The helminth fauna of brown trout (*Salmo trutta*) in the lake, Øvre Heimdalsvatn, before and after the establishment of a large population of the invasive species, European minnow (*Phoxinus phoxinus*)

Parasittfaunaen hjå aure (*Salmo trutta*) i Øvre Heimdalsvatn før og etter etablering av ein stor populasjon av invasjonsarten ørekyt (*Phoxinus phoxinus*)

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Preface

This thesis concludes my degree in Natural Resource Management at the Norwegian University of Life Science (UMB). The subject was chosen as a result of my interest in Natural Sciences and fish biology.

I would like to thank my supervisors Professor emeritus Reidar Borgstrøm (INA) for excellent guidance and help throughout the study, Professor Philip D. Harris (UiO) for help and supervising during the parasite identification, statistical analysis, and for sharing his vast knowledge on parasites, and Professor John E. Brittain (INA/UiO) for help during my fieldwork at the lake, Øvre Heimdalsvatn.

Special thanks are given to Leif Lien for giving me access to his unpublished manuscript, which made this thesis possible, and also thanks to Professor Jan T. Lifjeld for sharing his knowledge on the avifauna in Øvre Heimdalsvatn. As well I would like to thank my sister Liv Hatleli and my brother-in-law Geoffrey Gilpin for proofreading my English.

Last but not least I would like to thank my family and friends for support, and my significant other, Tina L. Nilsson for comfort and support throughout the study

Ås, 15 May 2012

Øyvind Hatleli

Abstract

The objective of this thesis is to compare the parasite fauna of brown trout (*Salmo trutta*) in the lake, Øvre Heimdalsvatn, before and after the establishment of a large population of the invasive species, the European minnow (*Phoxinus phoxinus*). Brown trout were collected in June, July and September of 2011, by means of gillnetting. A total of 392 fish were caught, of which 113 fish, evenly distributed on different length-classes from 9 cm to 39 cm, were examined for parasites. A study of the helminth fauna in the lake was done in 1969-72, just after the introduction of the European minnow, while the population density of the minnow was low. By repeating the study, 40 years later, we can gain valuable information on how introductions of the European minnow may affect the parasite fauna of brown trout in subalpine lakes.

Nine of the parasite species were the same as those found in 1969-72, while one species, *Eubothrium crassum,* was not found, though another species, *Diphyllobothrium dendriticum*, not recorded in 1969-72 was found in 2011. The mean intensity of some species showed major changes, especially the three species *Diphyllobothrium ditremum, Crepidostomum* sp. and *Diplostomum spathaceum,* these showed great changes in abundance from 1969-72 to 2011. Many of the parasite species found in brown trout are dependent on one or several intermediate hosts, both vertebrates and invertebrates, and changes in the availability of these will influence the success of the parasites. The increase in fish-eating birds in the lake, Øvre Heimdalsvatn, probably as a result of a large European minnow population, may also influence the transmission rates of parasites with birds as final host.

Documented changes in population dynamics of brown trout and the invertebrate community in the lake, Øvre Heimdalsvatn, has been linked to the establishment of the European minnow, and has effectively influenced the availability of intermediate hosts. As a conclusion, the European minnow has indirectly changed the premise for many of the helminth species in the lake, thus influencing the abundance of the parasite species. The changes in the parasite fauna of brown trout, demonstrate another effect on a subalpine lacustrine ecosystem, as a result of establishment of an invasive species, the European minnow.

Samandrag

Bakgrunnen for denne oppgåva er å samanlikne parasittfaunaen hjå aure (*Salmo trutta*) i Øvre Heimdalsvatn før og etter etableringa av ein stor populasjon av invasjonsarten ørekyt (*Phoxinus phoxinus*). Fisken vart fanga med ein fast garnserie i juni, juli og september 2011. Totalt vart 392 fisk fanga, og 113 av dei, jamt fordelt på lengdeklassar frå 9 cm til 39 cm, vart undersøkt for parasittar. Parasittfaunaen i vatnet er tidlegare undersøkt i 1969-72, før populasjonen av ørekyt i vatnet var stor. Ved å gjera same undersøkinga no, 40 år etter, kan ein få nyttig informasjon om endringar i parasittfaunaen i eit høgfjellsvatn som følgje av ei etablering av ørekyt.

Totalt ti parasittar vart funne i vatnet, inkludert ein ny art (*Diphyllobothrium dendriticum*). Samanlikninga av den gjennomsnittlege parasittinfeksjonen i 1969-1972 mot 2011 syner store skilnadar i parasittfaunaen i vatnet. Parasittane *Diphyllobothrium ditremum, Crepidostomum* sp. og *Diplostomum spathaceum* synte størst endring i gjennomsnittleg infeksjon. Fleire av parasittane i aure er avhengige av ein eller fleire mellomvertar for å fullføra livssyklusen sin, og ei endring i tettleiken av desse kan påverka suksessen til parasittane. Auken av fiskeetande fugl ved vatnet, truleg på grunn av ørekyt, kan ha påverka distribusjonen av parasittar som har fugl som endeleg vert.

Dei godt dokumenterte endringane i populasjonsdynamikken hjå aure og i evertebratsamfunnet i Øvre Heimdalsvatn har vorte kopla til etableringa av ørekyt i vatnet. Dette har som ein effekt endra tilgjenge av mellomvertar. Ørekyt har indirekte endra føresetnadane for mange av parasittane og dermed også utbreiinga i vatnet. Dei store endringane i parasittfaunaen er openberre og syner endå ein effekt av invasjonsarten ørekyt.

Content

1 Introduction

Fish parasites have developed a variety of life history strategies. Although some species have a simple life cycle without intermediate hosts, for example in the Monogenea (Smyth 2012), many have complicated life cycles, including one or several intermediate hosts (Hoffman 1999). Since fish may serve as both final host and intermediate hosts for parasites, changes in density of invertebrate prey species that serve as intermediate hosts, changes in the fish population density, as well as changes in abundance of piscivorous predators may all influence the parasite burden in a fish population.

During the last century, the European minnow (*Phoxinus phoxinus*) was introduced to many lakes in Norway (Saltveit & Brabrand 1991), and is now found in all counties of Norway (Hesthagen & Sandlund 1997). According to Næstad and Brittain (2010) the benthic invertebrate community may be strongly influenced by the establishment of a European minnow population, as observed in the subalpine lake, Øvre Heimdalsvatn. In this lake, recruitment and individual growth rates of brown trout (*Salmo trutta*) were also affected by the minnow's establishment (Museth et al. 2007; Borgstrøm et al. 2010). Although extensive studies of the brown trout population in lake Øvre Heimdalsvatn had been performed from 1958 (Jensen 1977), European minnow was not recorded before 1969 (Larsson et al. 1978). The minnow population had a low density in 1969 and for some years afterwards, but increased considerably during the next decades (Lien 1981; Museth et al. 2002).

The summer diet of brown trout during the years 1969-1972 included a high proportion of *Gammarus lacustris* and *Lepidurus arcticus* (Lien 1978b). *L. arcticus* has been almost non-existent in their diet during the last decades (Borgstrøm et al. 2010). *G. lacustris* is still an important part of their diet (Borgstrøm et al. 2010), but the species has declined considerably in abundance in the shallow part of the littoral zone (Næstad & Brittain 2010). Lien (1978b) did not find fish as part of the brown trout diet during the years 1969-1972, but during the last decades, both European minnow and brown trout, have become an important part of the diet (Borgstrøm et al. 2010). The large changes in both the invertebrate fauna and the fish community in the lake Øvre Heimdalsvatn after the increase in the European minnow population may have considerably influenced the parasite fauna of brown trout.

The helminth parasites of brown trout in the lake Øvre Heimdalsvatn were studied by Lien (1978b) by monthly sampling of trout during the period December 1969 to December 1971, as well as some sampling in 1972, i.e. during the period when European minnow still had a low population density. He recorded the following species: *Discocotyle sagittata, Phyllodistomum umblae, Crepidostomum farionis, C. metoecus, Diplostomum spathaceum, Proteocephalus* sp*., Cyathocephalus truncates, Diphyllobothrium ditremum, Capillaria* sp., and *Eustrongylides* sp. (Lien 1978b) i.e. species with both direct and indirect life cycles, and brown trout as both final host and intermediate host, with fish eating birds as final hosts for some of the parasites. Except for the species list published by Lien (1978b), his recordings were not published, but were placed at my disposal, making an excellent basis for a study of changes in the helminth parasites of brown trout around forty years after the introduction of European minnow in the lake.

Considering factors influencing the parasite fauna, and based on the changes in the population dynamics of brown trout, I expect that small changes have occurred regarding parasites with direct life cycles. Parasites with invertebrates as intermediate hosts and trout as final or intermediate host may show marked changes from 1969-1972 until today.

2 Materials and Methods

2.1 The Lake, Øvre Heimdalsvatn

Øvre Heimdalsvatn is a subalpine lake situated in Øystre Slidre municipality, in Oppland County (Fig. 1). The lake is located 1088 m.s.l., and is normally covered with ice from the end of October until early June (Kvambekk & Melvold 2010). It has a total area of 0.78 km^2 and an average depth of 4.7 meters (Grøterud & Kloster 1978; Vik 1978).

Figure 1 The eastern part of the lake, Øvre Heimdalsvatn, with the outflow river Hinøgla. Photo: Øyvind Hatleli

Øvre Heimdalsvatn is one of the most studied lakes in Norway, and is used as a reference lake on subalpine ecosystems (Brittain & Borgstrøm 2010). The brown trout population has been intensively studied from 1957 when Jensen (1977) started his studies on the brown trout dynamics. In 1957, the brown trout population was overpopulated, with high recruitment and early growth stagnation of the individual fish. High exploitation had a positive effect on the annual individual growth rates (Jensen 1977). In the last few decades there have been major changes in the population, with decrease in annual individual growth rates, as well as decreased recruitment (Borgstrøm et al. 2010). In 1957, brown trout was the only fish species in the lake. However, in 1969 the European minnow was observed for the first time in the lake (Lien 1978b). It was probably introduced to the lake by poachers using minnows as live bait, or mixed with stocked juvenile brown trout in the upstream lake Brurskardstjørn (Lien 1981). The minnow quickly established a population in the lake (Lien 1981), and the establishment of this species is probably the main reason for the ecological changes occurring in the lake over the last 50 years (Museth et al. 2002; Borgstrøm et al. 2010; Museth et al. 2010; Næstad & Brittain 2010). Young brown trout and minnows share the same shallow littoral areas probably to avoid predation, but minnows are also found in the streams (Museth et al. 2010). Overlapping diet and habitat use of the two species suggest a negative influence on the trout population (Borgstrøm et al. 2010; Museth et al. 2010; Næstad & Brittain 2010).

