	Diplostomum sp.	AL	Eye	No	Gastropod	Fish	Bird	100	23.2±3.4	96.7	14.5 ± 2.4	NS	**Lake: LT>ST**, **Length: positive**
	Phyllodistomum umblae	AU	Kidney, ureters	?	Bivalve	?	Fish	6.7	0.3 ±0.2	60	4.8 ± 1.6	**Lake: ST>LT** Length: NS	**Lake: ST>LT** Length: NS
	Tylodelphys sp.	AL	Eye	No	Gastropod	Fish	Bird	6.7	0.1 ± 0.1	6.7	0.1 ± 0.1	-	-
Cestoda	*Dibothriocephalus* spp.	AL	Intestine, caeca, cavity	Yes	Copepod	Fish	Bird /mammal	80	15.4 ±3.6	73.3	44.4 ± 17.7 (30.1 ± 10.7)	Lake: NS **Length: positive** Lake x length: NS	**Lake: ST>LT (**Lake: NS) **Length: positive**
	Eubothrium crassum	AU	Intestine/caeca	Yes	Copepod	-	Fish	63.3	1.47 ±0.3	73.3	2.5 ± 0.7	NS	NS
	Proteocephalus longicolllis	AU	Intestine/caeca	Yes	Copepod	-	Fish	13.3	0.1 ± 0.1	0	0	-	-
Acanthocephala	*Neoechynorhynchus* sp.	AU	Intestine		Arthropod	-	Fish	6.7	0.1 ± 0.1	0	0	-	-
Nematoda	*Eustrongylides* sp.	AL	Cavity, intestine	Yes	Oligochaeta	Fish	Bird	70	2.9 ±0.6	33.3	2.1 ± 1.0	**Lake: LT>ST** **Length: positive**	**Lake: LT>ST** **Lake x length: int**
	Nematode sp. 1[b]	AL	Stomach, intestine	Yes	?	?	?	13.3	0.2 ± 0.1	26.7	2.5 ± 1.2	**Lake: ST>LT** **Lake x length: int**	**Lake: ST>LT** **Length: positive**
	Eustrongylides + sp1 [b]	?	-	-	-	-	-	76.7	3.1 ± 0.6	36.7	3.6 ± 1.5	**Lake: LT>ST** **Lake x length: int**	NS
	Pseudocapillaria sp.	AU	Intestine	?	?	?	Fish	43.3	1.2 ± 0.4	23.3	0.8 ± 0.6	Lake: NS **Length: positive**	NS
	Nematode sp. 2[b]	AU	Stomach	?	?	?	Fish	0	0	6.7	0.3 ± 0.2	-	-
	Nematode sp. 3[b]	?	Intestine	?	?	?	?	3.3	0.1 ± 0.1	0	0	-	-
Myxozoa	-	AU	Cavity	No	Annelida	-	Fish	56.7	-	33.3	-	Lake: NS **Length: positive**	-
Crustaceans	*Argulus coregoni*	AU	Skin	No	-	-	Fish	13.3	0.3 ± 0.2	0	0	-	-
	Salmincola sp.	AU	Gills	No	-	-	Fish	6.7	0.1 ± 0.1	10	0.1 ± 0.1	-	-

a – summary of contrast analysis. NS=P-value > 0.05, int=interaction b – See Appendix B for information on nematodes.
Values parentheses refer to calculation with one highly infected trout in Storvatnet removed.
IMH = intermediate host, AU/AG=autogenic/allogenic, T.T = trophic transmitted to fish. Supplementary information: table S2 and S3 in Appendix A

4 Discussion

The parasite communities of the trout populations from the two lakes did not differ in taxon richness, total abundance, diversity, nor evenness (Table 2). Thus, *the first hypothesis considering* higher parasite taxon diversity in the lake with most eutrophic characteristics was not supported. Furthermore, *the second hypothesis* was also not supported since the diversity and total abundance of allogenic and autogenic taxa were similar in trout between the lakes. These results were surprising since both diet and stable isotopes differed significantly between the lakes, which indicates that trout occupy different trophic niches between Litlvatnet and Storvatnet.

Host diversity is one important driver of parasite diversity as the host are both the parasites habitat and the agent dispersing the parasite (Hechinger et al., 2005). These relatively close similarities in parasite diversity and evenness, as well as abundance of allogenic and autogenic parasites may be due to the lakes having similar fish assemblages (Fernandez et al., 2010) with trout as the dominant species. Brown trout are known to migrate between the lakes in the Fremstad catchment, which is a common behaviour of trout in other systems (Ferguson et al., 2019; Boel et al., 2014). Additionally, Litlvatnet and Storvatnet are located close to each other and belong to the same drainage (Paterson et al., 2021). Therefore, the birds acting as final hosts for several of the allogenic parasite taxa can easily move between the lakes. These factors may cause these similarities in observed parasite taxa community as in other studies (Baerger et al., 2006; Esch et al., 1990). Host diversity may be driven by habitat heterogenity (MacArthur & MacArthur, 1961; Rosenzweig, 1995). It was hypothesized that Litlvatnet had higher diversity than Storvatnet. This was due to the higher impact of agriculture and higher phosphorus levels suggesting that the lake was probably is more eutrophic than Storvatnet. Productivity can alter species richness if the lake is not too polluted (Valtonen et al., 1997; Marcogliese & Cone, 1991; Suthar et al., 2022). The similarity of diversity between the lakes may be caused from that Storvatnet could be more heterogenic due to the lake being bigger and having a greater pelagic zone with different light conditions and bottom vegetation, in contrast to Litlvatnet which could be more homogenous. This could annul the diversity differences that degree of productivity could lead to. It can also be a sign that Litlvatnet are more polluted than expected, which have resulted in a decrease in species richness in Litlvatnet, making the diversity more similar between the lakes (Valtonen et al., 1997).

Regarding the parasite community, differences were revealed. The parasite taxa more abundant and/or prevalent in Litlvatnet were the larval nematode *Eustrongylides* sp. and the metacercaria stage eye fluke *Diplostomum* sp., which are both allogenic. According to Esch (1971) and Wisniewski (1958) allogenic parasites tend to be more abundant in eutrophic lakes. This may suggest that some taxa of allogenic parasites favour Litlvatnet, even though the total abundance of allogenic parasites did not differ. *Eustrongylides* sp. is often associated with shallow, eutrophic waters (Menconi et al., 2020; Rusconi et al., 2022; Spalding & Forrester, 1993), and uses oligochaete as their intermediate host and birds as final hosts. Oligochaeta thrive in eutrophic waters (Djukic et al., 1993), which supports this high abundance and prevalence of *Eustrongylides* sp. The entire body of water are also easier assessable to the final host being birds which makes the trophic transmission of the parasite between these hosts more frequent (Esch, 1971, Wisniewski, 1958). *Eustrongylides* sp. may cause severe consequences for its final host and can in some cases case be lethal (Cole & Friend, 1999; Spalding & Forrester, 1993, Coyner et al., 2002). This makes this parasite important to monitor to avoid dramatic consequences for the birds using Litlvatnet as an important feeding and breading ground. Marcogliese and Cone (1991) states that shallower lakes often have high abundances of metacercaria stage digeneans with birds or mammals as final hosts. This can support that *Diplostomum* sp. prefer Litlvatnet. In addition to accessibility for birds, this is also probably due to gastropods, being the first intermediate host are littoral distributed.

In Storvatnet three taxa showed higher prevalence and/or abundance than Litlvatnet; the copepod transmitted, allogenic tapeworm larvae *Dibothriocephalus* spp., the autogenic kidney fluke *Phyllodistomum umblae,* and an unknown nematode (sp. 1, Appendix B). This high abundance of *Dibothriocephalus* spp. in Storvatnet could be one of the reasons that the total abundance of allogenic parasites was similar between the lakes since this parasite taxa contradicts the prediction of more allogenic parasites in the nutrient rich lake (Esch, 1971; Wisniewski, 1958). According to Marcogliese & Cone (1991) on the other hand, deeper lakes often have a parasite community consisting of more copepod transmitted parasites like *Dibothriocephalus* spp. which supports the findings in this study. When it comes to *P. umblae* the life cycle is still not fully revealed. The first intermediate host is most likely sphaeriid bivalves (Petkevičiūtė et al., 2015). Oligotrophic waters are suitable habitats for sphaeriid clams, making Storvatnet a preferable habitat for *P. umblae* (Kubíková et al., 2011). Some studies suggests that stone flies may be the second intermediate host (Faltýnková, et al., 2020).

P. umblae is also the one contributing most to the parasite infracommunity differences between the lakes, with low numbers for both prevalence and abundance in Litlvatnet and higher numbers in Storvatnet. In comparison to two other, relative deep oligotrophic inland lakes located in the same geographical area, the abundance and prevalence of *P. umblae* are relatively high in both lakes (Paterson et al., 2019b). Other parasitological studies on salmonids in Norway performed in oligotrophic waters also show the same pattern for *P. umblae* (Knudsen et al., 2008; Knudsen et al., 1997; Paterson et al., 2019a). This may indicate that *P. umblae* is better adapted to oligotrophic environments, regardless of whether they are coastal- or inland lakes. Shallower, more eutrophic water bodies may not offer the right environmental conditions and suitable hosts for *P. umblae.* Unfortunately, few studies have been conducted in eutrophic water to support a pattern of low abundance and prevalence of *P. umblae* in water bodies like Litlvatnet.

The high prevalence and abundance of *Diplostomum* sp., *Dibothriocephalus* spp. and *Eubothrium crassum* in Storvatnet/Litlvatnet is quite similar as nearby, inland lakes (Paterson et al., 2019b). However, a key difference between these studies is the absence of nematodes in the inland lakes. Nematodes is one of the dominating taxa in Fremstadvassdraget. This could be due to the eutrophic characteristics of Litlvatnet which probably drive the presence of the dominating nematode *Eustrongylides* sp. These allogenic parasites get dispersed with both birds and trout that move over to Storvatnet (Paterson et al., 2021), which also probably are the reason why they are present in oligotrophic Storvatnet, but not the inland oligotrophic lakes (Esch et al., 1990).

The parasite intracommunity analysis showed that the adult intestinal digenean *Crepidostomum brinkmanni* was one of the five taxa that contributed most to the infracommunity difference between the study lakes. Even though *C. brinkmanni* did not differ in prevalence nor abundance, they were distributed differently between the lakes, with a more aggregated distribution in Litlvatnet (Table 4, Table S4 Appendix A). *Crepidostomum brinkmanni* have been reported in stoneflies and mayflies as the second intermediate host, whilst sphaeriid clams (*Pisidium*) acts as the first intermediate host (Faltynkova et al., 2020). Benthos was abundant prey in both study lakes, this similarity in prevalence and abundance makes sense. *C. brinkmanni* is a newly discovered species (Faltynkova et al., 2020), reported from Switzerland, northern Norway, and Iceland (Rochat et al., 2021; Vainutis et al., 2021; Faltynkova et al., 2020), and now also from

central Norway (this study). In localities where this parasite occurs, it is both prevalent and abundant (Rochat et al., 2021), which is also usually the case for other *Crepidostomum* sp. studies (Paterson et al 2019b; Knudsen et al., 2008; Prati et al., 2020). These locations are usually oligotrophic, but this study suggests that *Crepidostomum* sp. are also well adapted for more nutrient rich environments as the parasite are quite prevalent and abundant in both lakes, in contrast to *Phyllodistomum umblae*. However, the differences in distribution pattern between the lakes does suggests differences in conditions favouring *C. brinkmanni* between deep, oligotrophic lake habitats and shallower lakes with high amounts of nutrient input.

