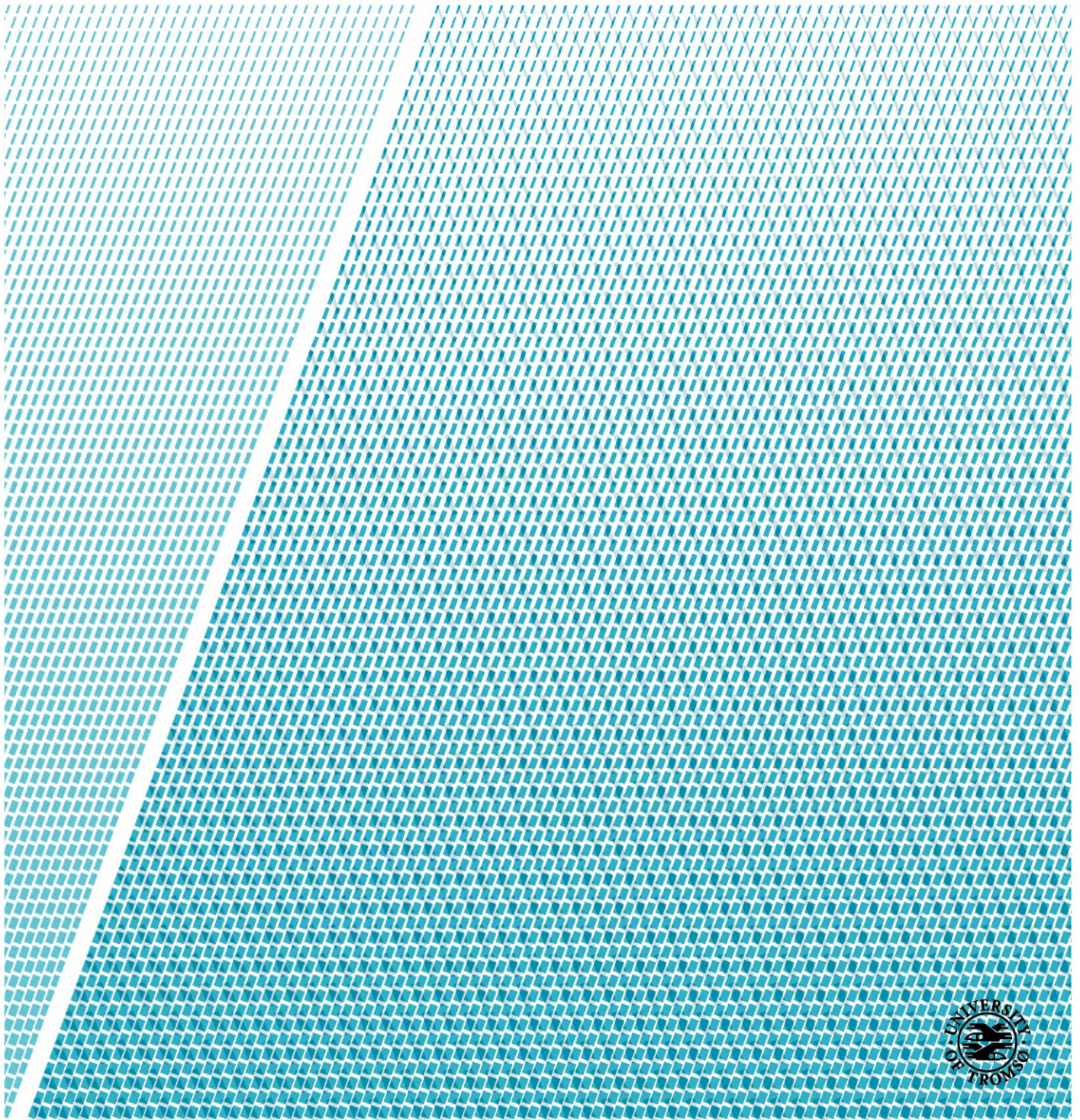# Seasonal and ontogenetic variation in the infection of intestinal parasites in Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) in a subarctic lake.

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UiT

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Summary

The vast majority of studies in northern lake systems have been carried out on a short-term scale, principally during spring and summer and mostly focusing on a single parasite species. There are few winter studies of fish parasites in the subarctic area, and even less regarding seasonal variations in the sub-communities of intestinal parasites in Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*). Seasonal and ontogenetic variations of intestinal parasites in Arctic charr and brown trout were therefore investigated. A total of 354 Arctic charr and 203 brown trout were sampled from the littoral habitat between June 2017 and May 2018, in lake Takvatn, northern Norway. I calculated the prevalence, mean intensity and mean abundance of all intestinal parasite species. A total of fife adult parasites taxa were found in the fish intestines. These parasites are trophically transmitted to the fish via zooplanktivory (*Eubothrium salvelini, E. crassum, Proteocephalus* sp.), benthivory (*Crepidostomum* spp. and *Cyathocephalus truncatus*) and piscivory (*E. crassum* and *Proteocephalus* sp.). Additionally, unencysted larvae (plerocercoids) of *Dibothriocephalus* spp. were recorded in the intestines of both salmonids, showing a high correlation with fish consumption. Diet was assessed using the frequency of occurence of prey items in the stomach and intestine. Intestinal parasites infections in Arctic charr displayed marked seasonal variations as a result of temporal changes in prey availability and host feeding behavior, whereas seasonal pattern in intestinal parasite infections in brown trout were mainly driven by host body size. Parasite infections increased with increasing fish size, leading to an accumulation through time of long-lived parasites, which was particularly evident in Arctic charr. Most intestinal parasites seemed to indicate the dietary preferences of Arctic charr and brown trout. Arctic charr exhibited the richest intestinal parasite community, which apparently was related to a broader dietary niche. Moreover, Arctic charr had a higher contribution of copepod- and amphipod-transmitted parasites as they included a greater proportion of these prey in their diet while trout had a higher contribution of helminths potentially transmitted through piscivory.

1 Introduction

Parasite communities of terrestrial and aquatic organisms are known to experience seasonal variations due to temporal changes in abiotic and biotic factors (Holmes, 1987, 1990; Altizer *et al.*, 2006; Kuhn *et al.*, 2016). Several processes may influence seasonality in parasite communities. These include environmental factors such as temperature (Chubb, 1979; Esch and Fernández, 1993; Pietrock and Marcogliese, 2003), the abundance of intermediate hosts (Esch and Fernández, 1993), the occurence of infective stages in the environment (Esch and Fernández, 1993), the feeding behaviour of the host (Curtis *et al.*, 1995; Knudsen *et al.*, 1996, 2008) and host immune responses (Dezfuli *et al.*, 2016). The importance of these factors likely depend on the life cycle of the parasite. Many parasites, especially helminths, possess complex life cycles involving one or several intermediate hosts (Chubb, 1979; Holmes, 1990). The transmission of parasites from one host to another take place through the consumption of an infected intermediate host. Resource availability, feeding activity, diet preferences and interactions between the host species may consequently influence the exposure to parasite infections (Carney and Dick, 2000; Knudsen *et al.* 2004, 2008; Fernández *et al.*, 2010). Moreover, while growing, many fish species experience ontogenetic shifts in diet due to changes in size, morphological constraints and physiological needs (Werner and Gilliam, 1984; Sánchez-Hernández *et al.*, 2019), that should influence parasite transmission. Furthermore, long-lived parasites will typically accumulate in the host through time and thus tend to increase extensively in numbers throughout the ontogeny, while short-lived parasites can be expected to display more extensive seasonal fluctuations.

Subarctic lakes along the west coast of northern Norway are typically oligotrophic and experience long winter conditions, being ice covered for six to seven months each year. Compared to boreal and temperate systems, subarctic lakes are species-poor. Arctic charr (*Salvelinus aplinus*) and brown trout (*Salmo trutta*), the focal host species of the present study, are the most common fish species in lakes along the west coast of northern Norway. The zooplankton and benthic invertebrate communities in subarctic lakes are typically species poor (Primicerio and Klemetsen, 1999; Frainer *et al.*, 2016). The lower diversity of potential intermediate and final host species inhabiting these lakes may consequently limit the occurrence of parasite species. Changes in temperature and light during the year can influence the productivity of the system, and consequently both benthic and zooplanktonic communities experience seasonal variations in species composition and abundance (Primicerio and Klemetsen, 1999; Klemetsen and Elliott, 2010; Frainer *et al.*, 2016).

Moreover, abiotic factors also influence the availability of infective stages in the environment, for instance, shedding of infective cercaria stages from mollusk intermediate hosts are usually positively correlated with temperature (Pietrock and Marcogliese, 2003; Poulin, 2006). Seasonal changes in the availability of intermediate hosts and infective stages could therefore produce seasonal pulses in parasite transmission (Esch and Fernández, 1993; Thieltges *et al.*, 2008). To better understand how intestinal parasite infections in Arctic charr and brown trout changes over time and in their structure, it is important to conduct seasonal studies. The vast majority of studies in northern lake systems have been carried out on a short-term scale, principally during spring and summer and mostly focusing on a single parasite species (Tedla and Fernando, 1969; Knudsen *et al.*, 1996; Amundsen and Knudsen, 2009). There are few winter studies of fish parasites in the subarctic area, and even less regarding seasonal variations in the sub-communities of intestinal parasites in Arctic charr and brown trout.

Trophically transmitted intestinal parasites of Arctic charr and brown trout typically have complex life cycles. Most of their intestinal parasites utilize copepods as intermediate hosts, others utilize amphipods, insect larvae or fish. Hence, the parasites are transmitted to Arctic charr and brown trout via zooplanktivory, benthivory or piscivory (Amundsen *et al.*, 1997; Scholz *et al.*, 2003; Knudsen *et al.*, 2008). Seasonal variation in the availability of prey can lead to seasonal changes in diet, thereby also influencing the structure of the parasite communities in the two salmonids. Diet differences between Arctic charr and brown trout during summer (Eloranta *et al.*, 2013) and winter (Klemetsen *et al.*, 2003; Amundsen and Knudsen, 2009) can therefore influence their exposure to parasite infections. A previous study conducted in two Norwegian subarctic lakes (Fjellfroskvatn and Buttelvatn), revealed that brown trout mostly used the littoral habitat and preyed upon small charr during the winter, amphipods during summer and early autumn, and insects all year round (Knudsen *et al.*, 2008). Arctic charr, in contrast, used all habitats and predominantly preyed upon zooplankton during summer and autumn, amphipods during autumn and winter and insects during late winter and early summer (Knudsen *et al.*, 2008). This difference in diet and habitat use was reflected by the parasite community composition of the two fish species. For instance Arctic charr possessed a richer and more diversified parasite community than brown trout due to the exposure to a wider range of copepod- transmitted parasites. Fish with a broad diet feeding on large variety of prey types thus generally have a richer parasite community than those with a narrower diet (Kennedy *et al.*, 1986; Locke *et al.*, 2014). Arctic charr in comparison to brown trout, is generally considered an opportunistic feeder. However, within a charr population, some individuals can

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exhibit strong dietary specialization (Amundsen, 1995; Klemetsen *et al.*, 2003). Individual feeding specialization can lead to accumulation of a large number of parasites in individual hosts that mainly feed on the parasite's intermediate host (Kennedy *et al.*, 1986; Marques *et al.*, 2011).