The number of fish-eating birds on the lake has changed over the last 50 years. In 1970-1972 the Common gull (*Larus canus*) was sighted, but had little dominance (Lien 1978c), and both *Mergus merganser* and *M. serrator* were observed fishing in the lake (Lien 1978a). In the last twenty years the population of fish-eating birds has been stable. There are three to five females of both *M. merganser* and *M. serrator* feeding and nesting in close proximity of the lake, and a pair of *Gavia arctica* foraging in the lake. Sightings of *Sterna paradisaea* have increased, and there are one to three individuals foraging in the lake, and about five nesting pairs of the Common gull around the lake (Prof Jan T. Lifjeld pers. comm. 2012).

2.2 Methods

The brown trout was sampled by means of gillnetting in June, July and September of 2011. Each gillnet had a length of 25 m and was 1.5 m in height, and the whole fleet consisted of nine different mesh sizes; 16, 19.5, 22.5, 26, 29, 31, 35, 39 and 45mm in order to capture fish from various length- and age-classes. A total of 392 brown trout were caught, of which 113 were examined with regard to parasites (Fig. 2). To ensure samplings from a spectrum of age- and length-classes, the examined fish were collected from the following length classes: <10 (1), 10-14.9 (13), 15-19.9 (18), 20-24.9 (20), 25-29.9 (18), 30-34.9 (32) and 35-39.9cm (11), with numbers in parenthesis indicating the number of examined fish.

Gills, eyes, body cavity, kidney, stomach, pyloric region, and the rest of the intestine of the sampled fish were all examined with regard to parasites. The pylorus was placed in water overnight to release *Proteocephalus* sp. and *Cyathocephalus truncatus*. Each organ was thoroughly examined, and parasites were counted and conserved in ethanol. In addition, the otholits and scales of the fish were taken for age determination, and the stomach contents were conserved in ethanol for diet analysis.

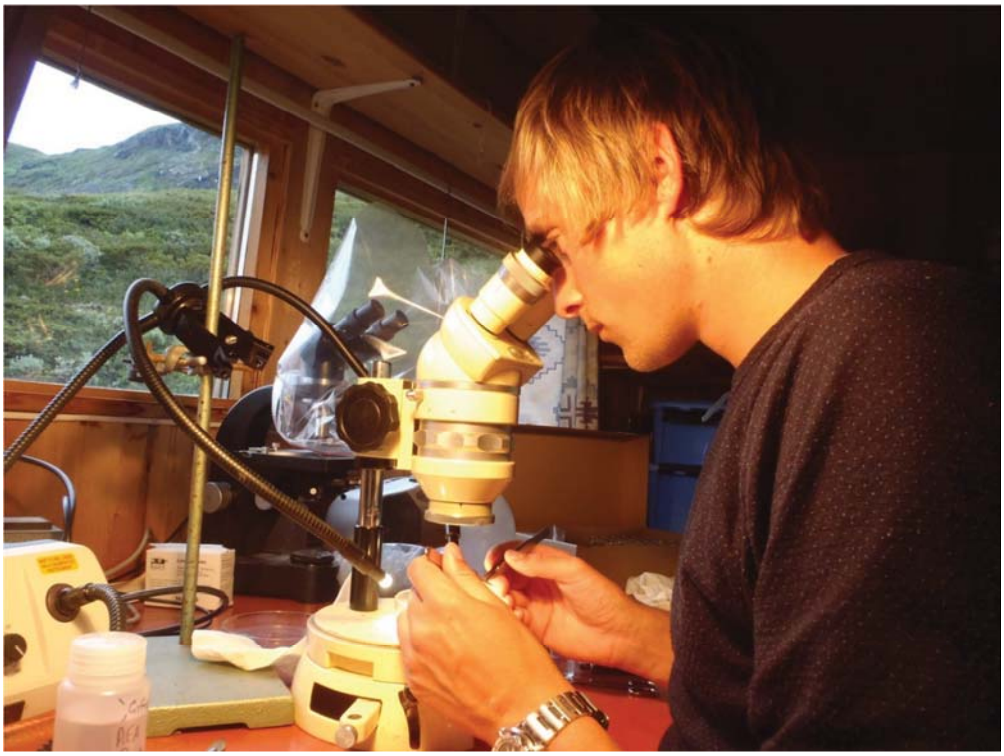

Figure 2 The sampling of the parasites from brown trout was performed at the research lab in Øvre Heimdalsvatn, July 2011. Photo: Øyvind Hatleli

The stomach contents were studied in the laboratory of the Department of Ecology and Natural Resource Management, at the University of Life Science in Ås. The volume per cent of the different prey item categories found in each stomach were recorded according to Hynes (1950).

2.3 Age determination of brown trout

Small otoliths were studied whole after clearing in ethanol. Otoliths from older fish were cut in half through the nucleus and burnt before reading the age by use of a binocular microscope (Power 1978) (Fig. 3). If otoliths were hyaline, the fish scales were used to determine age.

Figure 3 Burnt and cut otolith of a brown trout, aged 6 winters, from the lake Øvre Heimdalsvatn, captured in September 2011,

2.4 Parasite identification

Most of the parasites were separated by species and counted during the fieldwork. Some were counted and identified to species in the laboratory at the Zoological museum in Oslo (UiO).

Crepidostomum farionis and *C. metoecus* were identified and separated by morphology (Moravec 2002) in the laboratory using a binocular microscope.

Microscope slides were made of individual *C. farionis, C. metoecus, Discocotyle sagittata* and *Proteocephalus sp.* Parasites were washed in distilled water and stained for 2 weeks in alum carmine until an even dark purple in colour (Humason 1967). They were rinsed in tap water to blue the stain before destaining in ethanol with 1% 0.1M Hydrochloric acid. Parasites were destained with constant monitoring using a stereomicroscope until most of the stain had been removed from the outer layers, and the internal organs were clearly visible. Specimens were placed in 70 % ethanol overnight, and then dehydrated in 96 % ethanol for 2 hours. Finally the specimen was placed in acetone for 2 hours before clearing in xylene. The specimens were infiltrated with a 50:50 mixture of xylene and Canada Balsam overnight before mounting on a slide using Canada Balsam. Slides and cover glass were first rinsed briefly with xylene to remove grease.

Nematodes (*Capillaria* sp.) were mounted in TL buffer (1mM Tris, 0.1%SDS, pH8.5) on microscope slides and left overnight to clear before viewing.

2.5 DNA- analyses

DNA- analyses were made for *Diphyllobothrium* sp. and *Proteocephalus* sp. in cooperation with Professor P. D. Harris at Zoological museum, UiO. *Diphyllobothrium* sp. has larval forms in fish, and morphological identification is unreliable. *Proteocephalus* sp. was not identified to species by Lien, and as a character-poor group, the molecular examination will give a more confident identification.

Appropriate tissue samples were rinsed in distilled water prior to extraction to remove ethanol. The DNA extraction was done using the *Omega Bio Tek* E.Z.N.A. Tissue DNA kit as described in the manufacturers manual. DNA was eluted from the columns in 200µl elution buffer (1mM Tris buffer pH 8.3, 1mM EDTA) and stored at -20°C prior to PCR amplification. PCR amplification used Ampli-Taq Gold ® (Applied Biosystems) and 10µmoles of each primer with 2 µl of DNA template in a total reaction volume of 20µl.

Amplification was carried out in a GenAmp 9700 thermo cycler using primers and amplification conditions as outlined in Table 1.

Table 1 PCR primers and conditions for amplification.

Primer	**Bases**	**PCR conditions,** sample size: **20µl**.
Proteo1	5'-CGG TGG ATC ACT CGG CTC-3'	15 min at 95°C; then 30 cycles of: denaturation, 1 min at 94°C, annealing 1 min at 60°C, 2 min at 72°C and final extension for 10 min at 68°C (Scholz et al. 2007).
Proteo2	5'-TCC TCC GCT TAT TGA TAT GC-3'	

PCR products were analysed using 0.8% Agarose gels in TBE buffer (Ogden & Adams 1987) and electrophoresed at 80V for 1h. Products were visualised using Gel Red and viewed using a Gel Logic 200 system UV transilluminator. Products were treated with 1:10 dilution of ExoSapIt (Affymetrix), to degrade remaining primers, diluted for sequencing based on product yield established by gel electrophoresis, and sequenced on an Abi automated sequencer (Biologisk Institutt Blindern) using the same primers used for initial amplification. Chromatograms were viewed and edited using FinchTV (2011) (http://www.geospiza.com), before the sequences were aligned using Mega 5.1 (Tamura et al. 2011). The sequences were compared to available sequences for related parasites deposited in GenBank using Blast (Zhang et al. 2000).

2.6 Statistical analyses

Crude parasite counts were recorded in Excel spread sheets and used to compute abundance, mean intensity and prevalence (Margolis et al. 1982). Inspection of variance mean ratios (usually >1) revealed that parasite counts were over-dispersed and so $\log_{10}(x + 1)$ transformations were used for Analysis of Variance in the statistical programme R (R Development Core Team 2011). The data was also analysed using Generalised Linear Models, with the quasi family function. Models were simplified by elimination of factors stepwise using the Step function in R, and comparing the fit of models using the Aikake Information Criterion (AIC). The Minimum Sufficient Model was taken as the model which achieved a difference in AIC (ΔAIC) of less than 2 over the next most complex model.

Log_{10} mean and $\log_{10}$ variance were also calculated and plotted against each other to determine the slope and shape of the relationship for parasite burdens in fish of different length-classes (Taylor's Power Law -(Taylor 1961)). Graphics were prepared using Excel.

3 Results

3.1 Brown trout diet

The brown trout diet showed clear seasonal variation, as well as variations with regard to fish size (Fig. 4). In June the dominant prey items for length-class 10-19.9 cm were chironomids (31 %), *Eurycercus* (26 %), terrestrial insects (20 %) and planktonic cladocerans (17 %). In July, the main prey items were Trichoptera, Ephemeroptera and Plecoptera (TEP), making up 32 % of stomach volume, chironomids (26 %), and Cladocera (mainly *Eurycercus*) (22 %). In September, planktonic cladocerans completely dominated in the diet of this length-class, making up 60 % of the stomach content volume (Fig. 4).