Adult, intestinal cestode *Eubothrium crassum* and adult nematode *Pseudocapillaria* sp. does not differ significant between the lakes. Even distribution of *Eubothrium crassum* between lakes is a pattern observed in several other studies (Prati et al., 2020; Paterson et al., 2019b; Knudsen et al., 2008). *E. crassum* is copepod transmitted and autogenic. For *Pseudocapillaria* sp. the life cycle is not yet described (Moravec, 2004, p. 225). Siwertson et al., 2016 found results through investigating the parasite community of Arctic charr morphs suggesting that the closely related *Capellaria salvelini* are transmitted through piscivory. This could also be the case for trout in Fremstadvassdraget, where the prevalence of *Pseudocapillaria* was positively correlated to fish length. This may indicate that the larger fish eat more, and bigger prey like other fishes which increases the likelihood of getting infected (Poulin & Morand, 2000).

Besides the prevalence and/or abundance of *Pseudocapillaria* sp., Dibothriocephalus sp, *Eustrongylides* sp. and *Eubothrium crassum* are all also positively correlated to fish length. All these taxa seem to have the ability to re-establish from prey fish and therefore may be transmitted through piscivory (Siwertson et al., 2016; Henriksen et al., 2016; Sattari, 2004; Goncharov et al., 2018; Kuhn et al., 2016). This is supported by looking at nitrogen isotope signatures in the current study, which suggest that fish highly infected by these parasites are located at the upper part of the ellipses for each lake. This can indicate that these fish have been infected through piscivory since high nitrogen levels indicates a higher trophic position. This can also explain why copepods show such a low prey abundance in the stomachs of the trout, since the copepod transmitted parasites being *Dibothriocephalus* spp., *E. crassum* and *Proteocephalus longicollis* could be transferred through fish prey. The prey abundance of fish from the stomach contents is low, which can indicate that they consume other abundant prey like surface insects at this time of year instead of cannibalism and preying on other fish species

like three-spined stickleback (*Gasterosteus aculeatus).* Three-spined-spined stickleback often acts as an intermediate host for *Dibothriocephalus* spp. (Henriksen et al., 2016). There exists anadromous stickleback (Arai et al., 2020; Raeymaekers et al., 2005) which may also be the case in in Fremstadvassdraget, especially since the field team struggled with catching stickleback in June (Pers. ops). This may explain the low occurrence of potential prey fish at this time of year in brown trout highly infected by *Dibothriocephalus* spp. larvae.

The stable isotope values in Litlvatnet were generally higher for both nitrogen and carbon. This might be linked to higher marine and agricultural input to Litlvatnet (Schulting, 1998, Oczkowski et al., 2014), being the downstream located lake with more croplands in its catchment area (Winnem, 2010). Litlvatnet also has a smaller body of water making it more affected by both marine and agricultural input. Therefore, the piscivorous individuals in Storvatnet will probably have a lower δ15N signatures than the piscivorous individuals in Litlvatnet.

Several relatively rare parasite taxa were observed in the current study system. Out of these, three taxa were only present in Litlvatnet (the acanthocephalan *Neoechynorhynchus* sp., the cestode *Proteocephalus longicollis*, and the fish louse *Argulus coregoni).* The remaining three were present in low numbers in both lakes. (digeneans *Apatemon* sp. and *Tylodelphys* sp. and the gill louse *Salmincola* sp.) The acanthocephalan was shown through molecular methods to belong to the genus *Neoechynorhynchus* sp. According to Moravec (2004) the only species of this genus in salmonids in Europe is *N. rutili,* which often has ostracods, leeches, alder flies or crayfish as (one of the) intermediate host(s), and fish as final host. This parasite was previously observed in salmonids in several British lakes and in Switzerland (Kennedy et al., 1978; Lassierre & Crompton, 1988; Lasserie, 1989; Rochat et al., 2021). *Neoechynorhynchus* sp. are often generalists, present in low numbers and are common in European eel (Kennedy & Hartvigsen, 2000) which can explain them being present only in Litlvatnet. Adult cestode *Proteocephalus longicollis* are found in several sites in Norway (Paterson et al., 2019a; Knudsen et al., 2019; Andersen & Valtonen, 1990). *Argulus coregoni* are also found in several Scandinavian lakes including closely located inland lakes (Knudsen et al., 2019; Paterson et al 2019b, Moravec 2004), where it is only distributed in the lower located lake. *Apatemon* sp. and *Tylodelphys* sp. are also commonly found in trout in Norway, but *Apatemon* sp. metacercaria are more commonly found in the eyes rather than in the body cavity where the parasite is found

in this study (Paterson et al., 2019a, Paterson et al., 2019b). The only *Salmincola* species recorded in brown trout according to Moravec (2004) is *Salmincola salmoneus,* which is probably the species found in Fremstadvassdraget as well. This parasite is commonly found in anadromous fish including sea trout (Kusterle et al., 2013; Byrne et al., 1999). All these taxa are rare and in low intensities, and most likely have low impacts of their hosts.

4.1 Future research

Since this is a system with characteristics that are little researched in this part of the world a long-term monitoring program could with advantage be conducted. This can give us valuable information about parasite communities in costal lakes with eutrophic characteristics. Other similar systems should also be investigated if available to see if there is a similar parasite community pattern like in this study. One example of such a lake is Rusasetvatn in Ørland county (Baadsvik & Suul, 1977), which is a eutrophic, anthropogenic impacted, coastal lake located nearby Fremstadvassdraget. There were sampled 30 fish from each lake, which is the minimum number required from Poulin (1996) which make this data good to compare other samplings with. One September sampling are already conducted, and hopefully more monitoring to come, which would help reveal more general information about the parasite communities in the lakes. Pelagic caught fish from Storvatnet, and out-migrating fish from Fremstadelva were also sampled, which would also contribute to a better understanding of the system. Further sampling at different times of the year to see what the stomach content are throughout could also help explain the patterns in parasite community discussed, like for instance the possibility of seasonally predation on migratory stickleback.

Examination of some final hosts for allogenic parasites would be something for further research. Especially based on research on the effect *Eustrongylides* sp. infections can have on herons and other birds (Cole & Friend, 1999; Spalding & Forrester, 1993; Coyner et al.,2002), and that a lot of vulnerable migratory birds pass this system (Ulsund et al., 2013). More research on intermediate hosts is, as always also helpful since a lot of parasite species still are not fully described. Taxonomic identification of parasite taxa, especially nematodes, has been a challenge during this study. Genetic analyses have been used for many of the taxa to confirm morphological identification, however most nematode classifications were based on morphological features as genetic analysis was inconclusive. Therefore, this should be looked further into. Pictures and descriptions of nematodes are provided in appendix B.

Freshwater ecology all over the world should also include investigation of parasites in more projects. However, parasite examination and identification are a time-consuming process. By working on efficiency of current methods, more research can also be done with less resources and in a shorter period which makes it easier to include parasites in more of the freshwater ecology studies. This would help us get a better understanding of patterns and drivers in all ecosystems, which would help us manage our lakes in a sustainable way while still exploiting the many resources that they can offer.

4.2 Relevance for schools

As a future science teacher, the relevance of this study in schools will be discussed. Knowledge about parasites and their importance to our world ecosystems should be more distributed to the masses. This is a field that has the potential to fascinate and engage people, and that should be disseminated in a way that makes people more aware the importance of parasites. It is also an important part of understanding the complexity and see the bigger picture when it comes to ecology. Therefore, this work is relevant for current and future teachers, to include in teaching ecology to school children and youth.

5 Concluding remarks

The hypotheses investigated in this thesis were the following:

1. The parasite diversity will be higher in the downstream located lake with eutrophic characteristics
2. Parasite species with birds or mammals as final host (allogenic parasites) will be more abundant and/or prevalent in the downstream located lake with eutrophic characteristics. Parasites with brown trout as final host (autogenic parasites) will be more abundant in the upstream located, oligo/mesotrophic lake.

Trophic niche through stable isotope and diet analysis have also been investigated to support the parasite data. The hypothesises investigated were not supported, since both the diversity and total abundance of allogenic and autogenic parasites did not differ between the lakes. However, key differences in the parasite community composition were revealed. Factors like trophic gradient, lake size and depth and degree of marine and terrestrial influence may affect the parasite community in coastal lakes, even though they are located close to each other. Based on comparison with other studies, there are signs that some parasites abundant in oligotrophic lakes do not thrive in the nutrient rich lakes such as Litlvatnet (e.g. *P. umblae*), whereas other parasites may not thrive in oligotrophic inland systems (e.g. *Eustrongylides* sp.). Moreover, some parasite taxa may be well adapted for a wider spectre of lake characteristics (e.g. *Dibothriocephalus* spp., *Crepidostomum brinkmanni*). *Eustrongylides* sp. that thrive in Litlvatnet also has the possibility to have severe impact on the wildlife community both in and around the lake, which makes this parasite important to monitor considering the status of Litlvatnet as a nature reserve. The stable isotope and diet, together with the parasite community also shows that Litlvatnet has higher degree of marine and agricultural input than Storvatnet and that Fremstadvassdraget trout may consume different prey throughout the year. Long term monitoring in Fremstadvasssdraget may help to reveal general parasite community patterns in coastal, eutrophicated lake systems.

Works cited

Abdussalam, M., Käferstein, F. K., & Mott, K. E. (1995). Food safety measures for the control of foodborne trematode infections. *Food Control*, 6(2), 71-79. https://doi.org/10.1016/0956-7135(95)98910-S

Amundsen, P. A. (1995). Feeding strategy of Arctic charr (Salvelinus alpinus): general opportunist, but individual specialist. *Nordic Journal of Freshwater Research*, 150-156.

Amundsen, P. A., Gabler, H. M., & Staldvik, F. J. (1996). A new approach to graphical analysis of feeding strategy from stomach contents data—modification of the Costello (1990) method. *Journal of fish biology*, 48(4), 607-614. https://doi.org/10.1111/j.1095-8649.1996.tb01455.x

Andersen, K. I., & Valtonen, E. T. (1990). On the infracommunity structure of adult cestodes in freshwater fishes. *Parasitology*, 101(2), 257-264.https://doi.org.10.1017/S0031182000063319

Arai, T., Ueno, D., Kitamura, T., & Goto, A. (2020). Habitat preference and diverse migration in threespine sticklebacks, Gasterosteus aculeatus and G. nipponicus. *Scientific Reports*, 10(1), 1-15. https://doi.org/10.1038/s41598-020-71400-4

Baadsvik, K. & Suul J., *Biologiske registreringer og verneinteresser i Litlvatnet, Agdenes i Sør-Trønderlag*. (Botanisk serie 1977-4). NTNU Vitenskapsmuseet. http://hdl.handle.net/11250/272147

Barger, M. A. (2006). Spatial heterogeneity in the parasite communities of creek chub (Semotilus atromaculatus) in southeastern Nebraska. *Journal of Parasitology*, 92(2), 230-235. https://doi.org/10.1645/GE-645R1.1

Bennett, E. M., Carpenter, S. R., & Caraco, N. F. (2001). Human impact on erodable phosphorus and eutrophication: a global perspective: increasing accumulation of phosphorus in soil threatens rivers, lakes, and coastal oceans with eutrophication. *BioScience*, 51(3), 227-234. https://doi.org/10.1641/0006-3568(2001)051[0227:HIOEPA]2.0.CO;2

Berger, W. H., & Parker, F. L. (1970). Diversity of planktonic foraminifera in deep-sea sediments. *Science*, 168(3937), 1345-1347. https://doi.org.10.1126/science.168.3937.1345

Bjelić-Čabrilo, O., Novakov, N., Ćirković, M., Kostić, D., Popović, E., Aleksić, N., & Lujić, J. (2013). The first determination of Eustrongylides excisus Jägerskiöld, 1909—larvae (Nematoda: Dioctophymatidae) in the pike-perch Sander lucioperca in Vojvodina (Serbia). *Helminthologia*, 50(4), 291-294. https://doi.org/10.2478/s11687-013-0143-1

Blasco-Costa, I., Cutmore, S. C., Miller, T. L., & Nolan, M. J. (2016). Molecular approaches to trematode systematics:'best practice'and implications for future study. Systematic *Parasitology*, 93(3), 295-306. https://doi.org/10.1007/s11230-016-9631-2

Blasco-Costa, I., Balbuena, J. A., Kostadinova, A., & Olson, P. D. (2009). Interrelationships of the Haploporinae (Digenea: Haploporidae): a molecular test of the taxonomic framework based on morphology. *Parasitology International*, 58(3), 263-269. https://doi.org/10.1016/j.parint.2009.03.006

Boel, M., Aarestrup, K., Baktoft, H., Larsen, T., Søndergaard Madsen, S., Malte, H., ... & Koed, A. (2014). The physiological basis of the migration continuum in brown trout (Salmo trutta). *Physiological and Biochemical Zoology*, 87(2), 334-345.