Body size affects the feeding ability and size range of prey consumed by fish (Werner and Gilliam, 1984; Sánchez-Hernández *et al.*, 2019). For instance, gape size/mouth dimensions which are related to body size, limit the ability to handle prey in fish that consume the whole prey (Sánchez-Hernández *et al.*, 2013). This in turn, leads to different size classes being exposed to a changing range of trophically transmitted parasites (Poulin and Leung, 2011). Additionally, as individual growth is directly correlated with food consumption (Forseth *et al.,* 1994; Amundsen *et al.*, 2007; Sánchez-Hernández *et al.*, 2019), an increase in food intake may increase the exposure to trophically transmitted parasites. Furthermore, age and size of the host can influence the exposure to parasites as larger fish (*i.e.,* older individuals) are expected to harbor more parasites as they might accumulate in the host over time. Throughout their ontogeny, the general tendency in Arctic charr and brown trout is to feed on bigger prey as their sizes increase, shifting from a zooplankton-benthos based diet toward a more benthos-fish based diet (Klemetsen *et al.*, 2003). This ontogenetic diet shift should affect their exposure to trophically transmitted parasites.

The aim of this study was to investigate seasonal patterns in the infections of intestinal parasites and their association with the diet in Arctic charr and brown trout in the subarctic lake Takvatn to explore whether: (I) parasite communities differed between Arctic charr and brown trout, (II) intestinal parasite communities experienced seasonal changes in their composition, and (III) hosts ontogenesis influenced the exposure and accumulation of tropically transmitted intestinal parasites.

Firstly, it was hypothesized that Arctic charr possessed a higher diversity of parasites than brown trout due to a broader diet that might increase the exposure to parasites at the population level. Secondly, it was hypothesized that parasite infections varied throughout the year in relation to the resource availability, feeding activity and dietary choices of the hosts. Lastly, it was hypothesized that larger fish had a higher abundance of parasites due to higher intake of potential intermediate hosts and an accumulation of long-lived parasites over time.

2 Materials and methods

2.1 Study site

The study was conducted in Takvatn, a dimictic and oligotrophic subarctic lake located 214 m above the sea level in Troms country. The lake have a surface area of 15,2 km² and a maximum depth of 88 m. Secchi depths range between 14 and 17 m and phosphorous levels do not exceed five micrograms per liter (Eloranta *et al.*, 2013). The lake is usually ice-covered from November to early June (Amundsen *et al.*, 2007, 2009). In the winter of 2017-2018, the lake surface froze during the last week of November and the ice melted in the second week of May (Appendix Tab.1). The only fish species present in the lake are brown trout, Arctic charr and three-spined stickleback (*Gasterosteus aculeatus*). Originally only brown trout was present in the lake, but in 1930 Arctic charr was introduced from the nearby lake Fjellfrøsvatn, and around 1950, three-spined sticklebacks were introduced to provide a potential prey fish for both salmonids (Jørgensen and Klemetsen, 1995; Amundsen *et al.,* 2007).

2.2 Fish sampling

In total 354 Arctic charr and 203 brown trout were sampled from the littoral habitat (<15 m depth) between June 2017 and May 2018 (Tab. 1) using 40m long and 1.5 m depth bottom multi-mesh gillnets (eight randomly distributed 5m panels of 10, 12.5, 15, 18.5, 22, 26, 35 and 45 mm, knot to knot). The sampling was carried out monthly during the ice-free season (June to November) and every second month during the ice-covered period (December to May). During the ice-covered period, gill nets were pulled out and retrieved through holes in the ice by means of submerged ropes that were positioned in the lake in December when the ice thickness was still modest. The nets were left in the lake overnight for approximately 12 hours during the ice-free period and approximately 16 hours during the ice-covered period. In the field, fork length, weight, sex and gonad maturation were recorded. Stomachs were opened, and the total fullness was visually determined on a percentage scale ranging from empty (0%) to full (100%). The stomach contents were preserved in 96% alcohol, and the intestines were frozen to preserve the content, allowing parasitological and dietary analyses at a later time in the laboratory.

Table 1 – Number and average fork length (in mm) ± SD of sampled fish individuals throughout the sampling period. No trout were captured in June 2017.

Sampling	Season	Arctic charr	Brown trout
June 2017	Summer	n = 50, X = 304.7 mm ± 41.9	-
August 2018		n = 50, X = 232.9 mm ± 81.6	n = 50, X = 204.9 mm ± 42.0
September 2018	Autumn	n = 24, X = 316.5 mm ± 50.0	n = 36, X = 274.0 mm ± 72.3
October 2018		n = 36, X = 277.5 mm ± 87.7	n = 48, X = 245.3 mm ± 105.7
November 2018	Early winter	n = 50, X = 243.3 mm ± 54.2	n = 41, X = 247.6 mm ± 99.8
January 2018		n = 50, X = 273.8 mm ± 60.5	n = 11, X = 352.9 mm ± 122.5
March 2018	Late winter	n = 50, X = 256.3 mm ± 60.7	n = 7, X = 459.6 mm ± 125.1
May 2018		n = 44, X = 261.9 mm ± 73.7	n = 10, X = 379.8 mm ± 110.1

2.3 Parasites

The intestinal parasites were sampled by cutting the intestines open and sieving the contents including that of the pyloric caeca under running water with a 120-micron mesh size nylon net. The cleaned matter was then transferred to a Petri dish and immersed in a physiological saltwater solution (9% NaCl) for identification and counting of the occurring parasites. Several adult parasite taxa were recorded from the intestines (Tab. 2). These are transmitted to the fish via zooplanktivory (*Eubothrium salvelini, E. crassum, Proteocephalus* sp.), benthivory (*Crepidostomum* spp. and *Cyathocephalus truncatus*) and piscivory (*E. crassum* and *Proteocephalus* sp.). *E. salvelini* and *E. crassum* are host specific tapeworms, the first infects Arctic charr while the former brown trout (Scholz *et al.*, 2003). *E. salvelini* is transmitted to Arctic charr throught ingestion of infected copepods which act as first intermediate host. However, it was suggested, that there is a possibility that this parasite might also be able to re-establish in cannibalistic Arctic charr (Hammar, 2000). The transmission of *E. crassum* to brown trout, appear to occur by ingestion of infected copepods and infested fish prey such as three-spined sticklebacks (Vik, 1963; Kristmundsson and Richter, 2009). *Proteocephalus* sp. is transmitted to both salmonid species through consumption of infected copepods. However, species belonging to this genus are also reported to successfully re-establish in piscivorous fish (Lien and Borgstrøm, 1973; Scholz, 1999). The helminth *C. truncatus* is transmitted to Arctic charr and brown trout through ingestion of amphipods belonging to the genus *Gammarus* (Okaka, 1984; Amundsen *et al.*, 2003). Transmission of *Crepidostomum* spp. to Arctic charr and brown trout occur through predation of amphipods (*Gammarus lacustris*) and insect larvae of the order Ephemeroptera (Curtis *et al.*, 1995; Knudsen *et al.*, 1997; Kristmundsson and Richter, 2009). At least four potentially different species belonging to the genus *Crepidostomum* are present in the lake (Soldánová *et al.*, 2017), but are here grouped as *Crepidostomum* spp. as they are only distinguishable via genetic analysis. Additionally, the larval stage of two species of *Dibothriocephalus* (formerly *Diphyllobothrium* (Waeschenbach *et al.* 2017)), which use copepods

as a first intermediate host, fish as second intermediate host and birds as their final host (Vik, 1964; Halvorsen, 1970) were also recorded in the intestines of both Arctic charr and brown trout. These parasites need special considerations as they were the only parasites found in a larval stage in the intestinal tract of Arctic charr and brown trout. The plerocercoids of *Dibothriocephalus* are typically found encysted on the stomach wall or other parts of the viscera or in the muscles of infected fish and are infective for birds but not for fish as final host. The *Dibothriocephalus* plerocericoids included in this thesis only include the unencysted larvae found in the intestine, not those encysted in the viscera. The presence of unencysted plerocercoids in the intestine have generally been considered accidental and thus overlooked in previous studies. However, the surprisingly high correlation between unencysted plerocercoids and piscivory, particulary in brown trout indicated that their presence was not casual but a rather the result of ingestion of infected fish prey. Therefore, they were taken in consideration for seasonal and diet-parasites analysis.

Table 2 – Parasite species recorded in the intestine of Arctic charr and brown trout.