Fish of length-class 20-29.9 cm had chironomids (32 %), terrestrial insects (28 %), *Gammarus* (15 %) and planktonic cladocerans (14 %) as their dominant food source in June. In July, *Gammarus* was the most common food item (39 %), together with chironomids and terrestrial insects (Fig. 4). In September, the three most important prey groups were planktonic cladocerans (32 %), TEP (25 %), and terrestrial insects (22 %).

Length-class 30-39.9 cm had terrestrial insects (29 %) as the main food source in June. Chironomids (18 %), TEP (14 %) and *Gammarus* (12 %) were also common. TEP (36 %), *Gammarus* (23 %), and terrestrial insects (18 %) were the main food items in July. In September, the main food items were again terrestrial insects, with 39 % of the stomach content volume, but TEP (28 %) and *Gammarus* (21 %) were also important (Fig. 4).

3.1.1 Intermediate hosts in the diet

Gammarus, which is an intermediate host for *Cyathocephalus truncatus* and *Crepidostomum* spp., was found primarily as a food item in brown trout with length > 19 cm, and was quite numerous in some individuals. Copepods which are intermediate hosts for *Proteocephalus* and species of *Diphyllobothrium* were almost non-existent in the diet of brown trout during the months June-September 2011 (Fig. 4). Aquatic insects, usually species of Ephemeroptera, are intermediate hosts for *Crepidostomum.* TEP was an important food source, but it was mostly composed of Trichoptera, and only a few fish had mayflies as part of their diet. A few fish had eaten fish, but only minnows were found in the diet.

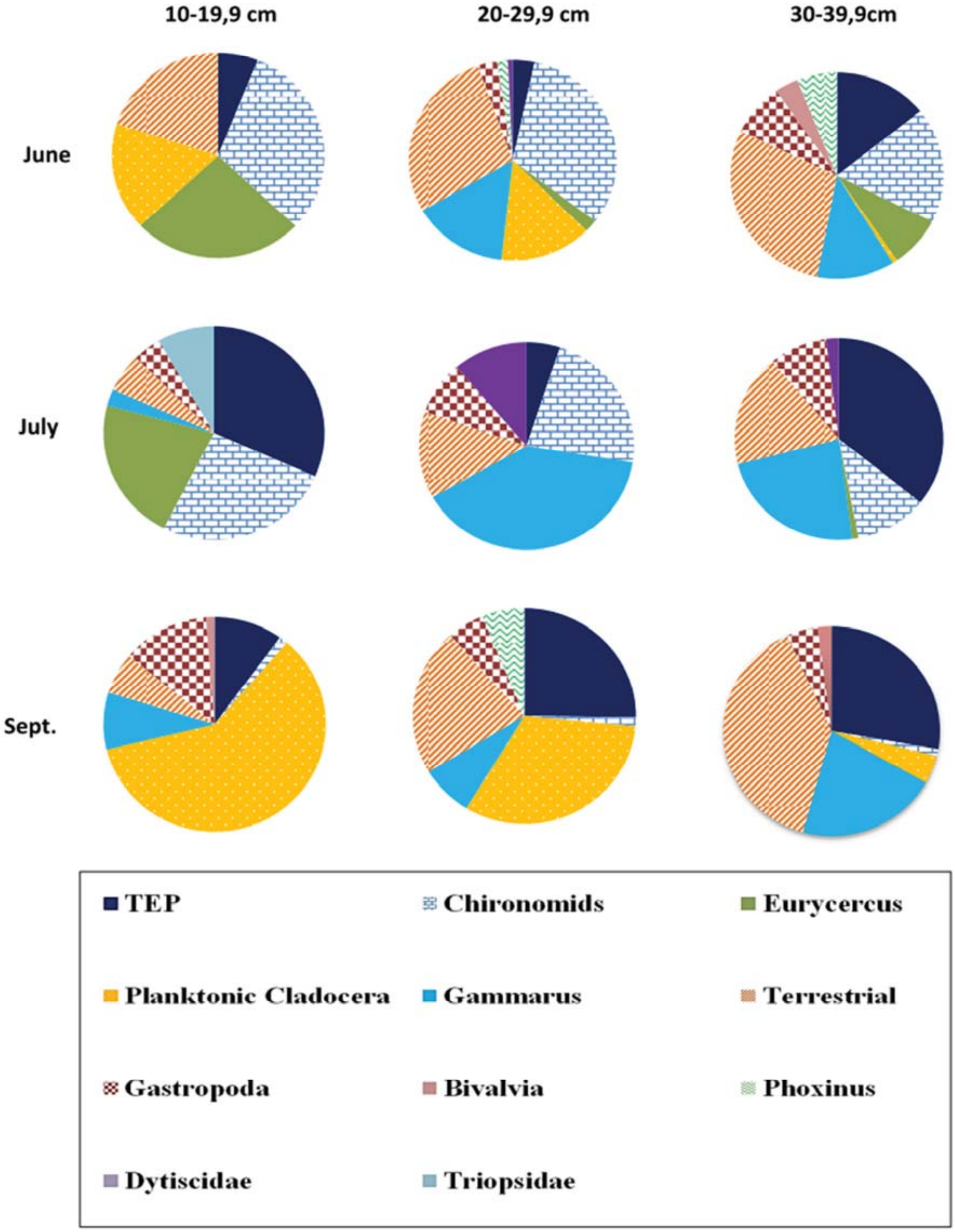

Figure 4 Diet (in volume percent) of three length-classes of brown trout, caught in the lake Øvre Heimdalsvatn during June, July and September 2011. TEP is *Trichoptera, Ephemeroptera* and *Plecoptera.*

3. 2 Parasites of brown trout

A total of ten helminth species were found as parasites of brown trout in Øvre Heimdalsvatn during the sampling in June, July and September 2011. All species present in 1969-1972 were found, except the nematode *Eubothrium crassum*. A single specimen of *Diphyllobothrium dendriticum* (ID not confirmed by molecular means) was identified as a new species when compared to 1969-72 (Table 1). The most numerous parasite group in 1969-72 was *Crepidostomum* sp., while in 2011 the most numerous parasite was *Diplostomum spathaceum* (Table 2).

Table 2 Mean intensity of parasites on different age-classes of brown trout in the lake Øvre Heidalsvatn in 1969-72 (Leif Liens unpublished) and in the months in June, July and September 2011.

	1969 - 1972				**2011**			
Age-class	**3-4 yrs.**	**5 yrs.**	**6 yrs.**	**≥ 7 yrs.**	**3-4 yrs.**	**5 yrs.**	**6 yrs.**	**≥ 7 yrs.**
Discocotyle sagittata	0.50	1.70	2.40	4.70	2.00	3.42	6.2	7.67
Phyllodistomum umblae	13.70	10.80	9.20	6.30	2.33	5.2	9.30	6.92
Crepidostomum sp.	**147.00**	**229.00**	**234.00**	**253.00**	**11.17**	**16.67**	**18.87**	**17.87**
Diplostomum spathaceum	**12.00**	**10.80**	**5.90**	**17.20**	**20,67**	**59.57**	**54.75**	**78.39**
Proteocephalus sp.	13.50	11.80	11.40	7.80	1.00	0.00	3.00	5.14
Cyathocephalus truncatus	3.10	4.90	7.30	9.70	1,60	1.67	3.50	4.30
Capillaria sp.	1.10	1.60	0.80	0.60	1.00	2.00	2.75	2.58
Diphyllobothrium ditremum	**0.00**	**0.00**	**0.00**	**0.10**	**1.66**	**10.33**	**9.75**	**26.00**
Diphyllobothrium dendriticum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

3.2.1 *Diphyllobothrium ditremum*

The identity of *D. ditremum* was confirmed by molecular means, and of seven parasites sequenced the most similar was GenBank accession DQ386126, with 99 % homology with species from Scotland.

The infection of *D.ditremum* was substantially higher in 2011 than in1969-72. In 1969 – 72, there was almost no infection of *D. ditremum* (Table 2), with only a few older fish infected, while in 2011 the prevalence was 42.7 per cent, and it was found in high numbers in some fish (Fig. 5).

