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Infection of *Diphyllobothrium ditremum* in brown trout, *Salmo trutta*, in Lake Øvre Heimdalsvatn, analysed in relation to diet, trophic status and mercury concentration

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Abstract

There have been profound alternations in the parasite fauna of brown trout in the subalpine lake Øvre Heimdalsvatn, since the first survey of the parasite fauna was conducted in 1969 - 71. One of the most prominent changes is the strong increase in infection of *Diphyllobothrium ditremum*. Accordingly the main objective of this study was to examine the cause and effect of the increased infection of *D. ditremum* in brown trout by looking at differences in feeding behaviour and parasite distribution. Since infection in brown trout with plerocercoids of *D. ditremum* may be caused by both feeding on infected copepods and by feeding on infected conspecifics, the diet of brown trout was studied with the aim of assessing the independent role of each of the two food items in transferring *D. ditremum* to the brown trout population. This was conducted by studying infection of *D. ditremum*, together with stomach content analysis, analyses of stable isotopes of nitrogen and carbon, as well as mercury concentrations in brown trout from Ø. Heimdalsvatn.

The results strongly suggests that copepods constitute the main source of *D. ditremum* to the brown trout population as there were no clear correlation between the trophic level of brown trout and the intensity of *D. ditremum*. Both prey items, however, constituted a small amount of the diet of the trout investigated. Even so, it is likely that there has been an increase in the proportion of infected copepods in the diet of brown trout following an increased number of birds, functioning as final hosts for *D. ditremum*, foraging in the lake. The increased number of final hosts will lead to a higher output of *D. ditremum* eggs into the water, most likely leading to a larger proportion of the copepod population being infected, thus increasing the number of *D. ditremum* larvae being transferred to the brown trout, when copepods are fed upon. Brown trout males had higher probability of being infected with *D. ditremum* and had higher intensity than females. This is most likely a result of males utilizing the near shore habitats to a greater extent than females. These areas have presumably higher proportion of infected copepods due uneven foraging activity of the final host.

Thus, European minnow seem to be the indirect reason for the increased infection of *D. ditremum* in brown trout in Lake Øvre Heimdalsvatn, by forming a larger food base for the final hosts.

Despite the substantial increase in infection, there were no clear signs of brown trout mortality caused by plerocercoids of *D. ditremum* in the present study.

Sammendrag

Det har skjedd store endringer i parasittfaunaen til brunørret i Øvre Heimdalsvatn siden parasittfaunaen for første gang ble undersøkt i 1969-71. En av de mest utpregede endringene er den store økningen i infeksjon av fiskandmakk. Følgelig har hovedformålet med oppgaven vært å undersøke årsak og virkning av økt fiskandmakkinfeksjon i brunørret, ved å se på ulikheter i diett og fordelingen av parasitten i brunørretpopulasjonen. Parasitten kan overføres til brunørret ved inntak av både infisert hoppekreps og infisert småørret. Dietten til brunørret har dermed blitt undersøkt ved hjelp av mageanalyser, analyser av stabile isotoper av nitrogen og karbon så vell som kvikksølvkonsentrasjon, for å undersøke byttedyrenes rolle i overføring av *D.ditremum* til brunørretpopulasjonen.

I følge resultatene er det sannsynlig at hoppekreps utgjør hovedkilden til fiskandmakk i brunørret ettersom det ikke var noen klar sammenheng mellom trofisk nivå og antall parasitter. Både småørret og hoppekreps utgjorde en liten del av dietten til brunørreten. Det er allikevel sannsynlig at det har skjedd en økning i mengden infiserte hoppekreps i dietten som følge av en økning i antall fiskespisende fugl ved vannet. Disse utgjør den endelige verten for parasitten og vil føre til at det slippes ut flere fiskandmakkegg i vannet nå enn tidligere. Dette har mest sannsynlig ført til at en større andel av hoppekrepspopulasjonen er blitt infisert, hvilket fører til at mer fiskandmakk vil overføres til brunørret i de tilfellene hvor brunørreten spiser hoppekreps.

Hannørret hadde større sannsynlighet for å være infisert med fiskandmakk og hadde større intensitet av parasitten enn hunnørret. Dette skyldes trolig at hannørret bruker de strandnære områdene i en større grad. Disse områdene har sannsynligvis en større konsentrasjon av infiserte hoppekreps enn andre deler av innsjøen som følge av ujevn aktivitet hos den endelige verten.

Den store økningen av fiskandmakk i brunørret i Øvre Heimdalsvatn ser ut til å være indirekte grunnet etableringen av den fremmede arten ørekyt, ved at den skaper et større næringsgrunnlag for de endelige vertene.

Til tross for den betydelige økningen i infeksjon av fiskandmakk, var det ingen tydelige indikasjoner på dødelighet hos brunørret som følge av parasitten.

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1. Introduction

Parasites occur in virtually all food webs and at all trophic levels (Marcogliese 2005). They often possess significant impact on the biology of their host population such as behaviour, reproduction and physiology and may constitute an important regulator of the host population (Dobson et al. 2008). Furthermore, parasites is suggested to play a vital role in structuring ecological communities (Marcogliese 2004; McCallum & Dobson 1995).

Fish serve as hosts to a range of parasites that are taxonomically diverse with a variety of life-history strategies (Barber et al. 2000). Some are trophically transmitted and have life cycles including several intermediate hosts (Amundsen et al. 2009). They thereby rely on trophic interactions to be transferred from one intermediate host to the next until they reach their final host. In freshwaters, fish often constitute the apex of the predator prey pyramid and thereby tend to be infected with a considerable amount of trophically transmitted parasites (Hoffman 1999). Since they are trophically transmitted, their presence in a host may provide valuable information of the long term feeding and habitat utilization of the host, as well as the presence of other hosts in the ecosystem that participate in the lifecycle of the parasites (Knudsen et al. 2014; Lafferty et al. 2008; Valtonen et al. 2010). Likewise, trophic parasites can be useful indicators of alternations in the ecosystem that affect the food web topology (Marcogliese 2005). Parasites may thereby provide valuable information of the stability, diversity and complexity of an ecosystem that are of great importance in conservation management (Lafferty et al. 2006; Marcogliese 2004).

Two common trophically transmitted parasite species in Scandinavian freshwater fish, primarily salmonids and sticklebacks; are the cestodes *Diphyllobothrium ditremum* (Creplin, 1825) and *Diphyllobothrium dendriticum* (Nitzh, 1824) (Andersen & Gibson 1989; Henricson 1977). These parasites have received much attention as a problem in fisheries since they may reduce condition and in some instances increase mortality of their second intermediate hosts, besides creating adverse effects on the recreational and commercial value of the fish (Andersen & Gibson 1989; Berube & Curtis 1986; Curtis 1984; Halvorsen & Andersen 1984; Kristoffersen et al. 1993; Rahkonen & Koski 1997;

Rodger 1991; Tolonen et al. 2000). Their detrimental effects on the fish intermediate host are mainly due to their penetration of body tissue and visceral organs causing inflammation and fibrosis (Curtis 1984; Rodger 1991; Sharp et al. 1989).

The plerocercoids of *D. dendriticum* may be encysted on most abdominal organs, such as the esophagus, stomach, liver, kidney and swim-bladder, and in some cases more or less totally cover the inner organs with cysts (Curtis 1984). The smaller plerocercoids of *D. ditremum* primarily encyst on the wall of the esophagus, stomach and pyloric caeca, and are suggested to be less detrimental to the fish host than *D. dendriticum* (Curtis 1984; Henricson 1977; Vik 1964b). High infections of *D. ditremum* have, however, been suggested to contribute significantly to mortalities of Arctic charr (*Salvelinus alpinus*) (Halvorsen & Andersen 1984; Kristoffersen et al. 1993), cage-reared juveniles of Atlantic salmon (*Salmo salar*) (Rodger 1991) and captive and transplanted Coho-salmon (*Oncorhynchus kisutch*)(Weiland & Meyers 1989).

Diphyllobothrium spp. possess complex life cycles including larval stages (procercoid and plerocercoid) in at least two intermediate hosts, i. e. copepods and fish, serving as first- and second intermediate host, respectively (Vik 1964a) (fig. 1). Since the larvae of *Diphyllobothrium* are trophically transmitted, they are dependent upon the next host to eat the current intermediate host, and so on, until they reach their final host, which may be birds or mammals (Henricson 1978). In the final host, they end up in the intestine where they develop into mature, egg-producing adults, the eggs are thereby shed out through the feces (Amundsen et al. 2009; Berube & Curtis 1986; Hartvigsen 1997; Henricson 1977).

Figur 1: Lifecycle of *D. ditremum*. a) *D. ditremum* eggs released into the water through bird droppings, b) free living larvae (coracidium), c) procercoids in copepods (first intermediate host), d) plerocercoids in arctic charr (second intermediate host) in which it can live for several years (Gallagher & Dick 2010) and e) mature *D. ditremum* larvae in *Gavia arctica* (final host). (From Kristoffersen (1989).

Brown trout (*Salmo trutta*) and arctic charr have also been found to serve as a third intermediate hosts for *Diphyllobothrium spp*. as a result of plerocercoid transmission through piscivory (Berube & Curtis 1986; Curtis 1984; Gallagher & Dick 2010; Hammar 2000; Haugstvedt Henriksen 2014; Knudsen et al. 2008). Since fish can host a significantly higher number of *Diphyllobothrium larvae* than copepods, it is reasonable to believe that piscivorous and cannibalistic brown trout and arctic charr, will obtain a higher amount of *Diphyllobothrium larvae* than non-piscivorous conspecifics. On the basis of experimental studies, however, plerocercoids of *D. ditremum* have demonstrated a much lower survival-rate when transferred from prey-fish to rainbow trout (*Oncorhynchus mykiss*), than *D. dendriticum* (Halvorsen & Andersen 1973; Halvorsen, O. & Wissler, S. K. 1973) which has been proposed to be a god indicator of piscivorous feeding (Knudsen et al. 2008). Nonetheless, transmission of *D. ditremum* through piscivory has been suggested to be of major importance for the infection rate in cannibalistic arctic charr (Gallagher & Dick 2010; Hammar 2000) and in arctic charr feeding upon sticklebacks (Berube & Curtis 1986; Curtis 1984; Gallagher & Dick 2010; Knudsen et al. 1996a). *D. ditremum* was also suggested to be transferred through piscivory in brown trout feeding upon arctic charr (Knudsen et al. 2008).

The long term habitat utilization and diet of fish has traditionally been estimated by looking at feeding behaviour or gut-content analyses (Atwell et al. 1998). Gut-content analyses, however, gives only information of what the fish has eaten most recently and does not necessarily reflect the long term feeding habits (Atwell et al. 1998; Gallagher & Dick 2010; Johnson et al. 2004). There might therefor be difficult to determine whether infection of *Diphyllobothrium* spp. is mainly due to piscivory of infected fish or feeding on infected copepods merely by looking at the stomach content.

An increasingly popular method which gives time integrated information of habitat use and diet is analysis of stable isotope ratios of nitrogen ($^{15}N/^{14}N$; $\delta^{15}N$) and carbon ($^{13}C/^{12}C$; $\delta^{13}C$) (Johnson et al. 2004; Layman et al. 2012; Peterson & Fry 1987; Post 2002; Rognerud et al. 2003; Vander Zanden & Rasmussen 1999).
As a general rule, the light isotopes (^{12}C, ^{14}N) form weaker bonds and reacts faster than heavier isotopes (^{13}C, ^{15}N) which lead to variations in $\delta^{15}N$ and $\delta^{13}C$-values among different chemical compounds (Rognerud et al. 2003).

When plants take up atmospheric CO_2 for use in photosynthesis, the carbon undergoes fractionation as a result of plants having a higher affinity for $^{12}CO_2$ than $^{13}CO_2$ (Farquhar et al. 1989; Rognerud et al. 2003). As different plant species have evolved different photosynthetic pathways, the uptake and fractionation varies between species, leading to differences in the $\delta^{13}C$-values at the base of the food web (France 1995; Rognerud et al. 2003). As there are only minor changes in the carbon isotope as it moves up through the food chain, usually an enrichment factor of less than 1 ‰, it makes a useful tool in providing information of the carbon sources in higher consumers (Post 2002; Rognerud et al. 2003). This technique is useful in lakes for distinguishing between two major energy-sources; littoral production from attached algae and detritus, and pelagic production from phytoplankton which tends to be diminished in ^{13}C (more negative $\delta^{13}C$) relative to the base of the littoral food web (France 1995; Post 2002).

The nitrogen isotope, ^{15}N, however, increases with an average of 3 – 4 ‰ from prey to predator as a result of the lighter isotope is more readily excreted out through metabolic processes (Kidd et al. 1995). This makes $\delta^{15}N$ a good tool for estimating the trophic position of organisms (Post 2002). Mercury (Hg) level can also give information of the trophic level of fish since it biomagnify, that is, increase in concentration with increasing trophic level of the organism, thus leading to accumulated Hg values in the

predator (Cabana & Rasmussen 1996; Desta et al. 2008; Hall et al. 1997; Power et al. 2002; Rognerud et al. 2002). Analysis of stable isotopes and Hg gives several advantages over traditional gut-content analysis as they are unaffected by sampling errors such as temporal changes in availability and different digestion time of prey items (Eloranta et al. 2013; Gallagher & Dick 2010; Johnson et al. 2004). Traditional gut analysis, however, is useful for identifying individual prey items in contrast to isotope and mercury analysis (Johnson et al. 2004).

Typically, *Diphyllobothrium spp*. show clumped (over – dispersed) distribution in fish intermediate hosts where the majority of hosts tend to harbor few parasites while a few hosts harbor the major proportion of the parasite population (Anderson & Gordon 1982; Halvorsen & Andersen 1973; Knudsen & Klemetsen 1994; Kristoffersen et al. 1993; Tolonen et al. 2000; Valtonen & Julkunen 1995). This pattern is commonly found for helminthes in nature (Halvorsen & Andersen 1984; Henricson 1977; Knudsen 1997; Shaw et al. 1998; Wilson et al. 2002) and is known to enhance the density-dependent regulation of both host and parasites (Anderson & Gordon 1982). It is in the hosts that harbor the major proportion of parasites, the density dependent processes exert their regulatory influence, such as the impact on host survival and fertility (Anderson & Gordon 1982) . This distribution pattern may further provide information about parasite induced host mortality, which will be discussed later in the method chapter.

Even though the proximate causes are poorly understood, the principal factors responsible for the generation of over-dispersion in natural populations of hosts are commonly thought to be heterogeneity among hosts in the exposure to infective parasite stages and differences in susceptibility or defensive capabilities (Halvorsen & Andersen 1973; Henricson 1977; Munger et al. 1989; Wassom et al. 1986). Heterogeneity in host traits and environmental conditions, however, has been suggested to be of greater importance than immunity and genetics under natural conditions (Knudsen et al. 2004). Heterogeneity in exposure is to a large extent due to different habitat use of individual fish and their feeding habits (Knudsen et al. 1996b; Knudsen et al. 2004; Valtonen & Julkunen 1995).

Both the pattern and level of parasite infection in a host population is determined by the rate of parasite flow through an intermediate host system (Henricson 1978). The main factors controlling this parasite transmission are suggested to be the availability of infective larvae and host feeding habits (Henricson 1978).
It is therefore reasonable to expect that several factors, amongst which changes in density of invertebrate prey species that serve as intermediate hosts, changes in fish population density, in abundance of piscivorous predators, as well as changes in the number of final hosts, may all influence the parasite burden with *Diphyllobothrium* plerocercoids in a fish population.

Introduced species can lead to severe alternations in a food web by acting as a consumer or prey for the existing species (Amundsen et al. 2013; Strayer 2010). They may further aid the arrival of other organisms using the new species as a resource. Thus, introduced species may create considerable changes in parasite fauna and abundance (Gozlan et al. 2010).
An example of a human assisted trans-location of a freshwater fish species in Norway is the extensive and severe spread of European minnow (*Phoxinus phoxinus*) during the last 100 –140 years (Hestehagen & Sandlund 2010; Museth et al. 2007). This species has demonstrated a great phenotypic and ecological plasticity which has made it successful in a range of new locations (Museth et al. 2007).
In the Norwegian subalpine Lake Øvre Heimdalsvatn, European minnow was first observed in 1969 (Lien 1981). One decade after the first observation, the population had increased significantly (Lien 1981), and some decades later, considerable changes in the lake ecosystem were evident, especially the brown trout and European minnow dynamics (Borgstrøm et al. 2010; Museth et al. 2002; Museth et al. 2010) and the benthic community (Næstad & Brittain 2010). Fish (mainly European minnow) has become an important part of the summer diet of brown trout in Ø. Heimdalsvatn (Bilstad & Bilstad 2006; Borgstrøm et al. 2010; Hagen 2003; Hasle & Skjølås 1995; Hatleli 2012; Museth et al. 2002; Museth et al. 2010), and is thereby expected to possess a higher trophic level than during the initial period where fish were not detected as part of the trout diet (Lien 1978b). Further has there been significant changes in the parasite fauna and abundance in the brown trout population (Hatleli 2012). One of the most prominent changes is the significant increase in the infection of *D. ditremum* (Hatleli 2012).

Øvre Heimdalsvatn has been the object of extensive studies both before and after the observed increase in *D. ditremum* which provide an excellent opportunity for studying the cause and effect of *D. ditremum* infection in a brown trout population.
Accordingly, the main objective of this study is to examine the substantial increase in infection (prevalence and intensity) of *D. ditremum* in brown trout in Ø. Heimdalsvatn by looking at differences in feeding behavior and the distribution pattern of *D. ditremum*

Since infection in brown trout with plerocercoids of *D. ditremum* may be caused by both feeding on infected copepods (*Cyclops* spp.) and by feeding on infected conspecifics, it is expected i) that there might be a positive relationship between both $\delta^{15}N$ and Hg concentration and the occurrence and intensity of plerocercoids of *D. ditremum* in brown trout from Ø. Heimdalsvatn, and ii) if not, it is more likely that the main plerocercoid burden in brown trout is caused mainly through feeding on copepods. Accordingly, I have studied infection of *D. ditremum*, together with stomach content analyses, analyses of stable isotopes of nitrogen and carbon, as well as mercury concentrations in brown trout from Ø. Heimdalsvatn.

2. Materials and methods

2.1 Lake Øvre Heimdalsvatn

The subalpine lake Øvre Heimdalsvatn lies in the valley Øvre Heimdalen, located on the eastern slope of Jotunheimen, 1088 m a.s.l., in Øystre Slidre municipality, Oppland County (fig. 2). The lake has a surface area of 0,775 km^2 and average depth of 4,7 m (Grøterud & Kloster 1978; Vik 1978). The catchment area covers 23,6 km^2 (Vik 1978), extending up over 1.800 m a.s.l, into the high alpine zone (Østhagen & Egelie 1978). Several streams enter the lake, but the main inlet stream is Brurskardbekken which rises from the small lake, Brurskardtjern 1309 m a.s.l. (Vik 1978). The period of ice-cover usually lasts from about the end of October to early June (Grøterud & Kloster 1978; Kvambekk & Melvold 2010). During the ice free season the water column is well mixed due to strong winds, thus the lake rarely experience thermal and chemical stratification, but have quite even temperatures and chemical concentrations throughout the lake (Grøterud & Kloster 1978; Kloster 1978; Vik 1978). Except for grazing by domestic livestock during summer, the valley is little influenced by human activity, and there is no permanent habitation (Vik 1978). On the other hand, the catchment received major radionuclide fallout from the Chernobyl accident in 1986 (Brittain & Bjørnstad 2010).

Figure 2: The geographical position of Lake Øvre Heimdalsvatn, situated in Oppland County, in south central Norway, is marked with a red circle (from Norgeskart.no).

The lake, Øvre Heimdalsvatn (fig. 3), has been the subject of extensive studies covering a wide range of disciplines since 1957 when Jensen (1977) started up his work on brown trout dynamics. The extensive knowledge of this ecosystem coupled with little influence as regards to its catchment area and no significant local sources of pollution, the lake have become an important reference site on subalpine ecosystems (Brittain & Borgstrøm 2010; Vik 1978).

Figure 3: The lake Øvre Heimdalsvatn during July 2013, looking westwards (photo: J. Trømborg).

Before the introduction and establishment of the European minnow (fig. 4), brown trout were the sole fish species in the lake. The brown trout population was dense and characterized by small individuals with stunted growth (Jensen 1977). After a stock-depletion program carried out in 1958 – 1969, the growth rate of the brown trout increased significantly, but showed a significant decrease again in the period 1993 – 2006 despite no significant change in the population density of brown trout (Borgstrøm et al. 2010). During the same period, a substantial decrease in the recruitment to the brown trout population was evident, resulting in a skewed aged distribution towards older individuals (Borgstrøm et al. 2010).

In the period after the establishment of the European minnow, there have been changes in both the summer diet and parasitic fauna of brown trout (Borgstrøm et al. 2010; Hatleli 2012), as well as considerable changes in the macroinvertebrate benthos, the

most prominent change being the major reduction in the population of *G. lacustris,* while the proportions of smaller forms, especially chironomids and Oligochaeta have increased (Brittain et al. 1988; Næstad & Brittain 2010). In terms of the summer diet of brown trout, it has changed from a dominance of the large and easily available *Lepidurus arcticus* to contain a considerable amount of fish (mainly minnows) which was not found as a food item in the initial period (Bilstad & Bilstad 2006; Borgstrøm et al. 2010; Hagen 2003; Hasle & Skjølås 1995; Hatleli 2012; Lien 1978b).

There has been a significant change in the abundance of several parasite species of brown trout from the period 1969-71 to 2011, including a strong increase of *D. ditremum* (Hatleli 2012). During the same time period there has been a substantial increase in the number of fish eating birds foraging in the lake (Hatleli 2012), including Common merganser (*Mergus merganser*), Red-breasted merganser (*Mergus serrator*) and Black-throated loon (*Gavia arctica*) which all serve as final hosts for *D. ditremum* (Henricson 1977). During 1971 – 1972 only three individuals of Common merganser and Red-breasted merganser were spotted during the ice-free season (Lien 1978c). The common gull (*Larus canus*) was sighted a couple of times within the shore zone of Ø. Heimdalsvatn through the summer period of 1978 (Lien 1978a). In the latter years, about five nesting pairs of Common gull have been observed regularly around the lake, as well as sightings of one to three individuals of Arctic tern (*Sterna paradisaea*), and a pair of Black-throated loon (*Gavia arctica*) foraging in the lake (Hatleli 2012). In addition, three to five females of both Common merganser and Red-breasted merganser have regularly been nesting and foraging in close proximity of the lake during the last 20 years (Hatleli 2012). During the present study, June – October 2013, several individuals of merganser as well as loon were observed fishing in the lake.

Figure 4: Male of European minnow from Øvre Heimdalsvatn, with conspicuous breeding coloration; dark back, golden sides and bright red abdomen (Kekäläinen et al. 2010) (photo: R. Borgstrøm).

2.2 Brown trout sampling

A total of 181 brown trout was sampled by means of gillnetting in June, July, August and October 2013 (fig. 5). Nine different mesh sizes (16, 19.5, 22.5, 26, 31, 35, 39 and 45 millimeter bar mesh) were used in order to capture a wide range of size classes present in the lake. To do so, individual fish were selected for dissection on the criteria length with the goal to obtain a representative sample. Each gill net, 25 meter long and 1.5 meter high, was set from shallow water to deeper water in the littoral area around the whole lake (fig. 6). The total brown trout sample consisted of 106 males and 73 females, the age span was 3 to 19 years (winters), the length ranged from 13 to 45 cm and the weight ranged from 21 to 869 gram (table 1).