Parasite species	Taxonomic group	Stage in intestine	Intermediate host	Re-infection through piscivory	Presence in Arctic charr/brown trout
Eubothrium salvelini	Cestode	Adult	Copepod	No (?)	Arctic charr
Eubothrium crassum	Cestode	Adult	Copepod	Yes	Brown trout
Cyathocephalus truncatus	Cestode	Adult	Amphipod	No	Both
Proteocephalus sp.	Cestode	Adult	Copepod	Yes	Both
Crepidostomum spp.	Trematode	Adult	Amphipod/insect larvae	No	Both
Dibothriocephalus spp.	Cestode	**Larvae**	Copepod	Yes	Both

2.4 Diet

In the laboratory, the stomach contents of each fish was analyzed by identifying prey items to the species, genus or family level and estimating their contribution to the total stomach contents according to the method described by Amundsen (1995). The identified prey types were subsequently grouped in the following diet categories: amphipods, zooplankton, mollusks, insect larvae, surface insects and fish. The frequency of occurrence of prey types was calculated to investigate niche differentiation between Arctic charr and brown trout and to contrast their trophic ecology with their infection rates of trophically transmitted parasites. From the six initial prey categories, only amphipods, insect larvae, zooplankton, and fish were considered for the parasite–diet comparison/analysis, as they are potential intermediate hosts of the identified intestinal parasites (Tab. 2). The diet composition of the fish was initially analyzed from the frequency of occurrence of prey types found in the stomachs. However, dietary information was partially missing

for individual fish due to a relatively high presence of empty stomachs in both Arctic charr (32,5% of the total sample) and brown trout (14,3%), especially during winter time, when the frequency of empty stomachs was particularly high (67% for Arctic charr, and 30% for brown trout). In order to overcome this issue, the intestinal contents of each fish were also carefully examined for the presence of identifiable prey remains. Differences in the presence/absence of each prey type found in the stomach versus those found in the intestine was tested in a 2x2 contingency table using χ^2 test with Yates correction. For both salmonid species, amphipods emerged to be more frequently represented in the intestines than in the stomachs (P <0.01), whereas for all other prey categories no significant differences were found. The data were combined to provide enhanced information from the whole gastrointestinal tract about the diet utilization of each individual fish. Overall, the implementation of the intestinal prey data covered missing information for 40% of the empty stomachs, which was particularly important for the winter material. The combined frequency of occurrence of prey types was then used for statistical analysis of parasite-diet relationships.

2.5 Statistical analysis

Descriptive and statistical analyses were performed with the open source software Rstudio (version 1.1.423, Rstudio Inc.) based on R (version 3.5.1, R Core Team) and QPweb (version 1.0.14, Reiczigel *et al.* 2019).

2.5.1 Quantification and comparison of parasite infections between host species

To investigate differences in parasite load between Arctic charr and brown trout, four quantitative parameters (mean number of species, prevalence, mean intensity and mean abundance) were analyzed according to Bush *et al.* (1997) and Poulin (1998). Mean number of species is defined as the mean number of parasite species per host individual. The mean number of parasite species was compared between Arctic charr and brown trout using the Mann-Withney U-test. Prevalence is defined as the proportion of host individuals infected by a particular parasite among the examined sample of a specific host species, usually expressed in percentage. Prevalence was compared between host species using a χ^2 test with Yates correction. Mean intensity represents the average number of parasite individuals belonging to a particular species found in all hosts infected by that parasite (*i.e.*, uninfected hosts are excluded). Mean abundance is the average number of parasites per host in the examined host species including the uninfected hosts. Parasites usually have an aggregated distribution among their hosts, where few hosts have a lot of parasites while most of them have few or none, resulting in a skewed distribution (Shaw and Dobson 1995; Neuhäuser and

Poulin 2004). The use of non-parametric tests is therefore in most cases recommended for comparisons of infections across different samples. As none of the common parametric and non-parametric tests perform well to analyze mean abundance and mean intensity due to skewness and variance heterogeneity (Skovlund and Fenstad, 2001; Neuhäuser and Poulin, 2004; Fagerland and Sandvik, 2009), the use of a non-parametric maximum test was suggested by Welz, Ruxton and Neuhäuser (2018). The suggested maximum test combines the Brunner-Munzel and Welch U-tests, correcting for type 1 error thus making it a more robust way of dealing with such data (Welz *et al.* 2018). To perform the test the freely available R data package created by these authors and hosted on Dryad Digital Repository (https://datadryad.org/resource/doi:10.5061/dryad.8s574) was used.

2.5.2 Seasonal variation in parasite infections

To analyze seasonal variations in the infections of intestinal parasites, prevalence and mean abundance were used. Mean intensity was in contrast omitted as zero values of uninfected hosts are excluded with this parameter, which therefore may often be highly dependent on a few extremely infected hosts due to the aggregated distribution of parasites (Rózsa *et al.* 2000). Seasonal variations patterns in parasite infections (*i.e.*, between summer, autumn, and early and late winter) did not significantly deviate from the monthly variations (Appendix Tab. 1). It was therefore decided to merge the monthly data in to the four seasonal periods (Tab. 1) in order to strengthen the sample sizes, which was particularly important in respect to the low numbers of trout captured during the winter. Prevalence was compared for seasonal variations of parasites using the Fisher's exact test, while seasonality in mean abundance was tested with Kruskal-Wallis test. To assess seasonal variation in parasite infections of charr and trout, a Principal Component Analysis (PCA) was employed. PCA allows a simplified visualization of the data association between all parasites species and predictor variables simultaneously giving an overview of the most important correlations. PCAs were calculated separately for Arctic charr and brown trout, using parasite abundance as response variable and seasons and length as predictor variables. As a one-way ANOVA test revealed that the length of both Arctic charr and brown trout showed significant differences among sampling seasons (Appendix Tab. 2), possible size effects on the seasonal variation in parasite infections was tested using a negative binomial generalized linear model (GLM). Negative binomial GLM is best suited to model the overdispersion of parasites distributions among hosts wich is typically aggregated, with high variance-to-mean-ratio ($s^2/\bar{x} > 1$; Appendix Tab.6; Wilson and Grenfell, 1997; Rózsa *et al.*, 2000; Paterson and Lello, 2003; Lindén

and Mäntyniemi, 2011). The model included parasites counts of infected hosts as the respose variable with seasons and fish length as predictors. The function glm.nb from the MASS package in R was used to run the model, and Anova (type II) function from the Car package in R was adopted to assess the main effects (Appendix Tab. 3). Size turned out to have a significant effect (P <0.05) on all intestinal parasites hosted by Arctic charr and brown trout, with the exceptions of *E. crassum* and *Crepidostomum* spp. in brown trout, and consequently, size effect has been taken into consideration for the interpretation of the results.

2.5.3 Ontogenetic variation in parasite infections

To visually and statistically asses if parasite load increased with increasing fish size, graphs with a fitted negative binomial GLM curve were generated. Separate GLMs were performed for each parasite species using fish length and species (Arctic charr or brown trout) as predictor variables and intensity of infection as the response variable.

2.5.4 Diet and diet-parasite relationship

To assess the effect of season and length on diet a logistic regression was used. Separate logistic regressions were performed for each prey type using season and fish length as predictor variables and presence-absence of prey as the response variable (Appendix Tab. 4). A correlation matrix with the Winsorized correlation coefficient (Wilcox, 2001) was used to analize potential correlations between parasite prevalence and frequency of occurrence of prey types. This method was preferred over the widely used Spearman-Rank and Kendall-Tau correlation coefficients as it is more robust to distribution shape, sample size and outliers (Wilcox, 2001; Tuğran *et al.*, 2015). Moreover, to assess any relationships between diet and intestinal parasite infections of Arctic charr and brown trout visually and statistically, PCA was employed. This multivariate analysis was implemented using parasite abundance as the response variable and the presence-absence of prey as a predictor variable.

3 Results

3.1 The intestinal parasite communities of Arctic charr and brown trout

Of all Arctic charr examined, 98% were infected with at least one intestinal parasite species and the mean number of parasites species per fish was 2.3 (± 0.95 SD, Fig. 1). In total, five intestinal parasites species were recorded in Arctic charr (Fig. 2). Of these, the most common were *E. salvelini* with 92% and *Crepidostomum* spp. with 71% prevalence, whereas *C. truncatus*, *Proteocephalus* sp. and *Dibothriocephalus* spp. had a much lower prevalences (<40%).

Of all brown trout examined, 88% were infected with at least one intestinal parasite species and the mean number of parasites species per fish was 1.5 (± 0.9 SD), which was significantly less than in Arctic charr (Mann–Whitney U-test, P<0.01; Fig. 1). Overall, five intestinal parasites species were found. The most common parasite was *Crepidostomum* spp. with a prevalence of 71%, whereas *E. crassum*, *C. truncatus*, *Proteocephalus* sp. and *Dibothriocephalus* spp. were less common (<25% prevalence; Fig. 2).
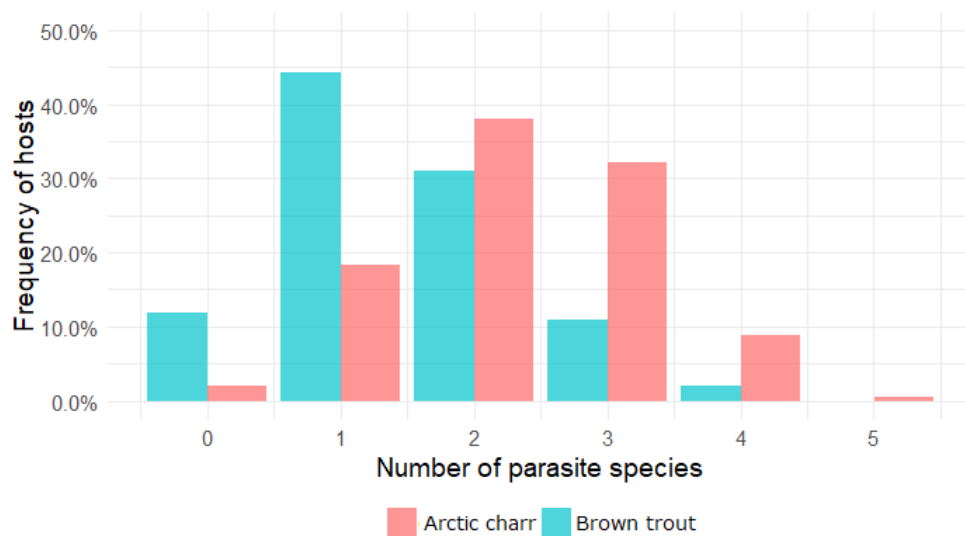

Fig. 1. Frequency distribution of the number of intestinal parasites species in Arctic charr and brown trout
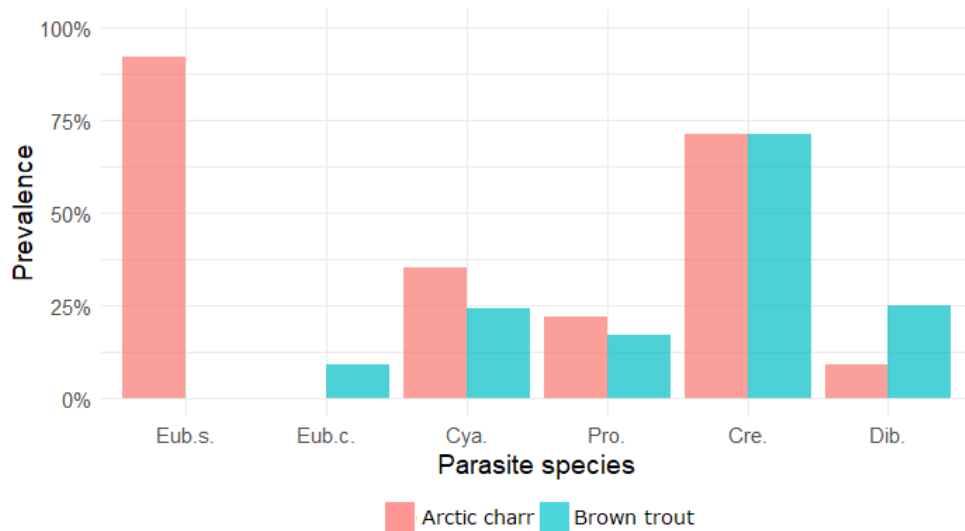
Fig. 2. Prevalence of trophically transmitted intestinal parasite species in Arctic charr and brown trout (Eub.s. = *E. salvelini*, Eub.c. = *E. crassum*, Cre.= *Crepidostomum* spp., Cya. = *C. truncatus*, Pro.= *Proteocephalus* sp., Dib. = *Dibothriocephalus* spp.)