Bolar, K., (2019). Package 'STAT.'. R Package Version, 2-5.

Bonsdorff, E. (2021). Eutrophication: Early warning signals, ecosystem-level and societal responses, and ways forward. *Ambio*, 50(4), 753-758. https://doi.org/10.1007/s13280-020-01432-7

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of parasitology*, 575-583. https://doi.org/10.2307/3284227

Byrne, C. J., Holland, C., & Tully, O. (1999). Metazoan parasite community structure of sea trout on the west coast of Ireland. *Journal of Fish Biology*, 55(1), 127-134. https://doi.org/10.1111/j.1095-8649.1999.tb00662.x

Cardinale, B. J., Bennett, D. M., Nelson, C. E., & Gross, K. (2009). Does productivity drive diversity or vice versa? A test of the multivariate productivity–diversity hypothesis in streams. *Ecology*, 90(5), 1227-1241. https://doi.org/10.1890/08-1038.1

Černotíková, E., Horák, A., & Moravec, F. (2011). Phylogenetic relationships of some spirurine nematodes (Nematoda: Chromadorea: Rhabditida: Spirurina) parasitic in fishes inferred from SSU rRNA gene sequences. *Folia Parasitologica*, 58(2), 135.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian journal of ecology*, 18(1), 117-143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Cole, R. A., & Friend, M. (1999). Parasites and Parisitic Diseases (Field Manual of Wildlife Diseases). *Other Publications in Zoonotics and Wildlife Disease*, 15.

Coyner, D. F., Spalding, M. G., & Forrester, D. J. (2002). Epizootiology of Eustrongylides ignotus in Florida: Distribution, density, and natural infections in intermediate hosts. *Journal of Wildlife Diseases*, 38(3), 483-499. https://doi.org/10.7589/0090-3558-38.3.483

Djukic, N., Maletin, S., & Miljanovic, B. (1993). Oligochaeta community as indicator of eutrophication in lower stream of River Tisza. *Tiscia (Szeged)*, 27, 49-52.

Dogiel, V. A. (1961). Ecology of the parasites of freshwater fishes. Dogiel, V.A., Petrushevski, G.K. & Polyanski. Yu.I. (Ed.). *Parasitology of Fishes* (p. 1-47). Oliver and Boyd LTD.

Downing, J. A. (2014). Limnology and oceanography: two estranged twins reuniting by global change. *Inland Waters*, 4(2), 215-232. https://doi.org.10.5268/IW-4.2.753

Eloranta, A. P., Knudsen, R., & AMUNDSEN, P. A. (2013). Niche segregation of coexisting Arctic charr (Salvelinus alpinus) and brown trout (Salmo trutta) constrains food web coupling in subarctic lakes. *Freshwater Biology*, 58(1), 207-221. https://doi.org/10.1111/fwb.12052

Esch, G. W. (1971). Impact of ecological succession on the parasite fauna in centrarchids from oligotrophic and eutrophic ecosystems. *American Midland Naturalist*, 160-168. https://doi.org/10.2307/2423696

Esch, G. W., Hazen, T. C., Marcogliese, D. J., Goater, T. M., & Crews, A. E. (1986). A long-term study on the population biology of Crepidostomum cooperi (Trematoda: Allocreadidae) in the burrowing mayfly, Hexagenia limbata (Ephemeroptera). *American Midland Naturalist*, 304-314. https://doi.org/10.2307/2425738

Esch, G. W., Shostak, A. W., Marcogliese, D. J. & Goater, T.M. (1990) Patterns and processes in helminth parasite communities: an overview. Esch, G.W., Bush, A. & Aho, J. (Ed.) *Parasite communities: Patterns and processes.* (p. 1-19). Chapman and Hall.

Faltýnková, A., Pantoja, C., Skírnisson, K., & Kudlai, O. (2020). Unexpected diversity in northern Europe: trematodes from salmonid fishes in Iceland with two new species of Crepidostomum Braun, 1900. *Parasitology Research*, 119(8), 2439-2462. https://doi.org/10.1007/s00436-020-06724-1

Ferguson, A., Reed, T. E., Cross, T. F., McGinnity, P., & Prodöhl, P. A. (2019). Anadromy, potamodromy and residency in brown trout Salmo trutta: the role of genes and the environment. *Journal of Fish Biology*, 95(3), 692-718. https://doi.org/10.1111/jfb.14005

Fernández, M. V., Brugni, N. L., Viozzi, G. P., & Semenas, L. (2010). The relationship between fish assemblages and the helminth communities of a prey fish, in a group of small shallow lakes. *Journal of Parasitology*, 96(6), 1066-1071. https://doi.org/10.1645/GE-2380.1

Freeland, W. J. (1983). Parasites and the coexistence of animal host species. *The American Naturalist*, 121(2), 223-236. https://doi.org/10.1086/284052

Gamito, S. (2010). Caution is needed when applying Margalef diversity index. *Ecological Indicators*, 10(2), 550-551.https://doi.org/10.1016/j.ecolind.2009.07.006

Goncharov, S. L., Soroka, N. M., Pashkevich, I. Y., Dubovyi, A. I., & Bondar, A. (2018). Infection of predatory fish with larvae of Eustrongylides excisus (Nematoda, Dioctophymatidae) in the Delta of the Dnipro River and the Dnipro-Buh Estuary in Southern Ukraine. http://doi.org.10.2478/vzoo-2018-0015

Hechinger, R. F., & Lafferty, K. D. (2005). Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. *Proceedings of the Royal Society B: Biological Sciences*, 272(1567), 1059-1066. https://doi.org/10.1098/rspb.2005.3070

Henriksen, E. H., Knudsen, R., Kristoffersen, R., Kuris, A. M., Lafferty, K. D., Siwertsson, A., & Amundsen, P. A. (2016). Ontogenetic dynamics of infection with Diphyllobothrium spp. cestodes in sympatric Arctic charr Salvelinus alpinus (L.) and brown trout Salmo trutta L. *Hydrobiologia*, 783(1), 37-46. https://doi.org/10.1007/s10750-015-2589-2

Holtan, H. (1988). *Undersøkelse av forurensningssituasjonen i Litlvatn, Agdenes kommune.* (NIVA Rapport; 2089). Norsk institutt for vannforskning. http://hdl.handle.net/11250/205082

Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, 80(3), 595-602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Johnson, P. T., Wood, C. L., Joseph, M. B., Preston, D. L., Haas, S. E., & Springer, Y. P. (2016). Habitat heterogeneity drives the host-diversity-begets-parasite-diversity relationship: evidence from experimental and field studies. *Ecology Letters*, 19(7), 752-761. https://doi.org/10.1111/ele.12609

Kamiya, T., O'Dwyer, K., Nakagawa, S., & Poulin, R. (2014). What determines species richness of parasitic organisms? A meta-analysis across animal, plant and fungal hosts. *Biological Reviews*, 89(1), 123-134. https://doi.org/10.1111/brv.12046

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. and Drummond, A. (2012). Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649. https://doi.org.10.1093/bioinformatics/bts199

Kennedy, C. R. (1975). The natural history of Slapton Ley Nature reserve. VIII. The parasites of fish, with special reference to their use as a source of information about the aquatic community. *Field studies*, 1975(4), 177-189.

Kennedy, C. R. (1978). An analysis of the metazoan parasitocoenoses of brown trout Salmo trutta from British lakes. *Journal of Fish Biology*, 13(2), 255-263. https://doi.org/10.1111/j.1095-8649.1978.tb03433.x

Kennedy, C.R. (1990) Helminth communities in freshwater fish: structured communities of stochastic assemblages? Esch, G.W., Bush, A. & Aho, J. (Ed.) *Parasite communities: Patterns and processes.* (p. 131-156). Chapman and Hall.

Kennedy, C. R., & Hartvigsen, R. A. (2000). Richness and diversity of intestinal metazoan communities in brown trout Salmo trutta compared to those of eels Anguilla anguilla in their European heartlands. *Parasitology*, 121(1), 55-64. http://doi.org.10.1017/S0031182099006046

Kleiber, C., Zeileis, A., & Zeileis, M. A. (2020). Package 'aer'. R package version, 12(4). https://CRAN.R-project.org/package=AER

Knudsen, R., Eloranta, A. P., Siwertsson, A., Paterson, R. A., Power, M., & Sandlund, O. T. (2019). Introduction of Mysis relicta (Mysida) reduces niche segregation between deep-water Arctic charr morphs. *Hydrobiologia*, 840(1), 245-260. https://doi.org/10.1007/s10750-019-3953-4

Knudsen, R., Kristoffersen, R., & Amundsen, P. A. (1997). Parasite communities in two sympatric morphs of Arctic charr, Salvelinus alpinus (L.), in northern Norway. *Canadian Journal of Zoology*, 75(12), 2003-2009. https://doi.org/10.1139/z97-833

Knudsen, R., Amundsen, P.A., Nilsen, R. & Klementsen, A. (2008). Food borne parasites as indicators of trophic segregation between Arctic charr and brown trout. *Environmental Biology of Fishes*, 83(107) https://doi.org/10.1007/s10641-007-9216-7

Knudsen, R., Siwertsson, A., Adams, C. E., Newton, J., & Amundsen, P. A. (2014). Similar patterns of individual niche use are revealed by different time-integrated trophic tracers (stable isotopes and parasites). *Ecology of Freshwater Fish*, 23(2), 259-268. https://doi.org/10.1111/eff.12074

Kortelainen, P., Rantakari, M., Huttunen, J. T., Mattsson, T., Alm, J., Juutinen, S., Larmola, T., Silvola, J. & Martikainen, P. J. (2006). Sediment respiration and lake trophic state are important predictors of large CO2 evasion from small boreal lakes. *Global Change Biology*, 12(8), 1554-1567. https://doi.org/10.1111/j.1365-2486.2006.01167.x

Kubíková, L., Simon, O., & Fricová, K. (2011). The occurrence of Pisidium species (Bivalvia: Sphaeriidae) in oligotrophic springs of the Blanice River catchment (Czech Republic) in relation to ecological conditions. *Biologia*, 66(2), 299-307. https://doi.org/10.2478/s11756-011-0012-7

Kuhn, J. A., Knudsen, R., Kristoffersen, R., & Amundsen, P. A. (2017). A simplified method to estimate Diphyllobothrium spp. infection in salmonids. *Journal of fish diseases*, 40(7), 863-871. https://doi.org/10.1111/jfd.12566