Figure 5: Fishing for brown trout by means of gillnet in the outflow of Øvre Heimdalsvatn October 2014 (photo: J. Trømborg).

Table 1: Sample characteristics showing mean, median, and max for age, length and weight of the total sample of trout caught in Øvre Heimdalsvatn during the present study.

Species	Age (winters)				Length (cm)				Weight (g)				Sex	
	Min	Med	Max	n	Min	Med	Max	n	Min	Med	Max	n	Male	Female
S. trutta	3	7	19	177	13.4	28	45.2	181	21	186	869	181	73	106

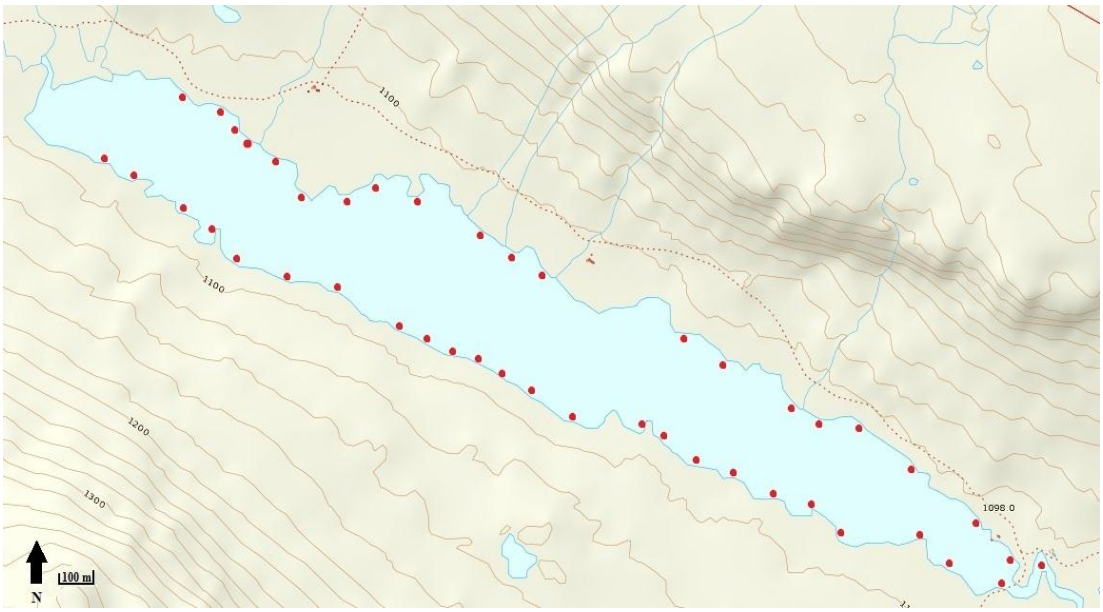


Figure 6: Map of Lake Øvre Heimdalsvatn with used gillnet position (Map copied from Norgeskart.no).

2.3 Zooplankton sampling

For studying the zooplankton composition in the lake, plankton samples were collected each month (June, July, August and October) by means of plankton net with mesh size 20 – 45 µm, operated after a row boat for a distance of about 30 – 50 m in the open water. The plankton samples were conserved by adding approximately 15 drops of Lugol`s iodine solution.

2.4 Sample preparation, age and diet of brown trout

The body cavity of each fish was thoroughly examined for plerocercoids of *D. ditremum* encysted on the esophagus-wall, stomach-wall, and on and between the pyloric caeca, in accordance with Vik (1964b) (fig. 7). The cysts were punctured and the parasite inside was pressed out using a tweezers. Plerocercoids were also found as free worms in the coelom. Each parasite were counted and placed in tap water for some hours before they were preserved in 70% ethanol. All sampled trout were weighted to nearest 0.1 gram on an electronic balance, and total length measured to nearest millimeter. Collection of esophagus/stomach contents, muscle sample for stable isotopes, as well as for mercury analyses, and sampling of otoliths and scales were done in accordance with EMERGE sampling manual for live fish (Rosseland et al. 2001). Otoliths and scales were stored in

paper (scale) envelopes for later age determination. Muscle samples of each fish were wrapped in Al-foil, placed in plastic zip bags, and frozen shortly after dissection. Determination of sex and maturation stage (I – VII) were done according to Sømme (1941 s. 223) (fig. 8). Fish age-, parasite- and zooplankton determination, and examination of stomach contents were carried out in the Ecology laboratory of the Department of Ecology and Natural Resource Management, at the Norwegian University of Life Sciences (NMBU).

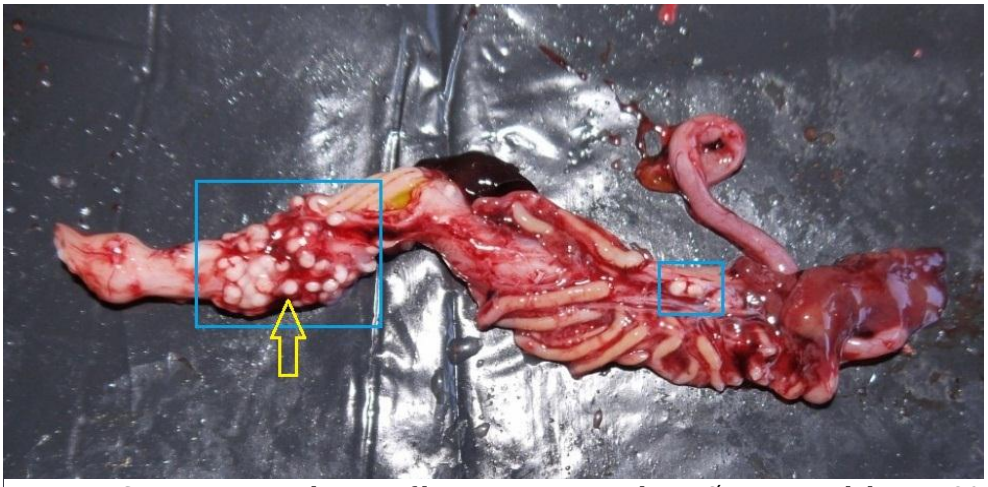


Figure 7: Gastrointestinal tract of brown trout caught in Øvre Heimdalsvatn 2013, with cysts of *D. ditremum* on oesophagus- and stomach wall (left) and between pyloric caeca (right) (picture: J. Trømborg).

Figure 8: Left: female stage II, and to the right a female in stage V/VI (photos: J. Trømborg).

2.4.1 Age determination

A total of 177 trout were age determined by means of otoliths and fish scales (fig. 9). The majority were age determined by reading of otoliths which, in contrast to fish-scales, continue to grow and produce new winter and summer-zones regardless of growth stagnation, though giving a more accurate result than fish scales (Jonnson 1976). Otoliths from fish longer than 20 centimeters were cut in half through the nucleus by use of a scalpel-blade, the half to be read were then polished by use of no1200 micro-mesh polishing sheet to make a planar surface, and then burned for some seconds until it reached a nut-brown colour. The burned otolith where thereafter placed in piece of plasticine with the polished surface pointing upwards, and immersed in 1, 2 – propandiol ($C_3H_8O_2$), to enhance clarity and prevent reflections. The otolith was then read under a Leica binocular microscope (fig. 9 b and d). A few individuals had hyaline otoliths, and in these cases the fish scales were used for age determination. Fish scales for age determination were pressed firmly onto a celluloid–strip and studied under a Micron 780A microfilm reader. For smaller trout (< 20 cm), the otoliths were placed whole in propandiol, and read directly against a dark background, under a binocular microscope (fig. 9 a and c). Otoliths from such small fish which were difficult to read directly, were placed in ethanol for clearing prior to the ageing, or treated as described for larger fish, i.e. by burning.

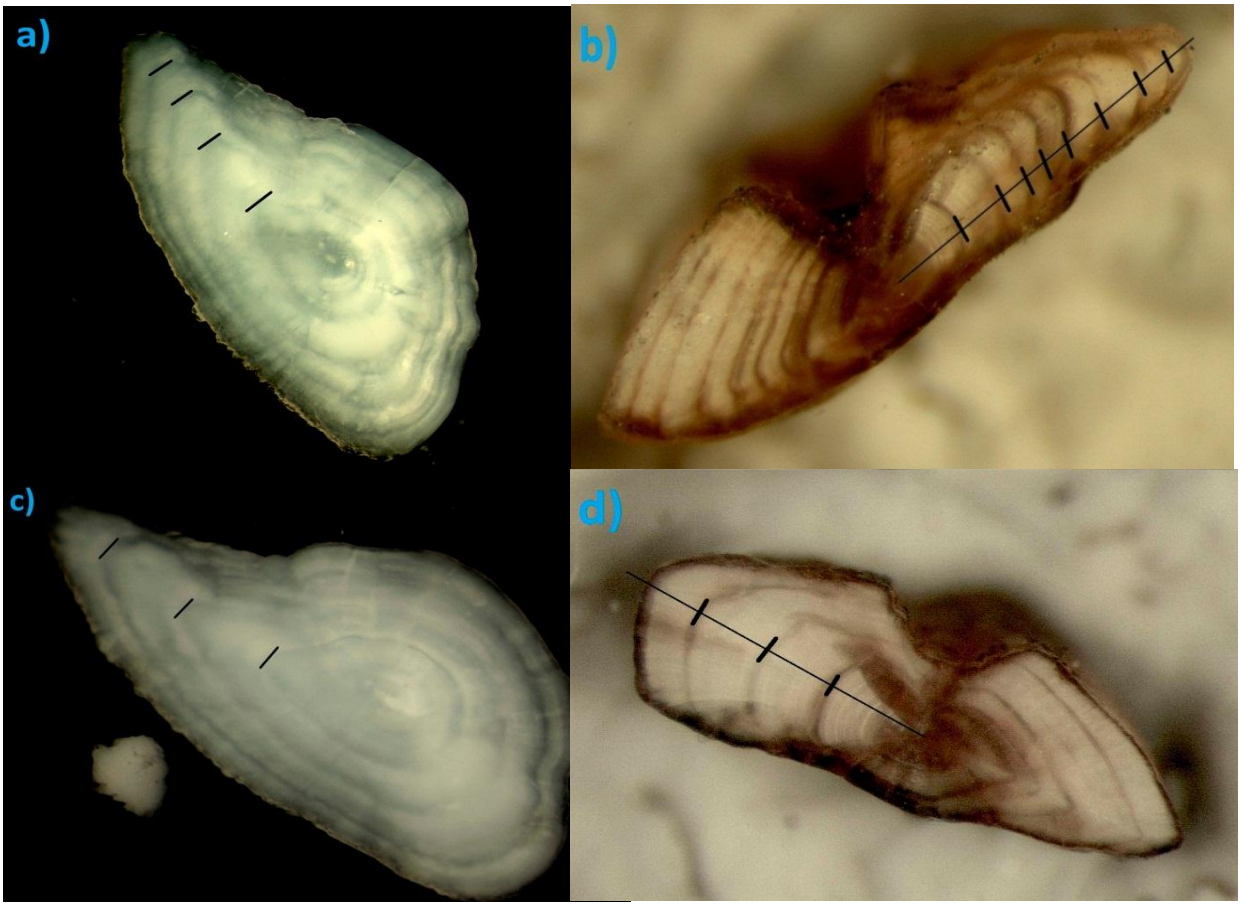


Figure 9: Pictures of otoliths, marked with winter-zones (short black lines), from brown trout sampled in Øvre Heimdalsvatn in June (c and d) and October (a and b) 2013. In the otoliths from October the zone representing last winter are clearly visible. Otolith a) and b) comes from 4- and 8 – winters old trout respectively. Otolith b) and c) are taken from two 4 – winters old trout. For the brown trout captured in June, the last winter-zone is still not visible and must be added when determining age (photo: J. Trømborg).

2.4.2 Diet analysis

The fullness of the oesophagus and stomach was classified in the field laboratory, according to a scale from 0 to 1, where 0 represents an empty oesophagus/stomach, and 1 equals a full oesophagus/stomach, i e. a modified Hynes point method (Hynes 1950). The stomach contents were then stored and preserved in small glass vials with 70% ethanol for later analysis.

Subsequently the stomach content of each brown trout examined was placed in separate Petri dishes. In the Petri dish, the food items were classified and sorted into the following categories: Chironomidae, Cladocera, Dytiscidae, Ephemeroptera, Trichoptera, Megaloptera, Oligochatea, Pisidium, Gastropoda, *Gammarus lacustris*, *Lepidurus arcticus*, Plecoptera, other (minor) benthic invertebrates, terrestrial insects, fish (brown trout or

European minnow), and trout eggs (fig. 10). Each category was assigned a percentage depending on how much it constituted of the total volume of that specific stomach content (P) (1 – 100 %). To eliminate the confounding effect of variations in stomach fullness among the different stomach samples, the percentage of each category was multiplied with the assigned level of stomach fullness (F). This gave a point reflecting the relation between the stomach fullness and how much the specific category amounted for (V). This was done for each of the separate stomach contents in the following way:

$$V = P \times F$$

For each category, the assigned points (V) from each of the stomach contents were summarized and the percentage each category amounted for in the total diet of brown trout examined were calculated.
Preyed fish which were found in the stomach contents were determined to the species (brown trout or European minnow) by use of jaw or pharyngeal bone (fig. 11). The jaw of brown trout contains sharp teeth in contrast to the toothless jaw of minnow, making them easy do identify. Total length (T) of prey minnow were estimated according to the method of Prenda et al. (2002), using the formula;

$$T = a + b * BL$$

Total bone length (BL) are the length of the pharyngeal bone shank (fig. 11 a) while a and b are constant values, a = 3, 31 and b = 17, 11.

Prey fish found in two stomach contents of piscivorous brown trout could not be identified by means of the jaw or pharyngeal bone. There was, however, possible to determine the age by studying the spine under a light microscope, in which the winter and summer zones became apparent. The two prey fishes were determined to age 5 and 6 years, respectively. The bone structure also reflected that these specimens were smaller than expected for 5 and 6 year old brown trout. In light of these observations, the two prey fishes were assumed to be minnows.

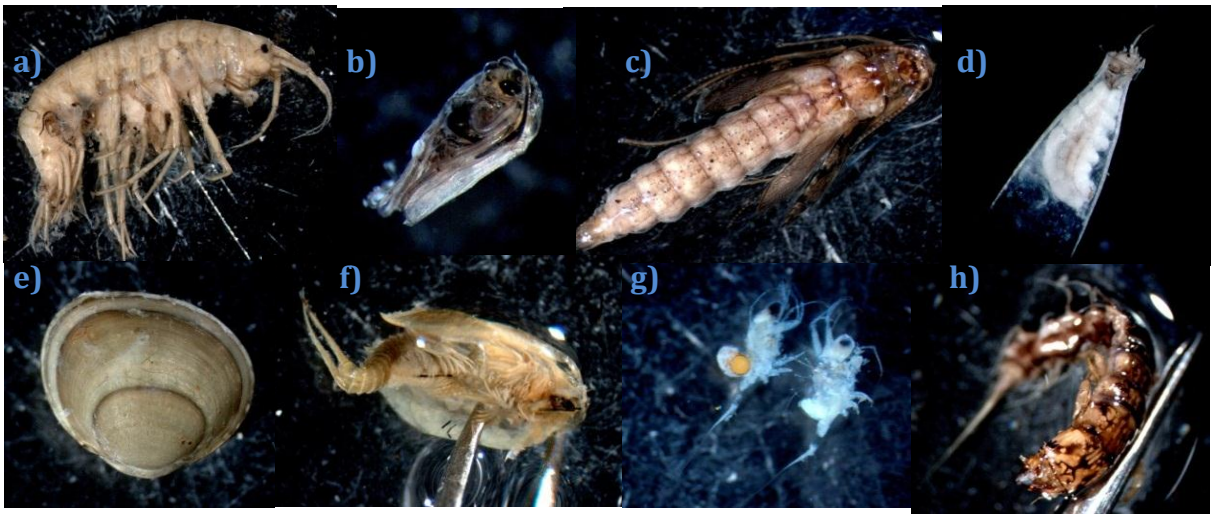


Figure 10: Some of the prey items found in the stomach content of brown trout in Øvre Heimdalsvatn sampled in June – October 2013. a) *Gammarus lacustris*, b) Chironomidae puppae, c & d) Trichoptera pupae, e) Sphaerium, f) *Lepidurus arcticus*, g) *Bythotrepes longimanus*, h) megaloptera. Photos J. Trømborg.

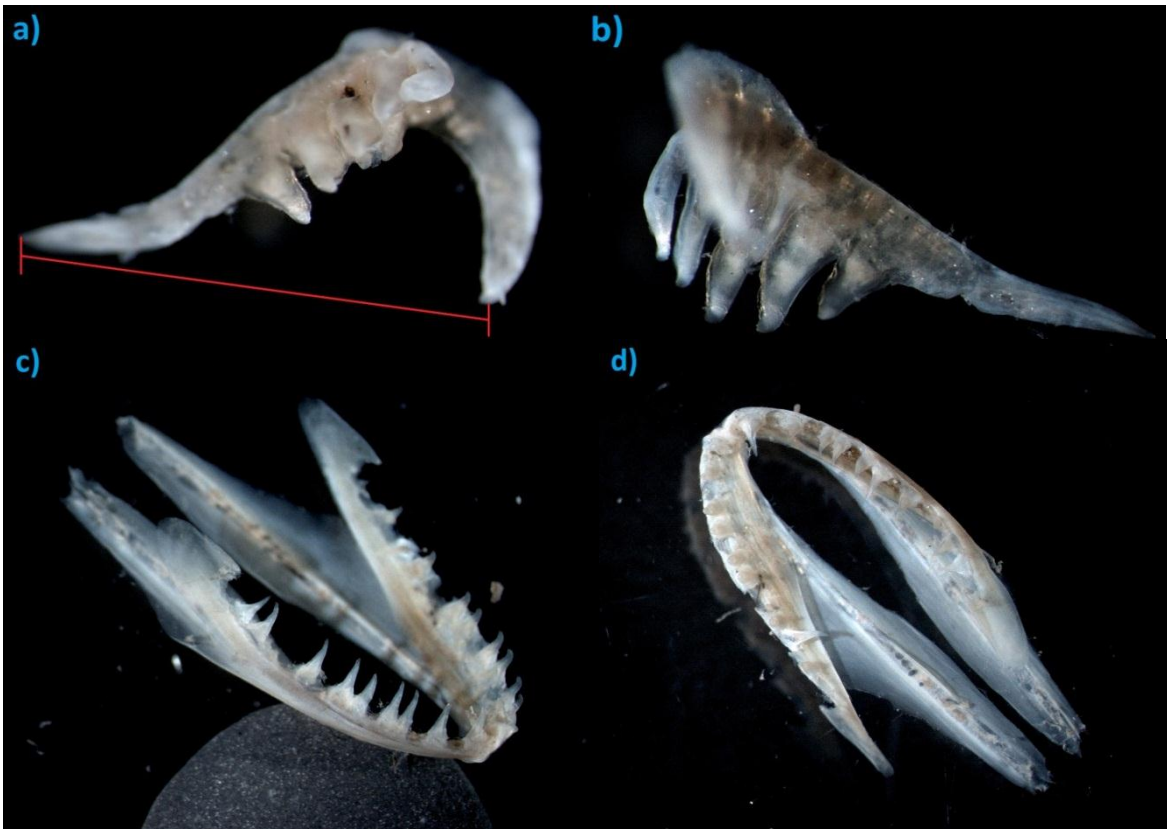


Figure 11: Pharyngeal bone of European minnow (a and b) and jaw of brown trout (c and d) found in the stomach of brown trout sampled in Øvre Heimdalsvatn in June – October 2013. In picture a) is the pharyngeal bone shank marked with a red line. The length of the shank can be used to estimate the total body length of the preyed minnow. Picture c) and d) jaw of brown trout which contains sharp teeth, in contrast to the toothless jaw of European minnow (All photos: J. Trømborg).

2.5 Zooplankton analyses

Zooplankton samples were counted and classified using a counting chamber under a binocular microscope (fig. 12). For each month the average percentage of each group was estimated by counting at least 80 individuals from each sample placed in a counting chamber.

Figure 12: : Zooplankton from Lake Øvre Heimdalsvatn sampled in June – October 2013: a) *Bosmina* sp., b) *Holopedium gibberum*, c) Cyclopoid copepod (*Cyclops* sp.), d) Calanoid copepod, e) *Eurycercus lamellatus*, f) *Daphnia* sp., and g) *Polyphemus pediculus* (all photos: J. Trømborg).

2.6 Parasite identification

Plerocercoids of *D. ditremum* (fig. 13) were roughly counted in the field laboratory, before a more thorough counting and identification were done in accordance with Andersen *et al*. (1987) and Andersen & Gibson (1989), in the department laboratory by means of a binocular microscope. Since some individuals were divided, usually in two pieces, each scolex was regarded as one *D. ditremum* individual.

Figure 13: Plerocercoids of *Diphyllobothrium ditremum* found in the body-cavity of brown trout captured in Øvre Heimdalsvatn in October 2013. The two longest ones are ca. 1.8 cm and 1 cm, respectively, and the smallest one is ca. 0.19 cm indicating a quite recent infection (Photo: Julie Trømborg).

2.7 Mercury and stable isotope analysis

A total of 60 brown trout were selected for mercury- and stable isotope analysis (fig. 14). The individuals were selected on the criteria age and number of *D. ditremum*. In each age group, the individuals were sorted into three groups according to plerocercoid number; 0, 1 – 15 and >15 plerocercoids. Individuals with high numbers of *D. ditremum* (≥ 15 plerocercoids), were irrespective of age, automatically selected for analysis. The rest were chosen based on infection group and age with the goal to obtain a representative sample including all age classes across each of the infection groups.

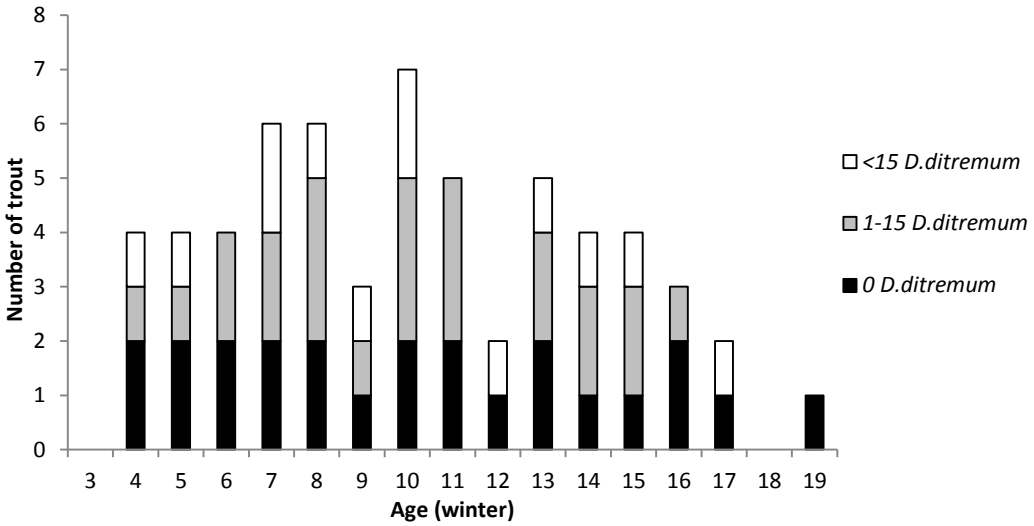


Figure 14: The number of individuals selected for isotope- and mercury analysis based on intensity of plerocercoids and age of brown trout sampled in Øvre Heimdalsvatn in June – October 2013.