3.2 Seasonal variations of intestinal parasites in Arctic charr and brown trout

Arctic charr displayed significant variations in the prevalence of several intestinal parasites throughout the seasons, whereas fluctuations in mean abundance were less distinct and not statistically significant (Fig. 3, Appendix Tab. 5). Seasonal variations in prevalence were evident for *C. truncatus*, *Crepidostomum* spp. and *Proteocephalus* sp. (Fisher's exact test, P<0.01; appendix Tab. 5). The prevalence and mean abundance of *C. truncatus* and *Crepidostomum* spp. were at the lowest during the summer and reached a peak in early winter, while for *Proteocephalus* sp. they were at a minimum in late winter and at a maximum in autumn. Both prevalence and mean abundance of *Dibothriocephalus* spp. were very low and stable throughout the seasons. The prevalence of *E. salvelini* remained steadily high in all seasons, while the mean abundance persisted at a constant low level. The frequenecy of distributions of all parasites species indicated by variance-to-mean-ratios were highly aggregated (Appendix Tab.6).

In brown trout, the prevalence of *E. crassum*, *C. truncatus,* and *Dibothriocephalus* spp. displayed a pronounced seasonality, while *Crepidostomum* spp. and *Proteocephalus* spp. had non-significant variations between seasons (Fig. 3, Appendix Tab. 5). The seasonal variations in mean abundance were in contrast statistically significant only in *E. crassum* (Kruskal-Wallis test, P<0.01; appendix Tab. 5). Both prevalence and abundance of *E. crassum* were at the lowest in autumn and reached a peak in late winter. The prevalence and mean abundance of *C. truncatus* were at the minimum in autumn, but peaked in early winter and in late winter, respectively. Both prevalence and mean

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abundance of *Dibothriocephalus* spp. were at the lowest in summer and at the maximum in late winter. The prevalence of *Crepidostomum* spp. and *Proteocephalus* sp. remained fairly stable throughout the year, although the mean abundance had a more pronounced peak in early winter. Intestinal parasites in brown trout had a highly aggregated distribution (Appendix Tab.6).
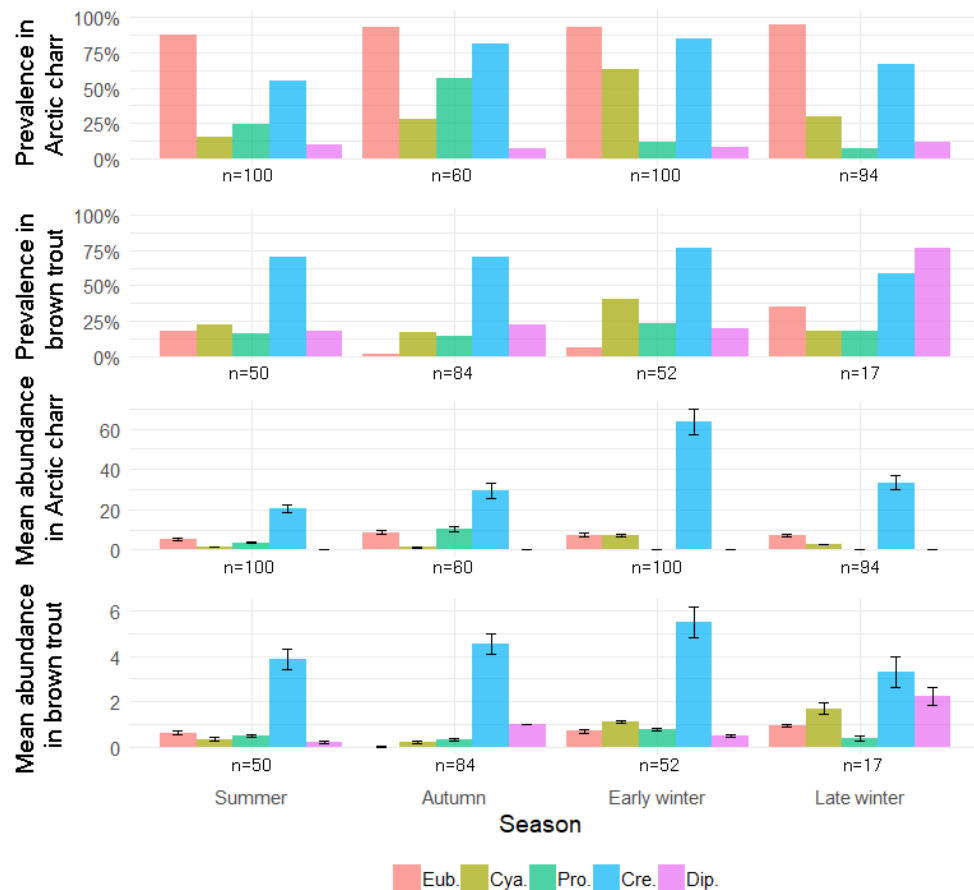

Fig. 3. Prevalence and mean abundance (with SE) of intestinal parasites in Arctic charr and brown trout throughout the seasons (Eub. = *E. salvelini*/*E. crassum*, Cya. = *C. truncatus*, Pro.= *Proteocephalus* sp., Cre.= *Crepidostomum* spp., Dib. = *Dibothriocephalus* spp.). Note differences in scales in the y-axes.

Seasonal patterns in parasite prevalence between Arctic charr and brown trout proved to be significantly different only in *Proteocephalus* sp. and *Dibothriocephalus* spp. (χ^2 test, P<0.05; Appendix Tab. 5). Temporal differences in mean abundance of *Crepidostomum* spp. and *Dibothriocephalus* spp. between Arctic charr and brown trout also resulted to be significant (maximum test, P<0.05; Appendix Tab. 5). However, a significant within-month disparity was seen in the prevalence and mean abundance of *C. truncatus,* although apparently suppressed by merging the months into seasons.

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A PCA (Fig. 4) for between-season variation in the parasite communities of both host species reflected the previously described pattern. Seasons are here represented as 95% confidence interval ellipses. In Arctic charr, the two-axis globally explained 47.8% of the variance. Dimension 1, which explained 28.9% of the variance, was driven mainly by the variability in *Crepidostomum* spp., while Dimension 2, which accounted for 18.9% of the variance, was driven mainly by *Proteocephalus* sp. Moreover, the PCA revealed a potential correlation between fish body length and infections of the helminths *Crepidostomum* spp. and *C. truncatus* in particular during early winter. In brown trout, the two-axis globally explained 45.5% of the variance. Dimension 1, which represented 26.3% of the variance, was driven mainly by variability in *Dibothriocephalus* spp. and *Proteocephalus* sp., while Dimension 2, which represented 19.2% of the variance, was driven mainly by *Crepidostomum* spp. Fish body length indicated possible correlations with infections of *Dibothriocephalus* spp. and *E. crassum*.
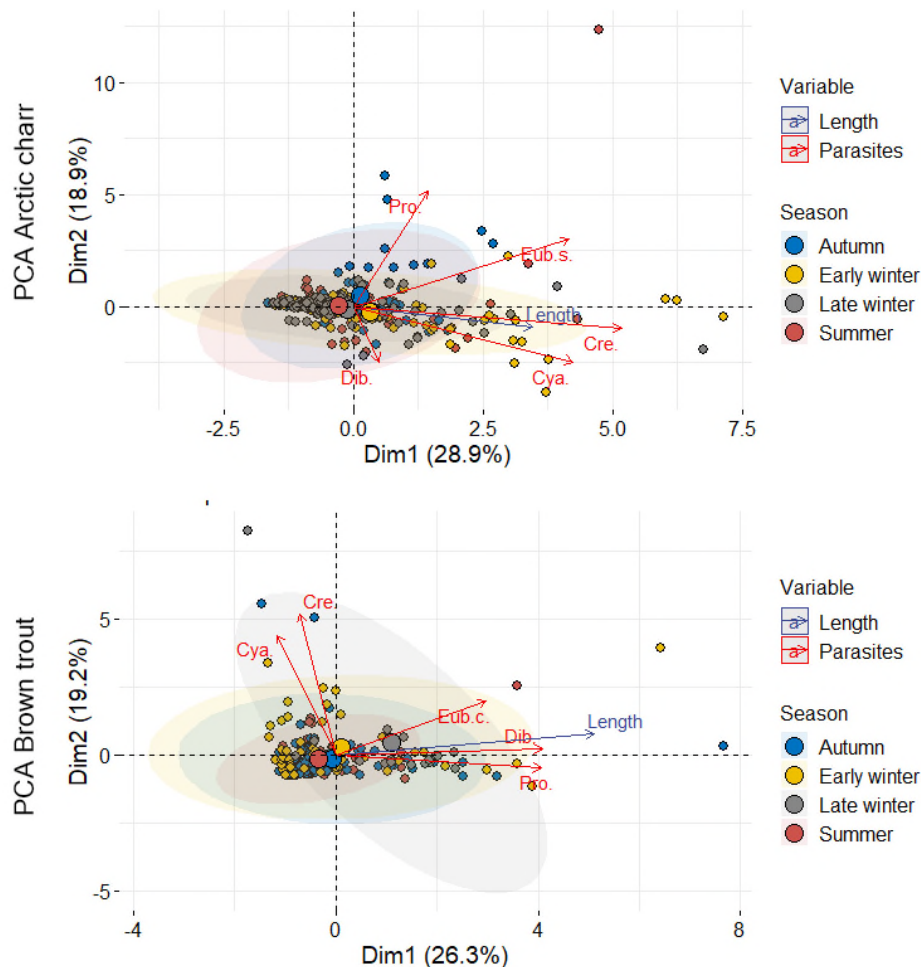

Fig. 4. Scatterplot scores of the PCA on factor influencing the parasite sub-communities and length of Arctic charr (upper) and brown trout (lower) throughout the seasons including a 95% confidence interval (Eub.s. = *E. salvelini*, Eub.c. = *E. crassum*, Cre.= *Crepidostomum* spp., Cya. = *C. truncatus*, Pro.= *Proteocephalus* sp., Dib. = *Dibothriocephalus* spp.).