Kuhn, J. A., Frainer, A., Knudsen, R., Kristoffersen, R., & Amundsen, P. A. (2016). Effects of fish species composition on Diphyllobothrium spp. infections in brown trout–is three-spined stickleback a key species?. *Journal of Fish Diseases*, 39(11), 1313-1323. https://doi.org/10.1111/jfd.12467

Kusterle, S., Halttunen, E., Thorstad, E. B., Næsje, T. F., Jensen, J. L. A., Gallo-Bueno, A., Olague, E. & Rikardsen, A. H. (2013). The gill maggot Salmincola salmoneus as an indicator of repeat spawning in Atlantic salmon Salmo salar. *Journal of fish biology*, 82(3), 1068-1073. https://doi.org/10.1111/jfb.12040

Lafferty, K. D. (2008a). Ecosystem consequences of fish parasites. *Journal of fish Biology*, 73(9), 2083-2093. https://doi.org/10.1111/j.1095-8649.2008.02059.x

Lafferty, K. D., Allesina, S., Arim, M., Briggs, C. J., De Leo, G., Dobson, A. P., Dunne, J. A., Johnson, P. T. J., Kuris, A. M., Marcogliese, D. J., Martinez, N. D., Memmott, J., Marquet, P.A., McLaughlin, J.P., Mordecai, E. A., Pascual, M., Poulin, R. & Thieltges, D. W. (2008b). Parasites in food webs: the ultimate missing links. *Ecology letters*, 11(6), 533-546. https://doi.org/10.1111/j.1461-0248.2008.01174.x

Lassiere, O. L., & Crompton, D. W. T. (1988). Evidence for post-cyclic transmission in the life-history of Neoechinorhynchus rutili (Acanthocephala). *Parasitology*, 97(2), 339-343. https://doi.org.10.1017/S0031182000058534

Lassiere, O. L. (1989). The ecology of Neoechinorhynchus rutili (Acanthocephala) in Scottish freshwater lochs (Doctoral dissertation, University of Glasgow). http://theses.gla.ac.uk/id/eprint/2440

Littlewood, D. T. J., Curini-Galletti, M., & Herniou, E. A. (2000). The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Molecular Phylogenetics and Evolution*, *16*(3), 449-466. https://doi.org/10.1006/mpev.2000.0802

Locke, S. A., Bulte, G., Forbes, M. R., & Marcogliese, D. J. (2013). Estimating diet in individual pumpkinseed sunfish Lepomis gibbosus using stomach contents, stable isotopes and parasites. *Journal of Fish Biology*, 82(2), 522-537. https://doi.org/10.1111/j.1095-8649.2012.03497.x

Lockyer, A. E., Olson, P. D., & Littlewood, D. T. J. (2003). Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. *Biological Journal of the Linnean Society*, *78*(2), 155-171. https://doi.org/10.1046/j.1095-8312.2003.00141.x

MacArthur, R. H., & MacArthur, J. W. (1961). On bird species diversity. *Ecology*, 42(3), 594-598. https://doi.org/10.2307/1932254

Marcogliese, D. J. (2005). Parasites of the superorganism: are they indicators of ecosystem health?. *International journal for parasitology*, 35(7), 705-716. https://doi.org/10.1016/j.ijpara.2005.01.015

Marcogliese, D. J., & Cone, D. K. (1991). Importance of lake characteristics in structuring parasite communities of salmonids from insular Newfoundland. *Canadian Journal of Zoology*, 69(12), 2962-2967. https://doi.org/10.1139/z91-417

Magurran, A. E. (2003). An index of diversity. In Magurran, A. E. (Ed.) *Measuring biological diversity*. (p. 100- 130). John Wiley & Sons.

Margalef, R. (1958). Information theory in biology. *General Systems Yearbook*, 3, 36-71.

Meerhoff, M., Audet, J., Davidson, T. A., De Meester, L., Hilt, S., Kosten, S., Liu, Z., Mazzea, N., Paerl, H., Scheffer, M. & Jeppesen, E. (2022). Feedback between climate change and eutrophication: revisiting the allied attack concept and how to strike back. *Inland Waters*, 1-18. https://doi.org/10.1080/20442041.2022.2029317

Menconi, V., Riina, M. V., Pastorino, P., Mugetti, D., Canola, S., Pizzul, E., Bona, M.C., Dondo, A., Acutis, P.L., & Prearo, M. (2020). First occurrence of Eustrongylides spp.(Nematoda: Dioctophymatidae) in a subalpine lake in Northwest Italy: new data on distribution and host range. *International journal of environmental research and public health*, 17(11), 4171. https://doi.org/10.3390/ijerph17114171

Miljødirektoratet, Lakseregisteret: Fremstadelva. Retrieved June 11 2022 from: https://lakseregisteret.fylkesmannen.no/visElv.aspx?vassdrag=Fremstadelva&id=119.9Z

Molloy, S., Holland, C., & Poole, R. (1995). Metazoan parasite community structure in brown trout from two lakes in western Ireland. *Journal of Helminthology*, 69(3), 237-242. https://doi.org.10.1017/S0022149X0001419X

Moravec, F. (2004). *Metazoan Parasites of Salmonid Fishes of Europe*. Academia Praha.

Nazari-Sharabian, M., Ahmad, S., & Karakouzian, M. (2018). Climate change and eutrophication: a short review. *Engineering, Technology and Applied Science Research*, 8(6), 3668. https://digitalscholarship.unlv.edu/fac_articles/562

Norges vassdrags og energidirektorat NVE Atlas. Retrieved June 11 2022 from https://atlas.nve.no/Html5Viewer/index.html?viewer=nveatlas#

Oczkowski, A., Markham, E., Hanson, A., & Wigand, C. (2014). Carbon stable isotopes as indicators of coastal eutrophication. Ecological Applications, 24(3), 457-466. https://doi.org/10.1890/13-0365.1

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., ... & Oksanen, M. J. (2013). Package 'vegan'. Community ecology package, version, 2(9), 1-295. https://CRAN.R-project.org/package=vegan

Olson, P. D., Cribb, T. H., Tkach, V. V., Bray, R. A., & Littlewood, D. T. J. (2003). Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International journal for parasitology*, 33(7), 733-755. https://doi.org/10.1016/S0020-7519(03)00049-3

Palm, H. W. (2011). Fish parasites as biological indicators in a changing world: can we monitor environmental impact and climate change?. In: Mehlhorn, H. (Ed.) Progress in Parasitology. *Parasitology Research Monographs*, vol 2. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-21396-0_12

Paterson, R. A., Knudsen, R., Blasco-Costa, I., Dunn, A. M., Hytterød, S., & Hansen, H. (2019a). Determinants of parasite distribution in Arctic charr populations: catchment structure versus dispersal potential. *Journal of Helminthology*, 93(5), 559-566. https://doi.org.10.1017/S0022149X18000482

Paterson, R. A., Nefjodova, J., Salis, R. K., & Knudsen, R. (2019b). Exploring trophic niches and parasite communities of sympatric Arctic charr and brown trout populations of southern Norway. *Hydrobiologia*, 840(1), 271-280. https://doi.org/10.1007/s10750-019-3956-1

Paterson, R. A., Berntsen, H. H., Næsje, T. F., Berg, M., & Finstad, B. (2021). Factors influencing return rate and marine residence duration in sea trout populations in Central Norway. *Journal of Fish Biology*, 99(3), 875-887. https://doi.org/10.1111/jfb.14770

Petkevičiūtė, R., Kudlai, O., Stunžėnas, V., & Stanevičiūtė, G. (2015). Molecular and karyological identification and morphological description of cystocercous cercariae of Phyllodistomum umblae and Phyllodistomum folium (Digenea, Gorgoderidae) developing in European sphaeriid bivalves. *Parasitology international*, 64(5), 441-447. https://doi.org/10.1016/j.parint.2015.06.007

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83(3), 703-718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Poulin, R. (2007). Are there general laws in parasite ecology?. *Parasitology*, 134(6), 763-776. https://doi.org/10.1017/S0031182006002150

Poulin, R. (1996). Measuring parasite aggregation: defending the index of discrepancy. *International Journal for Parasitology*, 26(2), 227-229. https://doi.org/10.1016/0020-7519(95)00117-4

Poulin, R., Closs, G. P., Lill, A. W., Hicks, A. S., Herrmann, K. K., & Kelly, D. W. (2012). Migration as an escape from parasitism in New Zealand galaxiid fishes. *Oecologia*, 169(4), 955-963. https://doi.org/10.1007/s00442-012-2251-x

Poulin, R., & Morand, S. (1999). Geographical distances and the similarity among parasite communities of conspecific host populations. *Parasitology*, 119(4), 369-374. https://doi.org,10.1017/S0031182099004795

Poulin, R., & Morand, S. (2000). The diversity of parasites. *The quarterly review of biology*, 75(3), 277-293.

Prati, S., Henriksen, E. H., Knudsen, R., & Amundsen, P. A. (2020). Impacts of ontogenetic dietary shifts on the food-transmitted intestinal parasite communities of two lake salmonids. *International Journal for Parasitology: Parasites and Wildlife*, 12, 155-164. https://doi.org/10.1016/j.ijppaw.2020.06.002

Raeymaekers, J. A. M., Maes, G. E., Audenaert, E., & Volckaert, F. A. M. (2005). Detecting Holocene divergence in the anadromous–freshwater three-spined stickleback (Gasterosteus aculeatus) system. *Molecular ecology*, 14(4), 1001-1014. https://doi.org/10.1111/j.1365-294X.2005.02456.x

Rochat, E., Brodersen, J., & Blasco-Costa, I. (2021). Conspecific migration and environmental setting determine parasite infracommunities of non-migratory individual fish. *Parasitology*, 148(9), 1057-1066. https://doi.org/10.1017/S0031182021000780

Rosenzweig, M. (1995). Coevolution of habitat diversity and species diversity. In *Species Diversity in Space and Time* (pp. 151-189). Cambridge: Cambridge University Press. https://doi:10.1017/CBO9780511623387.009

Rusconi, A., Prati, P., Bragoni, R., Castelli, M., Postiglione, U., Rigamonti, S., ... & Olivieri, E. (2022). Occurrence of Eustrongylides excisus (Nematoda: Dioctophymatidae) in European Perch (Perca fluviatilis) and Great Cormorant (Phalacrocorax carbo) in Lake Annone, Northern Italy. *The Journal of Parasitology*, 108(2), 209-216. https://doi.org/10.1645/20-175

Sattari, M. (2004). The occurrence and intensity of Eustrongylides excisus (Nematoda: Dioctophymidae) in some bony fish species of the Caspian Sea and its basin. *Caspian Journal of Environmental Sciences*, 2(1), 9-12. https://doi.org.10.22124/CJES.2004.3830

Schoener, T. W. (1970). Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology*, 51(3), 408-418. https://doi.org/10.2307/1935376

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, 52(3/4), 591-611. https://doi.org/10.2307/2333709

Schulting, R. J. (1998). Slighting the sea: stable isotope evidence for the transition to farming in northwestern Europe. *Documenta Praehistorica*, 25(203), 18.

Siwertsson, A., Refsnes, B., Frainer, A., Amundsen, PA., Knudsen, R. (2016). Divergence and parallelism of parasite infections in Arctic charr morphs from deep and shallow lake habitats. *Hydrobiologia*, 783, 131–143 https://doi.org/10.1007/s10750-015-2563-z

Spalding, R. F., & Exner, M. E. (1993). Occurrence of nitrate in groundwater—a review. *Journal of environmental quality*, 22(3), 392-402. https://doi.org/10.2134/jeq1993.00472425002200030002x

Spalding, M. G., & Forrester, D. J. (1993). Pathogenesis of Eustrongylides ignotus (Nematoda: Dioctophymatoidea) in ciconiiformes. *Journal of Wildlife Diseases*, 29(2), 250-260. https://doi.org/10.7589/0090-3558-29.2.250

Suthar, J., Unger, P. & Palm, H.W. (2022). Fish Parasite Community of Three Lakes with Different Trophic Status in Mecklenburg-Western Pomerania, Germany. *Acta Parasit*. 67, 340–350. https://doi.org/10.1007/s11686-021-00465-6

Timi, J. T., & Poulin, R. (2020). Why ignoring parasites in fish ecology is a mistake. *International Journal for Parasitology*, 50(10-11), 755-761. https://doi.org/10.1016/j.ijpara.2020.04.007

Ulsund, C. (2013). *Forvaltningsplan for Litlvatnet naturreservat i Agdenes kommune.* (Trønderlag Rapport 1-2013). Fylkesmannen i Sør-Trønderlag.