2.7.1 $\delta^{15}N$ and $\delta^{13}C$ analyses

2.7.1 a) Analytical methods

Analyses of $\delta^{15}N$ and $\delta^{13}C$ signatures in brown trout were undertaken at the Institute of Energy Technology (IFE) at the Department of Environmental Technology, Kjeller. The analysis followed their procedures, shortly described here.

The muscle samples of brown trout were dried in an oven for more than 12 hours at 80 ºC and crushed and homogenized in an agat mortar. The samples where then weighed and transferred to a 5 x 8 mm tin capsule. Approximately 1.0 mg of the samples was used. The combustion of the samples in the presence of O_2 and Cr_2O_3 at 1700 ºC was done in a Eurovector EA3028 element analyser. Reduction of NO_x to N_2 was done in a Cu oven at 650 ºC. H_2O is removed in a chemical trap of $Mg(ClO_4)_2$ before separation of N_2 and CO_2 on a 2 m Poraplot Q GC column. The C/N ratio was quantified on the basis of the TCD results from the GC. N_2 and CO_2 are directly injected on-line to a Horizon Isotope Ratio Mass Spectrometer (IRMS) from Nu-Instruments, for determination of $\delta^{13}C$ and $\delta^{15}N$.

2.7.1 b) Accuracy and precision

The accuracy and precision of $\delta^{13}C$ and $\delta^{15}N$ analyses was measured by replicate analysis of the internal standard (IFE trout). The standard was prepared by Soxhlet extraction with CH_2Cl_2: 7 % CH_3OH for approximately 2 hours, cleansed with 2N HCl and rinsed with distilled water to neutral pH. The $\delta^{15}N$ composition of IFE trout was calibrated against IAEA-N-1 and IAEA-N-2. The $\delta^{13}C$ composition of IFE trout was calibrated against USGS-24 standard. Average value for IFE trout is:

- $\delta^{15}N_{AIR}$: 11.45 ‰ ± 0.20 (1 sigma)
- $\delta^{13}C_{VPDB}$: -20.22 ‰ ± 0.19 (1 sigma)

2.7.1 c) Baseline adjustments for $\delta^{15}N$ and trophic adjustments for $\delta^{13}C$

Since there are considerable variations between different lake systems in $\delta^{13}C$ and $\delta^{15}N$ at the base of the food web, both isotopes must be adjusted to enable comparisons across different lake systems (Rognerud et al. 2003). Baseline adjustments for $\delta^{15}N$-values and trophic adjustments for $\delta^{13}C$-values were conducted according to Rognerud et al. (2003). In the present study periphyton from the lake bottom were used as baseline for both carbon and nitrogen isotopes.

The following calculations were used for all isotope values to adjust for among system variations:

1) $\delta^{15}N - k$ (‰) = $\delta^{15}N$ (‰) - $\delta^{15}N$ (‰) (periphyton)
2) $\delta^{13}C - k$ (‰) = $\delta^{13}C$ – ($\delta^{15}N$-k (‰) / 3.4) x 0.5

$\delta^{15}N$ and $\delta^{13}C$ are the measured values while $\delta^{15}N$–k and $\delta^{13}C$–k represent the adjusted values.

2.7.2 Total mercury analysis

Analyses of total mercury concentrations were performed at IPM, NMBU. Their procedures are shortly described here.

Approximately 1 gram of muscle was weighed and added 5 mL ultra-pure (UP) HNO3 and 2 mL UP H2O2 PA-quality before decomposed in UltraClave (MILESTONE) at 260

degrees. The samples were stabilized with 1 mL of concentrated HCl (UP) and diluted to 50 mL with de-Ionized water. Both Hg and Se was analysed with ICP-MS (Agilent 8800) in oxygen reaction-mode. The instrument was calibrated against known certified standards. Internal standard was 72Ge+ => 72Ge16O+ (Se) 197Au+ (Hg)

To ensure the accuracy of the THg analyses, three separate samples of the certified reference material Dorm 2 (*Squalus acanthias*) and Dorm 3 (fish protein), from the natural Research Council Canada, were also analyzed. All samples were analyzed three times. Instrument drift was checked against an internal standard (*S. trutta*). Accuracy of the three species of THg analyses are presented in table 2. Mean value of the blank samples, limit of detection (LOD) and limit of quantification (LOQ) is presented in table 3.

Table 2. Expected concentrations of certified reference material Dorm – 2 and 3, mean concentration ± standard deviation of the measured concentrations.

	Series 1		**Series 2**	
Reference material	**Measured values THg**	**Certified value THg**	**Measured values THg**	**Certified value THg**
Dorm – 2	4.3	4.64 ± 0.26	-	4.64 ± 0.26
Dorm – 2	4.4	4.64 ± 0.26	4.4	4.64 ± 0.26
Dorm – 3	0.39	0.392 ± 0.06	0.38	0.392 ± 0.06

Table 3. Mean value of blank samples, limit of detection (LOD) and limit of quantification (LOC) for two series of THg analyses of brown trout muscle tissue from Øvre Heimdalsvatn 2013.

	Series 1	**Series 2**
Blank (n = 3) (mg Hg/kg w.w.)	**< LD (<0.003)**	**< LD**
LOD (mg Hg/kg w.w.)	0.004	0.004
LOQ (mg Hg/kg w.w.)	0.013	0.013

2.8 Statistical analyses

Statistical analysis and figures were executed in Microsoft Excel (2010) and in RStudio (R Development Core Team 2012).

2.8.1 Statistical parameters

The terms prevalence, intensity and mean abundance are used according to Bush et al. (1997).

Prevalence (P): is the percentage of trout infected with plerocercoids of *D.ditremum* (I) in the total sample of brown trout (n). Prevalence is intended to reveal presence or absence of infection.

$P = I/n \times 100$

Intensity (I): The number of plerocercoids of *D.ditremum* in infected individuals only.

Mean abundance (A): is the average number of plerocercoids of *D. ditremum* ($\bar{x}$) in the total sample of brown trout, divided by the total number of trout in that sample (n), including infected and non – infected individuals.

$A = \bar{x}/n$

2.8.2 Statistical tests

Linear regressions (e.g. $\delta^{15}N$ vs. length) were performed using the lm procedure in R.

The distribution pattern were examined by comparing the total sample variance (s^2) and total sample mean ($\bar{x}$) according to Anderson and Gordon (1982) and Whitlock and Schluter (2009 p.196-197). If the parasites are spread out randomly among the host populations, the parasite burden would be expected to follow a Poisson distribution, where the variance is equal to the mean ($s^2 = \bar{x}$). If, however, the variance is greater than the mean ($s^2/\bar{x} > 1$), the distribution is over-dispersed (aggregated) and if the variance is smaller than the mean ($s^2/\bar{x} < 1$), the distribution pattern is under-dispersed.

It is shown that for some host parasite associations, parasite induced host mortality tends to induce a decline in the degree of overdispersion (decrease in $s^2/\bar{x}$) and mean

abundance of parasites in the host population (Anderson & Gordon 1982; Henricson 1978). Potential mortality of brown trout induced by plerocercoids of *D. ditremum* were thereby examined by looking at intensity and variance to mean ratio ($s^2/\bar{x}$) of plerocercoids by host age according to Anderson and Gordon (1982).

2.8.3 Multiple regression model

The statistical analysis of the infection data, were done in cooperation with Thrond Oddvar Haugen at the Department of Ecology and Natural Resource Management, NMBU. The infection data was analyzed both as zero-inflated Poisson models (package pscl in R) and negative binomial GLM models (package MASS), but due to severe overdispersion (package AER) none of these analytical approaches proved relevant for the data sampled. The overdispersed pattern is often described empirically by the probability distribution the negative binomial (Andersen & Gordon 1982). We therefore proceeded with a multinomial approach using package nnet. The response data were grouped into four infection categories: 0 plerocercoids, 1 – 5 plerocercoids, 6 – 20 plerocercoids and >20 plerocercoids. The candidate models were then fitted with different prediction model structures to these multinomial response categories using the multinom function. Model selection was then undertaken by means of Akaike's Information Criterion (Akaike 1974) where models differing with less than 2 AIC units compared to the most supported model where presented and discussed further in the thesis, according to Burnham and Anderson (1998).

3. Results

3. 1 Plerocercoids of *D. ditremum* in brown trout

The total sample variance (s^2 = 398) of plerocercoids greatly exceeded the total mean ($\bar{x}$ = 6); which means that the pattern of plerocercoids is highly over-dispersed (aggregated) within the brown trout examined, thus a few individuals harbour the main proportion of plerocercoids while the majority harbour non or a few plerocercoids (fig. 16).

There was no clear decrease in the variance to mean ratio with increasing age of the brown trout (fig. 15). The large variance to mean value by age 4 and 10 is due to one heavily infected individual at age 4 (169 plerocercoids), and two heavily infected individuals at age 10 (104 and 128 plerocercoids). There was still no clear decrease in the variance to mean ratio when these heavily infected trout individuals were taken out of the sample.

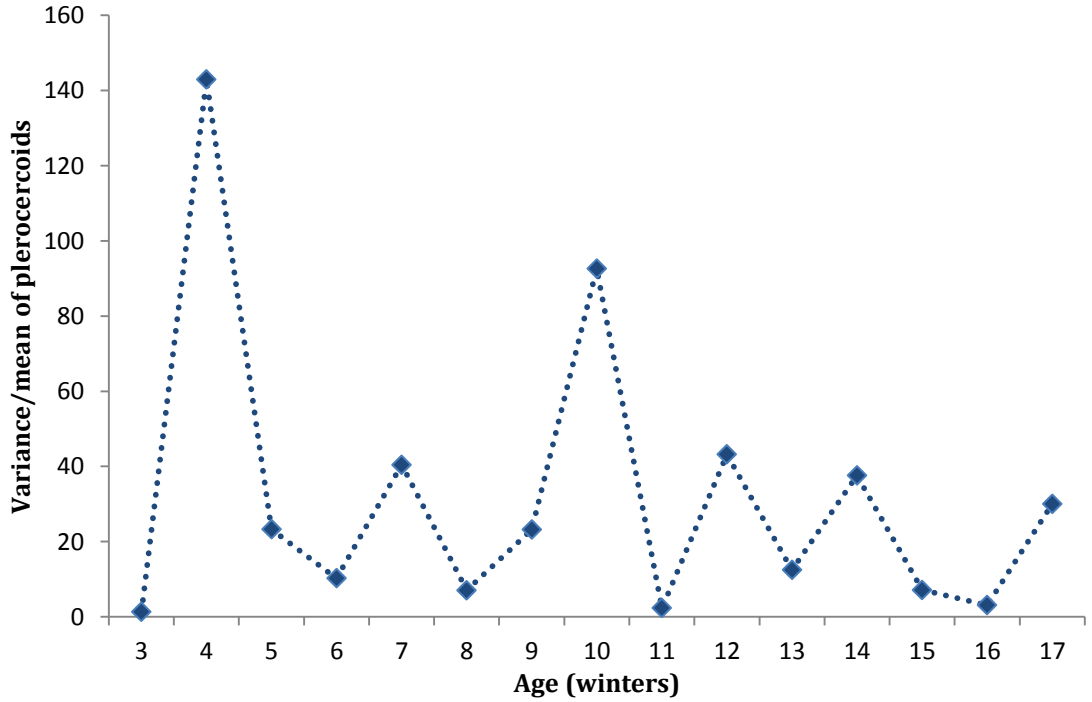


Figure 15: The variance of plerocercoids of *D. ditremum* divided by the mean number of plerocercoids in each age class of brown trout sampled in Øvre Heimdalsvatn from June – October 2013.

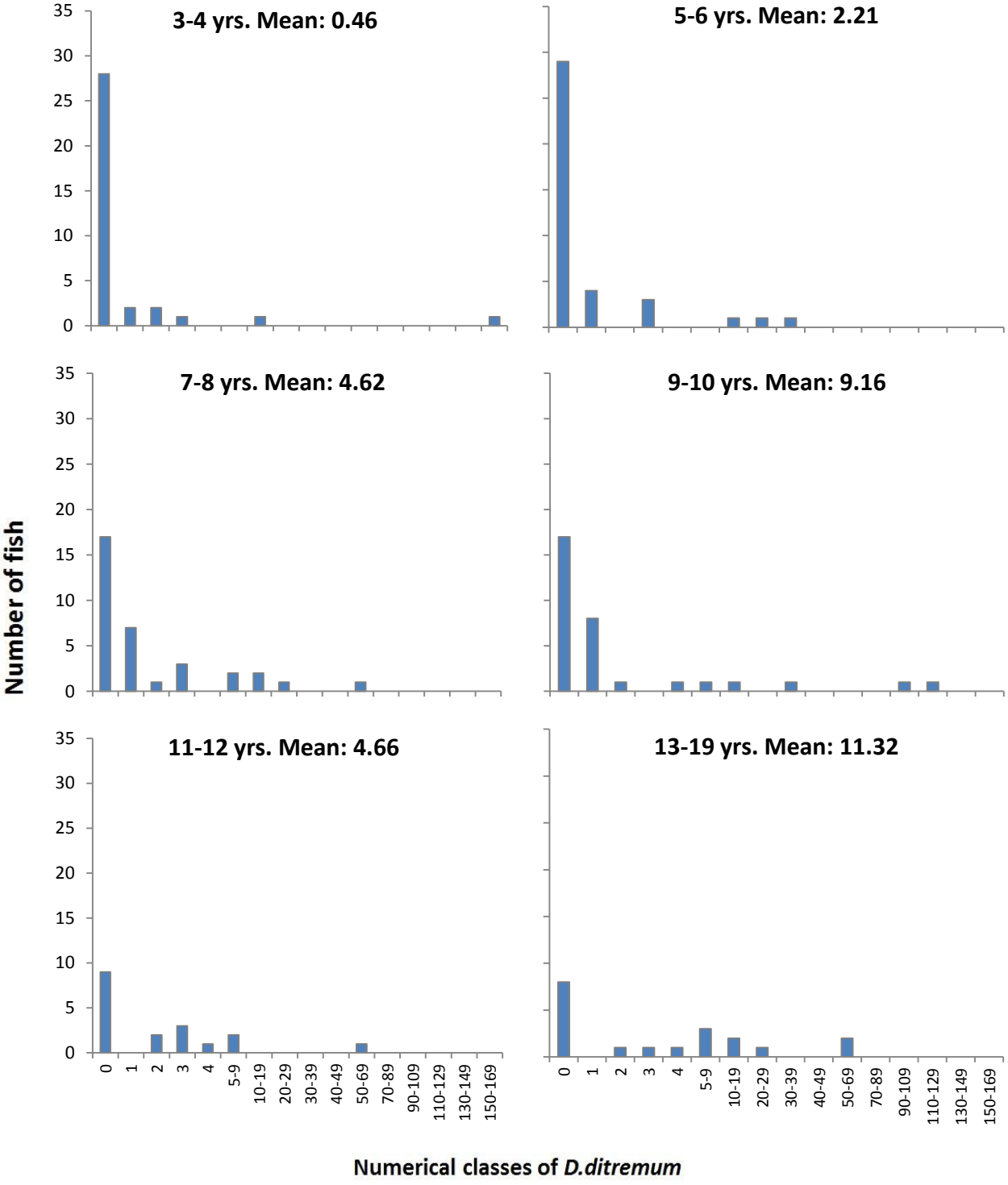


Figure 16: Frequency distribution of *D. ditremum* infection in various age classes of brown trout. Mean number of *D. ditremum* is given for each age class category. The figure represents the total sample of brown trout caught in Øvre Heimdalsvatn in June – October 2013.

Prevalence

Plerocercoids were found in 40 % of the total number of brown trout analysed. Brown trout were found to be infected throughout the age span 3 – 17 years (fig 17 & 19). The prevalence of infection increased steadily with increasing age (fig. 17 & 19) and length (fig. 18 and fig. 20) of brown trout. Up to 7 winters, between 20 – 30 % of the individuals examined were infected. From 8 winters and up, 50 % were infected, and after reaching 11 winters, over 60 % of the examined trout were infected. Prevalence of infected trout also differed according to sex, with males having a higher proportion of infected individuals (44 %) than females (35 %).

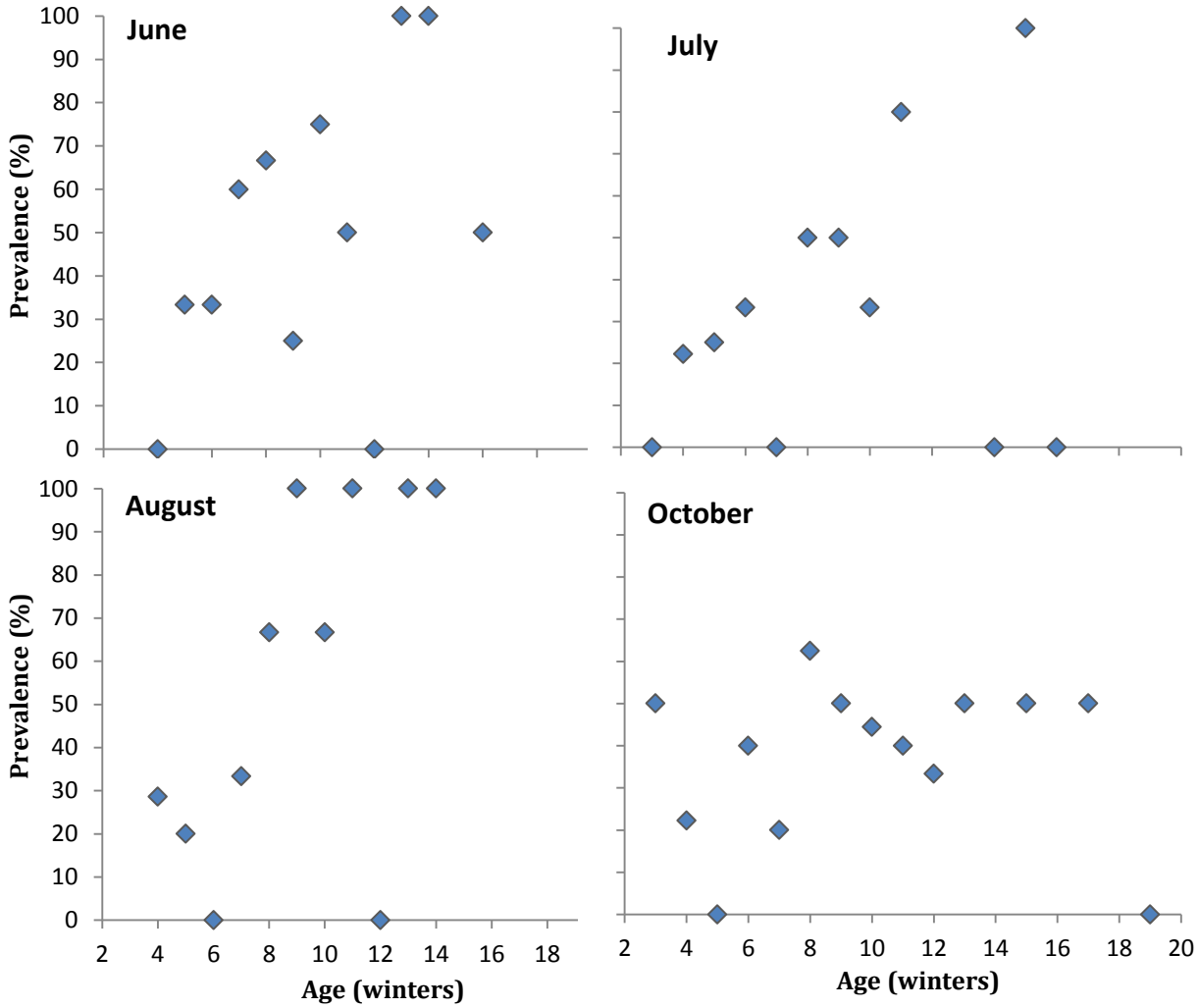


Figure 17: The mean percentage of brown trout (prevalence) infected with *D. ditremum* among each age class of brown trout sampled in Øvre Heimdalsvatn in June (a, n = 49), July (b, n = 38), August (c, n = 29) and October (d = 61) 2013.

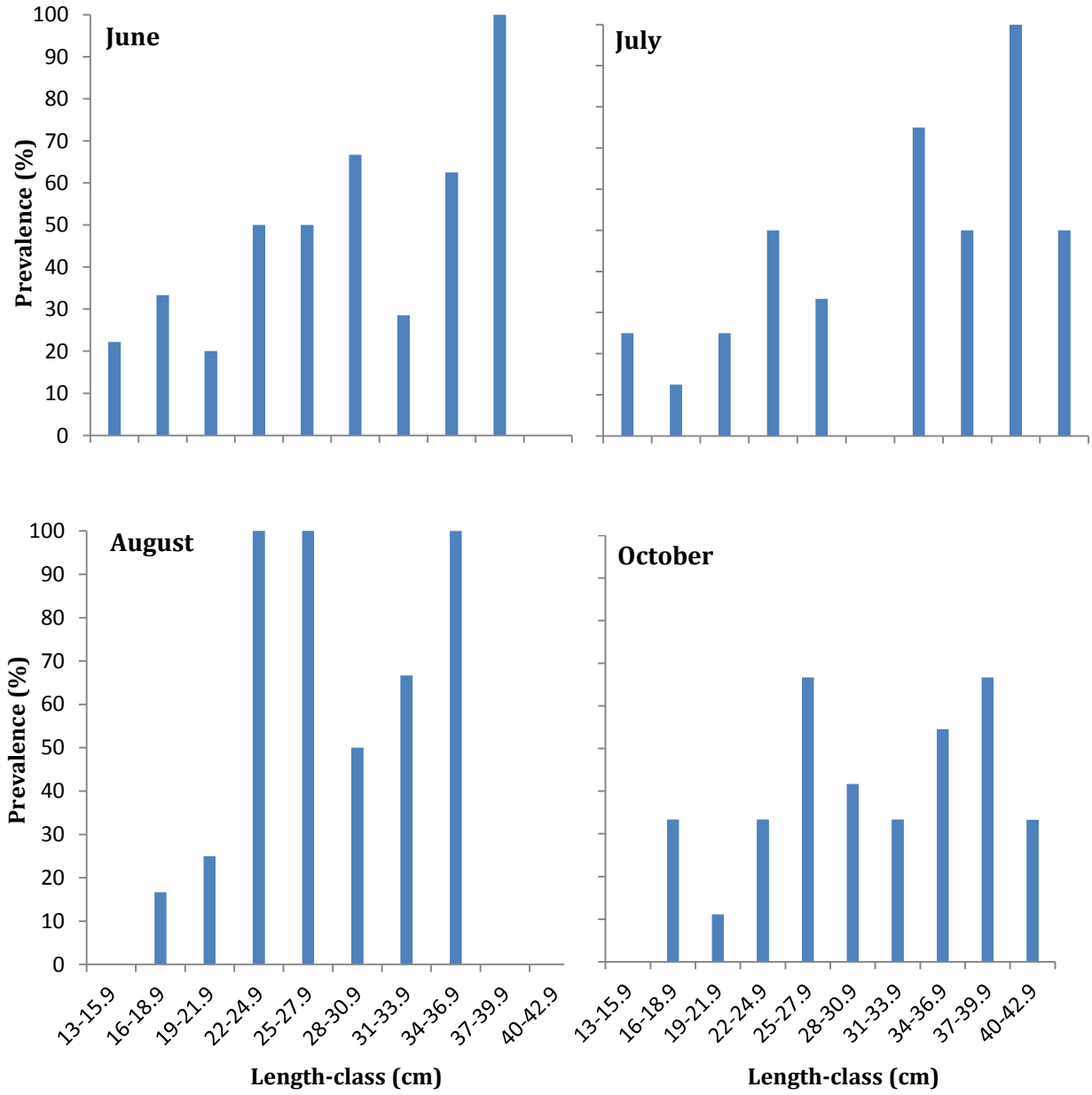


Figure 18: Prevalence of infected brown trout with *D. ditremum* among different length classes of brown trout sampled in Øvre Heimdalsvatn in June (a, n = 51), July (b, n = 38), August (c, n = 30) and October (d = 62) 2013.