3.3 Ontogenetic dynamics in parasite infections

The length distribution of both Arctic charr and brown trout varied significantly between seasons (One-way ANOVA, P<0.05; Appendix Tab. 2), influencing the parasite infections (GLM negative binomial P<0.05, Appendix Tab. 3). In the case of Arctic charr, individuals captured in autumn were larger than those of other sampling periods. Brown trout individuals captured during late winter were fewer and bigger than those caught in other seasons. A fitted negative binomial GLM underlined that in both hosts, parasite infections increased with increasing fish size (Fig. 5). In Arctic charr, the infection of *E. salvelini*, *C. truncatus,* and *Crepidostomum* spp. showed an exponential increase from around 20 cm fish length, whereas in *Proteocephalus* sp. and *Dibothriocephalus* spp. the increase was more linear.

In brown trout, the *Dibothriocephalus* spp. infections rose exponentially in fish over 40 cm, while the increase in mean intensity of *E. crassum* was distinct, but less pronounced. *Cyathocephalus truncatus* and *Crepidostomum* spp. infections were subject to minor increases with increasing fish length, whereas *Proteocephalus* sp. showed a slight decrease. Overall, with increased size, Arctic charr was more infected by *C. truncatus*, *Proteocephalus* sp. and *Crepidostomum* spp. than brown trout, while infections of *Dibothriocephalus* spp. rose to higher levels in brown trout.
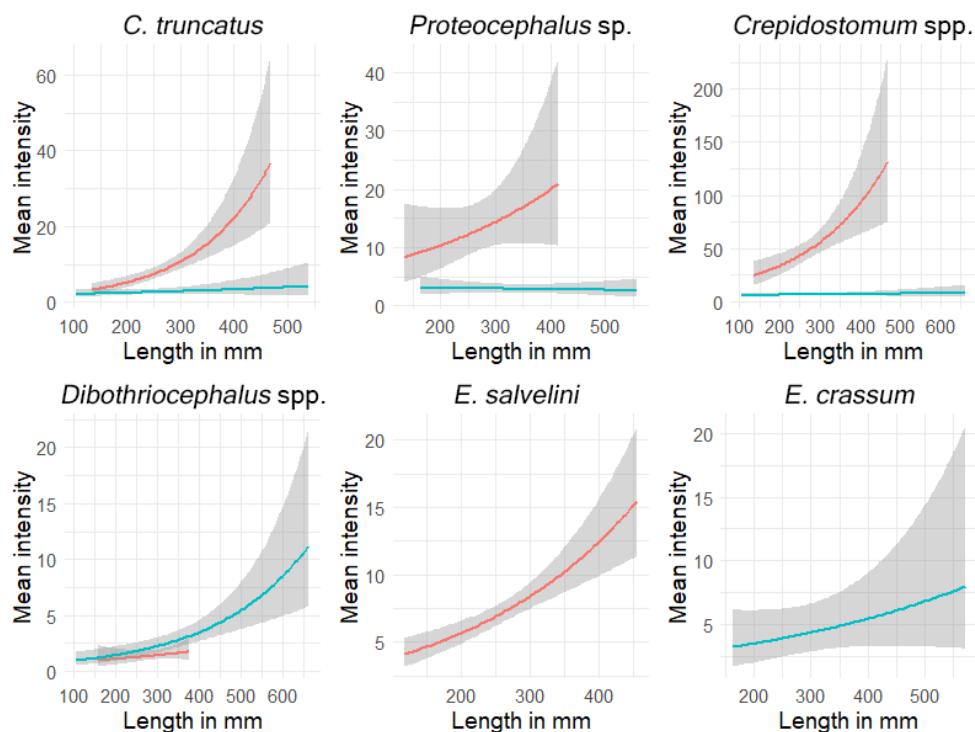

Fig. 5. Fitted GLM negative binomial for intensities of intestinal parasites infecting Arctic charr (red line) and brown trout (blue line) with 95% confidence intervals. Uninfected fish are excluded.

3.4 Association between parasites and diet

As trophically transmitted parasites are transmitted through consumption of infected intermediate hosts, correlations between parasites prevalence and frequency of occurrence of prey found in the stomach and intestine were investigated (Winsorized correlation coefficient, Appendix Fig. 1). In Arctic charr, the prevalence of *C. truncatus* (rw=0.85, P<0.05) and *Crepidostomum* spp. (rw=0.71, P=0.067) was highly correlated with the frequency of occurrence of amphipods, while *Proteocephalus* sp. was highly correlated with zooplankton (rw=0.91, P<0.01) and fish (rw=0.86, P<0.05). Other correlations were not significant. In brown trout, the prevalence of *Dibothiocephalus* spp. (rw=0.98, P<0.01) was highly correlated with the frequency of occurrence of fish. The prevalence of *Proteocephalus* sp. was highly correlated with amphipods (rw=0.90, P<0.05), even though this is not an intermediate host. Other correlations were not significant.

3.5 Seasonal variations in diet

The frequency of occurrence of the prey categories found in the stomachs and intestines throughout the sampling seasons revealed that insect larvae, zooplankton, and amphipods were the most common prey for Arctic charr (Fig. 6, Appendix Fig. 2). In Arctic charr, insect larvae were the most important prey during late winter and summer, whereas zooplankton was the most common prey during autumn and amphipods during early winter. In contrast, the fish contribution to the Arctic charr diet was minimal in all seasons.

In brown trout, insect larvae and mollusks were the most important prey throughout the year (Appendix Fig. 2). Among the prey categories associated with intestinal parasite transmission insect larvae and fish were the most frequent (Fig. 6). Insect larvae were the dominant prey from summer to early winter, while fish was the most important prey in late winter. The occurrence of amphipods in the brown trout diet was relatively modest throughout all seasons, whereas zooplankton was recorded only in autumn and early winter.

Fig. 6. Seasonal variations in the frequency of occurrence of prey categories in the diet of Arctic charr and brown trout. Prey categories not related to intestinal parasite transmission are excluded.

3.6 Ontogenetic variations in diet

Prey occurrence was influenced both by seasonality and host length (GLM logistic regression P<0.05, Appendix Tab. 4). The frequency of occurrence of the six prey categories revealed that insect larvae and zooplankton were the most common prey for Arctic charr among the different size classes followed by amphipods and mollusks. The importance of mollusks increased with increasing fish size (Appendix Fig. 3). Zooplankton was particularly important for Arctic charr between 10 and 20 cm, while amphipods were more frequent in fish larger than 20 cm. Fish prey were absent in the smallest size group of Arctic charr and remained at low levels in bigger fish (Fig. 7).

In brown trout, insect larvae were the most important prey among the different size classes, followed by surface insects and mollusks (Appendix Fig. 3). Fish consumption rose with increasing size becoming the main source of food for the largest brown trout. Amphipod prey were most common for fish between 30 and 35 cm, while their occurrence was at a modest level in the other size groups. Zooplankton was not commonly found in brown trout and was also completely absent in fish between 15-20 cm and over 35 cm (Fig. 7).
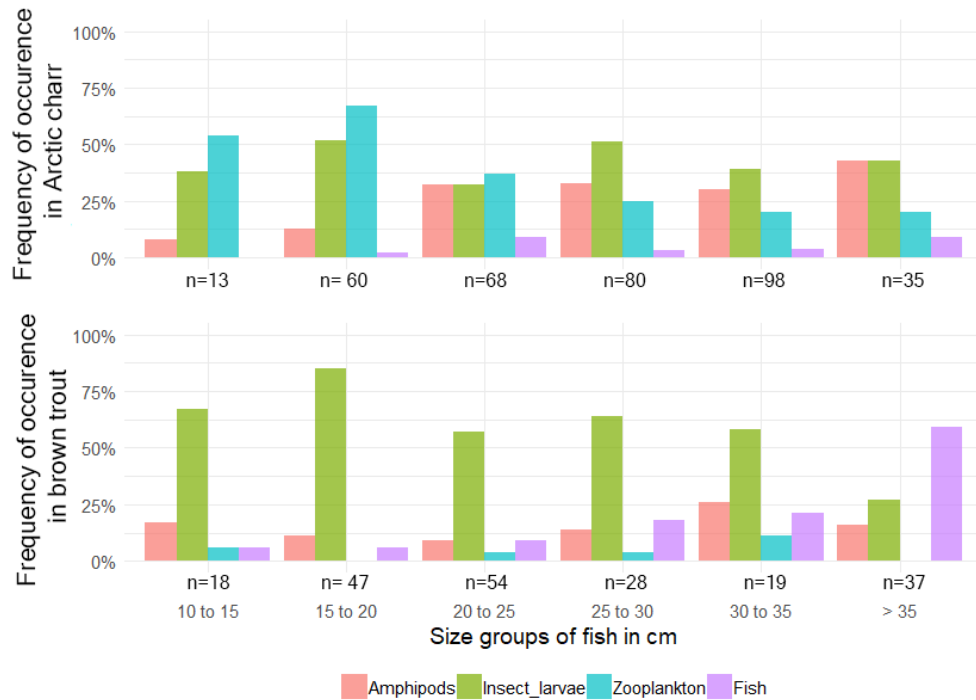
Fig. 7. Ontogenetic variations in the frequency of occurrence of prey categories in the diet of Arctic charr and brown trout. Prey categories not related to intestinal parasite transmission are excluded.