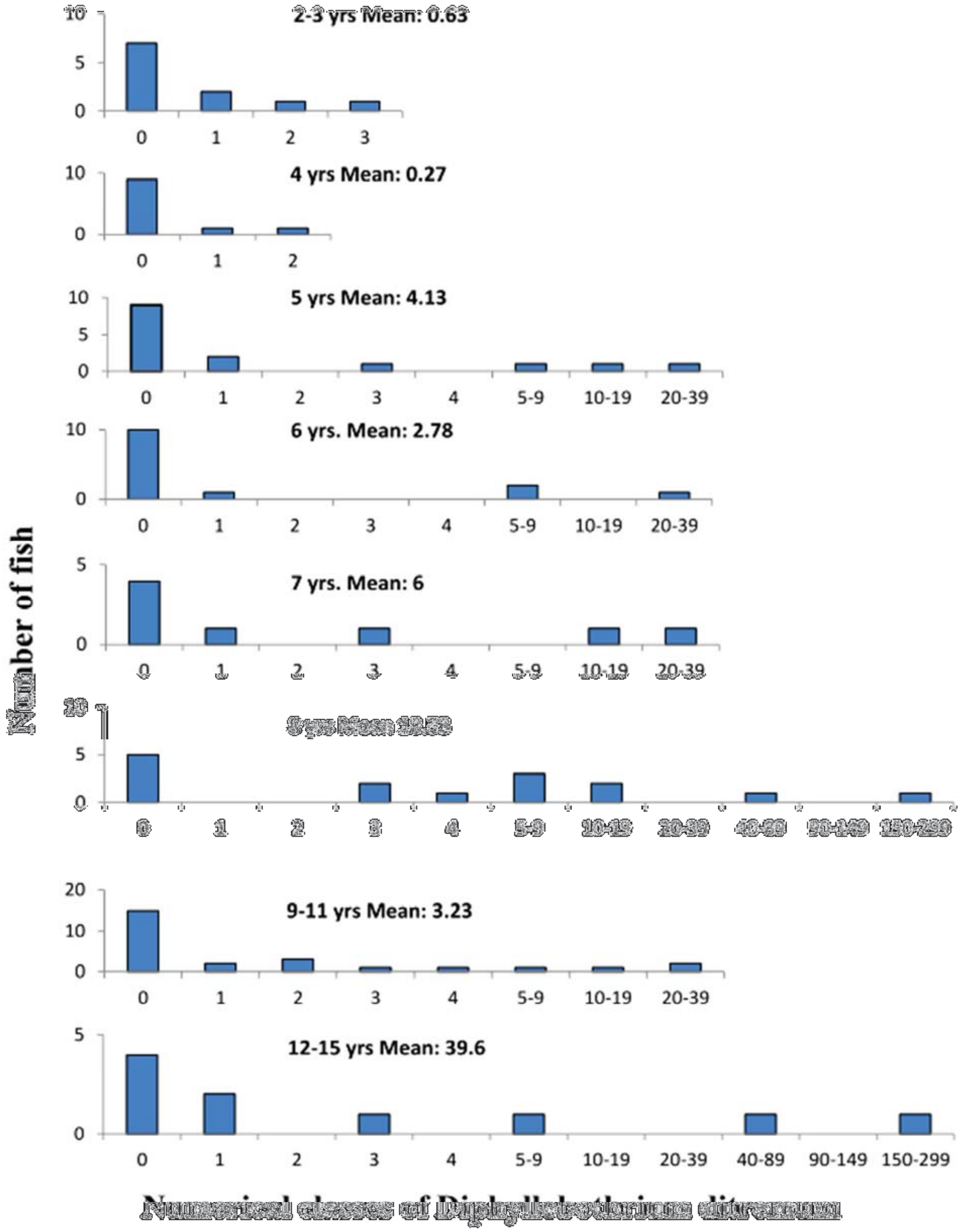

Figure 5 Numerical classes of *Diphyllobothrium ditremum* in various age-classes of brown trout, caught in the lake Øvre Heimdalsvatn in June, July and September in 2011. Mean number of *D. ditremum* is given for each age-class category.

The mean intensity was higher in older fish (Table 2), but even some small fish had heavy infections (Fig. 5). All age-classes had infected individuals, but the highest numbers were found in older fish (Fig. 5).

The distribution of *D. ditremum* showed significant relations to age, length and weight (Anova; glm F: 56.8, P<0.001).

The relationship between log mean and log variance (Taylor's power law) was linear (R^2 = 0.9749, b = 2.08) (Fig. 6), and there is no evidence of different infection, or mortality processes, affecting older fish infected with *D. ditremum* compared to younger fish. In particular, there is no evidence of variance being reduced relative to the mean of the largest fish group, which could be interpreted as evidence of *Diphyllobothrium*-induced host mortality (Anderson & Gordon 1982).

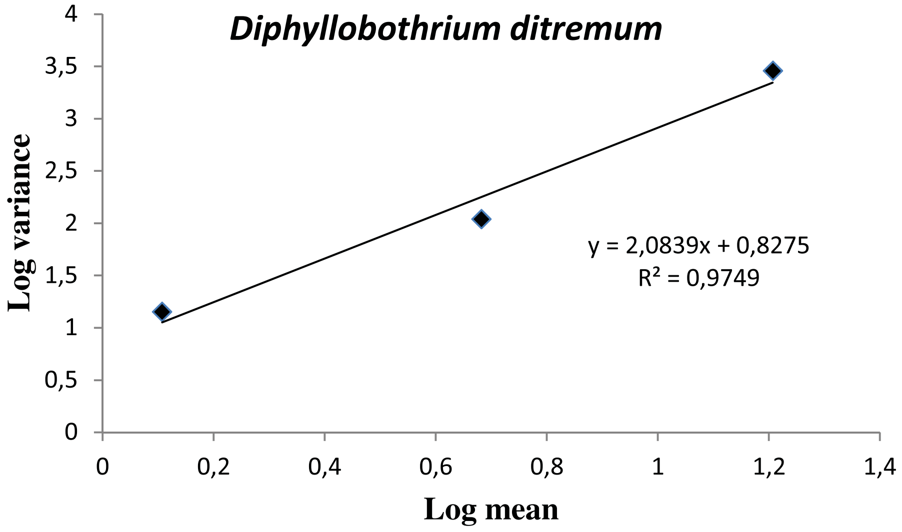

Figure 6 Relation between log variance and log mean of length-classes on the distribution of *Diphyllobothrium ditremum* in brown trout from the lake Øvre Heimdalsvatn caught in June, July and September 2011

3.2.2 *Crepidostomum* spp.

A total of 79.8 per cent of the examined brown trout were infected with *Crepidostomum sp.* The infections were mostly in small numbers, although a few fish showed a high level of infection. Two species of *Crepidostomum* were found; *C. farionis* and *C. metoecus* (Fig. 7, 8). The most numerous species was *C. metoecus,* while *C. farionis* was found in only a few fish.

Figure 7 ***Crepidostomum farionis*** **from brown trout caught in the lake Øvre Heimdalsvatn in 2011, stained with Canada Balsam. Picture taken with Differential Interference Microscopy (magnified 400X).**

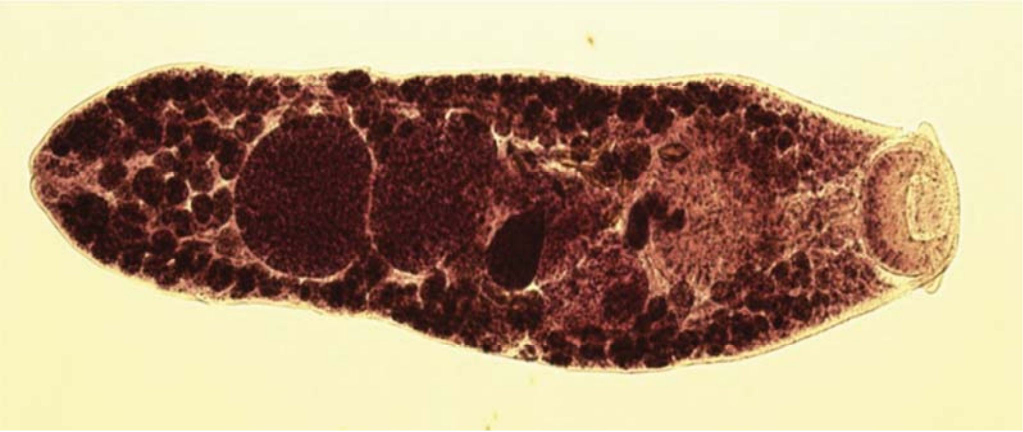

Figure 8 ***Crepidostomum metoecus*** **from brown trout caught in the lake Øvre Heimdalsvatn in 2011, stained with Canada Balsam Picture taken with Differential Interference Microscopy (magnified 400X).**

Compared to 1969-72, a clear decrease in mean intensity of *Crepidostomum sp.* was observed in 2011 (Table 2). There was also a significant decrease in infection throughout the summer (Anova; glm: F=53.32, P < 0.001) (Fig. 9). The level of infection increased from age-class 2-4 to 7-8, with a decrease in older fish (age-class 9-15) fish (Fig. 10).

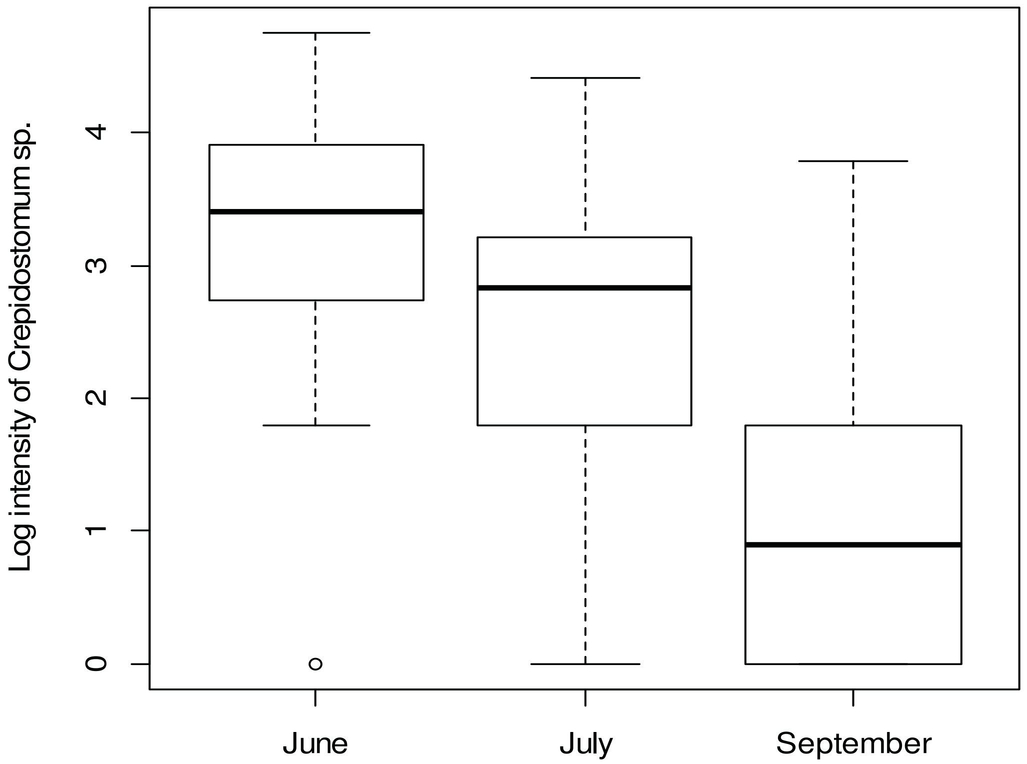

Figure 9 Log intensity of Crepidostomum sp. on age classes of brown trout from the lake Øvre Heimdalsvatn caught in June, July and September 2011