Vainutis, K. S., Voronova, A. N., & Urabe, M. (2021). Systematics of Crepidostomum species from the Russian Far East and northern Japan, with description of a new species and validation of the genus Stephanophiala. *Parasitology International*, 84, 102412. https://doi.org/10.1016/j.parint.2021.102412

Valtonen, E. T., Holmes, J. C., & Koskivaara, M. (1997). Eutrophication, pollution and fragmentation: effects on parasite communities in roach (Rutilus rutilus) and perch (Perca fluviatilis) in four lakes in central Finland. *Canadian Journal of Fisheries and Aquatic Sciences*, 54(3), 572-585. https://doi.org/10.1139/f96-306

Waeschenbach, A., Brabec, J., Scholz, T., Littlewood, D. T. J., & Kuchta, R. (2017). The catholic taste of broad tapeworms–multiple routes to human infection. *International Journal for Parasitology*, 47(13), 831-843.https://doi.org/10.1016/j.ijpara.2017.06.004

Werle, E., Schneider, C., Renner, M., Volker, M. and Fiehn, W. (1994). Convenient single–step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Research*, 22, 4354–4355. https://doi.org.10.1093/nar/22.20.4354.

Whitlock, M., & Schluter, D. (2015). *The analysis of biological data* (p. 768). Roberts Publishers.

Wickham, H., Chang, W., & Wickham, M. H. (2016). Package 'ggplot2'. *Create elegant data visualisations using the grammar of graphics*. Version, 2(1), 1-189. https://github.com/tidyverse/ggplot2

Winnem, A.M. (2010). *Tilstandsrapport for Litlvatnet i Agdenes kommune med forslag til restaureringstiltak*. (NOF Rapport 1-2010) Norsk Ornitologisk Forening.

Wisniewski, W.L., (1958). Characterization of the parasitofauna of an eutrophic lake.(Parasitofauna of the biocoenosis of Druzno Lake-part I). *Acta Parasitologica Polonica*, 6(1/7). 63-64.

Woolway, R. I., Kraemer, B. M., Lenters, J. D., Merchant, C. J., O'Reilly, C. M., & Sharma, S. (2020). Global lake responses to climate change. *Nature Reviews Earth & Environment*, 1(8), 388-403. https://doi.org/10.1038/s43017-020-0067-5

Xenopoulos, M. A., Lodge, D. M., Frentress, J., Kreps, T. A., Bridgham, S. D., Grossman, E., & Jackson, C. J. (2003). Regional comparisons of watershed determinants of dissolved organic carbon in temperate lakes from the Upper Great Lakes region and selected regions globally. *Limnology and Oceanography*, 48(6), 2321-2334. https://doi.org/10.4319/lo.2003.48.6.2321

Appendix A – supplementary tables and figures

Table S 1 - Influence of lake and fish length on the parasite taxa richness (S), total parasite abundance (N), diversity (Margalef's diversity index - DMg), evenness (Berger Parker evenness index - BP) and total abundance of autogenic (N_{AU}) and allogenic (N_{AL}) taxa of brown trout in Fremstadvassdraget, Trønderlag, Norway. Intercept is Litlvatnet, all models are simple (additive). Phyllodistomum is integrated as an autogenic parasite.

Index	Parameter	Contrast	SE	t-value	P [a]
Taxon richness	Intercept	-3.38	2.18	-1.56	0.125
	Storvatnet	-0.21	0.386	-0.550	0.584
	Fork length	0.0328	0.00800	4.10	**<0.001**
Total abundance	**Intercept**	-3.65 (-2.08)	1.65 (1.32)	-2.21 (-1.58)	**0.0313** 0.120
	Storvatnet	0.295 (0.0641)	0.234 (0.195)	1.26 (0.329)	0.212 0.744
	Fork length	0.0277 (0.0221)	0.00577 (0.00463)	4.79 (4.77)	**<0.001** **(<0.001)**
Diversity	**Intercept**	0.80 (0.724)	0.532 (0.540)	1.500 (1.340)	0.139 (0.186)
	Storvatnet	0.0055 (0.0155)	0.0942 (0.0952)	0.059 (0.163)	0.954 (0.871)
	Fork length	0.00145 (0.00172)	0.00196 (0.00199)	0.741 (0.867)	0.462 (0.390)
Evenness	**Intercept**	0.537 (0.581)	0.238 (0.240)	2.26 (2.42)	**0.028** **(0.0188)**
	Storvatnet	0.00965 (0.00368)	0.0421 (0.0423)	0.229 (0.087)	0.820 (0.931)
	Fork length	1.63×10^{-4} (-6.232×10^{-9})	8.73×10^{-4} (8.83×10^{-4})	0.187 (0.000)	0.853 (1.000)
Total abundance autogenic taxa	Intercept	-0.619 (-0.0244)	1.71 (1.70)	-0.363 (-0.014)	0.718 (0.989)
	Storvatnet	-0.0267 (-0.119)	0.278 (0.282)	-0.096 (-0.421)	0.924 (0.675)
	Fork length	0.0120 (0.00985)	0.00612 (0.00614)	1.96 (1.61)	0.0567 (0.114)
Total abundance allogenic taxa	**Intercept**	-5.38 (-3.55)	2.04 (1.71)	-2.64 (-2.08)	**0.0105** **(0.0426)**
	Storvatnet	0.354 (0.0852)	0.274 (0.244)	1.29 (0.349)	0.202 (0.728)
	Fork length	0.0327 (0.0263)	0.00706 (0.0060)	4.62 (4.38)	**<0.001** **(<0.001)**

Numbers in parentheses are with one highly infected individual removed.
a – p-value > 0.05 = significant. Marked in bold.

*Table S 2- Influence from lake and fish length on **prevalence** of parasite taxa in brown trout in Fremstadvassdraget, Trønderlag, Norway.*

Index	Model type [a]	Parameter	Contrast	SE	t-value	P [b]
Trematoda						
Crepidostomum sp.	Simple	Intercept	-2.81	3.33	-0.843	0.399
		Storvatnet	1.05	0.624	1.68	0.093
		Fork length	0.013	0.0123	1.01	0.313
Diplostomum sp.		Intercept	12.2	5108	0.002	0.998
		Storvatnet	-18.2	5108	-0.004	0.997
		Fork length	0.0358	0.0380	0.942	0.346
Phyllodistomum sp.		Intercept	-2.31	3.87	-0.596	0.551
		Storvatnet	3.05	0.823	3.71	**<0.001**
		Fork length	-0.00124	0.0141	-0.0870	0.930
Cestoda						
Dibothriocephalus spp.*	Complex	**Intercept**	-18.5	8.65	-2.14	**0.0321**
		Storvatnet	16.8	9.88	1.70	0.0892
		Fork length	0.0773	0.0348	2.23	**0.0260**
		Storvatnet: fork length	-0-067	0.0390	-1.73	0.0846
Eubothrium crassum	Simple	Intercept	0.470	3.127	-0.150	0.880
		Storvatnet	0.457	0.561	0.813	0.416
		Fork length	0.00377	0.0115	0.327	0.774
Nematoda						
Eustrongylides sp.	Simple	**Intercept**	-14.1	4.28	-3.30	**<0.001**
		Storvatnet	-2.30	0.746	-3.09	**<0.01**
		Fork length	0.0569	0.0165	3.44	**<0.001**
Nematode sp. 1	Complex	Intercept	-5.33	6.16	0.866	0.387
		Storvatnet	-19.3	9.16	-2.11	**0.0346**
		Fork length	-0.0273	0.0237	-1.15	0.251
		Storvatnet: fork length	0.0742	0.0338	2.20	**0.0281**
Eustrongylides +sp. 1	Simple	Intercept	5.34	6.16	0.866	0.387
		Storvatnet	-19.34	9.16	-2.11	**0.0346**
		Fork length	-0.0273	0.0238	-1.15	0.251
		Storvatnet: fork length	0.074	0.0338	2.20	**0.0281**
Pseudocapillaria sp.	Simple	**Intercept**	-14.4	4.38	-3.29	**<0.001**
		Storvatnet	-1.24	0.658	-1.91	0.0568
		Fork length	0.0520	0.0159	3.28	**<0.01**
Myxozoa		Intercept	-6.27	3.28	-1.91	0.0561
		Storvatnet	-1.10	0.0564	-1.94	0.0520
		Fork length	0.0243	0.0122	2.00	**0.0455**

** - Numbers in parentheses are with one highly infected individual removed.*
a – Simple model type refers to additive model. Complex model refers to interaction between fixed factors.
b – p-value > 0.05 = significant. Marked in bold.

*Table S 3 - Influence from lake and fish length on **abundance** of parasite taxa in brown trout in Fremstadvassdraget, Trønderlag, Norway.*

Index	Model type [a]	Parameter	Contrast	SE	t-value	P [b]
Trematoda						
Crepidostomum sp..	Simple	Intercept	-2.05	2.44	-0.843	0.403
		Storvatnet	-0.654	0.408	-1.60	0.115
		Fork length	0.0162	0.00870	1.86	0.0685
Diplostomum sp.	Simple	Intercept	-1.20	1.29	-0.935	0.354
		Storvatnet	-0.498	0.211	-2.37	**0.0214**
		Fork length	0.0158	0.0459	3.45	**<0.01**
Phyllodistomum sp.	Simple	Intercept	-1.31	3.25	-0.405	0.682
		Storvatnet	2.89	1.17	2.46	**0.0169**
		Fork length	2.92×10^{-5}	0.0112	-0.003	0.998
Cestoda						
Dibothriocephalus spp. *	Simple	**Intercept**	-10.6	3.13	-3.38	**<0.01**
			(-7.73)	(2.771)	(-2.79)	**(<0.01)**
		Storvatnet	1.03	0.404	2.54	**0.0138**
			(0.679)	(0.369)	(1.83)	(0.0708)
		Fork length	0.0472	0.0107	4.42	**<0.001**
			(0.0374)	(0.00954)	(3.91)	**(<0.001)**
Eubothrium crassum	Simple	Intercept	-2.21	2.23	-0.990	0.326
		Storvatnet	0.515	0.378	1.36	0.178
		Fork length	0.00950	0.00802	1.18	0.241
Nematoda						
Eustrongylides sp.	Complex	Intercept	-4.16	2.87	-1.45	0.153
		Storvatnet	-12.9	6.16	-2.09	**0.0407**
		Fork length	0.0190	0.0102	1.87	0.0674
		Storvatnet: fork length	0.0435	0.0211	2.06	**0.0439**
Nematode sp. 1	Simple	**Intercept**	-13.13	5.33	-2.46	**0.0168**
		Storvatnet	2.48	1.202	2.06	**0.0436**
		Fork length	0.0410	0.0180	2.28	**0.0262**
Eustrongylides sp. +sp. 1	Simple	Intercept	-3.65	3.85	-0.944	0.349
		Storvatnet	-10.0	6.15	-1.63	0.109
		Fork length	0.0174	0.0137	1.27	0.211
		Storvatnet: fork length	0.0362	0.0214	1.69	0.096
Pseudocapillaria sp.	Complex	Intercept	-4.02	4.05	-0.994	0.325
		Storvatnet	-14.6	9.52	-1.53	0.131
		Fork length	0.0154	0.0145	1.07	0.291
		Storvatnet: fork length	0.0489	0.0325	1.504	0.138

** - Numbers in parentheses are with one highly infected individual removed.*
a – simple model type refers to additive model. Complex model refers to interaction between fixed factors.
b – p-value > 0.05 = significant. Marked in bold.