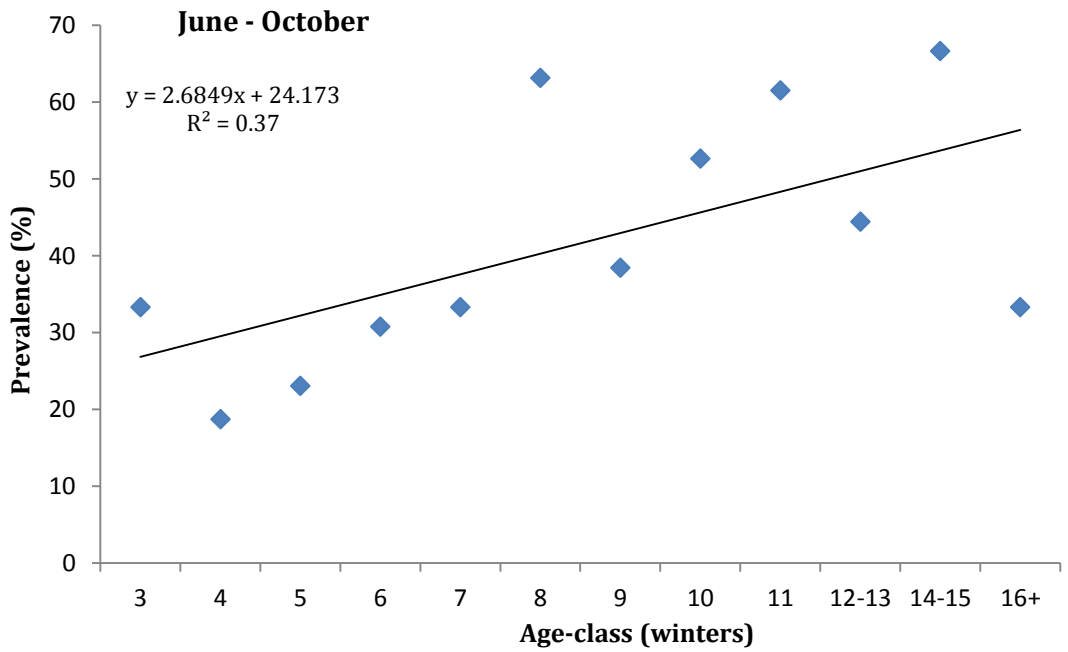


Figure 19: Prevalence (%) of infected brown trout for different age classes, sampled in in Lake Øvre Heimdalsvatn in June – October 2013 (n = 177). From age 11 and up, ageclasses are grouped together due to low sampling numbers.

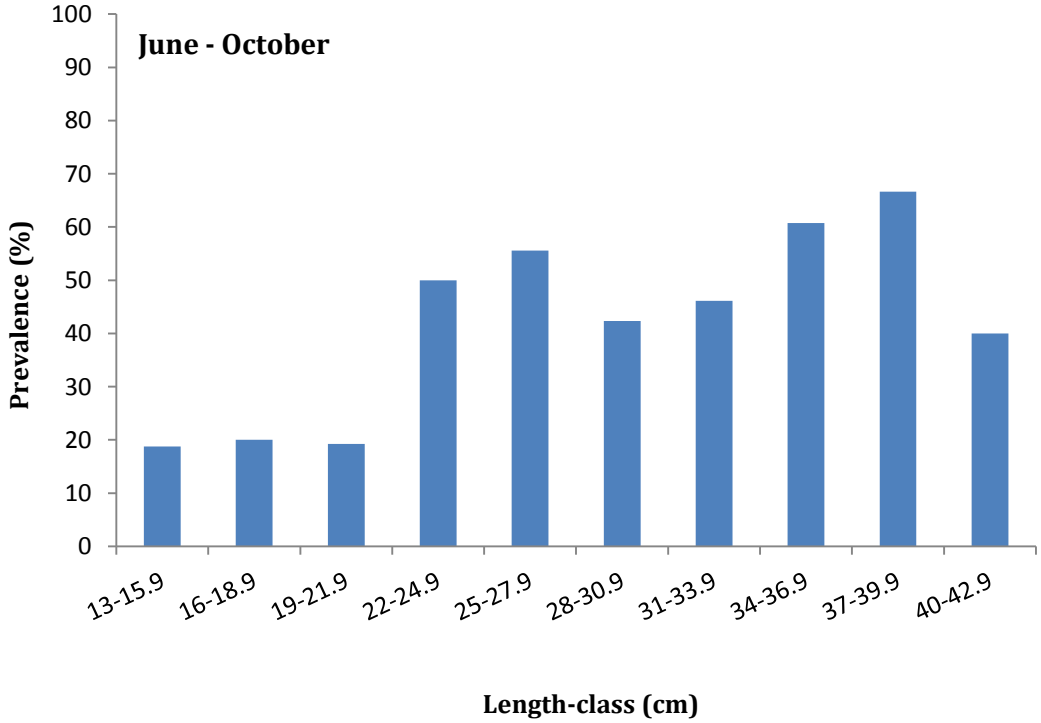


Figure 20: Prevalence of infection with *D. ditremum* among different length classes of brown trout sampled in Øvre Heimdalsvatn during June – October 2013 (n = 181).

Intensity

The intensity of infection varied bewteen 1 and 167 plerocercoids of *D. ditremum* (fig. 21), the highest number was found in a 4 year old trout (fig. 21). Except from this individual seven individuals exceeded 50 plerocercoids, the youngest of these being 7 years and the oldest 17 years old (fig. 21).

The highest intensities of plerocercoids was found in brown trout sampled in August and October, with 10 % and 11 % exceeding 20 plerocercoids, respectively (fig. 21). In October six of these individuals were infected by between 50 and 169 plerocercoids (9 % of the trout examined in October), while one of the trout individuals examined in August exceeded 50 plerocercoids (3 % of the trout examined in August). In June and July 6 % and 3% were infected with more than 20 plerocercoids, respectively.

Mean abundance

The mean abundance of plerocercoids increased slightly with both age (fig. 23) and length (fig. 22 and fig. 24), with no sign of levelling off at the highest age- and length classes. Males had higher mean abundance of plerocercoids ($\bar{x} = 8$) than females ($\bar{x} = 5$), with a mean abundance being approximately 20 percent higher in males. This was also the case when particularily large values (> 50 plerocercoids) were taken out of the sample. The mean abundance of *D. ditremum* infection did not show any distinct correlation with increasing total mercury (fig. 25). The correlation between $\delta^{15}N$ and infection will be discussed further down under the headline; infection probability.

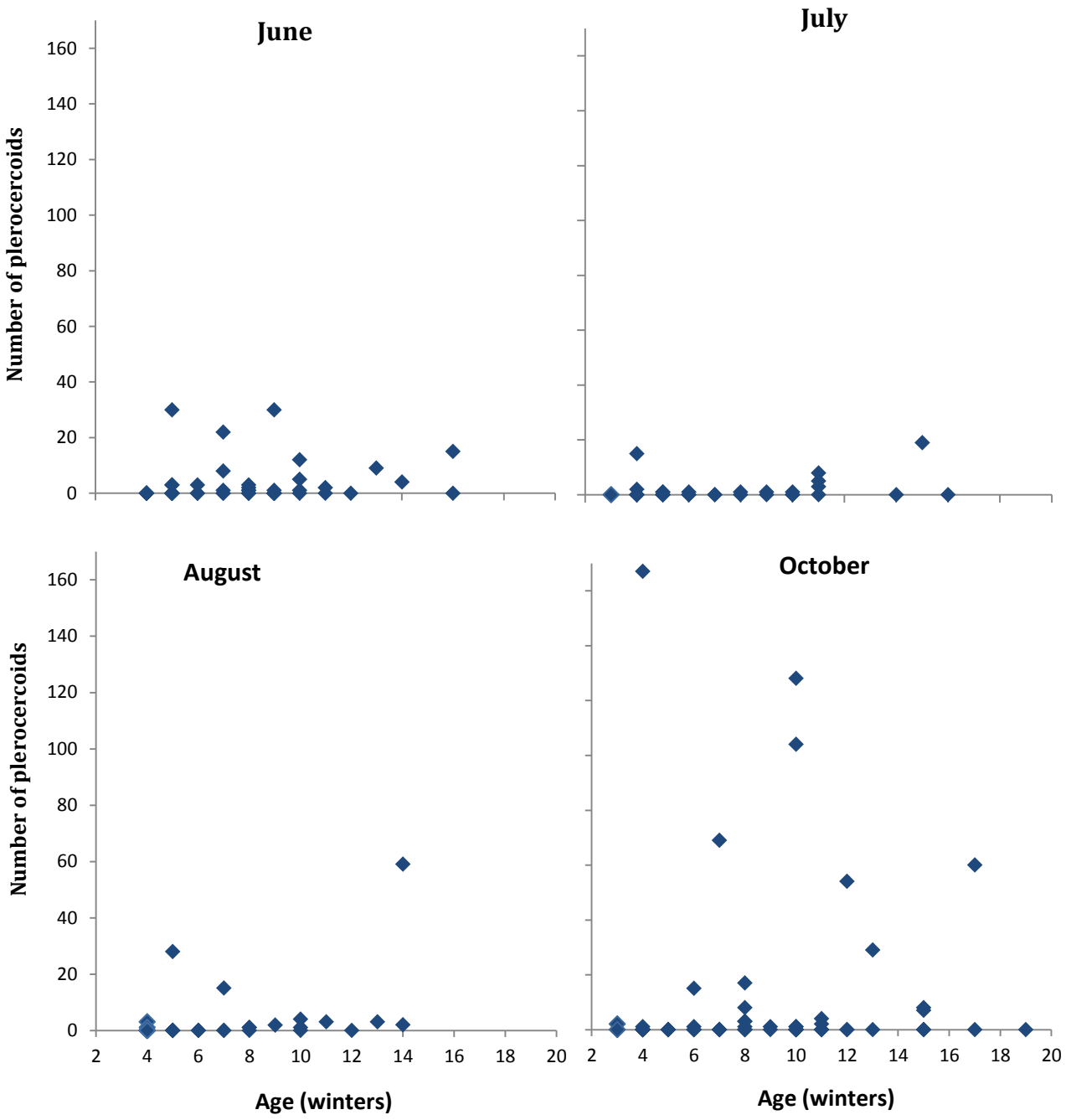


Figure 21: Number of plerocercoids of *D. ditremum* per brown trout individual across all age classes of brown trout caught Øvre Heimdalsvatn in a) June (n = 49), b) July (n = 38), c) August (n = 29) and d) October (n = 61) 2013. All brown trout individuals are presented in the figure (each blue dot represents one brown trout individual), but due to similar parasite numbers among some of the individuals, not all values are visible.

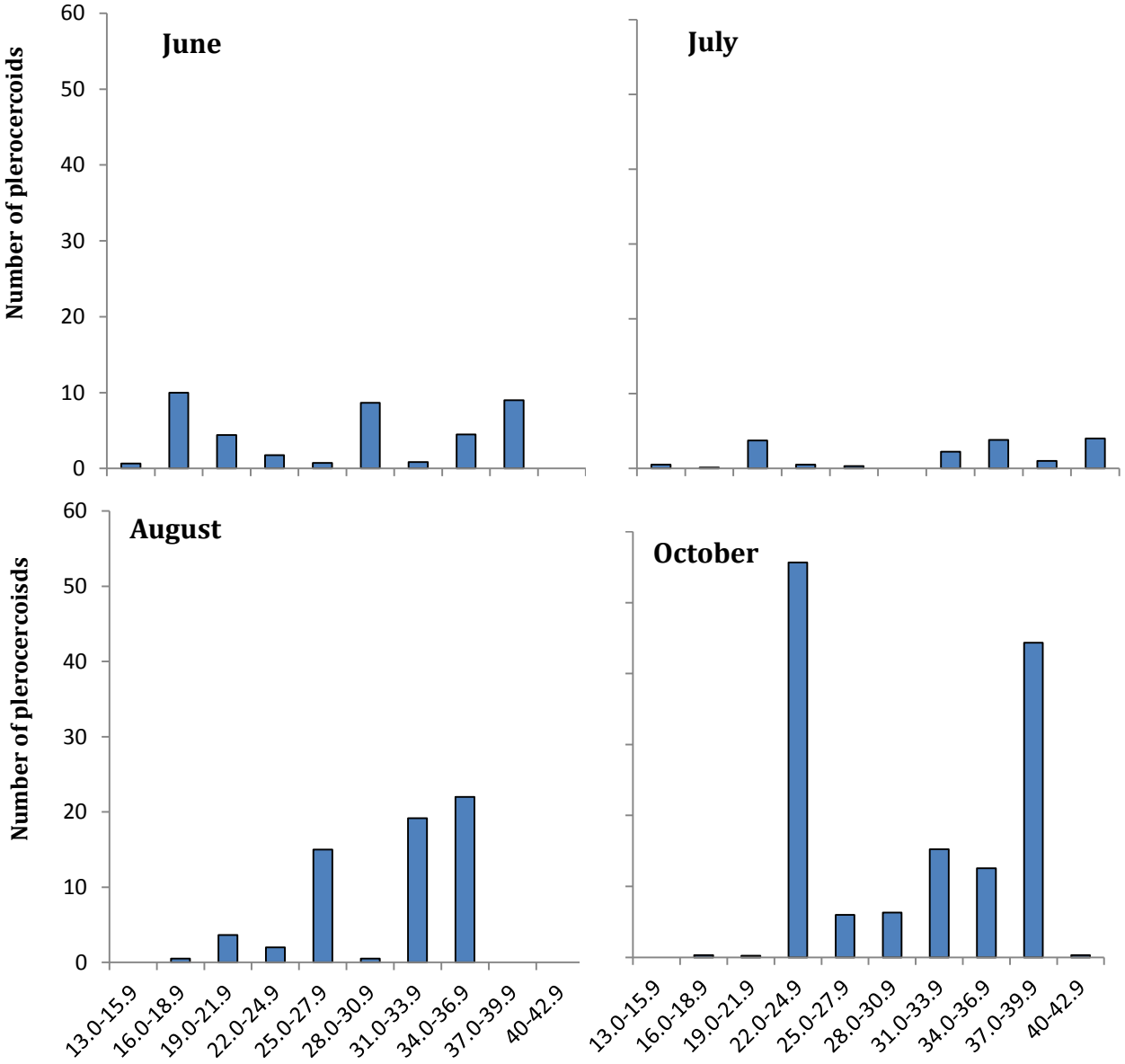


Figure 22: Mean abundance of plerocercoids of *D. ditremum* in different length classes of brown trout sampled in a) June (n = 51), b) July (n = 38), c) August (n = 30) and d) October (n = 62) 2013 in Øvre Heimdalsvatn.

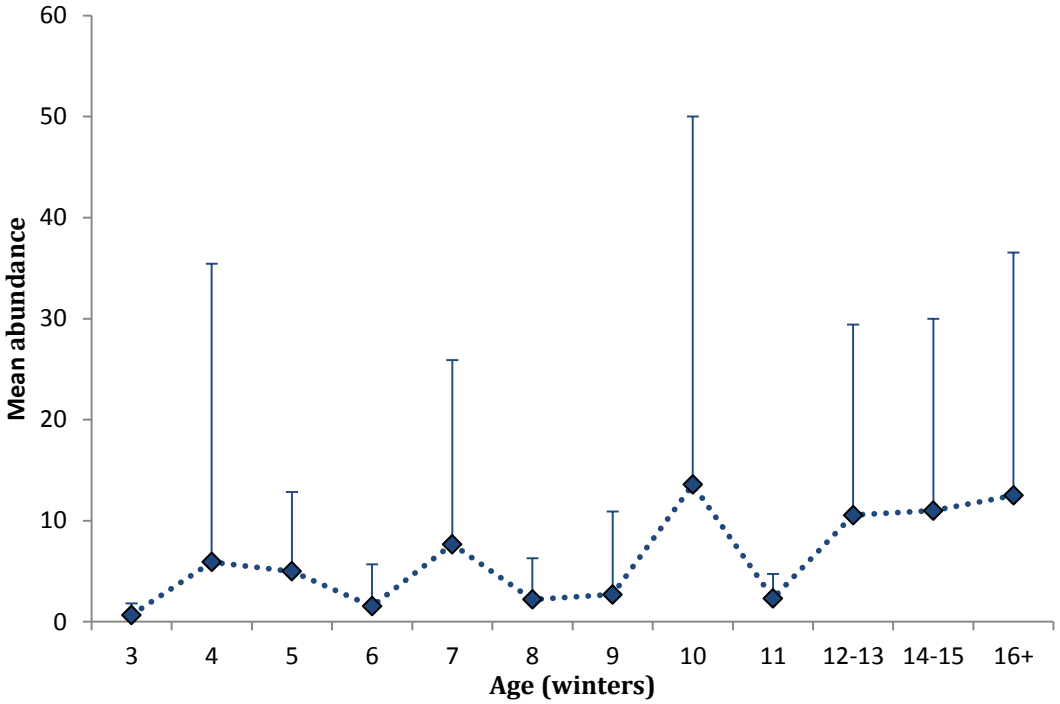


Figure 23: Mean abundance of plerocercoids of *D. ditremum* with standard deviation, in each age class from the total sample of brown trout caught in Øvre Heimdalsvatn in June – October (n = 181) 2013.

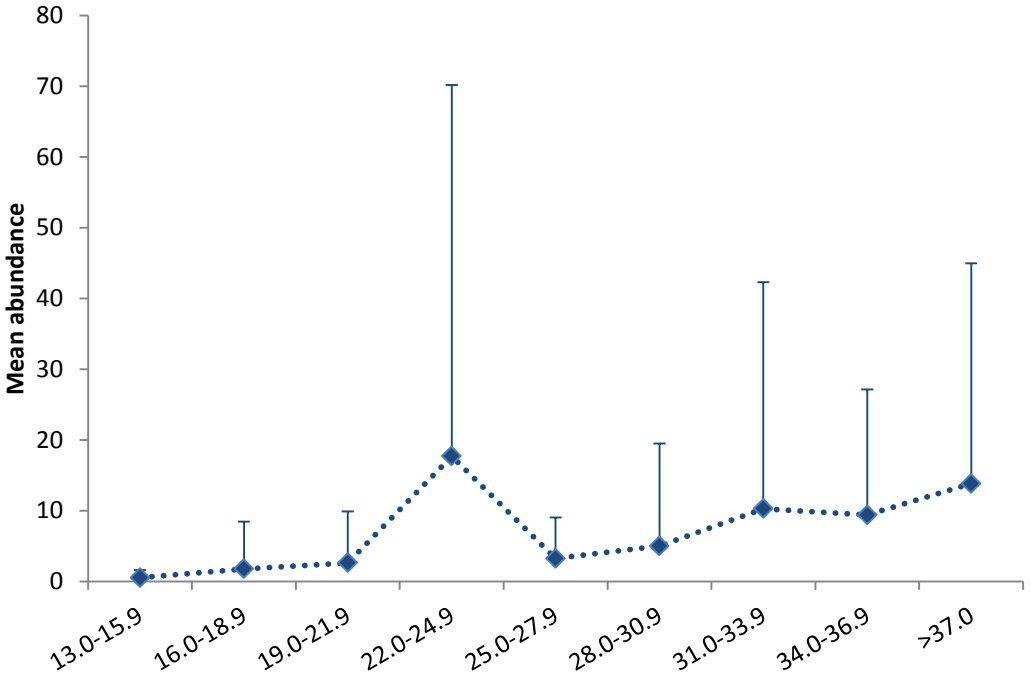


Figure 24: Mean abundance of plerocercoids of *D.ditremum* in total sample of brown trout sampled in Øvre Heimdalsvatn during the period June – October 2013.

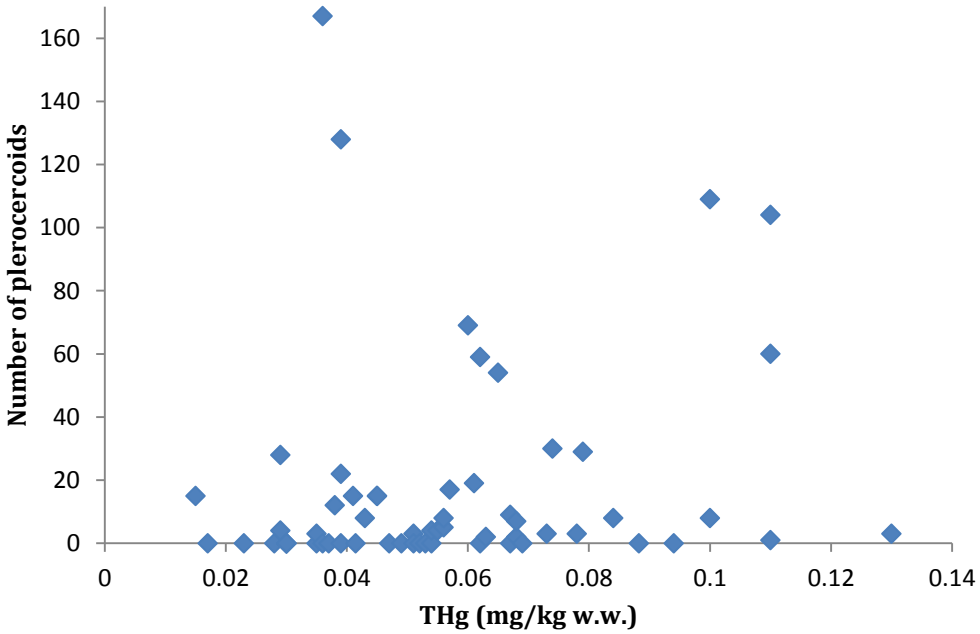


Figure 25: The correlation between number of plerocercoids of *D. ditremum* and THg in brown trout from Øvre Heimdalsvatn caught in June – October 2013.