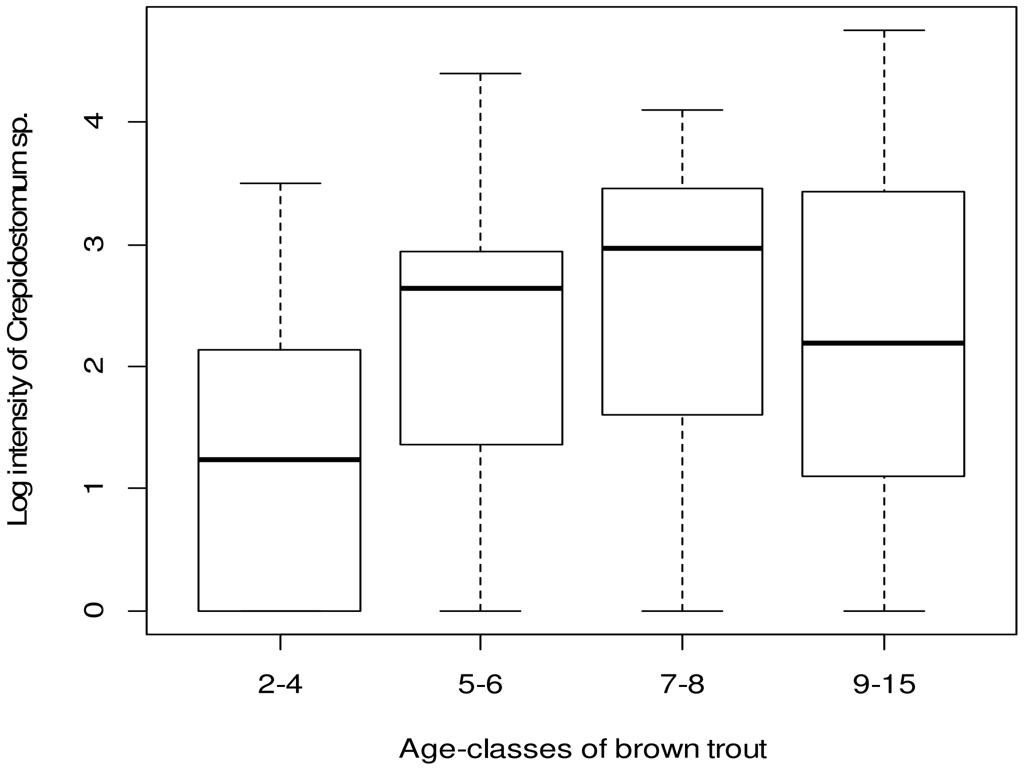

Figure 10 Log intensity of *Crepidostomum sp*. in brown trout from the lake Øvre Heimdalsvatn caught in June, July and September 2011

3.2.3 *Diplostomum spathaceum*

D. spathaceum was found to have a much higher mean intensity in 2011 than in 1969-72 (Table 2). The infection rate was considerably higher in every year class, and it was found in all examined fish. The data show significant correlation between the infection rate of *D. spathaceum* and age of the fish (Anova; multiple lm, F = 57.8, P<0.001) (Fig. 11). The relationship between log variance and log mean was linear (Fig. 12), again suggesting that parasite infection/death processes are consistent across all length-classes of fish sampled.

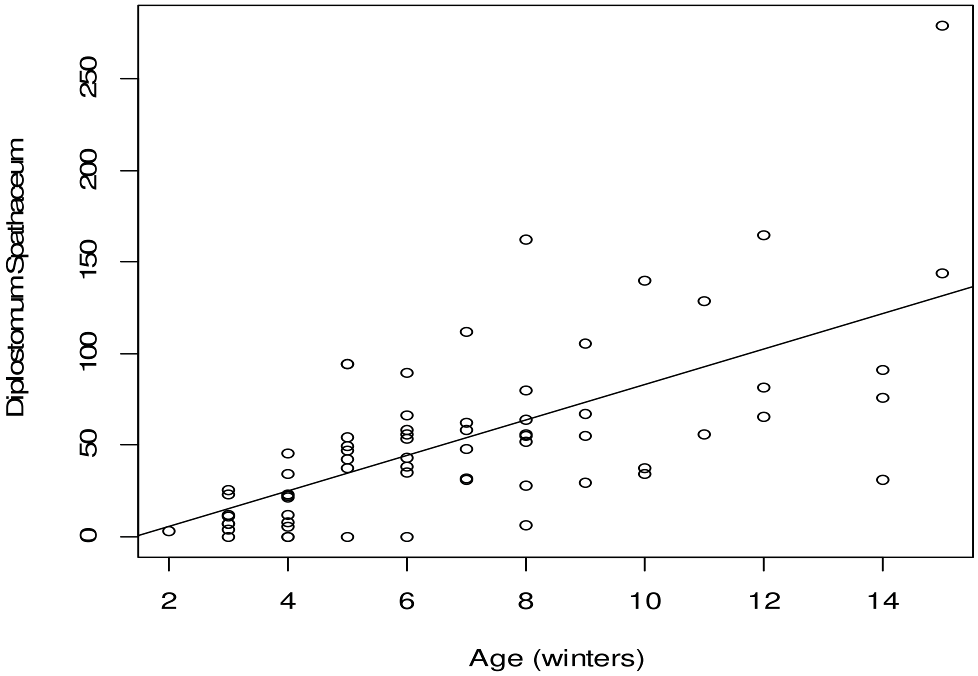

Figure 11 Number of *D. spathaceum* according to age of brown trout from the lake Øvre Heimdalsvatn, caught in June, July and September 2011

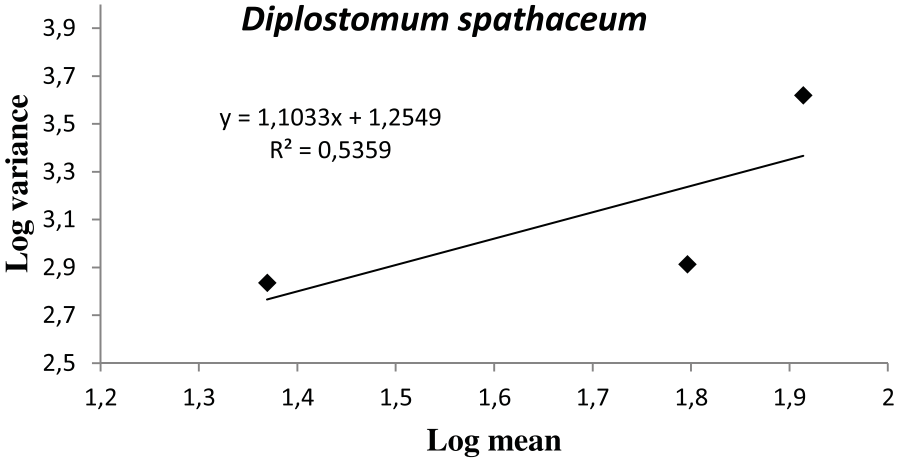

Figure 12 Relationship between log variance and log mean on the distribution of *Diplostomum spathaceum* in brown trout from the lake Øvre Heimdalsvatn caught in June, July and September 2011

3.2.4 *Discocotyle sagittata*

The gill fluke *D. sagittata* (Fig. 14) was quite common in Øvre Heimdalsvatn in 2011, with a prevalence of 75 per cent. The infections was significantly higher in larger fish (Anova; glm F=17.46, P <0.001) (Fig. 13).

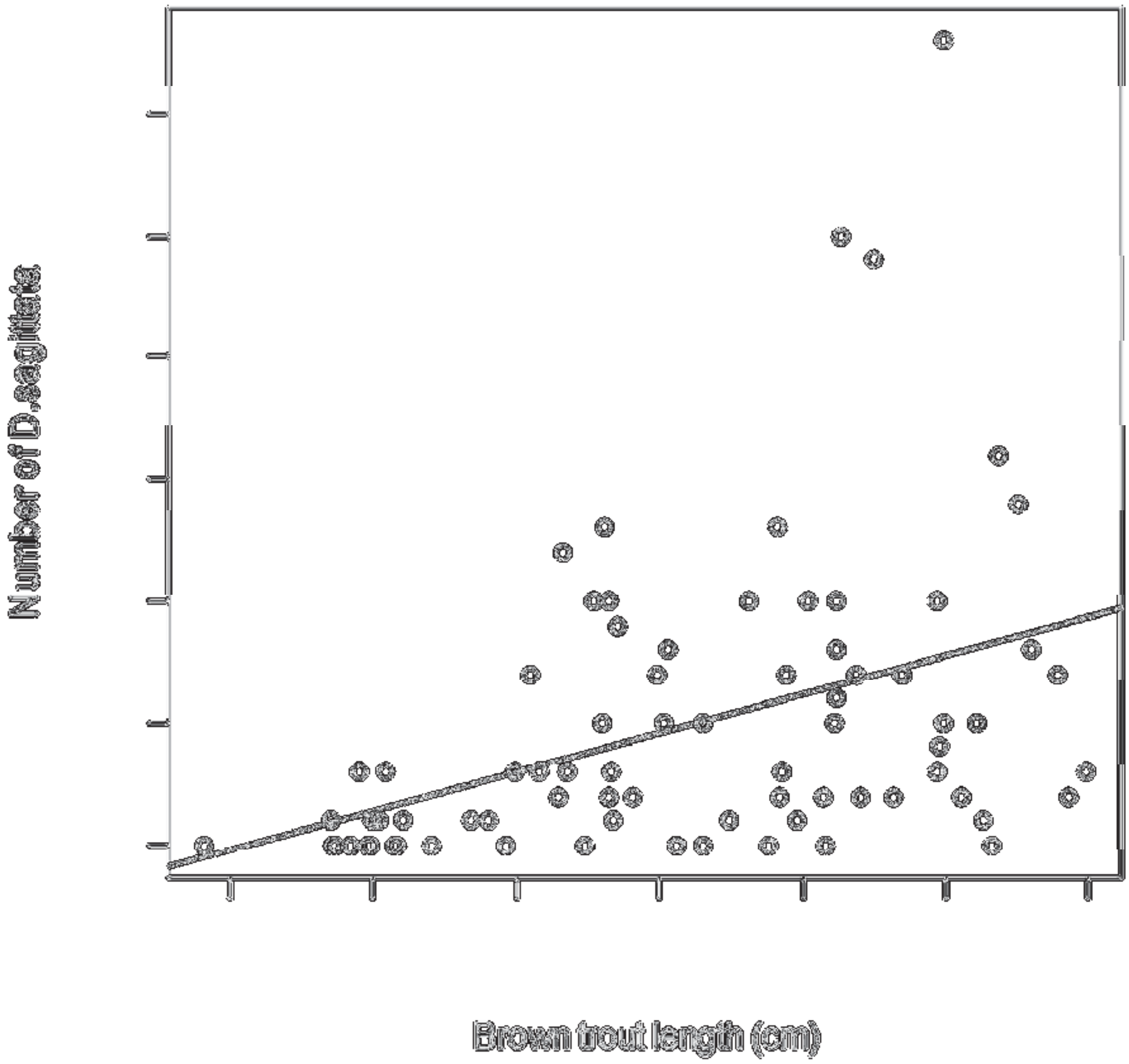

Figure 13 Number of *D. sagittata* according to length of brown trout caught in the lake Øvre Heimdalsvatn in June, July and September 2011

The relationship between log variance and log mean was linear (R² = 0.97, b = 1.66), again suggesting that parasite infection/death processes are consistent across all length-classes of fish sampled.