3.7 Parasite and diet differences between Arctic charr and brown trout

A PCA for variation of intestinal parasite communities between Arctic charr and brown trout individuals revealed parasite differences between the two host species. The use of prey (presence-absence data) as predictor variable additionally underlined potential correlations between parasites intensities and diet (Fig. 8). The first 3 dimensions explained in total 49% of the variance. Based on a visual inspection of the PCA, the 95% confidence intervals ellipses chiefly separated Arctic charr from brown trout along the Dimension 1 axis, which represented 21% of the total variation. This axis was driven mainly by the variability in *Crepidostomum* spp. and *C. truncatus,* which both are strongly correlated with the consumption of amphipods. There was also a distinct, but less marked separation on the Dimension 2 axis, which represented 15% of the variation. This axis was driven mainly by *Dibothriocephalus* spp. and *E. crassum,* which were strongly correlated with the consumption of fish prey. Dimension 3 axis, which represented 13% of the variation was driven mainly by *Proteocephalus* sp. which was highly correlated with the consumption of zooplankton. Overall, these findings suggests that the composition of intestinal parasites community in Arctic charr diverged from that in brown trout predominantly in *Crepidostomum* spp. and *C. truncatus,* which were chiefly correlated with the dietary contribution of amphipods. They also differed to a

smaller extent in *Dibothriocephalus* spp. and *E. crassum*, which were strongly associated with predation on fish and in *Proteocephalus* sp. which was highly related to the dietary contribution of zooplankton (Fig. 8).
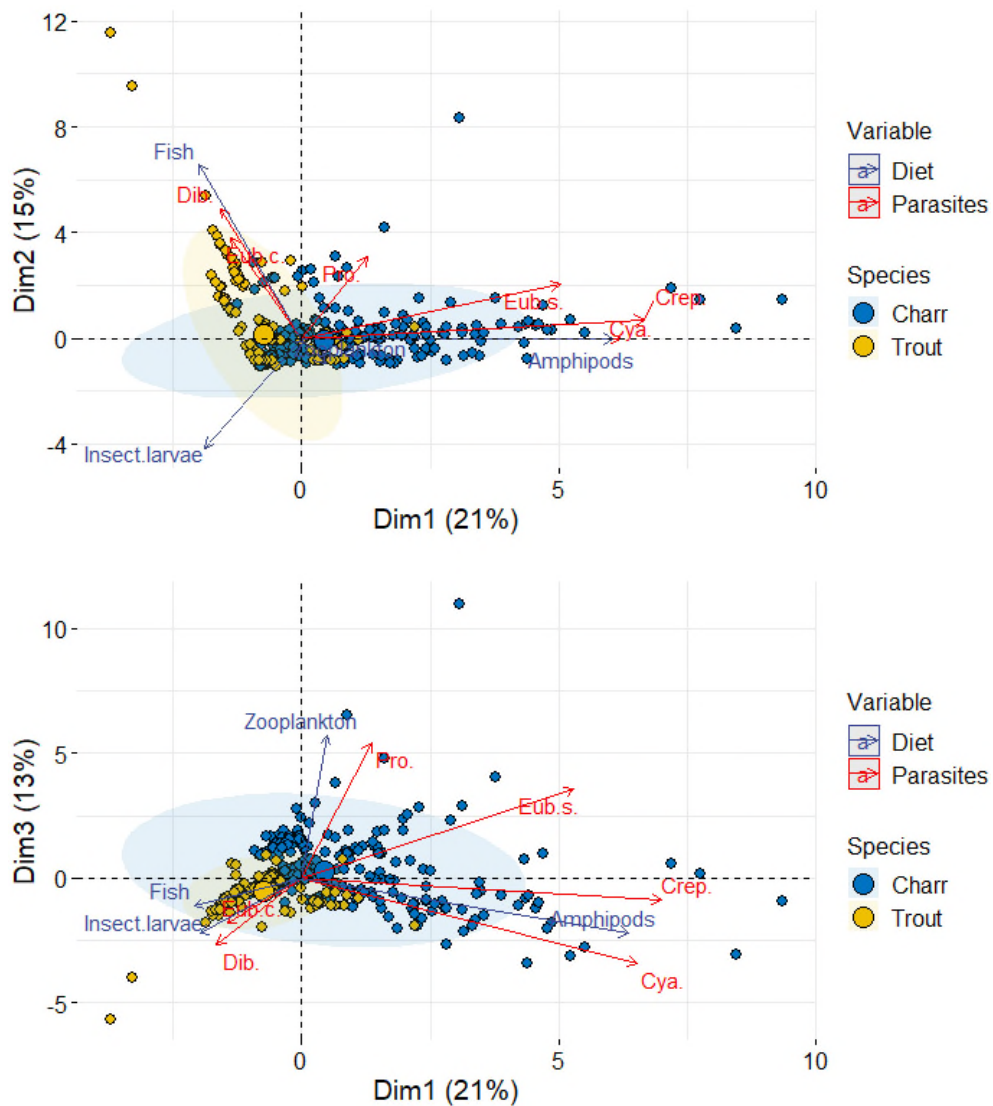

Fig. 8. Scatterplot scores of the PCA on factor influencing the parasite sub-community and diet of Arctic charr and brown trout with a 95% confidence interval. Dimension 1 and 2 (upper), Dimension 1 and 3 (lower). (Eub.s. = *E. salvelini*, Eub.c. = *E. crassum*, Cre.= *Crepidostomum* spp., Cya. = *C. truncatus*, Pro.= *Proteocephalus* sp., Dib. = *Dibothriocephalus* spp.).

4 Discussion

The community of intestinal parasites differed between the two salmonid species. As hypothesized, Arctic charr had the richest community of trophically transmitted intestinal parasites. Arctic charr not only hosted more parasite species but also had an overall higher abundance and prevalence of helminths compared to brown trout. Among the four shared intestinal parasites, *Crepidostomum* spp., *C. truncatus,* and *Proteocephalus* sp. were more common in Arctic charr, while *Dibothriocephalus* spp. was more common in brown trout. The fact that Arctic charr hosted the richest parasite community can be explained by its broader diet niche, which in contrast to brown trout also included a significant portion of zooplankton. The zooplankton prey group includes copepods which are known to transmit *Eubothrium* spp., *Dibothriocephalus* spp. and *Proteocephalus* sp. (Knudsen *et al.*, 2008; Kuhn *et al.*, 2016). Somewhat surprisingly, Arctic charr also fed more on the amphipod *G. lacustris* than brown trout. *G. lacustris* is known to transmit several parasites including *C. truncatus* and *Crepidostomum* spp., which explains the higher prevalence of these parasites in Arctic charr (Thomas, 1958; Vik, 1958; Okaka, 1984; Hoffman, 1999). The result also suggest that individual feeding specialization in Arctic charr might have contributed to a broader diet nice as some individuals specialized themselves on zooplankton in autumn while other on amphipods during winter. As hypothesized the host with the broadest nice (Arctic charr) should harbor the richest community of food transmitted parasites according to Kennedy *et al.* (1986) and Holmes (1990).

At first glance, it might seem contradictory that brown trout harbored several copepod-transmitted parasites given the low frequency of occurrence of zooplankton in their diet. However, this is likely due to re-infection processes taking place as brown trout frequently fed on potentially infected fish prey, in particular three-spined stickleback. The copepod-transmitted helminths *Dibothriocephalus* spp., *Proteocephalus* sp. and *E. crassum* do in fact have the ability to re-establish in piscivorous fish (Bylund, 1969; Halvorsen, 1970; Von Bonsdorff and Bylund, 1982; Williams and Jones, 1994; Scholz, 1999). The result from the present study, is in accordance with previous studies conducted in Takvatn, which revealed that piscivorous trout feed mainly on abundant sticklebacks that harbor several copepod transmitted parasites (Klemetsen *et al.*, 2002; Amundsen *et al.*, 2013; Henriksen *et al.*, 2016; Kuhn *et al.*, 2016).

In both Arctic charr and brown trout, several intestinal parasite species exhibited clear temporal variations in prevalence between seasons. In Arctic charr, the prevalence of *C. truncatus* and

Crepidostomum spp. reached a peak during early winter and *Proteocephalus* sp. in autumn. Their mean abundances did in contrast not show any significant seasonal variations. The high prevalence of *C. truncatus* and *Crepidostomum* spp. in early winter can be explained by a peak in predation on their intermediate host, *G. lacustris*. This is in accordance with studies carried out in the nearby lake Fjellfroskvatn (Amundsen *et al.*, 2003; Knudsen *et al.*, 2008; Amundsen and Knudsen, 2009). Similarly, the prevalence of *Proteocephalus* sp. reached a peak in autumn when zooplankton was the main prey and declined during the winter when zooplankton had the lowest contribution to the charr diet. Temporal changes in intestinal parasites communities can have several explanations. A shift in diet might be due to a relatively high availability of benthic invertebrates (*i.e.,* amphipods, insect larvae) in the littoral zone during the winter season, simultaneously co-occurring with a low availability of zooplankton (Klemetsen *et al.*, 2003; Amundsen and Knudsen, 2009) as most cladocerans enter the winter egg diapauses (Klemetsen *et al.*, 2002). A reduced zooplankton feeding will consequently diminish the exposure to *Proteocephalus* sp.*,* whereas an increased feeding on bigger and more abundant benthos favors the transmission of helminths like *Crepidostomum* spp. and *C. truncatus* that use amphipods and insect larvae as intermediate hosts. Furthermore, the establishment success and residence time of *C. truncatus* in the fish host is suspected to be controlled by temperature (Awachie, 1968; Amundsen *et al.*, 2003). A threshold of 10°C above which the establishment of the helminth in fish host might fail (Awachie, 1968), suggest that low water temperatures during winter might favor the establishment success of this parasite in the host species. Moreover, a decrease in water temperatures might simultaneously prolong the residence time in the fish host from less than four weeks in the summer to four or more weeks during the winter (Amundsen *et al.*, 2003). The prevalence of *E. salvelini* and unencysted *Dibotrhiocephalus* spp. plerocercoids in the intestine of Arctic charr, in contrast, had no significant correlation with the different prey categories and remained stable at high levels throughout the year. This suggests that the prevalence of *E. salvelini* and unencysted *Dibotrhiocephalus* spp. plerocercoids are less affected by seasonal changes in zooplankton availability. *E. salvelini* is able to survive up to one year and may accumulate over time in the final host (Hernandez and Muzzall, 2006; Smith, 2015). An eventual re-establishment of *E. salvelini* in Arctic charr through cannibalism seems low as no cannibalism was observed. The only fish prey found in the the gastrointestinal tract of Arctic charr were three-spined stickleback, which have not been reported as a suitable host for this parasite. The year-round low and stable prevalence of *Dibothriocephalus* spp. coincided with a likewise low inclusion of fish prey in the diet of Arctic charr. This parasite is found in a procercoid stage in its

first intermediate host, zooplankton. To become a plerocercoid the procercoid needs to penetrate the stomach or intestinal wall of a fish and get encysted in the viscera or in the muscles. This parasite was solely present as unencysted plerocercoids in the intestines suggesting that this infection is the result of recent predation upon infected three-spined stickleback rather than zooplankton.