3. 2 Infection probability

The most supported multinomial model, fitted to predict parasite infection probability and infection intensity of plerocercoids in brown trout from Øvre Heimdalsvatn, included just a sex effect, but the second-most supported model (ΔAIC = 1.6, table 4) included in addition an additive effect of $\delta^{15}N$ (table 4 and 5, fig 26). The sex effect model showed that males in general had higher infection probability and degree of infection than females. The sex+ $\delta^{15}N$ model shows that for both males and females the probability of being infected with zero plerocercoids of *D. ditremum* decreased substantially with increasing $\delta^{15}N$ (Figure 26). Higher $\delta^{15}N$ values increase the probability of being infected with 1 – 5 plerocercoids (Figure 26). The probability for further infections (being infected with > 6 plerocercoids) seems, however, to be less dependent on $\delta^{15}N$ (Figure 26). Females show a slightly higher correlation between the probabilities of being infected with more than 20 plerocercoids and increasing $\delta^{15}N$ (Figure 26). The logit parameter estimates for the sex+ $\delta^{15}N$ model (table 5), shows that males have a significantly higher probability of being infected with 6 – 20 plerocercoids (being in infection group 2), than females (confidence interval: 2.07 ± 0.89, containing

no zero values). The probability of being in infection group 1 (1 – 5 plerocercoids) is also higher for males than females (confidence interval: 1. 01 ± 0. 78). The probability of being infected by more than 20 plerocercoids (infection group 3), however, is quite similar for both male and female (sex coefficient males: 0.92 ± 0.77).

Table 4. Model selection table for candidate models fitted to predict parasite infection probability and infection intensity of *D.ditremum* in Øvre Heimdalsvatn brown trout.

Model structure	df	AIC	ΔAIC
sex	6	161.8	0
$\delta^{15}N$ + sex	9	163.3	1.6
$\delta^{15}N$	6	164.3	2.5
$\delta^{13}C$ + sex	9	165.7	3.9
$\delta^{13}C$	6	166.2	4.4
$\delta^{15}N$ * sex	12	166.2	4.5
Age + sex	9	167.6	5.8
Age + $\delta^{15}N$	9	168.0	6.2
$\delta^{15}N$ + $\delta^{13}C$	9	168.2	6.4
$\delta^{13}C$ * sex	12	169.3	7.6
age	6	169.7	7.9
$\delta^{15}N$ * $\delta^{13}C$	12	169.8	8.0
Age * $\delta^{15}N$	12	173.5	11.7
Age * $\delta^{15}N$ * sex	24	179.9	18.1
$\delta^{15}N$ * $\delta^{13}C$ * sex	24	180.4	18.7
Age * $\delta^{13}C$ * sex	24	183.6	21.8

Table 5. Logit parameter estimates for the second-most supported model fitted to predict parasite infection probability and infection intensity in Øvre Heimdalsvatn brown trout.

	Response level		
Term	**1 – 5 plerocercoids**	**6 – 20 plerocercoids**	**>20 plerocercoids**
Intercept	- 10.57 ± 4.93	- 6.54 ± 4.63	- 5.74 ± 4.5
$\delta^{15}N$	1.23 ± 0.64	0.62 ± 0.61	0.6 ± 0.59
Sex [male]	1.01 ± 0.78	2.07 ± 0.89	0.9 2 ± 0.77

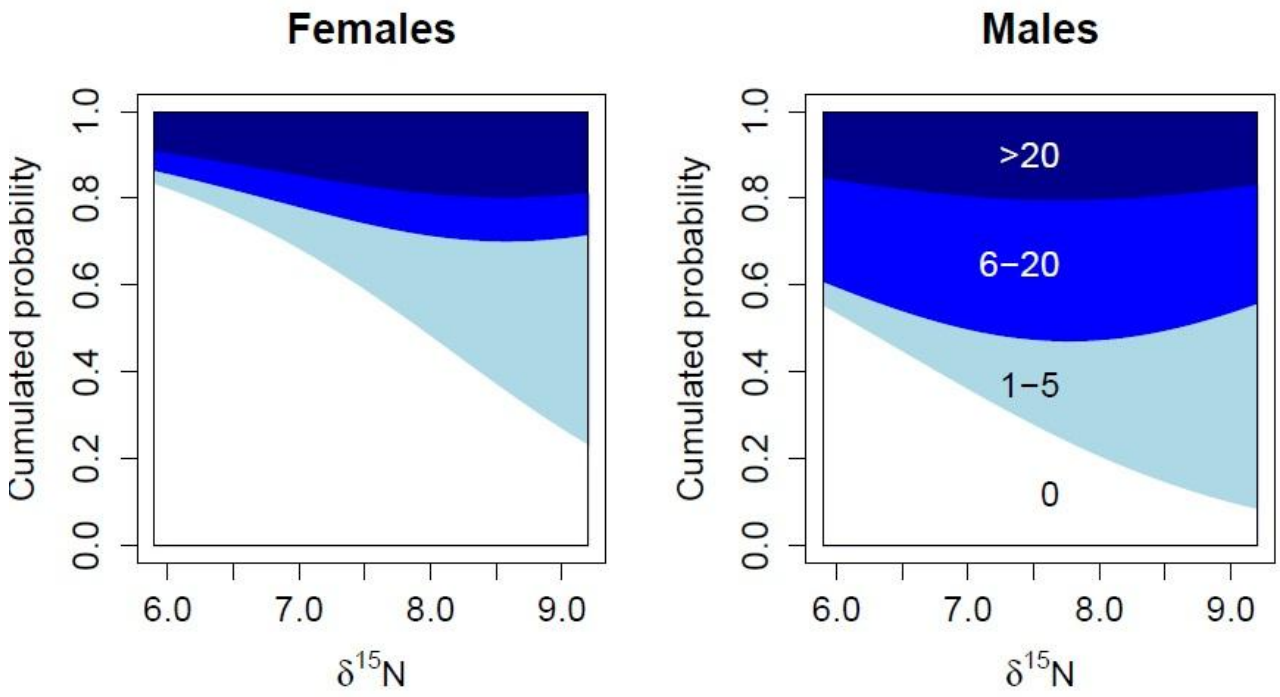


Figure 1: Multinomial model including both sex and $\delta^{15}N$ effects on the probability of brown trout being infected with plerocercoids of *D.ditremum* and the intensity of infection.

3.3 Analysis of stable isotopes and mercury in brown trout

3.3.1 $\delta^{15}N$

The mean and standard deviation of $\delta^{15}N$ was 7.610 ± 0.709 (table 6). The gap between the highest and lowest $\delta^{15}N$ values amounted for approximately one trophic level (3.4 ‰) (table 6). $\delta^{15}N$ increased significantly as a function of age (linear regression, $P < 0.001$, table 7), length (linear regression, $P < 0.001$, table 7) and weight (linear regression, $P < 0.01$, table 7). Approximately 22 – 23 % of the variability in $\delta^{15}N$ could be explained by the factors length and age, respectively, while weight accounted for 12% of the variability (table 7). The most important explanation variable for $\delta^{15}N$ values in the brown trout sample was THg ($P = 4.8\ e^{-10}$ table 7) with an explanation variable of 49 % (table 7). Males had slightly higher values (7.7 ‰) than females (7.5 ‰) (Figure 27).

3.3.2 $\delta^{13}C$

The $\delta^{13}C$ values of brown trout ranged from –30. 2 to –23.0 (mean ± SD = -26 ± 1.7, table 6). There was no significant relationship between $\delta^{13}C$ and age, length and weight ($p > 0.05$, table 7). When dividing the isotopic material into two groups based on sex, males showed slightly heavier $\delta^{13}C$ values (-26.2 ‰) than females (-25.6 ‰) (Figure 27).

3.3.3 Hg

Total mercury values in the sampled brown trout ranged from 0.02 to 0.13 mg/kg wet weight (w. w.), with a mean of 0.06 ± 0.03 mg/kg w.w (table 6). Total mercury increased significantly as a function of age (linear regression, $P < 0.001$, table 7), length (linear regression, $P < 0.01$, table 7) and weight (linear regression, $P = 0.01$, table 7). Approximately 16 to 20 % of the variability in THg could be explained by the factors length and age, respectively, while weight accounted for 11% of the variability (table 7).

Table 6. Mean and standard deviation of $\delta^{15}N$ and $\delta^{13}C$, together with minimum, medium and maximum values of $\delta^{15}N$ and $\delta^{13}C$ in brown trout from Øvre Heimdalsvatn sampled in June – October 2013.

	n	Mean ± SD	Min.	Med.	Max.
δ15N (‰)	60	7.610 ± 0.709	5.869	7.726	9.225
δ 13C (‰)	60	-25.985 ± 1.648	-30.193	-25.714	-22.886
Hg (mg/kg w.w.)	60	0.056 ± 0.025	0.015	0.0535	0.130

Table 7. Simple linear regression analysis for $\delta^{15}N$ (‰), $\delta^{13}C$ (‰) and THg (mg/kg w. w.), versus weight, length and age of brown trout sampled in Øvre Heimdalsvatn from June – October 2013. Significant relationships are presented in bold ($p < 0.05$).

Regression	n	Intercept ± Std. Error	Slope ± Std. Error	R^2	R^2_{adj}	P-value
$\delta^{15}N$ (‰) vs. weight	58	-435 ± 270	98.18 ± 35.51	0.12	0.10	**0.008**
$\delta^{15}N$ (‰) vs. length	58	-5.5 ± 8.9	4.7 ± 1.2	0.22	0.21	**0.000**
$\delta^{15}N$ (‰) vs. age	57	-10.90 ± 5.1	2.77 ± 0.66	0.23	0.22	**0.000**
$\delta^{15}N$ (‰) vs. THg (mg/kg w.w.)	58	-9.78 ± 0.92	3.37 ± 0.24	0.49	0.48	**$4.78e^{-10}$**
$\delta^{13}C$ (‰) vs. age	57	0.86 ± 8.04	-0.36 ± 0.31	0.02	0.01	0.255
$\delta^{13}C$ (‰) vs. length	57	25.06 ± 14.23	-0.21 ± 0.55	0.003	0.01	0.703
$\delta^{13}C$ (‰) vs. weight	57	298.41 ± 405.53	-0.41 ± 15.56	$1.22e^5$	0.02	0.979
log THg (mg/kg) w.w. vs. weight (g)	58	-3.97 ± 0.37	0.18 ± 0.07	0.11	0.10	**0.01**
log THg (mg/kg) w.w. vs. length (cm)	58	-5.40 ± 0.74	0.71 ± 0.22	0.16	0.14	**0.002**
log THg (mg/kg) w.w. vs. age (winters)	57	-4.06 ± 0.30	0.48 ± 0.13	0.20	0.19	**0.000**

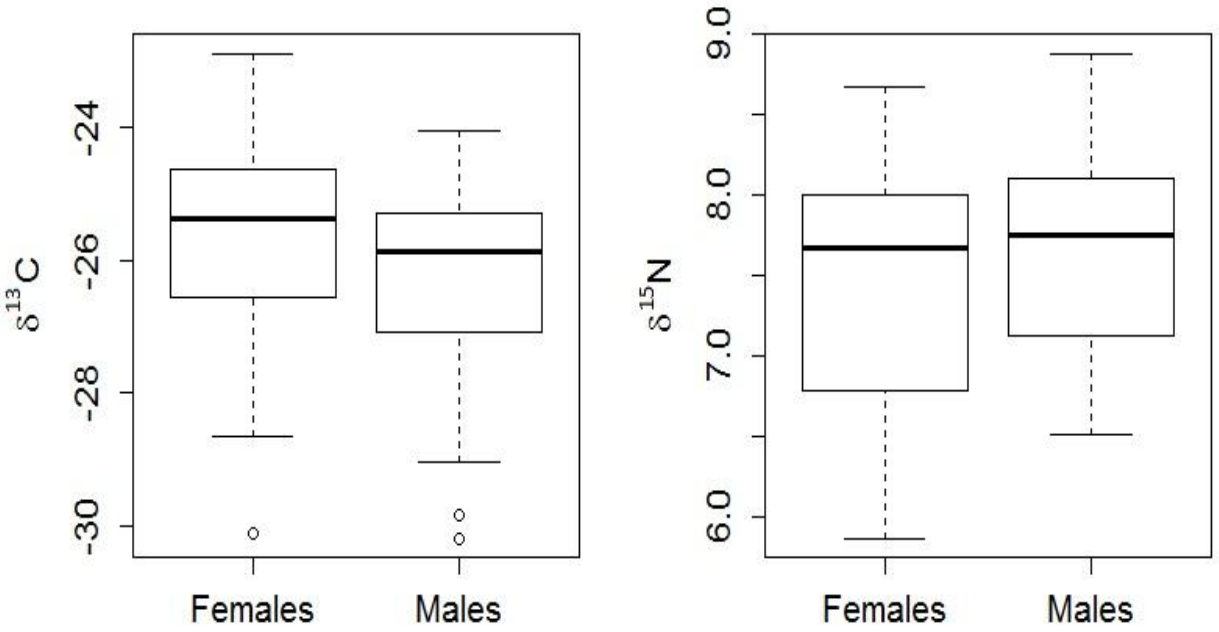


Figure 2: Box blot illustrating differences in both $\delta^{13}C$ (left box – plot) and $\delta^{15}N$ values (right box – plot) between male- and female- brown trout. The box-plot presents median, 1 σ error bars and maximum and minimum values of $\delta^{13}C$ and $\delta^{15}N$.

3. 4 Diet

Apart from August, fish were found in the diet of brown trout throughout the study period, but at a low frequency (Figure 3). A total of 7 out of 181 stomachs analysed contained fish. The majority of prey fish were found in June, when fish were found in 5 out of 51 stomachs analysed (Figure 3). Minnows constituted the main proportion of the prey fish (n = 5), while juvenile brown trout were found in two stomach contents, one in June and one in October. Brown trout that had eaten fish ranged from 4 – 13 years (mean 8, 5 years) and from 23-39 cm in length. The average weights of these individuals were 335 gram, ranging from 111 gram to 641 gram. Age of the preyed minnows (which could be aged) ranged between 5 and 6 years (n = 2), and the length ranged from 6.6 cm to 9.0 cm (n = 4). Copepods were only found in the stomach content of one brown trout which was captured in June. Only a few specimens were found, and because of the low volume, the copepods are not presented in Figure 3.

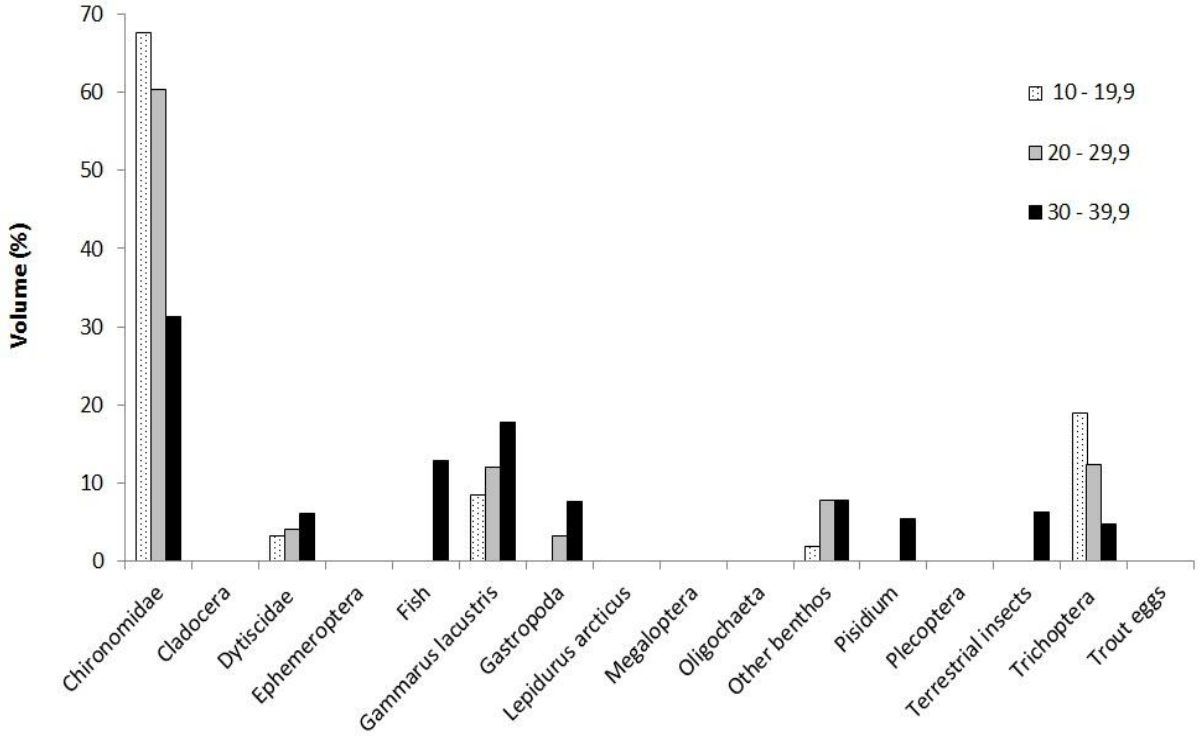
The diet of brown trout showed clear seasonal changes as well as changes with regard to length (Figure 3). Chironomids constituted the dominant food item of all three lengths classes (10-19.9, 20-29.9 and 30-39.9 cm) in June (Figure 3a). Trichoptera constituted approximately 15 – 20% of the total diet of the two smallest length classes in June (Figure 3 a). The second most important food item of length class 30-39.9 was *G. lacustris* (above 15%), which also constituted 10 – 15% of the diet of the two other length classes (Figure 3a). In June, fish were only found in length class 30-39.9 cm (Figure 3 a). The average stomach fullness (in percent) for each of the length classes analysed in June were 19% for length class 10-19.9 cm, 32% (n = 8) for length class 20-29.9 cm (n = 18), and 24% for length class 30-39.9 cm (n = 16). A total of 15,6% of the fish analysed had empty stomachs.

In July, cladocerans, mainly *Eurycercus lamellatus*, accounted for the main proportion of the diet of length class 10-19,9 cm (45%) and 30-39,9 cm (38%, Figure 3 b). Chironomidae still constituted a significant proportion for all three length classes (ca. 10% - 20%), however, at a much lower proportion than in June (Figure 3 b). Other important food items in July were Trichoptera (ca. 10 – 20%) and *G. lacustris* (Figure 3 b). Compared to June, most of the Trichopterans were now found in the two largest size

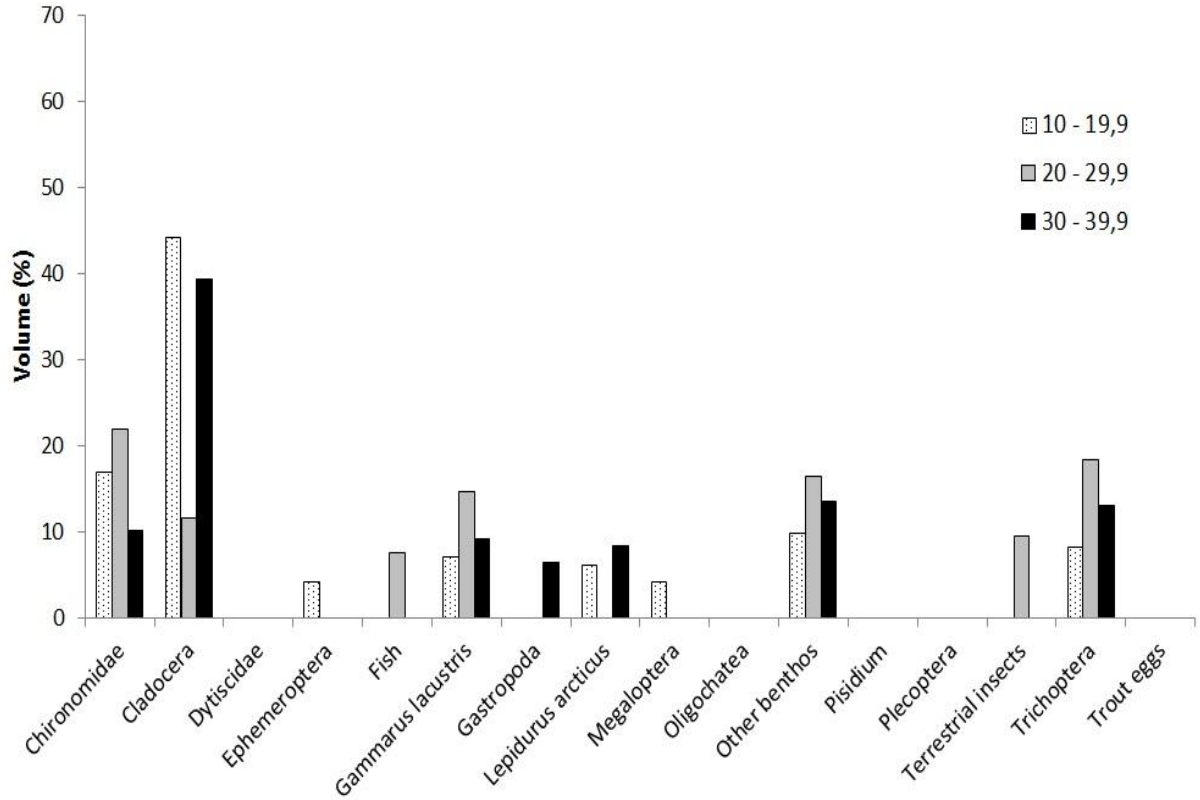
classes (Figure 3 b). The mean stomach fullness of fish analysed in July were 29% for length class 10-19.9 cm, 32% (n = 14) 46% for length class 20-29.9 cm (n = 10), and 28% for length class 30-39.9 cm (n = 12, Figure 3 b). In this month, 10, 5% of the stomachs analysed were empty (Figure 3 b).

In August, terrestrial insects were by far the most dominating food items for all three length classes, accounting for 38%, 42% and 65% of the total volume (Figure 3 c). Second most important food item for the two largest length classes in August was *Lepidurus arcticus* (ca. 20%, Figure 3 c). *L. arcticus* were also found in the longest length class in July, but in a much smaller amount (ca. 8%, Figure 3 c). Apart from terrestrial insects, the diet of size class 10-19.9 cm was largely made up by Gastropoda (ca. 20%), cladocerans (ca. 20%) and *G. lacustris* (ca. 10%) (Figure 3 c). The mean stomach fullness of fish analysed in July were 42% for length class 10-19.9 cm (n = 8), and 40% and 59% for length classes 20-29.9 cm (n = 8) and 30-39.9 cm (n = 10), respectively. In August, 6,6% of the stomachs analysed were empty.

In October, the diet of length class 30-39.9 cm was mainly comprised of trout eggs (ca. 50%, Figure 3 d), but also Plecoptera (ca. 16%) and Oligochaeta (ca. 10%) were important prey. Trout eggs were also found in length class 20-29.9 cm (ca. 20%, Figure 3 d). The main food item in this length class was, however, cladocerans (ca. 30%), which also comprised the most common prey group in the diet of length class 10-19.9 cm (ca. 52%, Figure 3 d). Except from cladocerans, length class 10-19.9 cm had eaten mainly trichopterans and Gastropoda (ca. 11% and 12%) this month (Figure 3 d). The mean stomach fullness in October were 15% for length class 10-19.9 cm (n = 8), and 32% and 22% for length classes 20-29.9 cm (n = 15) 30-39.9 cm (n = 15), respectively. By far, the highest number of empty stomachs was found in October, with 35% of the stomachs analysed being empty.