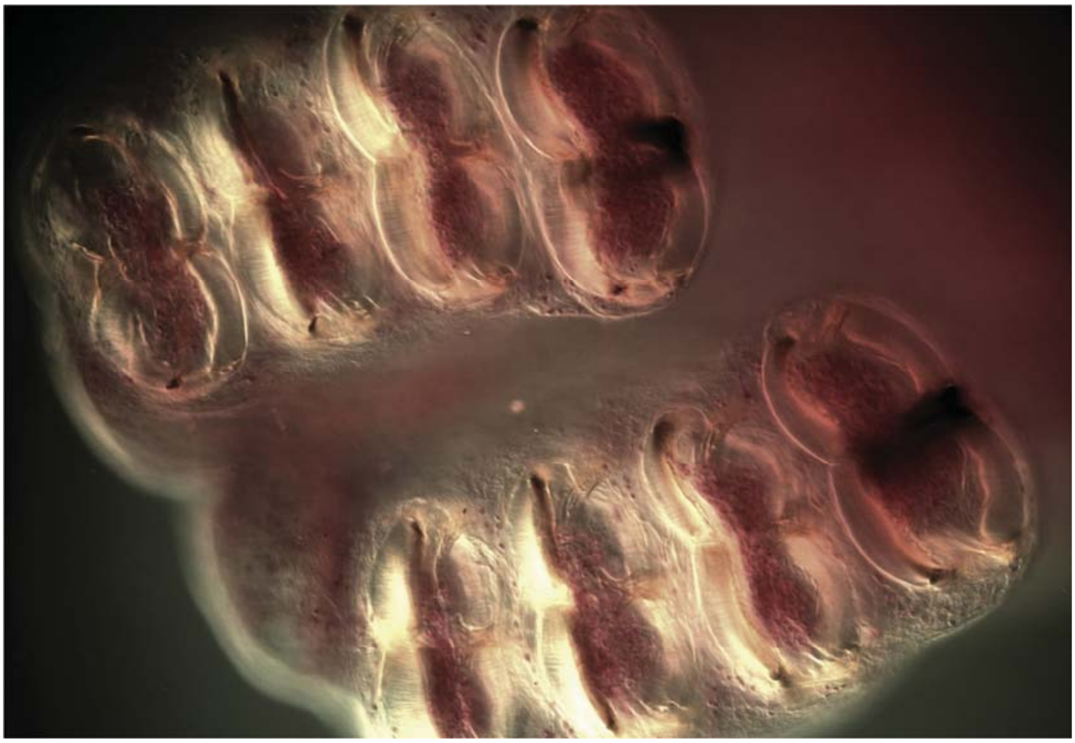

Figure 14 Clamps of *D. sagittata*, used for attachment on the gills of the fish host. Stained with Canada Balsam and viewed with Differential Interference Microscopy (magnified 400X)

3.2.5 *Phyllodistomum umblae*

The kidney fluke *P. umblae* was quite common in 2011, with a prevalence of 62.7 per cent. The mean intensity was similar to that found in 1969-72 (Table 2). The infection peaked in medium length fish (Fig. 15), and there was a significant relationship between mean intensity and length (Anova, multiple linear model, F value = 16.2748, P value< 0.001)

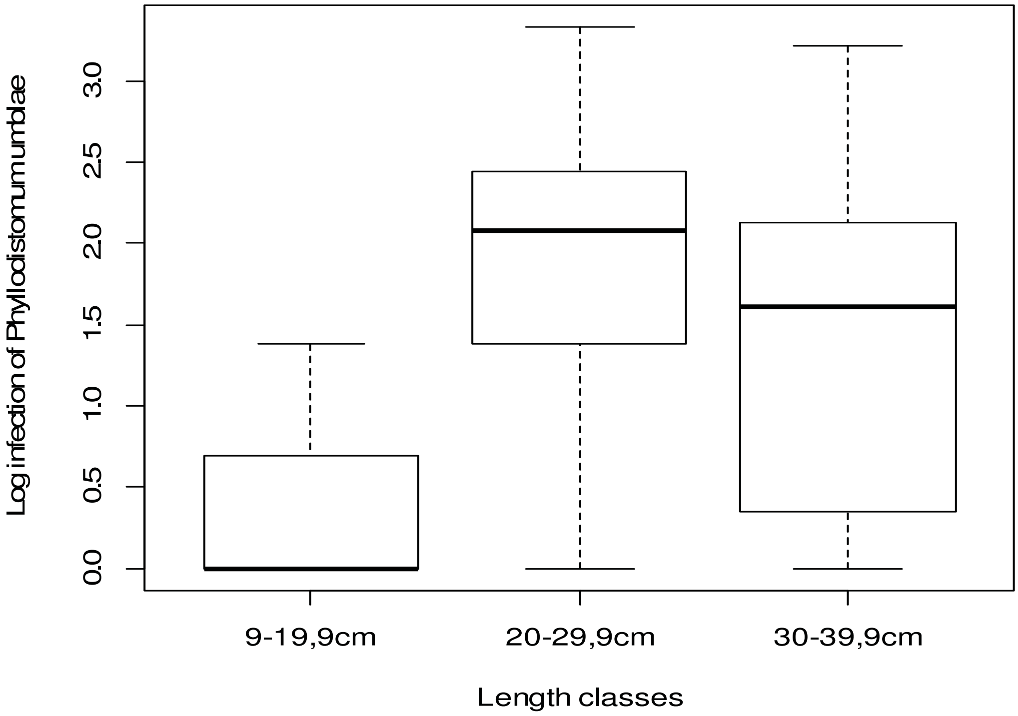

Figure 15 Log number of *P. umblae* in length classes of brown trout caught in the lake Øvre Heimdalsvatn in June, July and September in 2011

The relationship between log variance and log mean was linear ($R^2 = 0.98$, $b = 1.55$), again showing a normal population dynamic on length-classes of fish. In 1969-72 *P. umblae* had the highest mean infection in age-classes 3-4. In 2011, the highest mean infection was found in age-class 6 fish (Fig. 16).

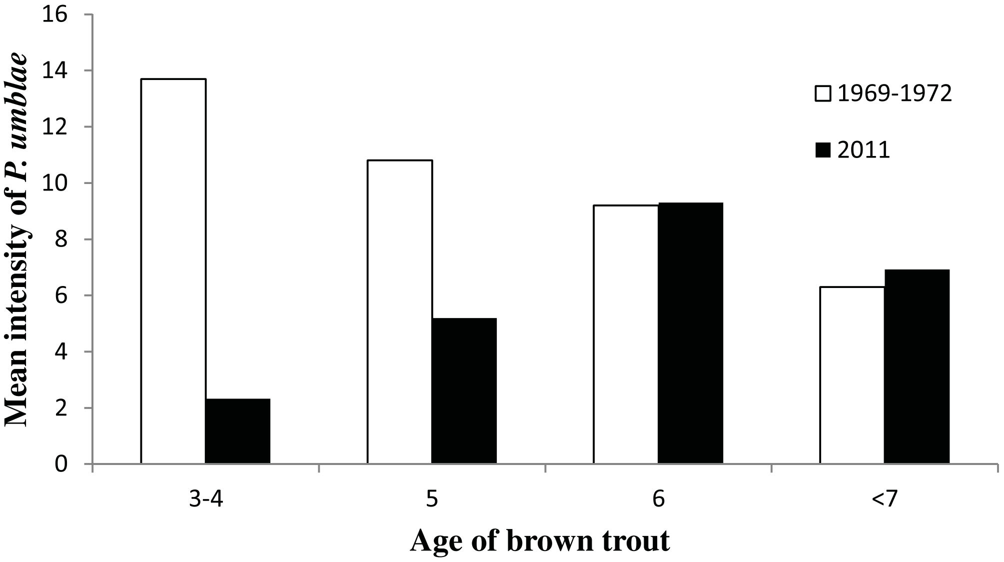

Figure 16 Mean intensity of P. umblae on age groups of brown trout from the lake Øvre Heimdalsvatn in 1969-72 and 2011

3.2.6 *Cyathocephalus truncatus*

The mean intensity of infection of *C. truncatus* was lower in 2011 than in 1969-72 (Table 2). In 2011, the prevalence was 37.5 per cent, with a mean intensity of 3.56. The frequency distribution was typically over-dispersed, with the majority of fish uninfected but with high infections in a few fish (Fig. 17). There was a significant relation between the volume percent of *Gammarus* and infection of *C. truncatus* (Anova, Lm, F-value = 8.72, P= 0.0042).