In brown trout, unencysted *Dibothriocephalus* spp. plerocercoids and *E. crassum* reached their highest levels during late winter and *C. truncatus* in early winter. The mean abundance of intestinal parasites in brown trout, in contrast, showed significant seasonal variation only in *E. crassum*. The peak in prevalence and mean abundance of *Dibothriocephalus* spp. and *E. crassum* coincided with a distinct rise in piscivory during late winter. The presence of *E. crassum* and *Dibotrhiocephalus* spp. can be related to their ability to re-establish in piscivorous fish (Vik, 1963; Henriksen *et al.*, 2016). Accordingly, the high prevalence of *Dibothriocephalus* spp. plerocercoids can be explained by the piscivorous behavior exhibited by large trout in this period of the year. As the prevalence of *Dibothriocephalus* spp. and the frequency of occurrence of fish prey were highly correlated, the presence of plerocercoids in the intestinal tract of brown trout appeared to be an immediate response to a piscivorous behavior. The peak in prevalence of *C. truncatus* between autumn and early winter corresponded to a higher frequency of occurrence of amphipods in the diet of brown trout. A prolonged residence time in the host caused by the cold water temperatures might have favored its accumulation. The prevalence of *Crepidostomum* spp. in brown trout remained fairly high in all the seasons. This is not surprising, given that brown trout exhibited a persistent feeding on insect larvae throughout the year and that *Crepidostomum* spp. might be able to survive in the host intestine up to one year (Thomas, 1958). Insect larvae (Ephemeroptera) are together with *G. lacustris* intermediate hosts for this parasite (Curtis *et al.*, 1995; Knudsen *et al.*, 1997; Kristmundsson and Richter, 2009). There was however no correlation between *Crepidostomum* spp. and insect larvae or amphipods. Insect larvae, however, harbor less *Crepidostomum* spp. metacercaria compared to gammarid hosts (Olsen, 1984), and in the present study the contribution of Ephemeroptera in the brown trout diet was only modest. Additionally, the low number of trout sampled during winter did potentially not give a correct representation of the importance of *G. lacustris* in their diet. Similarly, the low winter sample size might have influenced the correlations between *Proteocephalus* sp. and zooplankton, fish and *G. lacustris.* The presence of *Proteocephalus* sp. during the winter suggests that this helminth is mainly transmitted by zooplankton during autumn and early winter when the feeding activity on this prey is at the highest. This is in agreement with a study conducted by Scholz (1999), which suggested that the recruitment of a new

Proteocephalus sp. generation in principle take place during summer and autumn. Moreover, from the present findings, zooplankton appears to have a major role in the transmission of *Proteocephalus* sp. as the intensity of the parasite in brown trout decreased in fish larger than 40 cm, which are mainly piscivorous and do not include zooplankton in their diet. The data of the present study suggest that the structure of intestinal parasites communities of both salmonids varied throughout the season in relation to the resource availability, feeding activity and preferences of the hosts.

Both charr and trout, like many other fish species typically experience ontogenetic changes in diet as they grow (Sánchez-Hernández *et al.*, 2019). The present study revealed that parasite infections in both Arctic charr and brown trout generally increased with increasing fish size. As body size increases over time, there will also be a rise in energetic demands consequently larger hosts ingest a greater quantity of food and have had more time to accumulate parasites than smaller ones (Poulin, 2000). This is particularly evident in Arctic charr, where size emerged as a major factor in determining parasite infections, as the intensity of all intestinal parasite species significantly increased with body size. The young Arctic charr feeds primarily on zooplankton, whereas adults typically switch to larger prey such as mollusks, amphipods and in some cases fish, as seen from other studies (Forseth *et al.*, 1994). Host feeding behavior can partially explain why larger fish had higher infections than smaller ones. The frequency of occurrence of amphipods in the diet of Arctic charr, for instance, was higher in larger fish coinciding with a rise in mean intensity of *Crepidostomum* spp. and *C. truncatus.* Similarly the mean intensity of *Dibothriocephalus* spp. increased slightly in Arctic charr over 15 cm when they started to include a low amount of fish in their diet. This finding further supports the assumption that *Dibothriocephalus* spp. plerocercoids are transmitted through ingestion of infected fish prey rather than ingested copepods. The rise in mean intensity of *Proteocephalus* sp. and *E. salvelini* was, on the contrary, not related to an enhanced zooplankton predation. Zooplankton was, in fact, less important for larger Arctic charr. However, the prevalence of *Proteocephalus* sp. was highly correlated with the occurrence of fish prey, suggesting that the modest level of piscivory displayed by Arctic charr might to some extent contribute in the transmission and establishment of new parasite individuals of this species. As *Proteocephalus* sp. and *E. salvelini* can live for more than one year in the host (Scholz, 1999; Hernandez and Muzzall, 2006; Smith, 2015), the most plausible mechanism behind the elevated level of infections in larger fish is an accumulation through time. This process is then reinforced by a constant establishment of new parasite individuals through persistent but modest feeding on their

intermediate hosts. This is supported by the presence of zooplankton in all size classes of Arctic charr. Accordingly, accumulation of long-lived parasites in the final host is expected if predation on the intermediate host is not limited by fish size (Zelmer and Arai, 2006).

In brown trout, the most remarkable rises in mean intensities were seen in *Dibothriocephalus* spp. and *E. crassum*, in fish over 35 cm and corresponded with a shift toward piscivory. This indicates that three-spined stickleback, the only fish prey found in the gastrointestinal tract, might have a key role in the transmission of *E. crassum* as previously suggested by Vik (1963), and act as intermediate host for *Dibothriocephalus* spp. (Kristmundsson and Richter, 2009). Smaller size classes of brown trout feed mainly on benthic invertebrates and surface insects, while larger trout (>30 cm) may become piscivorous as commonly seen (Jensen *et al.*, 2012). The two helminths transmitted by *G. lacustris*: *C. truncatus* and *Crepidostomum* spp., had no significant increase in mean intensity over the ontogeny due to the modest importance of amphipods in the diet of brown trout. In contrast, the mean intensity of *Proteocephalus* sp. decreased toward the biggest size classes. The decrease in mean intensity of *Proteocephalus* sp. in brown trout over 35 cm can be explained by the absence of zooplankton in their diet.

In conclusion, Arctic charr exhibited the richest intestinal parasite community of the two studied salmonids, which apparently was related to a broader dietary niche. *Proteocephalus* sp. infections in Arctic charr were clearly associated with zooplankton, and *Crepidostomum* spp. and *C. truncatus* with amphipods, reflecting seasonality in diet and individual feeding specialization. In contrast, *Dibothriocephalus* spp. and *E. crassum* were clearly associated with piscivorous feeding in brown trout. Intestinal parasite infections in Arctic charr displayed marked seasonal variations as a result of temporal changes in prey availability and host feeding behavior. Seasonal pattern in intestinal parasite infections in brown trout were mainly driven by host body size. Ontogenetic diet shifts occurring in the hosts seem to be an important structuring force for the helminths communities of the two salmonids. Parasite infections increased with increasing fish size, leading to an accumulation through time of long-lived parasites, which was particularly evident in Arctic charr. Most intestinal parasites proved to be good indicators of the dietary preferences of Arctic charr and brown trout.

5 References

Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M., and Rohani, P. (2006) 'Seasonality and the dynamics of infectious diseases', *Ecology Letters*, 9, 467–484.

Amundsen, P.-A. (1995) 'Feeding strategy of Arctic charr (*Salvelinus alpinus*): General opportunist, but individual specialist', *Nordic Journal of Freshwater Research*, 71, 150–156.

Amundsen, P.-A. and Knudsen, R. (2009) 'Winter ecology of Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) in a subarctic lake, Norway', *Aquatic Ecology*, 43, 765–775.

Amundsen, P.-A., Knudsen, R., and Klemetsen, A. (2007) 'Intraspecific competition and density dependence of food consumption and growth in Arctic charr', *Journal of Animal Ecology*, 76, 149–158.

Amundsen, P.-A., Knudsen, R., Kuris, A. M., and Kristoffersen, R. (2003) 'Seasonal and ontogenetic dynamics in trophic transmission of parasites', *Oikos*, 102, 285–293.

Amundsen, P.-A., Kristoffersen, R., Knudsen, R., and Klemetsen, A. (1997) 'Infection of *Salmincola edwardsii* (Copepoda: Lernaeopodidae) in an age-structured population of Arctic charr - A long-term study', *Journal of Fish Biology*, 51, 1033–1046.

Amundsen, P.-A., Lafferty, K. D., Knudsen, R., Primicerio, R., Kristoffersen, R., Klemetsen, A., and 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, 993–1002.

Awachie, J. B. E. (1968) 'On the bionomics of *Crepidostomum metoecus* (Braun, 1900) and *Crepidostomum farionis* (Müller, 1784) (Trematoda: Allocreadiidae)', *Parasitology*, 58, 307–324.

Bush, A. O., Lafferty, K. D., Lotz, J. M., and Shostak, A. W. (1997) 'Parasitology meets ecology on its own terms: Margolis *et al.* revisited.', *Journal of Parasitology*, 83, 575–83.

Bylund, G. (1969) 'Experimentell undersökning av *Diphyllobothrium dendriticum* (= *D. norvegicum*) från norra Finland', *Tiedoksianto-Information*, 10, 3–17.