Table S 4 – Mean intensity ± standard error for each parasite taxa with count data from Fremstadvassdraget, Trønderlag, Norway

Lake		Litlvatnet	Storvatnet
Trematoda	*Apatemon* sp.	1 ± 0	2.7 ± 0.7
	Crepidostomum brinkmanni	17.1 ± 4.8	6.9 ± 1.7
	Diplostomum sp.	24.9 ± 3.4	15.5 ± 2.4
	Phyllodistomum umblae	4.0 ± 1	8.0 ± 2.4
	Tylodelphys sp.	2.0 ± 0	2.0 ± 0
Cestoda	*Dibothriocephalus* spp.	19.3 ± 4.2	60.5 ± 23.4 (41.6 ± 14.3)
	Eubothrium crassum	2.3 ± 0.4	2.4 ± 0.9
	Proteocephalus longicolllis	1 ± 0	0
Acanthocephala	*Neoechynorhynchus* sp.	1.5 ± 0.5	0
Nematoda	*Eustrongylides* sp.	4.2 ± 0.8	6.4 ± 2.2
	Nematode sp. 1[b]	1.5 ± 0.5	9.3 ± 3.7
	Eustrongylides + sp1 [b]	4.1 ± 0.7	12.5 ± 4.4
	Pseudocapillaria sp.	2.9 ± 0.5	3.4 ± 2.3
	Nematode sp. 2[b]	0	5.0 ± 0
	Nematode sp. 3[b]	2.0 ± 0	0
Crustaceans	*Argulus coregoni*	2.3 ± 0.5	0
	Salmincola sp.	1.0 ± 0	1.0 ± 0

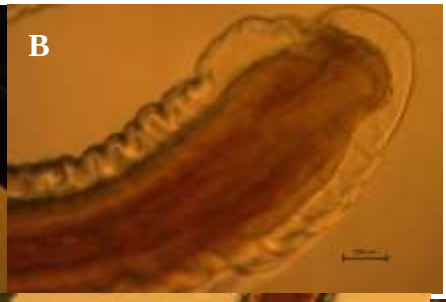
Appendix B – morphology nematodes

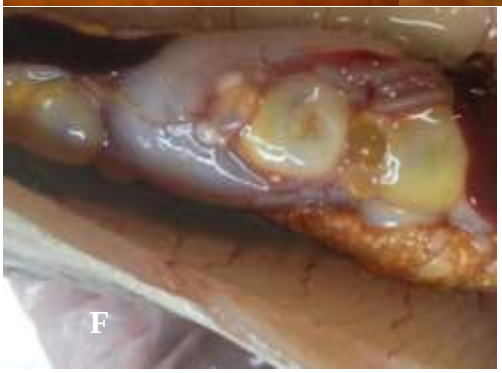
***Eustrongylides* sp. larvae**
(Jägerskiöld, 1909)

FEATURES:
Site of infection: encysted or free in body cavity, stomach and encysted in/on organ walls.
Length: > 15-60 mm
Surface: ribbed
Colour: red, occasionally brown
Anterior end: 1-2 circles of papillae. Alae around head, narrow then broader
Posterior end: Possible male (B) bulky alae around posterior part. Possibly female (D) narrow alae around posterior part. Rounded tail
Cysts: yellowich, often with red lines looking like blood vessels

Notations:
Scalebars for picture D and E are wrong.

PICTURES:



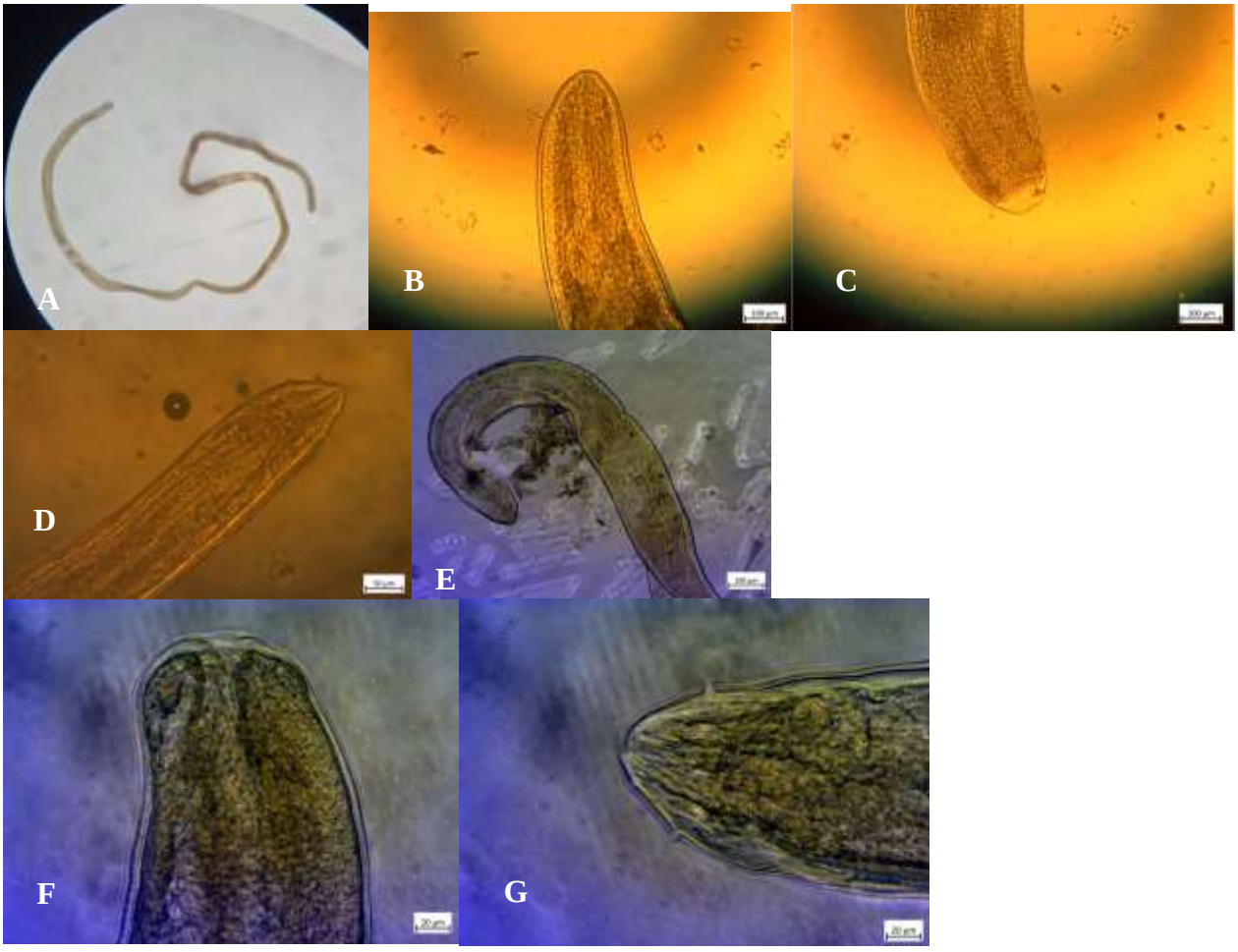


Nematode sp. 1
(Unknown)

FEATURES:
Site of infection: free or occasionally encysted in stomach and/or intestine
Length: 5-15 mm in intestine, 20-25 mm in stomach
Surface: Smooth
Colour: light red, pink, or brown
Anterior end: visible papillae for some specimen (picture C and F, most not visible
Posterior end: tail rounded, with narrow alae.

Notations:
May be young *Eustrongylides* sp. larvae that have not yet emerged from the digestive system. The correlation between *Eustrongylides* sp and sp1 in CCorA biplot also supports this. In that case younger individuals seems to be more abundant in Storvatnet at this time of year, whilst older in Litlvatnet. The intestine nematodes seems to have slightly different characteristics than stomach nematodes with visible anterior papilla on more of the intestine specimen (picture D and G).

PICTURES:
From intestine:

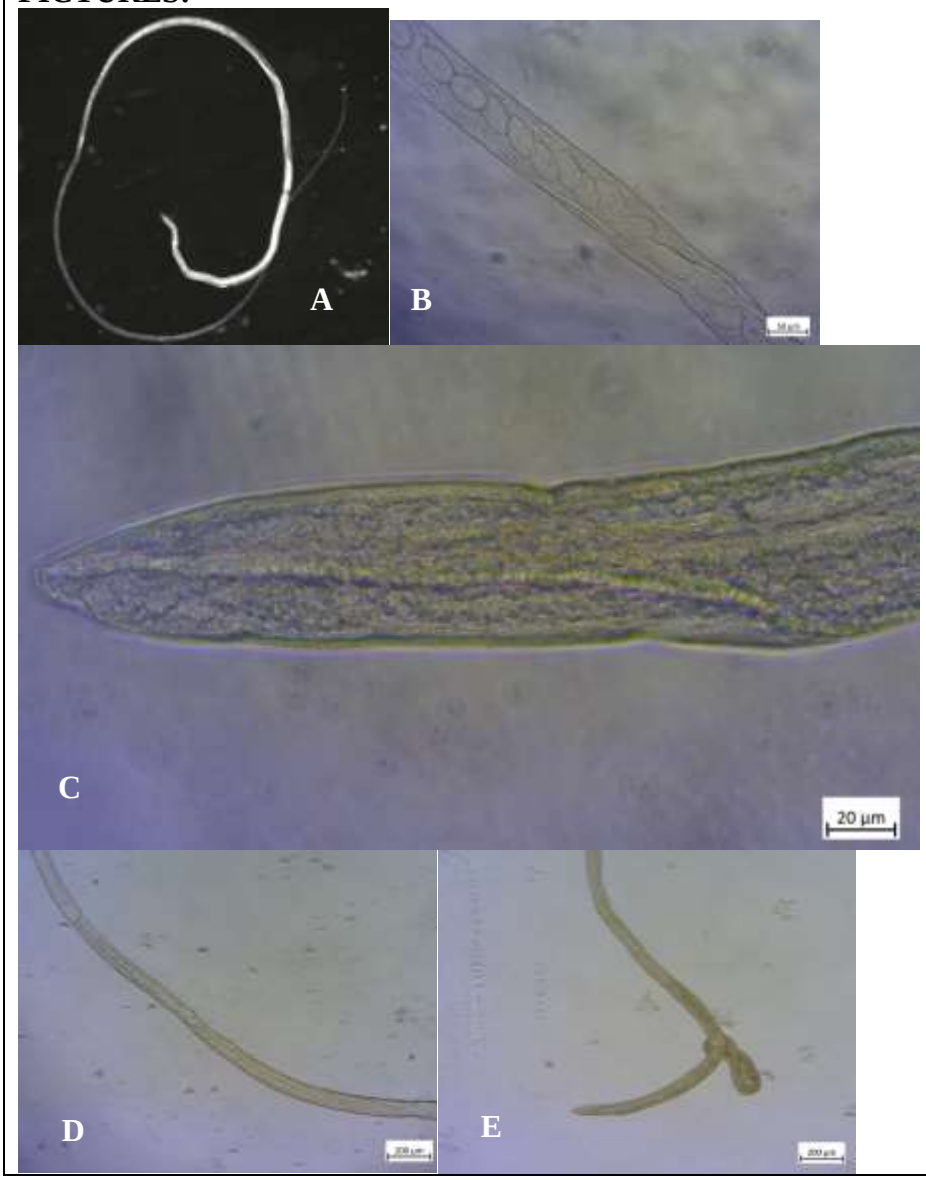


From stomach:

***Pseudocapillaria* sp.**
(Freitas, 1959)

FEATURES:
Site of infection: free in intestine and/or stomach
Length: 6.5-15 mm
Max width: 0.06 mm
Surface: Smooth
Colour: see through
Body: thicker anterior end, narrower in posterior end
Eggs: ca. 0.050 x 0.025 mm

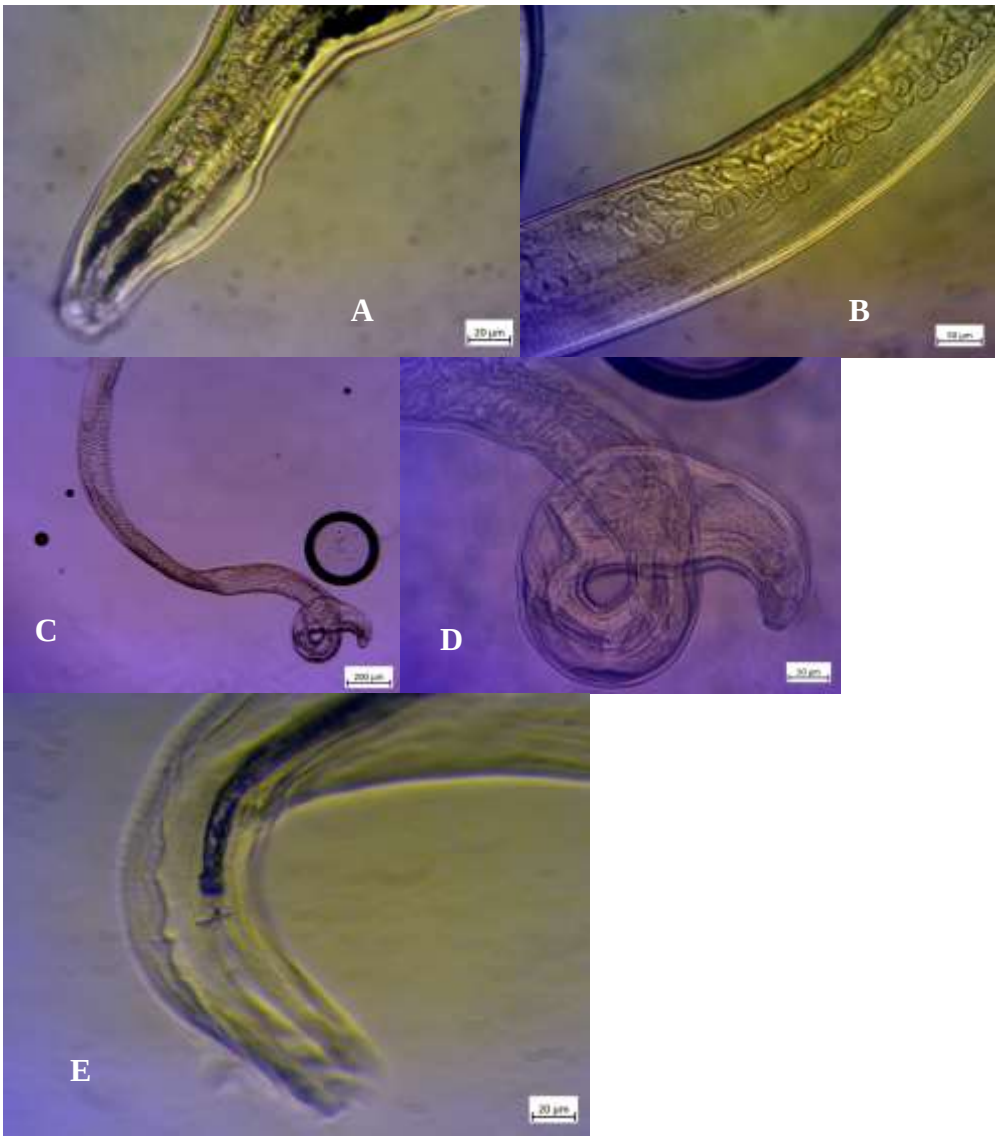
PICTURES:



Nematode sp. 2
(Unknown, adult)

FEATURES:
Site of infection: free in stomach
Length: 5-10 mm
Max width:
Surface: smooth
Colour: mostly see through
Antherior end: rounded
Posterior end: rounded
Eggs: ca. 0.028 x 0.015 mm

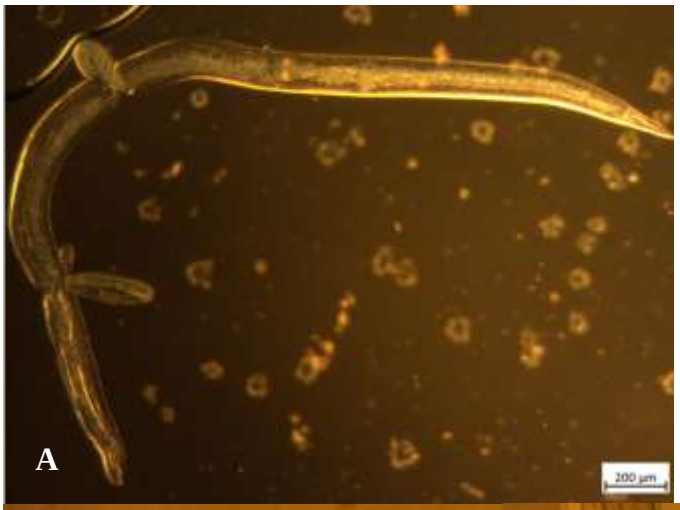
PICTURES:

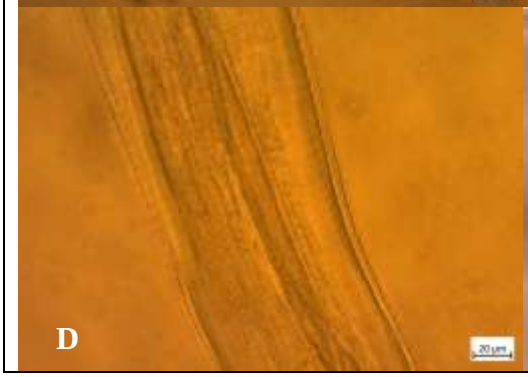


Nematode sp. 3
(Unknown)

FEATURES:
Site of infection: free in intestine
Length: 2.5 mm
Max width: 0.11 mm
Surface: slightly ribbed
Colour: see through
Antherior end: pointy. Well developed lips.
Posterior end: pointy

PICTURES:





Appendix C – codes

Calculation of indices

###Make columns and calculate mean and SE S, N, DMg and BP, NAL and NAU ((also calculated without outlier for those relevant)

```
Fremstadparasitter$S <- apply(Fremstadparasitter[,7:19, 21:26]!=0, 1, sum)
aggregate(Fremstadparasitter$S, list(Fremstadparasitter$Lake),FUN = function(x) c(mean = mean(x), se = std.error(x)))

Fremstadparasitter$N <- apply(Fremstadparasitter[,7:19, 21:25], 1, sum)
aggregate(Fremstadparasitter$N, list(Fremstadparasitter$Lake),FUN = function(x) c(mean = mean(x), se = std.error(x)))

Fremstadparasitter$DMg <- (Fremstadparasitter$S - 1) / (log(Fremstadparasitter$N))
aggregate(Fremstadparasitter$DMg, list(Fremstadparasitter$Lake),FUN = function(x) c(mean = mean(x), se = std.error(x)))

Fremstadparasitter$BP <- (Fremstadparasitter$Nmax / Fremstadparasitter$N)
aggregate(Fremstadparasitter$BP, list(Fremstadparasitter$Lake),FUN = function(x) c(mean = mean(x), se = std.error(x)))

AU <- subset(Fremstadparasitter, select=c(Fish_ID, Lake, Gaffel_lengde_mm, Vekt_g, argulus, salmincola, phyllo, crepidostomum, proteo, Pseudocapillaria, tot_e_crassum, tot_acantho, Nematode_adult_stomach_sp2,Amount, d13C, d15N, percent_C, percent_N, CN_ratio))

AL <- subset(Fremstadparasitter, select=c(Fish_ID, Lake, Gaffel_lengde_mm, Vekt_g, apatemon, Eustrongilydes,diplo_abundance, total_tylo, total_diboth, Amount, d13C, d15N, percent_C, percent_N, CN_ratio))

AU$N <- apply(AU[5:13], 1, sum)                AL$N <- apply(AL[5:9], 1, sum)

aggregate(AU$N, list(AU$Lake),FUN = function(x) c(mean = mean(x), se = std.error(x)))

aggregate(AU_withoutST21_017$AU_N_2, list(AU_withoutST21_017$AU_lake_2), FUN=function(x) c(mean = mean(x), se = std.error(x))

aggregate(AL$N, list(AL$Lake),FUN = function(x) c(mean = mean(x), se = std.error(x)))

aggregate(AL_withoutST21_017$AL_N_2, list(AL_withoutST21_017$AL_lake_2), FUN=function(x) c(mean = mean(x), se = std.error(x)))
```

Generalized linear models for indices

```
#PACKAGES NEEDED
library(AER) #GLM and dispersiontest

#TAXON RICHNESS
hist(Fremstadparasitter$S, breaks=10) ###### looks gaussian distributed, checking with test:
shapiro.test(Fremstadparasitter$S) #hypotesis of normality rejected (p-value=0,006), probably because the values are integer. Sticking to default setting of gaussian anyways.
S_glm_interactive  <- glm(S ~ Lake * Gaffel_lengde_mm, data = Fremstadparasitter)
S_glm_addative <- glm(S ~ Lake + Gaffel_lengde_mm, data = Fremstadparasitter)
anova(S_glm_interactive, S_glm_addative, test="F") #p-value>0,05 -> addative model best
summary(S_glm_addative)
anova(S_glm_interactive,  test="F")

#TOTAL ABUNDANCE (same procedure, NAL and NAU with and without outlier)
hist(Fremstadparasitter$N, breaks=60)  #looks poisson, so started with poison family. After the dispersiontest showed overdispersed so family was changed to quasipoisson.
N_glm_interactive <- glm(N ~ Lake * Gaffel_lengde_mm, data = Fremstadparasitter, family = quasipoisson)
N_glm_addative <- glm(N ~ Lake + Gaffel_lengde_mm, data = Fremstadparasitter, family = quasipoisson)
dispersiontest(N_glm_interactive)
#dispersion >1, overdispersed --> changing family in glm from poisson to quasipoisson and use F-test. If you want to run this test you need to change it back to poisson above first
anova(N_glm_interactive, N_glm_addative, test="F") #p-value > 0,05 - addative model best
summary(N_glm_addative)

#MARGALEF (DMg, same procedure for BP with and without outlie)
hist(Fremstadparasitter$DMg, breaks=60) #Gaussian, shapiro test to check
shapiro.test(Fremstadparasitter$DMg)  #p-value > 0,05, sticking to default
DMg_glm_interactive  <- glm(DMg ~ Lake * Gaffel_lengde_mm, data = Fremstadparasitter)
DMg_glm_addative <- glm(DMg ~ Lake + Gaffel_lengde_mm, data = Fremstadparasitter)
anova(DMg_glm_interactive, DMg_glm_addative, test="F")#p > 0,05 -> addative best
summary(DMg_glm_addative)
```