The relationship between log variance and log mean was linear ($R^2 = 0.995$, $b = 2.11$), suggesting that parasite infection/death processes are consistent across all length-classes of fish sampled

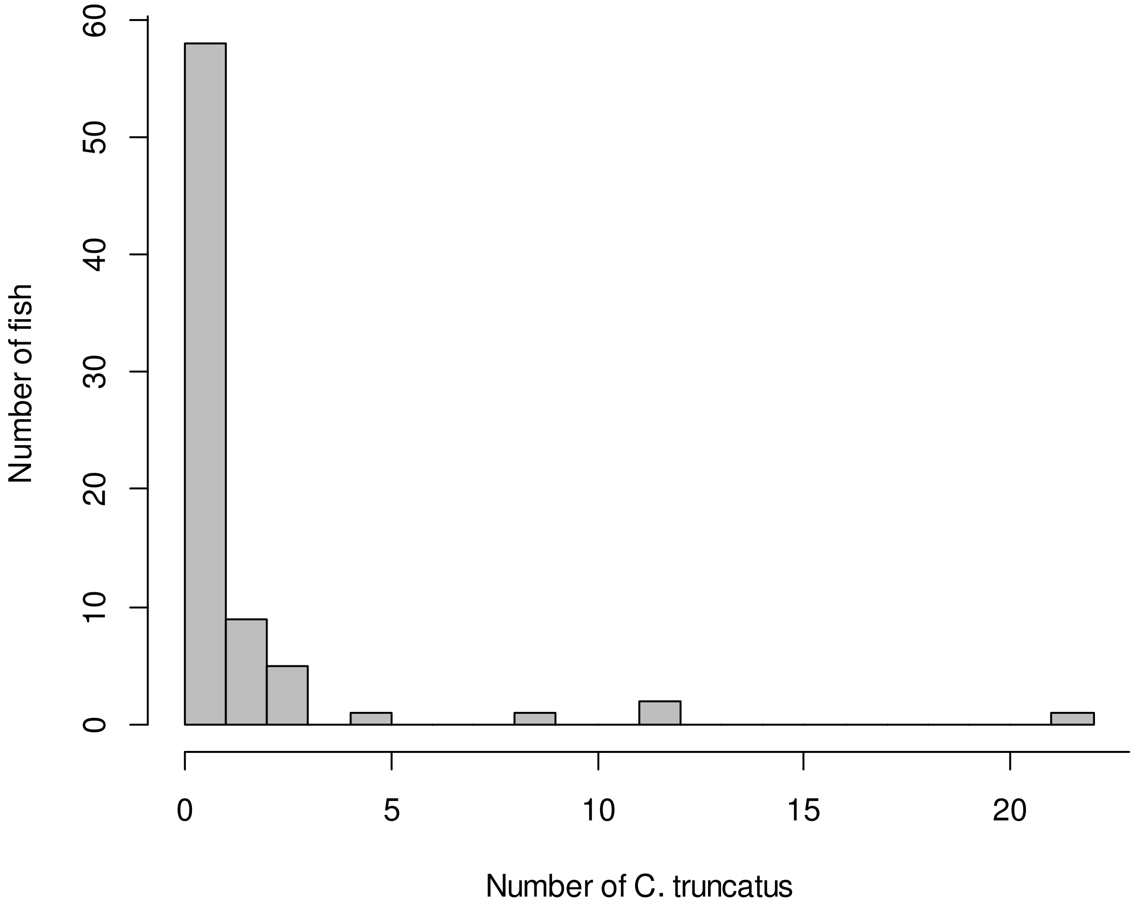

Figure 17 Distribution of *C. truncatus* in brown trout caught in the lake Øvre Heimdalsvatn in June, July and September 2011

3.2.7 *Proteocephalus longicollis*

The molecular analysis of the *Proteocephalus* species was confirmed as *P. longicollis* (Genbank: AY551166.1), with 99 % homology with species from Switzerland.

P. longicollis was primarily found in June, with only one specimen found in July and none in September. In June, 29 per cent of the examined fish were infected with *P. longicollis*, and the mean intensity was 4.7. The infection intensity ranged from 1 to 6 except in one fish which contained 20 worms (Fig. 18).

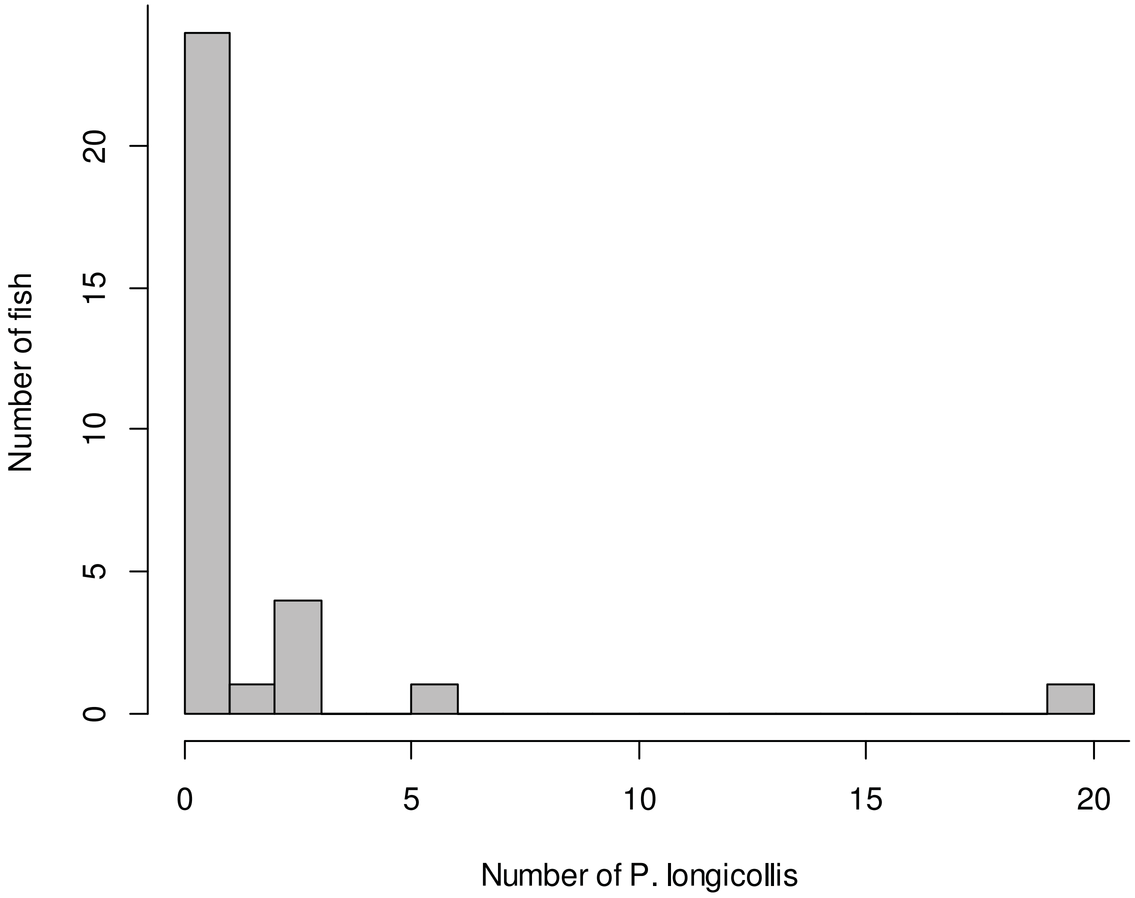

Figure 18 Distribution of *P. longicollis* in brown trout caught in the lake Øvre Heimdalsvatn in June 2011

3.2.8 *Capillaria* spp.

The mean intensity of *Capillaria sp*. (Fig. 20) for each age group of trout differed between 1969 -1972 and 2011 (Table 2). *Capillaria sp.* had the highest mean intensity in age-class 5 years in 1969-72, whilst in 2011 the highest mean intensity was in the age class 6 years (Fig.19). The relation between log variance and log mean ($R^2 = 0.95$, $b = 1.62$) suggest that parasite infection/death processes are consistent across all length-classes of fish sampled.

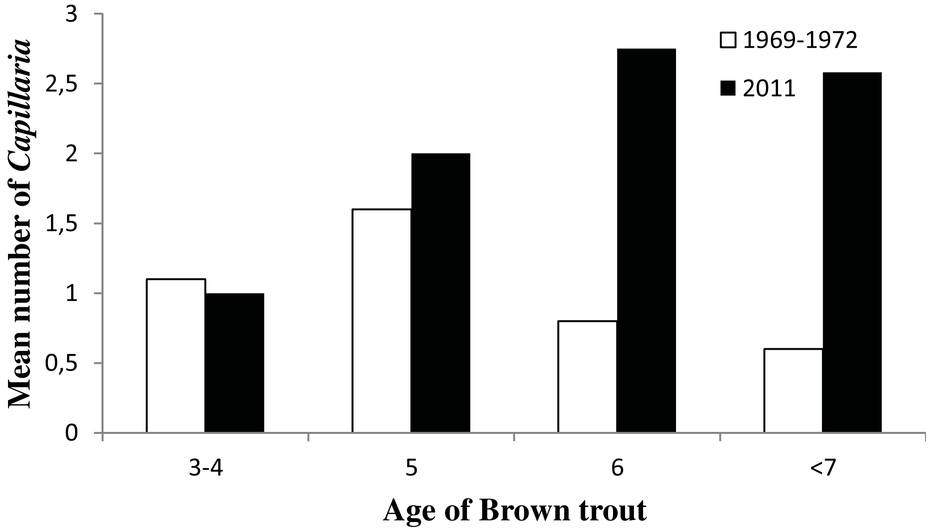

Figure 19 Mean intensity of *Capillaria* sp. on age groups of brown trout from the lake Øvre Heimdalsvatn in 1969-72 and 2011

Figure 20 Picture of *Capillaria* sp. male with spicule (left) and female with eggs (right), picture taken with Differential Interference Microscopy. Magnified 400X

4 Discussion

The abundance of some parasite species in 2011 showed a completely different picture than in 1969-1972, suggesting long term changes in the parasitic fauna in the lake. The most obvious differences are the much higher numbers of *Diphyllobothrium ditremum* and *Diplostomum spathaceum,* and the substantial decrease in the number of *Crepidostomum* spp. Other species, including *Phyllodistomum umblae, Discocotyle sagittata,* and *Cyathocephalus truncatus* did not show any marked differences in occurrence between 1969-72 and 2011. These observations may be explained by long-term changes in (i) the population density of both brown trout and European minnow, (ii) density of birds as hosts for parasites of which fish are intermediate hosts, and (iii) the availability of invertebrate prey functioning as intermediate hosts for parasites which have brown trout either as a host or as an intermediate host.

The identity of the *Diphyllobothrium* species in the lake was confirmed by molecular means as *D. ditremum*. This species was almost absent from the lake during the period 1969-72 (Lien pers. comm.), whereas in 2011 it was found in fish of every age-class, with high numbers (up to 279) in some fish.