70
60
50
40
30
20
10
0
Volume (%)
10 - 19,9
20 - 29,9
30 - 39,9
Chironomidae
Cladocera
Dytiscidae
Ephemeroptera
Fish
Gammarus lacustris
Gastropoda
Lepidurus arcticus
Megaloptera
Oligochaeta
Other benthos
Pisidium
Plecoptera
Terrestrial insects
Trichoptera
Trout eggs

b)


70
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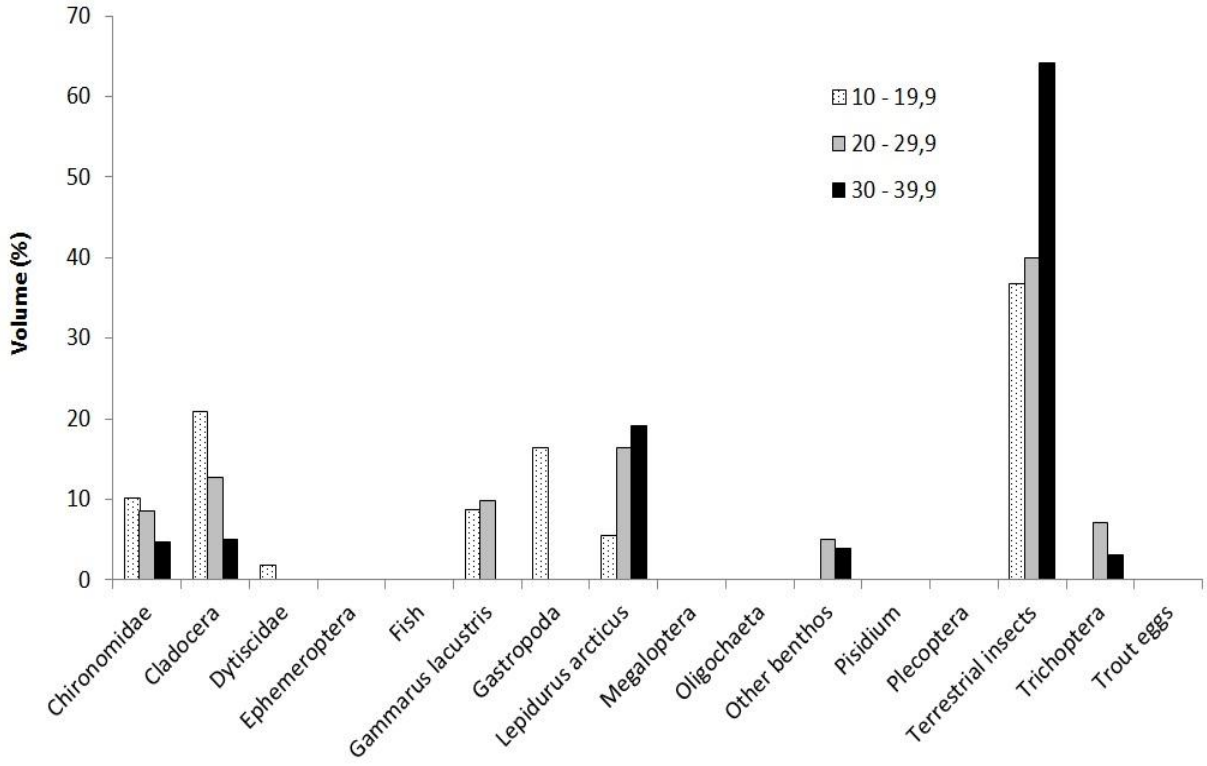
Volume (%)
10 - 19,9
20 - 29,9
30 - 39,9
Chironomidae
Cladocera
Dytiscidae
Ephemeroptera
Fish
Gammarus lacustris
Gastropoda
Lepidurus arcticus
Megaloptera
Oligochatea
Other benthos
Pisidium
Plecoptera
Terrestrial insects
Trichoptera
Trout eggs

c)



d)

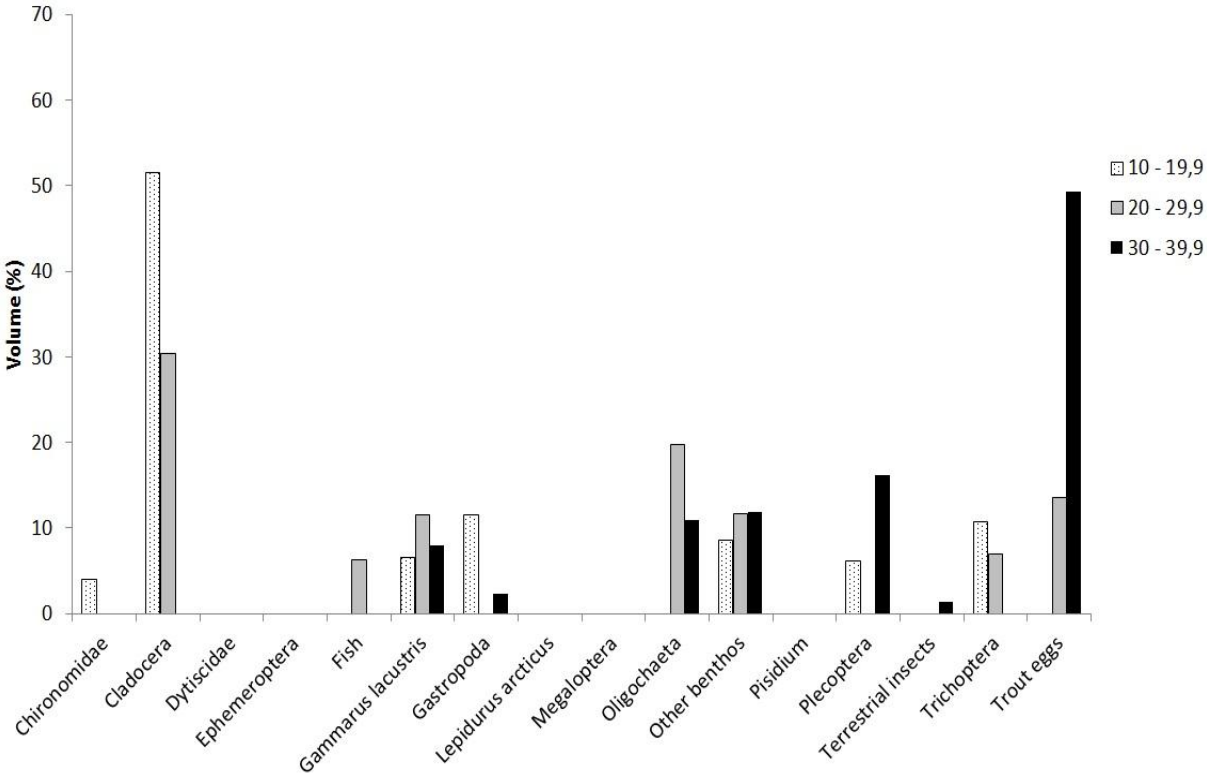


Figure 3: Diet of brown trout sampled in Øvre Heimdalsvatn in June (a, n = 51), July (b, n = 38), August (c, n = 30) and October (d, n = 62) 2013 for the three length classes; 10-19.9cm, 20-29.9cm and 30-39.9cm. The result are expressed as volume per cent. The

3.5 Zooplankton composition

The zooplankton samples from June – October were mainly made up by copepods (Cyclopoda and Calanoida) and the two cladocerans, *Holopedium gibberum* and *Bosmina* spp. (Figure 29). *Daphnia* sp. and *Polyphemus pediculus* represented a minor portion of the sample from June and July (1 - 2%, Figure 29 a and b).

Cyclopoid copepods, *Bosmina* spp. and *H. gibberum* were found throughout the sampling period. Cyclopoid copepods dominated the plankton sample in October (45%) (Figure 29 d), but did also constitute a large proportion of the July sample (23% Figure 29 b). No plankton sampling was performed in September. *Bosmina* spp. had its main peak in June where it constituted 87% of the plankton sample (Figure 29 a). Calanoid copepods were mainly found in July, 23%, but were also found to constitute a small amount in August (4%) (Figure 29 b and c). *H. gibberum* made up the highest proportion of the plankton sample in July (48%) and August (68%), and constituted also a significant part of the October sample (24%) (Figure 29 b to d).

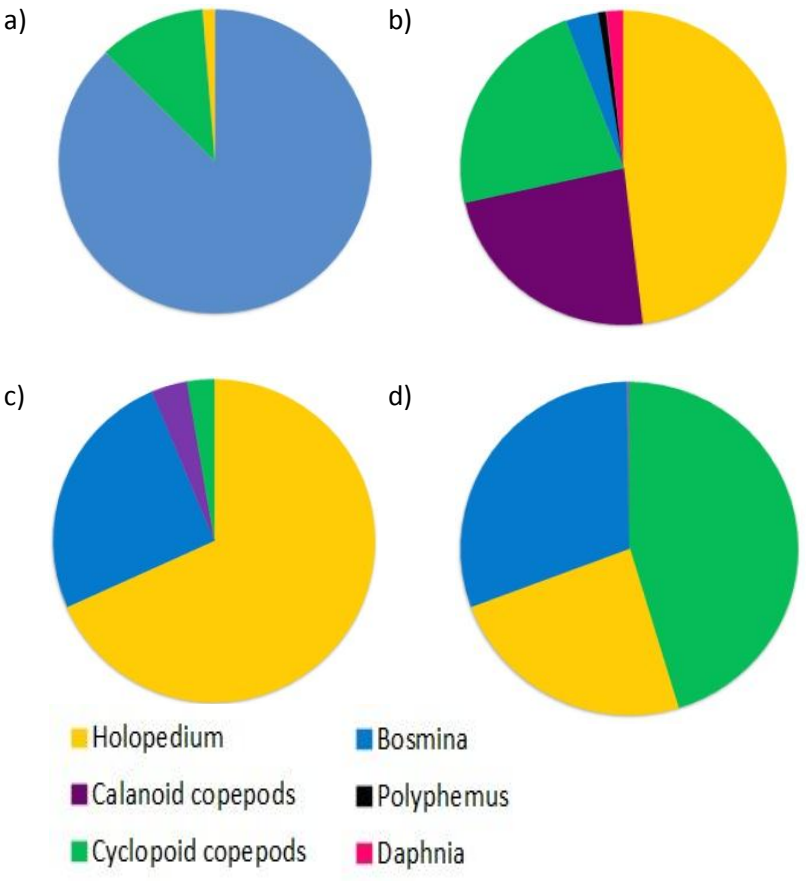


Figure 4: The zooplankton-composition of plankton samples collected in June (a), July (b), August (c) and October (d) in Øvre Heimdalsvatn 2013. The composition of each zooplankton sample is presented as percentage proportion (%).

4. Discussion

4.1 The effect of trophic position on *D. ditremum* infection in brown trout

If the high infections of *D. ditremum* found in some of the trout examined from Ø. Heimdalsvatn are due to cannibalism it would be expected that these specimens also possess higher δ^{15}N- and Hg-values than trout infected with few or zero plerocercoids of *D. ditremum*. This is further supported by earlier studies showing that *D. ditremum* has a low survival rate when transferred through piscivory, at least to rainbow trout (Halvorsen, O. & Wissler, K. 1973), which means that high numbers of plerocercoids of *D. ditremum* through cannibalism, acquires relatively high consumption of infected conspecifics, which would lead to distinctly higher δ^{15}N- and Hg-values than non-cannibalistic trout. Brown trout with higher intensity of *D. ditremum* in the present study, however, did not show distinctly higher δ^{15}N- and Hg-values than trout with low intensity, nor did specimens with fish in the stomach content possess any higher number of plerocercoids. The highest number of plerocercoids of *D. ditremum* was found in a 4 year old trout, which based upon its size (23.9 cm) has most likely gained the high infection through consumption of copepods, and not through cannibalism, since the smallest cannibal recorded in Ø. Heimdalsvatn has been above 32 cm (Bilstad & Bilstad 2006; Hagen 2003; Hasle & Skjølås 1995). This is further supported by the low mercury (0.036 mg/kg) and δ^{15}N-value (6.92‰) in this individual. In other words, high numbers of plerocercoids in the brown trout in Ø. Heimdalsvatn is most likely a result of consumption of infected copepods and not a result of cannibalism. High numbers of plerocercoids of *D. ditremum* gained through consumption of infected copepods alone has also been found for non piscivorous arctic charr in Lake Røyetjern (Halvorsen & Andersen 1984), and in benthic whitefish (*Coregonus lavaretus*), in northern Finnish Lapland (Tolonen et al. 2000).

The chance of being infected with between 1 – 5 plerocercoids of *D. ditremum* correlates, however, positively with increasing δ^{15}N-values. This can be explained more probably by the fact that as the trout grows older, it is more likely to have eaten both fish, and in that way gained higher δ^{15}N-value, and consumed infected copepods during its lifespan. Since the parasite can live in the second intermediate host for several years, it will

accumulate in the trout even though the trout have had low consumption of infected copepods (Halvorsen & Andersen 1984; Knudsen et al. 2004). The chance of being infected with 1 – 5 plerocercoids might therefor correlate positively with increasing $\delta^{15}N$ even though they are not directly linked together, but just as a fact of increasing chance of piscivory with age and size (Klemetsen et al. 2003), together with increased chance of having encountered and consumed infected copepods with increasing age.

An increase in both infection probability and intensity of plerocercoids with increasing age and size of the fish, as seen in the present study, is common for parasites that live more than one year in the host (Henricson 1977). This may be due to several factors such as older fish may have been exposed to infection over a more extended period of time than younger individuals, bigger fish will also have a larger consumption per time unit and might thereby accumulate more parasite individuals (Halvorsen & Andersen 1973; Halvorsen, O. & Wissler, K. 1973). The main factor creating this pattern, however, is commonly thought to be the longevity of the plerocercoids in the host (Halvorsen & Andersen 1984).

The $\delta^{15}N$-signatures in brown trout varied from 5.9 to 9.2 ‰, with all individuals having enrichment in $\delta^{15}N$ less than 3.4 ‰ from the lowest $\delta^{15}N$-value (5.9 ‰). Commonly there is an enrichment of 3.4 ‰ in the $\delta^{15}N$-signature in the predator relative to the $\delta^{15}N$-signature of their diet (Cabana & Rasmussen 1994). In other words, there is no distinctly piscivorous brown trout amongst the individuals sampled during the present study, probably as a result of fish (mostly minnows) as a food source is being restricted to a short period of the year (mainly in June during the spawning season of European minnow) as suggested by Lien (1981) and Museth et al. (2003). A temporal variation in the rate of piscivory by brown trout were also found during the present study were 5 out of 7 stomach contents containing fish residuals were found in June. Small levels of piscivory corresponds with the results of Jenssen et al. (2010) which also found brown trout in Ø. Heimdalsvatn to have enrichment factors lower than 3.4 ‰, indicating omnivory, in other words feeding at more than one trophic level (Cabana & Rasmussen 1994).

4.2 Factors influencing mean abundance of *D. ditremum* in brown trout

The present study and the results of Hatleli (2012) show that there has been a substantial increase in the infection of *D. ditremum* in brown trout since the first survey of the helminth fauna was conducted by Lien in 1969 – 1972 (unpublished manuscript). Lien found plerocercoids in only 2 % of the examined trout compared to 43 % in the study of Hatleli (2012), and 40 % in the present study. There has also been a substantial increase in the intensity of plerocercoids, with several brown trout exceeding 50 plerocercoids, up to a maximum of 167 plerocercoids in the present study, in sharp contrast to a maximum of 7 plerocercoids in the initial period (unpublished manuscript Lien).

The substantial increase in both prevalence and mean abundance of plerocercoids, reflects an increased temporal and/or spatial overlap between the parasite and the host population, in other words increased contact between host and parasite, which is a vital factor for the success of the parasite population (Henricson 1978). The extent of host-parasite contact will change with time as it is affected by both abiotic and biotic factors (Halvorsen & Andersen 1973).

One factor which has generally been shown to be of significance in determining the transmission success and thereby both the mean abundance and prevalence of parasites**,** is the densities of the different host populations (Arneberg et al. 1998; Kennedy et al. 2001; McCarthy 1990). For parasites with life cycles including several intermediate hosts there might, however, be difficult to determine which host density that is of most importance for the infection levels, and how altered density of one intermediate host influence the infection level in the other intermediate hosts (Amundsen & Kristoffersen 1989; Hansen & Poulin 2006). In Takvatn, northern Norway, both the intensity and prevalence of *D. dendriticum* in the second intermediate host, the arctic charr, decreased significantly as a result of heavy exploitation of charr (second intermediate host) (Haugstvedt Henriksen 2014). Infection of *D. ditremum*, on the other hand, did not start to decrease before some years after the fish removal program was terminated, indicating that other factors than density of the second intermediate host played a significant role in determining *D. ditremum* abundance in the lake (Haugstvedt Henriksen 2014). In Lake Stuorajavri, northern Norway, there was a substantial decrease in the amount of the helminth *Triaenophorus crassus* in both copepods and whitefish serving as first and second intermediate host respectively, after an extensive

removal of its final host, the pike (*Esox lucius*) (Amundsen & Kristoffersen 1989). The reduced infections were most likely a result of a reduced output of *T. crassus* eggs as whitefish continued to feed extensively upon copepods.

Cyclops scutifer and *Heterocope saliens* is the most common copepods in Ø. Heimdalsvatn (Larsson et al. 1978; Larsson et al. 2010). *Heterocope* spp. has not been reported as the intermediate hosts for *D. ditremum* (Henricson 1978), which strongly implies that *C. scutifer* represents the main copepod species responsible for transfer of *D. ditremum* to the brown trout population in Ø. Heimdalsvatn. There has, however, not been any distinct change in the population size of *C. scutifer* during the period of increased *D. ditremum* infections in brown trout (Larsson et al. 2010). The same is true for the brown trout population (Borgstrøm et al. 2010).

Although the plankton community and the brown trout population do not seem to have undergone large density changes in Ø. Heimdalsvatn during the last 50 years, it has been a substantial increase in the number of final hosts (Common- and Red – breasted merganser and Black throated loon) foraging in the lake during the ice free season, most likely as a result of increased food resources through the establishment of European minnow (Hatleli 2012). The increased number of final hosts will result in a substantial increase in the output of *D. ditremum* eggs in the lake, likely leading to a larger proportion of the copepod population being infected, thus be of significant importance for the observed increase in mean abundance of *D. ditremum* in brown trout, as also suggested by Hatleli (2012).

The availability of infected copepods, however, is also dependent upon the timing of the first intermediate host in relation to the occurrence of coracidium of *D. ditremum* (Henricson 1978). The population of *C. scutifer* in Ø. Heimdalsvatn is commonly higher in July and August compared to the rest of the year (Jensen 1978). Likewise, cyclopoid copepods constituted a significant proportion of the zooplankton sample from July (23%) in the present study. The highest proportion of cyclopoid copepods were found in October (45%). Thus, the period coracidium of *D. ditremum* are present in the lake, i.e. when the final hosts are present during the ice-free season, coincide with the population peak of *C. scutifer*, thereby increasing the availability of infected copepods.

4.3 The presence of intermediate hosts of *D.ditremum* in the diet of brown trout

Increased numbers of piscivorous birds and parasites as a result of fish introduction was also found for Lake Takvatn, where brown trout originally were the only fish species present in the lake until arctic charr and whitefish was introduced around 1930 and 1950, respectively (Amundsen et al. 2013). The introduced fish species also increased the competition altering the fish diet towards a higher proportion of small cructacean zooplankton forms, such as copepods, and increased piscivory, which furthermore increased the transmission rate of parasites including *D. ditremum*.

Likewise has it been markedly changes in the summer diet of brown trout in Ø. Heimdalsvatn in the period after the establishment of the European minnow (Borgstrøm et al. 2010), which have led to changes in the parasite composition and abundance (Hatleli 2012). The consumption of intermediate hosts of *D. ditremum*, however, seems to remain low. Juvenile brown trout constitute a small proportion of the diet as stated above. Likewise was copepods virtually absent in the stomach content of brown trout which correlates with the findings of both Hatleli (2012) and Lien (1978b), supporting the assumption that copepods constitute a minor part of the summer diet for all age classes of brown trout in Ø. Heimdalsvatn. It is, however, likely that there has been an increase in the proportion of infected copepods in the diet of brown trout following an increased number of final hosts, thereby leading to increased prevalence and intensity of *D. ditremum* infection in brown trout. Important, however, Lien (1978b) found that copepods were mainly foraged on during winter and spring and might therefore constitute a more important part of the brown trout diet than what appears to be the case in the present results.

4.4 Factors influencing the distribution pattern of *D. ditremum* in brown trout

The results of the present study and the study of Hatleli (2012) show a highly overdispersed distribution of plerocercoids of *D. ditremum* within the brown trout population ($s^2 > \bar{x}$) in Ø. Heimdalsvatn.

Clustered distribution of infected copepods

An overdispersed distribution of *D. ditremum* was also found for arctic charr in the lakes Bjellojaure in Sweden and Røyetjern in Norway, and was believed to be a result of

clustered distribution of infected copepods, leading to a few fish being exposed to a high rate of infection (Henricson 1977). Two factors of main importance in creating this pattern of infected copepods are; patchy distribution of plankton organisms, and uneven distribution of coracidium larvae in the water (Halvorsen & Andersen 1973) .

Plankton organisms may often show a more or less patchy distribution in terms of both vertical and horizontal distribution, which are believed to be due to numerous factors such as wind and current patterns, but also biological factors such as social behavior (Klemetsen 1970; Malone & McQueen 1983; Tessier 1983). One type of pattern involves high density swarms of plankton which can range from a few centimeters up to several meters (Tessier 1983). This swarm formation has been observed for copepods (Malone & McQueen 1983).

Heterogeneous distribution of parasite eggs or larvae has been suggested to be strongly dependent upon the behavioral patterns of the final host (Smith 2001). Since the final host is the source of parasites to the intermediate host, through e.g. faeces, it is expected that the parasite numbers in the intermediate host population, is highest in the areas most frequently used by the definite host (Smith 2001). Halvorsen and Andersen (1973) found that black throated diver, functioning as final host for *D. ditremum* in Lake Røyetjern, favoured some parts of the lake over others, thus creating uneven distribution of faeces, and thereby uneven distribution of coracidium of *D. ditremum*. Uneven activity distribution is commonly found for loon, one important factor being patchy distribution of prey organisms which are often found in highest densities in the littoral zone (Barr 1996; Dunker 1974). Thus, uneven activity pattern of piscivorous birds in Ø. Heimdalsvatn is likely to result in uneven distribution of coracidium and thereby uneven distribution of infected copepods. Since most of the foraging activity of the final hosts is expected to be concentrated in the littoral areas due to higher density of prey organisms; it is likely that the littoral zone has a higher abundance of infected copepods than other parts of the lake. “Hot spots” with high densities of infected copepods, might further be generated by patchy distribution of copepods.

Differential habitat utilization

Differences among individuals in the intermediate host population as regards to behavior and immunology has also been proven to be a determining factor for aggregated distribution of parasites (Shaw et al. 1998; Wilson et al. 2002). The most explanatory variable for both prevalence and intensity of *D. ditremum* in brown trout in the present study was sex, with males having significantly higher probability of being situated in infection group 2 (6 – 20 plerocercoids), and a higher probability of being in infection group 1 (1 – 5 plerocercoids). Higher rates of parasitism in males have commonly been found for a range of vertebrates and can be due to a number of biological mechanisms (Folstad & Karter 1992; Halvorsen & Andersen 1984). These mechanism are often divided into ecological mechanisms such as sex differences in behaviour, diet composition and body size, and physiological mechanisms such as different composition of hormones (Folstad & Karter 1992).