Generalized linear models for prevalence and abundance

```
########################PREVALENCE ###################################
#MYXO (same procedure for all taxa with additive model  as best fit)
#Make indicator variable/dummy variable like smoking, yes or no
Fremstadparasitter$crepido_dummy <- ifelse(Fremstadparasitter$crepidostomum==0, 0, 1)
glm_myxo_binom_add <- glm(myxo_dummy ~ Lake + Gaffel_lengde_mm, data=Fremstadparasitter, family="binomial")
```

```
glm_myxo_binom_int <- glm(myxo_dummy ~ Lake *Gaffel_lengde_mm,
data=Fremstadparasitter, family="binomial")
#addative or interactive?
anova(glm_myxo_binom_add, glm_myxo_binom_int, test="Chi") #p  > 0,05 -> addative
summary(glm_myxo_binom_add)

#DIBOTHRIOCEPHALUS SPP (example where interactive was best fit)
#first i have to make dummy data instead of count data (present or not present)
Fremstadparasitter$diboth_dummy <- ifelse(Fremstadparasitter$total_diboth==0, 0, 1)
glm_diboth_dummy_add <- glm(diboth_dummy ~ Lake + Gaffel_lengde_mm,
data=Fremstadparasitter, family="binomial")
glm_diboth_dummy_int <- glm(diboth_dummy ~ Lake * Gaffel_lengde_mm,
data=Fremstadparasitter, family="binomial")
#addative or interactive?
anova(glm_diboth_dummy_add, glm_diboth_dummy_int, test="Chi")
#p-value < 0,05 --> interactive best fit???
summary(glm_diboth_dummy_int)
anova(glm_diboth_dummy_int, test="Chi")
#no sign in prevalence between lakes. Fish size has an impact on prevalence and there is an
intercept between lake and fork lenght.

#########################ABUNDANCE#####################################
#CREPIDOSTOMUM (same procedure for all where addative is the best fit. IP < 0.05
interactive would be the best fit, but still same procedure)
hist(Fremstadparasitter$crepidostomum, breaks=20)
#Poisson dispersion test: H0=data are poisson dist, Ha not poisson
crepido_glm_interactive <- glm(crepidostomum ~ Lake * Gaffel_lengde_mm, data =
Fremstadparasitter, family = quasipoisson)
crepido_glm_addative <- glm(crepidostomum ~ Lake + Gaffel_lengde_mm, data =
Fremstadparasitter, family = quasipoisson)
dispersiontest(crepido_glm_interactive)
#disperison value --> overdispersed --> changing family to quasipoisson and use F-test
#is interactive or addative best?
anova(crepido_glm_interactive, crepido_glm_addative, test="F") #p> 0,05 --> addative
summary(crepido_glm_addative)
```

Stable isotope analysis

```
#MANN WHITNEY U TEST TO CHECK FOR DIFFERENCE BETWEEN LAKES
wilcox.test(Fremstadparasitter$d13C ~ Fremstadparasitter$Lake)
#p-value << 0.05 --> null hypotesis rejected
wilcox.test(Fremstadparasitter$d15N ~ Fremstadparasitter$Lake)
#p-value << 0.05 --> null hypotesis rejected,

#KRUSCALL WALLIS TO CHECK RELATIONSHIP WITH FISHLENGTH
kruskal.test(d13C ~ Gaffel_lengde_mm, data=Fremstadparasitter)
kruskal.test(d15N ~ Gaffel_lengde_mm, data=Fremstadparasitter)

#PACKAGES NEEDED
library(SIBER) #to calculate overlap etc, instead of nicherover
library(ggforce) #to make ellipses with ggplot
library(ggplot2)

#Make subset. Needs to be organized: "iso1", iso2", "group", "community" with those names and charactes needs to be numbers as factors
siber_group <- subset(Fremstadparasitter, select=c(d13C, d15N, Lake, Art), header=TRUE)
siber_group$iso1 <- siber_group$d13C
siber_group$iso2 <- siber_group$d15N
siber_group$group <- as.character(siber_group$Lake)
siber_group$community <- as.character(siber_group$Art)
siber_group_2 <- subset(siber_group, select=c(iso1, iso2, group, community), header=TRUE)
siber_group_2$group <- ifelse(siber_group_2$group=="Litjvatnet", 1, 2)
siber_group_2$community <- ifelse(siber_group$community=="Trout", 1, 2)
siber_group_2$group <- as.factor(siber_group_2$group)
siber_group_2$community <- as.factor(siber_group_2$community)
siber_group_2 <- as.data.frame(siber_group_2)

#make simper object
siber.g.example <- createSiberObject(siber_group_2)

#calculation summary statistics for each group : TA, SEA and SEAc figure more oput what this is but do overlap and variation and ellipse size first
groupMetricsML((siber.g.example))

#MAKE ELLIPSE PLOT
#plot of carbon vs nitrogen isotopes
ggplot(data=Fremstadparasitter, aes(x=d13C, y=d15N, color=Lake, label=Fish_ID))+
geom_point(size=3)+theme_bw()+
scale_color_manual(values=c("blue", "orange"))+
```

```
stat_ellipse(aes(color=Lake))+
stat_ellipse(geom = "polygon",aes(fill = Lake),   alpha = 0.25)+
scale_fill_manual(values=c("lightblue", "pink"))+
xlab(expression({delta}^13*C))+ ylab((expression({delta}^15*N)))+
theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())
 theme(axis.text = element_text(size = 15))+ theme(axis.title = element_text(size = 20))+
theme(legend.text = element_text(size = 17))+ theme(legend.title = element_text(size = 17))

#RELATIVE ELLIPSE OVERLAPS CALCULATION
overlap.0.95 <- maxLikOverlap("1.1", "1.2.", siber.g.example, p.interval=0.95, n=100)
print(overlap.0.95)
overlap.0.40 <-  maxLikOverlap("1.1", "1.2.", siber.g.example, p.interval=0.40, n=100)
print(overlap.0.40)
overlap.propotion <- overlap.0.95[3]/(overlap.0.95[2]+overlap.0.95[1]-overlap.0.95[3])
print(overlap.propotion)
```

Non-Metric multidimensional scaling (NMDS)

```
#packages needed for NMDS:
library(vegan
library(ggplot2)  #To make plot with ggplot      library(ggforce) #to make ellipses

all_para_subset <- subset(Fremstadparasitter, select=c(phyllo, Eustrongilydes,
Nematode_sp1, Pseudocapillaria, crepidostomum, diplo_abundance, tot_e_crassum,
total_diboth))

##First make plot to visiulize parasite comunity differences among lakes.
#Bray Curts dissimilarities of log transformed parasite abundances. Both trophically and non-
tropchically transmitted parasites
ord <- metaMDS(all_para_subset)
grp_abbr <- factor(rep(c("LT", "ST"), each = 30))
grp_long <- factor(rep(c("Litlvatnet", "Storvatnet"), each = 30))
cols <- c("blue", "orange")

plot(ord, type="n")
points(ord, display="sites", col="black", bg=cols[grp_long], pch=c(21), cex=1.8)
text(ord, display="species", col="black", cex=1.0)
ordiellipse(ord, grp_abbr, display = "sites", kind = "sd", label = T, conf = 0.0000001, col =
cols, cex=2.2) #Adding ellipse to fix mean, found it the easiest way. Made ellipses wery small
legend('bottomright', cex=1.5, legend = tools::toTitleCase(levels(grp_long)),  fill = cols, bty =
'n')
```

```
#Analyse with permanova to check for community differences between the lakes
dis <- vegdist(all_para_subset, method="bray")
#first 30 LV, next 30 ST, make groups
groups <- factor(c(rep(1,30), rep(2, 30)), labels=c("Litlvatnet", "Storvatnet"))

#calculate multivariate dispersion
mod <- betadisper(dis, groups)

#permutation test
permutest(mod, pairwise=TRUE, permutations=999)
#p-value <0.05 --> dispersions are not the same between the lakes -> assumption not met
#try with log transformation
library(dplyr)
log10plus <- function(x, na.rm = FALSE) log10(x + 1)
all_para_subset_logplus1 <- all_para_subset %>% mutate_if(is.numeric, log10plus, na.rm =
TRUE)

#permutation test with log transformed data
dis_log <- vegdist(all_para_subset_logplus1, method="bray")
mod_log <- betadisper(dis_log, groups)
permutest(mod_log, pairwise=TRUE, permutations=999) # p> 0.01 – can conduct analysis

#### Run PERMANOVA #####
para_perma_div_logtrans <- adonis2(all_para_subset_logplus1 ~ Lake, data =
Fremstadparasitter, permutations = 999, method="bray")

###SIMPER TO GET LIST OF PARASITE SPECIES THAT CONTRIBUTE TO THE
DIFFERENCES IN COMMUNITY###
simper(all_para_subset_logplus1, groups, permutations = 999, trace = FALSE)
```

Canonical correlation analysis (CCorrA)

```
#PACKAGES NEEDED
library(vegan)

#Make subset for diet and parasites (for trophically transmitted only)
troph_para_subset <- subset(Fremstadparasitter, select=c(crepidostomum, phyllo,
total_diboth, tot_e_crassum, Eustrongilydes, Nematode_sp1,  Pseudocapillaria))
diet_subset <- Fremstadparasitter[, c("Surface_insects", "Benthos", "Zooplankton", "Fish")]

#Transformating diet (normal) and parasites (log) since none were normal distributed
diet_subset_chordtrans <- decostand (diet_subset, method = "normalize")
troph_para_subset_logtrans <- decostand(troph_para_subset, method = "log")Corr <-
```

```
CCorA(troph_para_subset_logtrans, diet_subset_chordtrans, permutations=999)
#Look at canonical correlation values to see how many % each axis explains

#made ord to be able to make ellipses to plot the means like done for the stable isotope analysis. A little different solution that I have seen before but the best way I managed
#with finding another way so I made really small labeled ellipses instead

ord_troph <- metaMDS(troph_para_subset)
grp_abbr <- factor(rep(c("LT", "ST"), each = 30))  #seems like LT and ST got switced, change direction of factor
grp_long <-factor(rep(c("Litlvatnet", "Storvatnet"), each = 30))
data.frame(troph_para_subset)
grp_long <- factor(rep(c("Litlvatnet", "Storvatnet"), each = 30))
cols <- c("blue", "orange")

# Make biplot use lake as X-labs. Think it works because the data frames has the same order and R understands that it is the same. All that are white and really small is to make them unvisible. Might be other ways to do this as well
biplot(Corr$Cy, Corr$corr.Y.Cy, xlabs=Fremstadparasitter$Lake, col=c("white"))
colbg=c("blue", "orange")[as.factor(Fremstadparasitter$Lake)]
biplot(Corr$Cy,Corr$corr.Y.Cy, xlabs=Fremstadparasitter$Lake, xlim=c(-5,5),ylim=c(-5,5), col=c("white","red"), cex=c(0.7))
 points(Corr$Cy/1.7, pch=c(21, 22)[as.factor(Fremstadparasitter$Lake)], col="black", bg=colbg, cex=0.7)
legend('bottomright', cex=0.5, legend = tools::toTitleCase(levels(grp_long)),
   fill = cols, bty = 'n')
text(Corr$corr.X.Cy,labels=row.names(Corr$corr.X.Cy), col="black", cex=0.3)
points(Corr$corr.X.Cy, col="black",bg="black", cex=1.0)
text(Corr$corr.X.Cy, col="black",bg="black", cex=1.0)
box()
ordiellipse(ord_troph, grp_abbr_2, display = "sites", kind = "sd", label = T, conf = 0.000000000001, col = c("blue", "darkorange"), cex=2.2
title(xlab = "                              (49%)", ylab = "                              (31%)")
```