D. ditremum has copepods and trout as first and second intermediate hosts respectively, and fish-eating birds as final host (Andersen & Gibson 1989). Since the brown trout population density has not changed markedly during the intervening 40 years (Borgstrøm et al. 2010), the increase in infection is likely to be due to changes regarding infection of copepods as a result of an increased number of infected fish-eating birds, or increased predation on copepods by brown trout. The number of fish-eating birds is higher than in 1970-72 (Prof. Jan T. Lifjeld pers. comm.; Lien 1978a), probably as a result of the establishment of European minnow, making the lake able to sustain a larger number of breeding pairs. This may therefore be significant in increasing the abundance of *D. ditremum* in brown trout via an increased number of parasites in the final hosts (most importantly *Merganser merganser, M. serrator* and *Gavia arctica*). The high numbers of *D. ditremum* in some trout may also be a result of cannibalism, since brown trout have also included small individuals of their own species in the diet during the last few decades (Borgstrøm et al. 2010), while cannibalism was not recorded during the period 1969-1972 (Lien 1978b). However, only minnows were found in the diet during the present study. If an infected fish is eaten by a cannibal, the plerocercoids can survive and re-establish in the new host, although it has been demonstrated that *D. ditremum* has a poor ability to re-establish in fish (Halvorsen & Wissler 1973). According to the log variance, log mean ratio (Taylor's power law, Taylor, 1961), the infection of *D. ditremum* does not increase the mortality of the brown trout host (Anderson & Gordon 1982), a seen in Lake Røyetjern. In Lake Røyetjern, the high infections of *D. ditremum* increased the mortality of the Arctic charr population (Halvorsen & Andersen 1984). However, Arctic charr in Lake Røyetjern was overpopulated with individuals stagnating in growth at a small size, probably making them more available for loon predation, resulting in high mortality rates, especially of those individuals which had high parasite burdens (up to 200) (Halvorsen & Andersen 1984). Some fish may also specialize on certain prey species, such as copepods, as seen in Arctic charr (Knudsen et al. 1996), and thereby obtaining a high parasite burden.

The second *Diphyllobothrium* species found in the lake was not ID confirmed by molecular means, but are probably *D. dendriticum*. The marker used, Ribosomal ITS, is not good at discriminating *D. ditremum* and *D. dendriticum* (Yera et al. 2008). The mitochondrial marker Cytochrome c Oxidase 1 (*cox*1), is more suitable for distinguishing between the two species, and places them in distinct phylogenetic clades (Wicht et al. 2010a). Another possible method to distinguish between *Diphyllobothrium* species is using multiplex PCR, however more research is needed to optimize the diagnostic test (Wicht et al. 2010b). Multiplex PCR is a cheaper and faster molecular test, where one can determine the species only from the PCR, which could be of significant use in future studies. *D. dendriticum* was not found by Lien in 1969-72, while I found only one specimen. This species is most common along the coast, and especially in lakes with three-spined sticklebacks (*Gasterosteus aculeatus*) and with gulls as final hosts (Vik 1959). Common gulls are breeding around Øvre Heimdalsvatn, and they may already be infected upon arrival to the lake in the spring, but lack of sticklebacks may be an obstacle for establishment in brown trout in the lake Øvre Heimdalsvatn.

The increase of fish-eating birds may also have affected the number of *Diplostomum spathaceum*. The mean intensity is considerably higher in every year-class compared to 1969-72, with high numbers in some fish (up to 269). Fish-eating birds are the final host, while *Lymnaea* and a variety of fish are first and second intermediate hosts, respectively (Palmieri et al. 1976). *Lymnaea peregra* is the only suitable first intermediate host in Øvre Heimdalsvatn, and is mainly located in the shallows of the lake, although it can be found sporadically at all depths of the lake (Brittain 1978). Both brown trout and European minnow may be intermediate hosts for this parasite, and thus the number of intermediate hosts have increased considerably since 1969-72 (Museth et al. 2002). With an additional increase in number of fish-eating birds, the increased number of *D. spathaceum* in brown trout may be explained.

The distribution of the parasite in Øvre Heimdalsvatn shows a significant relationship with age of fish, which may be due to exposure to infection over time. Free-living cercariae penetrate the skin or gills of fish (Karvonen et al. 2003), and since an older fish would have been exposed for longer, the infection may build up in old fish. The free-swimming cercariae do not actively search for hosts, but are often found in concentrations around infected snails (Karvonen et al. 2003), which indicates that older brown trout are present in the shallow littoral of the lake, possibly to feed on minnows, where the risk of infection is higher. However, it is demonstrated that trout can recognize and perform avoiding behavior in areas with high densities of cercariae, and develop a physiological resistance to lower the infection (Karvonen et al. 2004).

The mean intensity of *Crepidostomum* sp. was substantially lower in all age-classes relative to 1969-72. Parasites were typically younger and smaller in September compared with June-July, and were more likely to be overlooked during sampling, which could be a source of error when comparing with the results from 1969-72, since Lien sampled throughout the year. *Crepidostomum* has molluscs, *Lymnaea* and *Pisidium*, as first host, mayflies (*Ephemeroptera* sp.) or amphipods (*G. lacustris*) as second intermediate host, and trout as final host (Awachie 1968). The cercariae emerge from the mollusc and penetrate the mayflies or amphipods, which in turn have to be eaten by trout to complete the life cycle. Since the density of the trout population has not changed much (Borgstrøm et al. 2010), the change in abundance of *Crepidostomum* is most likely due to changes in densities of one, or both of the intermediate hosts. A reduction in availability of mayflies, the second intermediate host, could be the reason for the decrease in abundance. Mayflies were common in the brown trout diet in 1969-1972 (Lien 1978b), while they were infrequently found as part of the stomach contents of brown trout during the present study. However, the relative frequency of *Ephemeroptera* in the lake has increased (Næstad & Brittain 2010), suggesting that trout may have switched to other prey.

There was a significant decrease in numbers of *Crepidostomum* throughout the summer, which could be related to the seasonality of new infections as found in other lakes. Fitzgerald (1983) found the highest occurrence during winter - early spring, and the lowest during mid-summer. Awachie (1968) found that *C. metoecus* could not establish itself in temperatures exceeding 10 °C. The water temperature in Øvre Heimdalsvatn has increased over the last decades and are frequently above 10°C during late summer (Kvambekk & Melvold 2010).

This could be an influencing factor to the low numbers in abundance in the present study, suggesting a higher transmission during early summer and late autumn.

As already stated, the population density of brown trout has not changed markedly from 1969-72 until today (Borgstrøm et al. 2010), and brown trout parasites with direct life cycles are therefore expected to show only small differences in abundance. The gill fluke, *D. sagittata,* was the only parasite recorded which has a direct life cycle. The mean intensity was slightly higher in 2011 than in1969-72, perhaps due to seasonal variations in occurrence. *D. sagittata* has a temperature dependent transmission, and is therefore able to reproduce and transmit most effectively during the warmer months (Rubio-Godoy & Tinsley 2008). Lien sampled throughout the year, whereas the present study was confined to the summer months, and the higher numbers of *D. sagittata* may therefore be due to the sampling procedure. This may also be the reason for the apparent decrease in the abundance of *P. longicollis*. *P. longicollis* also has a seasonal occurrence, and is typically at its lowest during the summer months (Lien & Borgstrøm 1973). Other cestodes in fish, like *Triaenophorus nodulosus* in pike (*Esox lucius*), also more or less disappear during the summer as a result of a release of mature worms in the spring (Borgstrøm 1970). This suggests that the low numbers of *P. longicollis* found during the summer 2011 may therefore be due to the loss of worms in spring- early summer, and with no sampling during late autumn and winter, my results regarding this parasite are not comparable with Lien's results. *P. longicollis* has copepods as intermediate host and the adult stage in fish (Hanzelova & Gerdeaux 2003). According to Lien (1978b), copepods are found in the trout diet during winter and spring, which coincides with the diet I found. Copepods were virtually absent from the diet of brown trout during June - September, and this may explain the disappearance of *P. longicollis* during the summer months, as also suggested by Hanzelova & Gerdaux (2003). This suggests that the brown trout gets infested by eating infected copepods during autumn and early winter, and that *P. longicollis* spend the winter in the trout host.

C. truncatus has amphipods (*G. lacustris*) as intermediate host, and fish as final host (Vik 1958). The decrease in infection of *C. truncatus* in all age-classes of brown trout from 1969 - 1972 may be explained by a reduction in population density of *G. lacustris*. *G. lacustris* has declined in the littoral of the lake, probably as a consequence of European minnow predation (Næstad & Brittain 2010). However, *G. lacustris* is still common in the brown trout summer diet of the larger fish (Borgstrøm et al. 2010), and was found during the present study. Seasonal variation in transmission of *C. truncatus* is known (Amundsen et al. 2003), but typically with the lowest infections during late winter and early summer. Since the density of *G. lacustris* is strongly reduced in the littoral area (Næstad & Brittain 2010), this may influence the success of eggs of *C. truncatus* in reaching the intermediate host, and thus result in a lower infection in the brown trout as well.

The kidney fluke *P. umblae* was found to have similar mean intensity as in Liens study, and it is not known to have seasonal occurrence (Rahkonen & Valtonen 1987). However, there was a shift in the age which showed the highest infection, from 3-4 years in 1969-72, to 6 years in 2011. This could be a peak shift, indicating a lower transmission rate (Anderson & May 1985), which once more may be explained by a decreased density of either *Sphaerium* or arthropods as first and second intermediate host respectively (Hoffman 1999). However, the life cycle is not completely understood regarding the cercariae, which either are ingested directly by the trout or via an arthropod. There is a similar shift in the infection of *Capillaria* sp. again suggesting a lower transmission of the parasite. *Capillaria* sp. has uncertainties regarding the life cycle, but it is thought to have an oligochaete as intermediate host (Hoffman 1999). Since both *Capillaria* sp. and *P. umblae* has uncertainties concerning their life cycles, it is difficult to discuss possible reasons for a potential change in transmission.

After the introduction of the European minnow to the lake, Øvre Heimdalsvatn, there have been major changes occurring in the lake. The dynamics of the brown trout population have changed with reduced individual growth and recruitment, the invertebrate community in the lake has been altered, and the number of fish-eating birds connected to the lake has increased markedly. The change in the invertebrate community is possibly most important regarding changes in the parasitic fauna, since most of the parasites rely on invertebrates as intermediate hosts. The difference in abundance of the parasite species over the last 40 years probably reflects long term changes in the parasitic fauna of the lake. Considering all the factors influencing the parasitic fauna, it is likely that most of the major changes in the parasitic fauna of brown trout over the last 40 years have occurred as a consequence of the introduction of the European minnow. The overall result is increased infection intensities of some species, and decrease in others, depending on the life cycle of each species.

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