In brown trout, both size and habitat use has been proven to differ between males and females (Haraldstad & Jonsson 1983; Klemetsen et al. 2003). Several studies have shown that male trout utilize near shore habitats to a greater extent than females which are more prone to exploit pelagic waters (Haraldstad & Jonsson 1983; Klemetsen et al. 2003). In a study on lake dwelling brown trout in Myrkdalsvatnet, Norway, habitat segregation by sex was especially evident during spring and summer, and was proposed to be due to females being less aggressive than males as the same trend was observed among all age groups (Haraldstad & Jonsson 1983). Further it was observed that mature males, but not females, congregated in onshore areas in September before entering the tributaries to spawn in October and November. If this pattern is the same for brown trout in Ø. Heimdalsvatn, it will be explanatory for the infection pattern of *D. ditremum* observed between male and female trout, as the highest densities of infected copepods is likely to be concentrated in the littoral zone.

The range in δ^{13}C-signatures (-30 to -23 ‰) in brown trout from Ø. Heimdalsvatn indicate that there are variability in food habits on an individual level. The δ^{13}C-signatures do not show any clear correlation with increasing burden of *D. ditremum* or increasing age and length, but there is a small difference in the δ^{13}C-signatures between males and females (males depleted with approximately 1 ‰), supporting the assumption that there are some segregation in feeding habits as a function of sex. The mean δ^{13}C-signature in males is – 26, 3 ‰ while female have a mean signature of – 25, 6

‰. $\delta^{13}C$ -signature below – 25 ‰ has been strongly suggested to be due to incorporation of planktonic and terrestrial organic carbon (Rognerud et al. 2002). The main proportion of plankton found in the stomach content of brown trout in the present study, however, consisted of *E. lamellatus* which will not lead to more depleted $\delta^{13}C$-signatures than other benthos due to similar carbon source (periphyton). This suggests that males have a higher intake of terrestrial organic carbon in means of terrestrial insects than females, which contribute to the somewhat more depleted $\delta^{13}C$-signature. This further supports the assumption that males stay more in near shore areas than female trout in Ø. Heimdalsvatn.

The mean $\delta^{13}C$-signature in the brown trout population (- 26 ‰) is quite similar to the $\delta^{13}C$-signature of the periphyton sample from the lake bottom (-27 ‰), which indicate that benthic algae is the main carbon source for the prey organisms of brown trout in Ø. Heimdalsvatn when considering the whole population. This correlates with other studies showing that periphyton often constitute the main carbon source for important prey organisms, such as *G. lacustris, L. arcticus, E. lamellatus*, gastropoda and insect larvae, in clear shallow mountain lakes such as Ø. Heimdalsvatn and lakes in the arctic (Rognerud et al. 2003).

4.5 *D. ditremum* induced mortality in brown trout

In the present study, the variance to mean ratio seem to decrease slightly with increasing age which might indicate parasite induced host mortality. The observed decrease in variance to mean ratio, however, is most likely due to two significantly high values in age group 4 and 10. When these two values were taken out of the sample there was no evident decrease in the variance to mean ratio. Likewise is there no clear decrease in intensity of plerocercoids with increasing age or length. In other words, there are no clear signs of brown trout mortality caused by plerocercoids of *D. ditremum,* which correlates with the results of Hatleli (2012). This indicates that the transmission success of *D. ditremum* in Ø. Heimdalsvatn is still limited similar to the results from Lake Bjellojaure (Henricson 1978), but that abiotic or biotic changes in the ecosystem that favor transmission can increase the plerocercoid burden in brown trout to lethal levels. Further might changes in the ecosystem that reduces the condition of brown trout or increase the activity of the plerocercoids, i.e. increase migration inside the host (Rodger 1991) lower the number of parasites needed to cause detrimental effects.

Since no individuals in age-classes >10 had very high infections, this may still indicate a higher mortality of fish with high parasite burdens, similar to the results from arctic charr in the Lake Røyetjern (Halvorsen & Andersen 1984).

4.6 Conclusion

The strong increase in prevalence and intensity of *D. ditremum* in brown trout in Ø. Heimdalsvatn from the period around 1970 until today, is most likely a result of the increased number of final hosts (merganser and black throated loon) foraging in the lake. The increased amount of piscivorous birds may be connected to the establishment of European minnow in the lake, giving increased availability of prey fish. The increased number of final hosts will lead to an increased output of *D. ditremum* eggs, and likely lead to a higher proportion of infected first intermediate host, the copepod *C. scutifer*, thus increasing the number of procercoids being transferred to the second intermediate host, the brown trout, when copepods are fed upon. If infection of *D. ditremum* in brown trout was due to cannibalism, it would be expected that individuals with high numbers of plerocercoids possessed higher $\delta^{15}N$-values, as well as elevated Hg-concentrations compared to trout with few or non plerocercoids. There is, however, no clear difference in $\delta^{15}N$ and Hg concentrations between these two groups.

Higher prevalence and intensity of plerocercoids in brown trout males is most likely a result of males utilizing the near shore habitats, with a supposed higher proportion of infected copepods. Thus, European minnow seem to be the indirect reason for the increased infection of *D. ditremum* in brown trout in Lake Øvre Heimdalsvatn, by forming a larger food base for the final hosts.

Despite the substantial increase in infection, there were no clear signs of brown trout mortality caused by plerocercoids of *D. ditremum* in the present study. Even so, the parasite is still likely to be unfavorable and may be detrimental to the trout host.

References

Akaike, H. A. (1974). New look at the statistical model identification. *IEEE Transactions on Automatic Control*, 19 (6): 716-723.

Amundsen, P.-A. & Kristoffersen, R. (1989). Infection of whitefish (*Coregonus lvaretus* L. s.l) by *Triaenophorus crassus* Forel (Cestoda: Pseudophyllidae): a case study in parasite control. *Can. J. Zool.* , 68: 1187-1192.

Amundsen, P.-A., Lafferty, K. D., Knudsen, R., Primicerio, R., Klemetsen, A. & Kuris, A. M. (2009). Food web topology and parasites in the pelagis zone of a subarctic lake. *Journal of Animal Ecology*, 78: 563 - 572.

Amundsen, P. A., Lafferty, K. D., Knudsen, R., Primicerio, R., Kristoffersen, R., Klemetsen, A. & Kuris, A. M. (2013). New parasites and predators follow the introduction of two fish species to a subarctic lake: implications for food-web structure and functioning. *Oecologia*, 171 (4): 993-1002.

Andersen, K. I. & Gibson, D. I. (1989). A key to three species of larval *Diphyllobothrium Cobbold*, 1858 (Cestoda: Preudophyllidae) occuring in European and North American freshwater fishes *Systematic Parasitology*, 13: 3-9.

Anderson, R. K. & Gordon, D. M. (1982). Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology*, 85: 373-398.

Arneberg, P., Skorping, A., Grenfell, B. & A.F, R. (1998). Host densities as determinants of abundance in parasite communities. *Proc. R. Soc. B Biol. Sci.* , 265: 1283-1289.

Atwell, L., Hobson, A. K. & Welch, E. H. (1998). Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. *Can. J. Fish. Aquat. Sci*, 55: 1114 - 1121.

Barber, I., Hoare, D. & Krause, J. (2000). Effects of parasites on fish behaviour: a review and evolutionary perspective. *Reviews in Fish Biology and Fisheries*, 10: 131-165.

Barr, J. F. (1996). Aspects of Common Loon (Gavia immer) feeding biology on its breeding ground. *Hydrobiologia*, 321: 119 - 144.

Berube, M. & Curtis, M. A. (1986). Transmission of *Diphyllobothrium ditremum* to Arctic charr (*Salvelinus alpinus*) in two subarctic Quebec lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 43: 1626 - 1634.

Bilstad, A. & Bilstad, B. (2006). Population dynamics in brown trout (*Salmo trutta*) in the lake Øvre Heimdalsvatn 36 years after establishment of European minnow (*Phoxinus phoxinus*). *Master thesis Norwegian University of Life Science*.

Borgstrøm, R., Museth, J. & Brittain, J. E. (2010). The brown trout (*Salmo trutta*) in the lake , Øvre Heimdalsvatn: long - term changes in population dynamics due to exploitation and the invasive species, European minnow (*Phoxinus phoxinus*). *Hydrobiologia* 642: 10.

Brittain, J. E., Brabrand, Å., Saltveit, S. J., Bremnes, T. & Røsten, E. (1988). The biology and population dynamics of *Gammarus lacustris* in relation to the introduction of minnows, *Phoxinus phoxinus*, into Øvre Heimdalsvatn, a Norwegian subalpine lake *Laboratorium for ferskvannsøkologi og innlandsfiske*, Rapport nr. 109.

Brittain, J. E. & Bjørnstad, H. E. (2010). A long-term study of catchment inputs of 137Cs to a subalpine lake in the form of allochthonous terrestrial plant material. *Hydrobiologia*, 642: 6.

Brittain, J. E. & Borgstrøm, R. (2010). The Norwegian reference lake ecosystem, Øvre Heimdalsvatn. *Hydrobiologia*, 642: 7.

Burnham, K. P. & Anderson, D. R. (1998). Model Selection and Inferences. *New York: Springer Verlag*: 353.

Bush, A. O., Lafferty, K., D. , Lotz, J., M. & Shostak, A. W. (1997). Parasitology Meets Ecology on its own Terms: Margolis at al. Revisited. *J. Parasittology*, 83(4): 575-583.

Cabana, G. & Rasmussen, B. J. (1994). Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature*, 372: 255 - 257.

Cabana, G. & Rasmussen, B. J. (1996). Comparison of aquatic food chains using nitrogen isotopes. *Proc. Natl. Acad. Sci. USA*, 93: 10844 - 10847.

Curtis, A. M. (1984). Diphyllobothrium spp. and the Arctic Charr: Parasite Acquisition and its Effects on a Lake-Resident Population. *L .Johnson and B.L. Burns [eds.] Biology of the Arctic charr, Proceedings of the International Symposium on Arctic Charr, Winnipeg, Manitoba, May 1981. Univ. Manitoba Press, Winnipeg*: 395-411.

Desta, Z., Borgstrøm, R., Gebremariam, Z. & Rosseland, B. O. (2008). Habitat use and trophic position determine mercury concentration in the straight fin barb *Barbus paludinosus*, a small fish species in Lake Awassa, Ethiopia. *Journal of Fish Biology*, 73 (3): 477-497.

Dobson, A., Lafferty, K. D., Kuris, A. M., Hechinger, R. F. & Jetz, W. (2008). Colloquium paper: homage to Linnaeus: how many parasites? How many hosts? *Proc Natl Acad Sci U S A*, 105 Suppl 1: 11482-9.

Dunker, H. (1974). Habitat selection and territory size of the Black-throated Diver, *Gavia arctica* (L.), in south Norway. *Norw. J. Zool.*, 22: 15-29.

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.

Farquhar, G. D., Ehleringer, J. R. & Hubick, K. T. (1989). Carbon isotope discrimination and photosynthesis. *Ann. Rev. Plant Physiol. Plant. Mol. Biol*, 40: 503 - 537.

Folstad, I. & Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. *The American Naturalist*, 139: 603 - 622.

France, L. R. (1995). Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnol. Oceanogr.*, 40 (7): 1310-1313.

Gallagher, C. P. & Dick, T. A. (2010). Trophic structure of a landlocked Arctic char *Salvelinus alpinus* population from southern Baffin Island, Canada. *Ecology of Freshwater Fish*, 19 (1): 39-50.

Gozlan, R. E., Britton, J. R., Cowx, I. & Copp, G. H. (2010). Current knowledge of non-native freshwater fish introductions. Review paper. *Journal of Fish Biology*, 76: 751-786.

Grøterud, O. & Kloster, A. E. (1978). Hypsography, meteorology and hydrology of the Øvre Heimdalen catchment. *Holarctic Ecology*, 1: 111 - 116.

Hagen, E. (2003). Piscivorous brown trout (*Salmo trutta*) in the high mountain lake Øvre Heimdalsvatn; Cannibalism and predation on minnows (*Phoxinus phoxinus*). *MSc thesis Institute of Biology and Nature Conservation, NHL, Ås*.

Hall, B. D., Bodaly, R. A., Fudge, R. J. P., Rudd, J. W. M. & Rosenberg, D. M. (1997). Food as the dominant pathway of methylmercury uptake by fish. *Water, Air, and Soil Pollution*, 100: 13 - 24.

Halvorsen, O. & Andersen, K. (1973). Parasitter i ferskvannsmiljøer, biologi og økologi. *Fauna*, 26: 165-189.

Halvorsen, O. & Wissler, K. (1973). Studies of the helminth fauna of Norway XXVIII. An experimental study of the ability of *Diphyllobothrium latum* (L.), *D. dendriticum* (Nitzsch), and *D.ditremum* (Creplin) (Cestoda, Pseudophyllidea) to infect paratenic hosts. *Norw. J. Zool.*, 21: 201-210.

Halvorsen, O. & Wissler, S. K. (1973). Studies of the helminth fauna of Norway. XXVIII: An experimental study of the availability of *Diphyllobothrium latum* (L.), *D.dendriticum* (Nitzsch) and *D.ditremum* (Creplin) (Cestoda: Pseudophyllodiae) to infect paratenic hosts. *Norw. J. Zool.* , 21: 201-210.

Halvorsen, O. & Andersen, K. (1984). The ecological interaction between arctic charr, *Salvelinus alpinus (L.)*, and the plerocercoid stage of *Diphyllobothrium ditremum*. *Journal of Fish Biology*, 25: 305 - 316.

Hammar, J. (2000). Cannibals and parasites: conflicting regulators of bimodality in high latitude Arctic char, *Salvelinus alpinus*. *OIKOS*, 88: 33-47.

Hansen, E. K. & Poulin, R. (2006). Spatial covariation between infection levels and intermediate host densities in two trematode species. *Journal of Helminthology* 80: 255-259.

Haraldstad, Ø. & Jonsson, B. (1983). Age and sex segregation in habitat utilization by brown trout in a Norwegian lake. *Transactions of the American Fisheries Society*, 112 (1): 27-37.

Hartvigsen, R. (1997). Spredning av parasitter ved innvandring og/eller introduksjon av nye fiskearter: spredning av øreky (*Phoxinus phoxinus*) til ørretvassdrag. *NINA Oppdragsmelding*, 466: 1-14.

Hasle, K. & Skjølås, S. (1995). Ørret (*Salmo trutta*) i Øvre Heimdalsvatn - dynamikk og endringer i bestanden etter etablering av ørekyt (*Phoxinus phoxinus*). *MSc thesis Institute of Biology and Nature Conservation, NLH, Ås*.

Hatleli, Ø. (2012). 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*). *Master thesis. Norwegian University of Life Sciences*.

Haugstvedt Henriksen, E. (2014). Long-term population dynamics of *Diphyllobothrium ditremum* and *D.dendriticum* (Cestoda: Pseudophyllidae) in their salmonid hosts following a fish removal experiment. *Master thesis. The Arctic University of Norway*.

Henricson, J. (1977). The abundance and distribution of *Diphyllobothrium dendriticum* (Nitzsch) and *D.ditremum* (Creplin) in the char *Salvelinus alpinus* (L.) in Sweden *Journal of Fish Biology*, 11: 231-248.

Henricson, J. (1978). The dynamics of infection of *Diphyllobothrium dendriticum* (Nitzsch) and *D.ditremum* (Creplin) in the char *Salvelinus alpinus* (L.) in Sweden. *Journal of Fish Biology*, 13: 51-71.

Hestehagen, T. & Sandlund, O. T. (2010). NOBANIS - Invasive alien species fact sheet - *Phoxinus phoxinus*. -From: Online Database of the European Network on Invasive Alien Species - NOBANIS www.nobanis.org, Date of access 24/02/2014.

Hoffman, G. L. (1999). Parasites of North American freshwater fishes. Second Edition. *Cornell University Press*: 1.

Hynes, H. B. N. (1950). The food of the freshwater stickle-backs (*Gasterosteus acculeatus* and *Pygosteus pungitius*) with a review of methods used in the studies of the food of fishes. *J. Anim. Ecol.*, 19.

Jensen, K. (1977). On the dynamics and exploitation of the population of brown trout (*Salmo trutta*) L., in Lake Øvre Heimdalsvatn, Southern Norway *Institute of Freshwater Researh Drottningholm Report*, 56: 18-69.

Jensen, K. W. (1978). The dynamics and exploitation of the brown trout population of Øvre Heimdalsvatn. *Holarctic Ecology*, 1: 277 - 278.

Jenssen, M. T., Borgstrøm, R., Salbu, B. & Rosseland, B. O. (2010). The importance of size and growth rate in determining mercury concentrations in European minnow (*Phoxinus phoxinus*) and brown trout (*Salmo trutta*) in the subalpine lake, Øvre Heimdalsvatn. *Hydrobiologia*, 642: 115 - 126.

Johnson, W. M., Hesslein, R., H. & Dick, T. A. (2004). Host length, age, diet, parasites and stable isotopes as predictors of yellow perch (*Perca flavesens* Mitchill), trophic status in nutrient poor Canadian Shield lakes. *Environmental Biology of Fishes*, 71: 379-388.

Jonnson, B. (1976). Comparison of scales and otoliths for age determination in brown trout, *Salmo trutta* L. . *Norw. J. Zool.*, 24 (295-301).

Kennedy, C. R., Shears, P. C. & Shears, J. A. (2001). Long-term dynamics of *Ligula intestinalis* and roach *Rutilus rutilus*: a study of tree epizootic cycles over thirty-one years. *Parasitology*, 123: 257-269.

Kidd, K. A., Hesslein, R., H., Fudge, R. J. P. & Hallard, K. A. (1995). The influence of trophic level as measured by $\delta^{15}N$ on mercury concentrations in freshwater organisms. *Water, Air, and Soil Pollution*, 80: 1011 - 1015.

Klemetsen, A. (1970). Plankton swarms in Lake Gjokvatn, East Finmark. *Astarte, Journal of Arctic biology*, 3: 83 - 85.

Klemetsen, A., Amundsen, P.-A., Dempson, J. B., Jonsson, B., Jonsson, N., O`Connell, M. F. & Mortensen, E. (2003). Atlantich salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic

charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecology of Freshwater Fish*, 12: 1-59.
Kloster, A. E. (1978). Physical and chemical properties of the waters of Øvre Heimdalen. *Holarctic Ecology*, 1: 117 - 123.
Knudsen, R. & Klemetsen, A. (1994). Infections of *Diphyllobothrium dendriticum*, *D. ditremum* (Cestoda), and *Cystidicola farionis* (Nematoda) in a north Norwegian population of Arctic charr (*Salvelinus alpinus*) during winter. *Can. J. Zool.*, 72: 1922 - 1930.
Knudsen, R., Klemetsen, A. & Staldvik, F. (1996a). Parasites as indicators of individual feeding specialization in Arctic charr during winter in northern Norway. *Journal of Fish Biology*, 48: 1256 - 1265.
Knudsen, R., Klemetsen, A. & Staldvik, F. (1996b). Parasites as indicators of individual feeding spesialization in Arctic charr during winter in northern Norway. *Journal of Fish Biology*, 48: 1256 - 1265.
Knudsen, R. (1997). Aggregation of Helminths: the role of feeding behaviour of fish hosts. *The Norwegian College of Fisheries Science, University of Tromsø*.
Knudsen, R., Curtis, A. M. & Kristoffersen, R. (2004). Aggregation of Helminths: the role of feeding behaviour of fish hosts. *J. Parasittology*, 90 (1): 1-7.
Knudsen, R., Amundsen, P.-A., Nilsen, R., Kristoffersen, R. & Klemetsen, A. (2008). Food borne parasites as indicators of trophic segregation between Arctic charr and brown trout. *Environmental Biology of Fishes*, 83 (1): 107-116.
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.
Kristoffersen, R. (1989). Parasittene hos Takvassrøya. *Ottar*, 176: 31 - 37.
Kristoffersen, R., Klemetsen, A. & Halvorsen, O. (1993). Epidemiology of two sympatric *Diphyllobothrium* species in arctic charr *Salvelinus alpinus* (L.): temporal and spatial patterns. *Ph.D. thesis, University of Tromsø*: 24-55.
Kvambekk, Å. S. & Melvold, K. (2010). Long-term trends in water temperature and ice cover in the subalpine lake, Øvre Heimdalsvatn, and nearby lakes and rivers. *Hydrobiologia*, 642: 14.
Lafferty, K. D., Dobson, A. P. & Kuris, A. M. (2006). Parasites dominate food web links. *Proc Natl Acad Sci U S A*, 103 (30): 11211-11216.
Lafferty, K. D., Allesina, S., Arim, M., Briggs, C. J., De Leo, G., Dobson, A. P., Dunne, J. A., Johnson, P. T., Kuris, A. M., Marcogliese, D. J., et al. (2008). Parasites in food webs: the ultimate missing links. *Ecol Lett*, 11 (6): 533-46.
Larsson, P., Brittain, J. E., Lien, L., Lillehammmer, A. & Tangen, K. (1978). The lake ecosystem of Øvre Heimdalsvatn. *Holarctic Ecology*, 1: 304 - 320.
Larsson, P., Hansen, H. & Bjørnstad Helland, L. K. (2010). Between-year variations in the development of crustacean zooplankton in the Norwegian subalpine lake, Øvre Heimdalsvatn. *Hydrobiologia*, 642: 10.
Layman, C. A., Araujo, M. S., Boucek, R., Hammerschlag-Peyer, C. M., Harrison, E., Jud, Z. R., Matich, P., Rosenblatt, A. E., Vaudo, J. J., Yeager, L. A., et al. (2012). Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biol Rev Camb Philos Soc*, 87 (3): 545-62.
Lien, L. (1978a). The vertebrates of the Øvre Heimdalen valley. *Holarctic Ecology*, 1: 107 - 110.
Lien, L. (1978b). The energy budget of the brown trout population of Øvre Heimdalsvatn. *Holarctic Ecology*, 1: 279 - 300.
Lien, L. (1978c). Energy consumption of ducks on Øvre Heimdalsvatn. *Holarctic Ecology*, 1: 301 - 303.
Lien, L. (1981). Biology of the minnow *Phoxinus phoxinus* and its interactions with brown trout *Salmo trutta* in Øvre Heimdalsvatn, Norway *Ecography*, 4 (3): 191-200.
Malone, B. J. & McQueen, D. J. (1983). Horizontal patchiness in zooplankton populations in two Ontario kettle lakes. *Hydrobiologia*, 99: 101 - 124.

Marcogliese, D. J. (2004). Parasites: Small Players with Crucial Roles in the Ecological Theater. *EcoHealth*, 1: 151-164.

Marcogliese, D. J. (2005). Parasites of the superorganism: are they indicators of ecosystem health? *Int J Parasitol*, 35 (7): 705-16.

McCallum, H. & Dobson, A. (1995). Detecting disease and parasite threats to endangered species and ecosystems. *TREE*, 10 (5): 190-194.

McCarthy, A. M. (1990). The influence of second intermediate host dispersion pattern upon the transmission of cercariae of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae). *Parasitology*, 101 (01): 47-43.

Munger, J., C., Karasov, W., H. & Chang, D. (1989). Host genetics as a cause of overdispersion of parasites among hosts: how general a phenomen. *J. Parasitol.*, 75 (5): 707 - 710.

Museth, J., Borgstrøm, R., Brittain, J. E., Herberg, I. & Naalsund, C. (2002). Introduction of the European minnow into a subalpine lake: habitat use and long-term changes in population dynamics. *Journal of Fish Biology*, 60: 1308-1321.

Museth, J., Borgstrøm, R., Hame, T. & Holen, Å. L. (2003). Predation by brown trout: a major mortality factor for sexually mature European minnows. *Journal of Fish Biology*, 62: 692-705.

Museth, J., Hestehagen, T., Sandlund, O. T., Thorstad, E. B. & Ugedal, O. (2007). The history of the minnow *Phoxinus phoxinus* (L.) in Norway: from harmless species to pest. *Journal of Fish Biology*, 71 (Supplement D): 184-195.

Museth, J., Borgstrøm, R. & Brittain, J. E. (2010). Diet overlap between introduced European minnows (*Phoxinus phoxinus*) and young brown trout (*Salmo trutta*) in the lake, Øvre Heimdalsvatn: a result og abundant resources or forced niche overlap? *Hydrobiologia*, 642: 93-100.

Næstad, F. & Brittain, J. E. (2010). Long-term changes in the littoral benthos of a Norwegian subalpine lake following the introduction of the European minnow (*Phoxinus phoxinus*). *Hydrobiologia*, 642: 71-79.

Peterson, J. B. & Fry, B. (1987). Stable Isotopes in Ecosystem studies. *Ann. Rev. Ecol. Syst.* , 18: 293-320.

Post, M. D. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83(3): 703-718.

Power, M., Klein, G. M., Guiguer, K. R. R. A. & Kwan, M. K. H. (2002). Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. *Journal of Applied Ecology*, 39: 819 -830.

Rahkonen, R. & Koski, P. (1997). Occurence of cestode larvea in brown trout after stocking in a large regulated lake in northern Finland. *Diseases in Aquatic organisms, Dis Aquat Org*, 31: 55 - 63.

Rodger, H. D. (1991). *Diphyllobothrium* sp. infections in freshwater-reared Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 95 (7-14).

Rognerud, S., Grimalt, J. O., Rosseland, B. O., Fernandez, P., Hofer, R., Lackner, R., Lauritzen, B., Lien, L., Massabuau, J. C. & Ribes, A. (2002). Mercury and organochlorine contamination in Brown trout (*Salmo trutta*) and Arctic charr (*Salvelinus alpinus*) from high mountain lakes in Europe and the Svalbard Archipelago. *Water, Air, and Soil Pollution*, 2: 209-232.

Rognerud, S., Borgstrøm, R., Qvenild, T. & Tysse, Å. (2003). Ørreten på Hardangervidda. Næringsnett, kvikksølvinnhold, ørekytspredning og klimavariasjoner - følger for fiske og forvaltning. *Rapport LNR. 4712-2003*: 1-68.

Rosseland, B. O., Massabuau, J.-C., Grimalt, J., Hofer, R., Lackner, R., Raddum, G., Rognerud, S. & Vives, I. (2001). The EMERGE fish sampling manual for live fish. *The EMERGE Project (European Mountain lake Ecosystems: Regionalisation, diaGnostic and socio-economic valuation)*.

Sharp, G. J. E., Pike, A. W. & Secombes, C. J. (1989). The immune response of wild rainbow trout, *Salmo gairdneri* Richarson, to naturally acquired plerocercoid infections of *Diphyllobothrium dendriticum* (Nitzsch, 1824) and *D.ditremum* (Creplin, 1825). *Journal of Fish Biology*, 35 (6): 781-794.

Shaw, D. J., Grenfell, B. T. & Dobson, A. P. (1998). Patterns of macroparasite aggregation in wildlife host populations. *Parasitology*, 117: 597 - 610.
Smith, N. F. (2001). Spatial heterogeneity in recruitment of larval trematodes to snail intermediate hosts. *Oecologia*, 127 (1): 115-122.
Strayer, D. L. (2010). Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology*, 55 (Suppl. 1) (152-174).
Sømme, I. D. (1941). Ørretboka. *Jakob Dybwards forlag, Oslo*: 1-223.
Team, R. D. C. (2012). R: A language and environment for statistical computing. *R foundation for statistical somputing (ed). Vienna, Austria*.
Tessier, A., J. (1983). Coherence and horizontal movements of patches pf *Holopedium gibberum* (Cladocera). *Oecologia*, 60: 71 - 75.
Tolonen, A., Rita, H. & Peltonen, H. (2000). Abundance and distribution of *Diphyllobothrium ditremum* Creplin (Cestoda: Pseudophyllidae) plreocercoids in behtic whitefish, in northern Finnish Lapland. *Journal of Fish Biology*, 57: 15-28.
Valtonen, E. T. & Julkunen, M. (1995). Influence of the transmission of parasites from prey fishes on the composition of the parasite community of a predatory fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 51(Suppl. 1): 233-245.
Valtonen, E. T., Marcogliese, D. J. & Julkunen, M. (2010). Vertebrate diets derived from trophically transmitted fish parasites in the Bothnian Bay. *Oecologia*, 162 (1): 139-52.
Vander Zanden, M. J. & Rasmussen, B. J. (1999). Primary consumer δ13C and δ15N and the trophic position of aquatic consumers. *Ecology*, 80 (4): 1395 - 1404.
Vik, R. (1964a). The genus *Diphyllobothrium*: An example of the interpendence of systematics and experimental biology. . *Exp. Parasitol*, 15: 361-380.
Vik, R. (1964b). Studies of the helminth fauna of Norway. V. Plerocercoids of Diphyllobothrium spp. from the Rössåga water system, Nordland county. *Nytt magasin for zoologi*, 12: 1-9.
Vik, R. (1978). The Lake Øvre Heimdalsvatn - a subalpine freshwater ecosystem: Introduction. *Holarctic Ecology*, 1: 84 - 88.
Wassom, D., L., Dick, T. A., Arnason, N., Stricland, D. & Grundmann, A., W. (1986). Host genetics: a key factor in regulating the distribution of parasites in natural host populations. *J. Parasit.*, 72 (2): 334 - 337.
Weiland, K. A. & Meyers, T. R. (1989). Histopathology of *Diphyllobothrium ditremum* plerocercoids in coho salmon *Oncorhynchys kisutch*. *Diseases in Aquatic organisms*, 6: 175-178.
Whitlock, C. M. & Schluter, D. (2009). The analysis of biological data. *Greenwood Village, Colorado, Roberts and Company Publishers*
Wilson, K., Bjørnstad, O. N., Dobson, A. P., Merler, S., Poglayen, G., Randolph, S. E., Read, A. F. & Skorping, A. (2002). Chapter 2: Heterogeneities in macroparasite infections: patterns and processes. *The Ecology og Wildlife Diseases*: 6 - 44.
Østhagen, H. & Egelie, K. (1978). The vegetation of the Øvre Heimdalen valley. *Holarctic Ecology*, 1 no. 2-3: 103 - 106.

Appendix 1

Raw data from Lake Øvre Heimdalsvatn.

Table 1: Sex, length, weight, age, stomach fullness, colour (R = red, LR = light red, W = white) and number of plerocercoids of *D.ditremum* in brown trout caught in Øvre Heimdalsvatn in June 2013. The sex column does also present the fertility stage, symbolized with roman numbers. Specimens with fish in the stomach content are symbolized with bold ID numbers and grey background.

ID	Sex	Length (cm)	Weight (g)	Age (w.)	Stomach	Colour	*D.ditremum*
1	♂ II	35.5	377	16	3/4	LR	0
2	♀ II	27.6	187	9	3/4	LR	1
3	♀ VII/II	36.3	395	14	1/3	LR	4
4	♂ II	33.8	352	10	1/4	R	0
5	♂ VII/II	38.7	641	13	1/3	LR	9
6	♂ VII/II	32.6	424	10	1/10	R	5
7	♀ I/II	21.1	71	7	1/5	W	22
8	♀ II	32.2	291	9	1/2	R	0
9	♀ VII/II	32.9	345	9	1/10	R	0
10	♂ II	29.8	223	8	1/4	LR	0
11	♂ VII/II	35.5	440	10	1/10	R	1
12	♀ VII/II	27	186	8	1/2	W	0
13	♀ II	27.6	181	8	1/3	LR	1
14	♂ VII/II	29.9	224	10	1/2	R	12
15	♂ VII/II	28.3	206	9	1/4	LR	0
16	♂ I	15.2	31	4	1/4	W	0
17	♀ II	26.7	159	7	1/4	R	1
18	♂ II	24.3	130	6	1/2	LR	0
19	♂ I	29.6	221	9	1/4	R	30
20	♂ I	24.1	128	8	1/4	LR	3
21	♂ I	16.4	36	5	0	W	30
22	♂ I	15.7	32	5	0	W	3
23	♂ I	14.2	25	4	0	W	0
24	♀ I	18.2	49	5	0	W	0
25	♂ I	14.5	26	5	1/15	W	0
26	♂ 1	17.2	44	5	1/4	W	0
27	♀ I	15.2	29	4	1/4	W	0
28	♂ II	20.5	75	6	1/6	W	0
29	♀ I	14.6	25	4	0	W	0
30	♂ I	15.2	28	4	1/20	W	0
31	♀ I	15.2	29	5	1/4	W	3
32	♂ I	13.4	21	4	1/10	W	0
33	♂ II	28.9	209	7	1/20	LR	8
34	♂ II	27.4	185	7	1/5	R	0
35	♂ VII/II	34.5	458	11	1/5	R	0

36	♀ VII/II	36.2	448	12	1/20	LR	0
37	♀ I	25.8	148	7	1/2	LR	0
38	♂ I	22.2	102	NA	0	W	4
39	♂ I	25	131	6	1/20	W	3
40	♀ I	19.6	64	5	1/3	W	0
41	♂ II	22.4	94	4	0	W	0
42	♀ I	20	66	5	0	W	0
43	♀ I	19.2	60	5	1/5	W	0
44	♀ II	36.1	469	NA	1/4	R	14
45	♂ VII/II	34.7	438	11	1/5	R	2
46	♂ II	29.8	227	8	1/3	W	2
47	♀ VII/II	31.6	245	8	1/5	LR	1
48	♂ VII/II	31.1	282	9	1/3	R	0
49	♂ VII/II	27	185	9	1/2	K	0
50	♂ VII/II	32.5	314	9	1/10	R	0
51	♂ VII/III	34.7	493	16	1/20	R	15

Table 2: Sex, length, weight, age, stomach fullness, colour (R = red, LR = light red, W = white) and number of plerocercoids of *D.ditremum* in brown trout caught in Øvre Heimdalsvatn in July 2013. The sex column does also present the fertility stage, symbolized with roman numbers. Specimens with fish in the stomach content are symbolized with bold ID numbers.

ID	Sex	Length (cm)	Weight (g)	Age (w.)	Stomach	Colour	*D.ditremum*
52	♂ III/IV	35.8	50	15	1/20	R	19
53	♂ III/IV	34.2	433	10	1/10	R	0
54	♀ II/III	30.4	252	7	1	LR	0
55	♂ II	24.6	137	5	1/6	LR	1
56	♂ I	15.4	37	4	0	W	0
57	♀ I	22.8	111	4	1	W/LR	0
58	♂ IV/V	40	642	11	1/20	R	8
59	♂ VII/V	35.7	430	8	2/5	R	1
60	♂ VII/VI	36	452	10	1/10	R	0
61	♀ II	28.2	187	7	2/3	LR	0
62	♂ VII/II	42.8	854	14	0	R	0
63	♀ II	19	61	6	1/20	W	0
64	♂ V	18.7	54	4	1/5	W/LR	0
65	♂ II	18.1	58	5	2/3	W	0
66	♂ II	18.5	55	3	2/3	W	0
67	♂ I	15.6	37	5	1/6	W	0
68	♀ V	28.4	241	11	1/6	R	0
69	♀ II	28.2	184	8	1/5	LR	0
70	♂ II	16.6	41	5	1/7	W	0
71	♂ I	14.3	29	4	1/5	W	2
72	♂ V/II	33.7	359	9	1/2	R	1
73	♂ VII/IV	31.7	346	9	1/5	R	0
74	♂ VII/IV	31.7	329	11	1/3	R	5
75	♂ II	18.5	60	4	1/10	W	0
76	♀ VII/V	35.7	403	11	2/3	R	3
77	NA	32.2	320	11	1/20	LR	3
78	♂ VII/II	37.4	515	10	0	LR	1
79	♀ II	26.1	152	6	2/3	LR	0
80	♀I	20.5	85	4	1/2	W/LR	15
81	♂ II	20.5	76	5	1/3	W	0
82	♂ I	19.5	62	4	1/3	W	0
83	♀ II	25.5	150	6	1/4	W/LR	1
84	♂ I	18.6	70	5	1/2	W	1
85	♀ I	18.8	55	5	1/20	W	0
86	♂ I	15	30	4	2/3	W	0
87	♀ I	18	46	4	1/6	W	0
88	♀ II	25.3	124	5	2/3	LR	0
89	♂ VII/V	36.2	494	16	1/20	R	0

Table 3: Sex, length, weight, age, stomach fullness, colour (R = red, LR = light red, W = white) and number of plerocercoids of *D.ditremum* in brown trout caught in Øvre Heimdalsvatn in August 2013. The sex column does also present the fertility stage, symbolized with roman numbers. Specimens with fish in the stomach content are symbolized with bold ID numbers.

ID	Sex	Length (cm)	Weight (g)	Age (w.)	Stomach	Colour	*D.ditremum*
90	♂ VII/II	24.5	388	14	1/5	R	2
91	♂ VII/VI	34.4	464	10	1/7	R	4
92	♂ I	18.2	65	4	1/10	W	0
93	♂ II	29.6	259	7	1/3	R	0
94	♂ VII/II	31	273	9	1/2	R	2
95	♀ IV	32.8	391	NA	1	R	109
96	♀ VI/VII	31.9	311	13	1	LR	3
97	♀ II	31.6	284	10	1/4	R	1
98	♂ III/II	29.5	266	8	1/5	LR	1
99	♀ II	29.5	230	8	1	R	0
100	♂ V	26	177	7	1/4	W	15
101	♂ VII/II	29.6	217	8	NA	LR	1
102	♂ VII/V	35.9	481	11	2/3	R	3
103	♂ VII/IV	36.2	527	14	2/3	LR	59
104	♂ VII/IV	37.6	601	7	1/3	R	0
105	♂ VII/II	33.6	352	10	0	R	0
106	♂ V	20.8	91	6	1/20	W	0
107	♂ II	18.8	61	4	1/6	W	3
108	♂ II	20.5	83	5	1/2	W	0
109	♀ I	20.3	78	5	2/3	W	0
110	♂ I	19.7	78	4	1/2	W	0
111	♀ I	19.4	72	5	2/3	W	28
112	♂ I	20.2	70	5	1/20	W	0
113	♀ V	32.5	348	12	1	R	0
114	♂ IV/V	18.6	72	4	1/4	W	0
115	♀ I	17.6	54	4	1/6	W	0
116	♂ I	20.3	87	4	1/3	W	1
117	♂ V	16.6	46	5	1/2	W	0
118	♂ I	18.2	60	4	1	W	0
119	♂ I	20.1	79	6	1/10	W	0

Table 4: Sex, length, weight, age, stomach fullness, colour (R = red, LR = light red, W = white) and number of plerocercoids of *D.ditremum* in brown trout caught in Øvre Heimdalsvatn in October 2013. The sex column does also present the fertility stage, symbolized with roman numbers. Specimens with fish in the stomach content are symbolized with bold ID numbers.

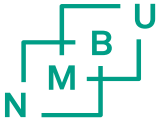
ID	Sex	Length (cm)	Weight (g)	Age (w.)	Stomach	Colour	*D.ditremum*
120	♀ VII	45.2	869	17	1/10	R	0
121	♂ VI/VII	35.6	464	15	1/10	R	8
122	♂ V/VI	37.5	484	10	0	LR	104
123	♂ V/VI	31.9	307	8	0	LR	0
124	♀ V	36	421	11	1/20	W	0
125	♀ II	33.5	299	10	1/4	R	0
126	♂ II	27.5	184	7	1/30	LR	0
127	♀ VII/II	34	312	17	0	R	60
128	♂ II	35.4	406	9	0	LR	0
129	♀ II	28.5	198	8	1/3	R	1
130	♀ V	28.9	216	7	0	W	0
131	♂ II	25.4	150	6	1/5	LR	15
132	♂ II	31.2	266	7	1/4	LR	0
133	♂ V	33.5	361	8	1/2	W	8
134	♀ VII/II	34.7	352	15	1/30	NA	7
135	♀ VII/II	32.2	275	12	0	W	0
136	♀ V	32.5	369	13	0	W	0
137	♀ II	28	194	8	1/3	LR	3
138	♀ I/II	19.4	61	5	1/4	W	0
139	♂ I	19.2	63	4	1/20	W	0
140	♀ I	18.2	50	6	1/5	W	0
141	♀ I	20.8	67	4	1/10	W	0
142	♂ I	20.5	79	3	0	W	2
143	♀ II	28	191	8	1/20	R	0
144	♂ II	26.7	154	8	0	W	17
145	♀ I	15.3	30	4	1/10	W	0
146	♂ I	13.6	24	3	0	W	0
147	♀ II	21.2	79	5	3/4	W	0
148	♂ I	20.3	74	4	0	W	0
149	♀ I	15.6	31	4	1/30	W	0
150	♀ I	16.5	36	4	1/5	W	1
151	♀ I	17.3	44	4	1/5	W	0
152	♀ I	24.9	125	6	0	LR	0
153	♀ II	24.3	148	7	1/20	LR	0
154	♀ II	21	83	5	1/20	LR/w	0
155	♂ II	23.9	121	4	0	LR	167
156	♀ II	19.6	66	4	1/5	NA	0
157	♂ V	28.8	238	11	1	LR	0

158	♀ V	29.8	266	10	0	LR	0
159	♀ VII/II	29.7	225	11	1/4	R	2
160	♂ II	29.3	224	10	1/5	R	0
161	♀ V	29.1	215	7	0	R	69
162	♂ VI	29.1	196	8	1/2	LR	0
163	♀ VII/II	29.4	205	11	0	LR	0
164	♀ VII/II	34	312	15	0	W	0
165	♂ V/VI	40.2	588	10	1/2	W	1
166	♀ II	26.9	165	6	1/5	LR	1
167	♀ II	25.3	150	6	0	LR	0
168	♀ II	28.3	182	9	0	LR	1
169	♀ II	25.8	148	8	3/4	LR	3
170	NA	40.2	635	19	1/20	W	0
171	♂ VI	34.8	532	11	2/3	R	4
172	♀ VII/II	35.5	347	15	0	LR	0
173	♂ V	37	450	10	1/20	LR	0
174	♂ V	36	434	NA	1/20	R	5
175	♂ VI	37.4	460	13	1/10	LR	29
176	♂ VI	33.8	356	10	1/10	R	1
177	♂ VI	34.3	402	12	0	LR	54
178	♂ VI	34	341	10	1/3	LR	0
179	♂ VI	33.6	382	12	1/30	LR	0
180	♂ VI	32.4	300	10	1/3	LR	128
181	♂ II	21	121	5	0	LR	0

Table 5: δ15N (‰), δ13C (‰), C/N, δ15N-k (‰), δ13C-k (‰), Hg (mg/kg w.w.) in the analyses of brown trout from Øvre Heimdalsvatn sampled in June – October 2013. Analysis of the stable isotopes was conducted by Ingar Johansen at the Institute for Energy Technology (IFE). Mercury analysis was performed by Solfrid Lohne at the Department of Environmental Sciences (IMV) at NMBU.

ID	δ15N (‰)	δ13C (‰)	C/N	δ15N-k (‰)	δ13C-k (‰)	Hg (mg/kg w.w.)
1	7.09	-26.81	2.92	8.01	-27.99	0.052
3	6.80	-24.41	2.98	7.72	-25.55	0.055
4	5.82	-24.01	3.15	6.74	-25.01	0.037
5	7.40	-24.62	3.41	8.33	-25.84	0.067
6	7.00	-24.29	3.38	7.92	-25.46	0.056
7	7.76	-25.60	3.08	8.68	-26.88	0.039
14	6.16	-24.74	2.88	7.08	-25.78	0.038
19	6.20	-23.85	2.86	7.12	-24.90	0.074
22	6.22	-25.36	2.83	7.14	-26.41	0.051
29	5.92	-23.50	2.85	6.84	-24.51	0.035
33	7.71	-22.78	2.84	8.63	-24.04	0.084
39	6.39	-23.68	2.78	7.31	-24.76	0.073
48	6.83	-26.18	3.06	7.76	-27.32	0.054
51	7.08	-26.30	3.11	8.00	-27.48	0.045
52	7.47	-28.96	3.28	8.39	-30.19	0.061
58	6.31	-25.79	2.88	7.23	-26.86	0.043
62	6.16	-23.30	3.00	7.09	-24.34	0.028
67	5.59	-25.36	2.82	6.51	-26.31	0.030
68	5.81	-21.90	2.99	6.74	-22.89	0.030
79	6.21	-25.38	2.88	7.14	-26.43	0.051
80	5.01	-26.42	2.81	5.93	-27.29	0.015
87	4.95	-24.09	2.99	5.87	-24.95	0.017
88	5.79	-24.00	2.86	6.71	-24.99	0.030
89	7.20	-25.33	3.13	8.12	-26.53	0.047
90	7.75	-28.56	4.02	8.67	-29.83	0.063
91	6.87	-27.90	3.43	7.79	-29.04	0.029
94	7.70	-24.46	2.85	8.62	-25.73	0.068
95	8.30	-23.24	3.12	9.23	-24.60	0.10
96	7.23	-26.99	2.96	8.15	-28.19	0.13
99	7.09	-25.51	2.90	8.02	-26.69	0.067
100	6.27	-25.01	2.84	7.19	-26.07	0.045
102	6.68	-25.68	3.09	7.60	-26.80	0.035
103	6.18	-25.47	2.97	7.10	-26.52	0.062
111	5.66	-24.73	2.83	6.58	-25.70	0.029
120	6.89	-24.66	2.83	7.81	-25.81	0.041
121	6.58	-26.05	3.05	7.50	-27.15	0.056
122	7.96	-22.94	2.90	8.88	-24.25	0.11
123	6.19	-24.35	2.97	7.11	-25.39	0.039

124	7.04	-24.25	3.36	7.97	-25.43	0.062
125	7.37	-22.44	2.80	8.29	-23.66	0.088
127	7.22	-23.59	2.87	8.14	-24.78	0.11
129	7.22	-23.60	2.82	8.14	-24.80	0.11
131	6.30	-24.51	2.80	7.22	-25.57	0.041
132	6.03	-24.00	2.96	6.95	-25.03	0.030
133	7.58	-23.38	2.94	8.50	-24.63	0.10
134	6.87	-24.45	2.90	7.80	-25.60	0.068
136	6.52	-21.82	3.08	7.44	-22.91	0.069
140	5.59	-22.65	2.77	6.51	-23.60	0.036
144	6.95	-24.93	2.84	7.88	-26.09	0.057
153	6.69	-28.98	2.90	7.62	-30.10	0.023
155	6.00	-24.22	2.87	6.92	-25.24	0.036
161	6.42	-27.58	2.94	7.34	-28.66	0.060
164	6.81	-24.16	3.10	7.73	-25.30	0.094
169	6.61	-23.63	2.84	7.54	-24.74	0.078
170	7.07	-23.20	3.16	8.00	-24.37	0.049
171	7.02	-24.83	2.92	7.95	-26.00	0.054
175	6.28	-24.31	3.00	7.20	-25.36	0.079
177	6.97	-24.71	2.86	7.90	-25.87	0.065
179	7.18	-27.35	2.87	8.10	-28.54	0.053
180	7.31	-27.19	2.83	8.23	-28.40	0.039
Periphyton	-0.92	-27.57	11.87			



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