Carney, J. P. and Dick, T. A. (2011) 'Helminth communities of yellow perch (*Perca flavescens* (Mitchill)): determinants of pattern', *Canadian Journal of Zoology*, 78, 538–555.

Chubb, J. C. (1979) 'Seasonal occurrence of helminths in freshwater fishes part II. Trematoda', *Advances in Parasitology*, 17, 141–313.

Curtis, M. A., Berube, M., and Stenzel, A. (1995) 'Parasitological evidence for specialized foraging behavior in lake-resident arctic char (*Salvelinus alpinus*)', *Canadian Journal of Fisheries and Aquatic Sciences*, 52, 186–194.

Dezfuli, B. S., Bosi, G., DePasquale, J. A., Manera, M., and Giari, L. (2016) 'Fish innate immunity against intestinal helminths', *Fish and Shellfish Immunology*, 50, 274–287.

Eloranta, A. P., Knudsen, R., Amundsen, and 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, 207–221.

Esch, G. W. and Fernández, J. C. (1993) 'Factors influencing parasite populations', in *A functional biology of parasitism: ecological and evolutionary implications*. Chapman & Hall, London, 61-102.

Fagerland, M. W. and Sandvik, L. (2009) 'The Wilcoxon-Mann-Whitney test under scrutiny', *Statistics in Medicine*, 28, 1487–1497.

Fernández, M. V., Brugni, N. L., Viozzi, G. P., and Semenas, L. (2010) 'The relationship between fish assemblages and the helminth communities of a prey fish, in a group of small shallow lakes', *Journal of Parasitology*, 96, 1066–1071.

Forseth, T., Ugedal, O., and Jonsson, B. (1994) 'The energy budget, niche shift, reproduction and growth in a population of Arctic charr, *Salvelinus alpinus*', *Journal of Animal Ecology*, 63, 116-126.

Frainer, A., Johansen, K. S., Siwertsson, A., Mousavi, S. K., Brittain, J. E., Klemetsen, A., Knudsen, R., and Amundsen, P.-A. (2016) 'Variation in functional trait composition of benthic invertebrates across depths and seasons in a subarctic lake', *Fundamental and Applied Limnology*, 188, 103–112.

Halvorsen, O. (1970) 'Studies of the helminth fauna of Norway XV: on the taxonomy and biology of plerocercoids of *Diphyllobothrium* Cobbold, 1858 (Cestoda, Pseudophyllidea) from north-western Europe.', *Nytt Magasin for Zoologi*, 18, 113–174.

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

Henriksen, E. H., Knudsen, R., Kristoffersen, R., Kuris, A. M., Lafferty, K. D., Siwertsson, A., and Amundsen, P.-A. (2016) 'Ontogenetic dynamics of infection with *Diphyllobothrium spp.* cestodes in sympatric Arctic charr *Salvelinus alpinus* (L.) and brown trout *Salmo trutta* L', *Hydrobiologia*, 783, 37–46.

Hernandez, A. D. and Muzzall, P. M. (2006) 'Seasonal patterns in the biology of eubothrium salvelini infecting brook trout in a creek in lower Michigan', *Journal of Parasitology*, 84, 1119-1123.

Hoffman, G. L. (1999) *Parasites of North American freshwater fishes*. Comstock Publishing Associates.

Holmes, J. C. (1987) 'The structure of helminth communities', *International Journal for Parasitology*, 17, 203–208.

Holmes, J. C. (1990) 'Helminth communities in marine fishes', in *Parasite Communities: Patterns and Processes*. Springer, Dordrecht, 101–130.

Jensen, H., Kiljunen, M., and Amundsen, P.-A. (2012) 'Dietary ontogeny and niche shift to piscivory in lacustrine brown trout *Salmo trutta* revealed by stomach content and stable isotope analyses', *Journal of Fish Biology*, 80, 2448–2462.

Jørgensen, L. and Klemetsen, A. (1995) 'Food resource partitioning of Arctic charr, *Salvelinus alpinus* (L.) and three-spined stickleback, *Gasterosteus aculeatus* L., in the littoral zone of lake Takvatn in northern Norway', *Ecology of Freshwater Fish*, 4, 77–84.

Kennedy, C. R., Bush, A. O., and Aho, J. M. (1986) 'Patterns in helminth communities: Why are birds and fish different?', *Parasitology*, 93, 205–215.

Klemetsen, A. and Elliott, M. J. (2010) 'Spatial distribution and diversity of macroinvertebrates on the stony shore of a subarctic lake', *International Review of Hydrobiology*, 95, 190–206.

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

Klemetsen, A., Amundsen, P.-A., Grotnes, P. E., Knudsen, R., Kristoffersen, R., and Svenning, M. A. (2002) 'Takvatn through 20 years: long-term effects of an experimental mass removal of Arctic charr, *Salvelinus alpinus*, from a subarctic lake', *Environmental Biology of Fishes*, 64, 39–47.

Klemetsen, A., Knudsen, R., Staldvik, F. J., and Amundsen, P.-A. (2003) 'Habitat, diet and food assimilation of Arctic charr under the winter ice in two subarctic lakes', *Journal of Fish Biology*, 62, 1082–1098.

Knudsen, R., Klemetsen, A., and Staldvik, F. (1996) 'Parasites as indicators of individual feeding specialization in Arctic charr during winter in northern Norway', *Journal of Fish Biology*, 48, 1256–1265.

Knudsen, R., Amundsen, P.-A., Nilsen, R., Kristoffersen, R., and Klemetsen, Anders (2008) 'Food borne parasites as indicators of trophic segregation between Arctic charr and brown trout', *Environmental Biology of Fishes*, 83, 107–116.

Knudsen, R., Curtis, M. A., and Kristoffersen, Roar (2004) 'Aggregation of helminths: the role of feeding behavior of fish hosts', *Journal of Parasitology*, 90, 1–7.

Knudsen, R., Kristoffersen, R., and Amundsen, P.-A. (1997) 'Parasite communities in two sympatric morphs of Arctic charr, *Salvelinus alpinus* (L.), in northern Norway', *Canadian Journal of Zoology*, 75, 2003–2009.

Kristmundsson, A. and Richter, S. H. (2009) 'Parasites of resident arctic charr, *Salvelinus alpinus*, and brown trout, *Salmo trutta*, in two lakes in Iceland', *Icelandic Agricultural Sciences*, 22, 5–18.

Kuhn, J. A., Amundsen, P.-A., Kristoffersen, R., Frainer, A., and Knudsen, R. (2016) 'Effects of fish species composition on *Diphyllobothrium spp.* infections in brown trout - is three-spined stickleback a key species?', *Journal of Fish Diseases*, 39, 1313–1323.

Kuhn, J. A., Knudsen, R., Kristoffersen, R., Primicerio, R. and Amundsen, P.-A. (2016) 'Temporal changes and between-host variation in the intestinal parasite community of Arctic charr in a subarctic lake', *Hydrobiologia*, 783, 79–91.

Lien, L. and Borgstrøm, R. (1973) 'Studies of the helminth fauna of Norway. XXXI: distribution and seasonal occurrence of *Proteocephalus sp.* Weinland, 1858 (Cestoda: Proteocephala) in brown trout, *Salmo trutta* L., from southern Norway', *Norwegian Journal of Zoology*, 21, 293–297.

Lindén, A. and Mäntyniemi, S. (2011) 'Using the negative binomial distribution to model overdispersion in ecological count data', *Ecology*, 92, 1414–1421.

Locke, S. A., Marcogliese, D. J. and Valtonen, E. T. (2014) 'Vulnerability and diet breadth predict larval and adult parasite diversity in fish of the Bothnian Bay', *Oecologia*, 174, 253–262.

Marques, J. F., Santos, M. J., Teixeira, C. M., Batista, M. I., and Cabral, H. N. (2011) 'Host-parasite relationships in flatfish (Pleuronectiformes) – the relative importance of host biology, ecology and phylogeny', *Parasitology*, 138, 107–121.

Neuhäuser, M. and Poulin, R. (2004) 'Comparing parasite numbers between samples of hosts', *Journal of Parasitology*, 90, 689–691.

Okaka, C. E. (1984) Studies on the biology of *Cyathocephalus truncatus* (Pallas, 1781) (Cestoda: Spathebothridea) in its fish and crustacean hosts. PhD thesis. University of Leeds.

Olsen, O. W. (1986) *Animal parasites: their life cycles and ecology*. Dover, 308

Paterson, S. and Lello, J. (2003) 'Mixed models: Getting the best use of parasitological data', *Trends in Parasitology*, 19, 370–375.

Pietrock, M. and Marcogliese, D. J. (2003) 'Free-living endohelminth stages: at the mercy of environmental conditions.', *Trends in Parasitology*, 19, 293–9.

Poulin, R. (1998) 'Comparison of three estimators of species richness in parasite component communities', *Journal of Parasitology*, 84, 485-490.

Poulin, R. (2000) 'Variation in the intraspecific relationship between fish length and intensity of parasitic infection: biological and statistical causes', *Journal of Fish Biology*, 56, 123–137.

Poulin, R. (2006) 'Global warming and temperature-mediated increases in cercarial emergence in trematode parasites', *Parasitology*, 132, 143–151.

Poulin, R. and Leung, T. L. F. (2011) 'Body size, trophic level, and the use of fish as transmission routes by parasites', *Oecologia*, 166, 731–738.

Primicerio, R. and Klemetsen, A. (1999) 'Zooplankton seasonal dynamics in the neighbouring lakes Takvatn and Lombola (northern Norway)', *Hydrobiologia*, 411, 19–29.

R Core Team (2018) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Available from: https://www.R-project.org

Reiczigel, J., Marozzi, M., Fábián, I. and Rózsa, L. (2019) 'Biostatistic for parasitologists – a primer to quantitative parasitology', *Trends in Parasitology,* 35, 277-281.

Rózsa, L., Reiczigel, J., and Majoros, G. (2000) 'Quantifying parasites in samples of hosts.', *Journal of parasitology*, 86, 228–32.

Sánchez-Hernández, J., Servia, M. J.,Vieira-Lanero, R., and Cobo, F. (2013) 'Ontogenetic dietary shifts in a predatory freshwater fish species: the brown trout as an example of a dynamic fish species', in *New Advances and Contributions to Fish Biology*. InTech, Croatia, 271–298.

Sánchez-Hernández, J., Nunn, A. D., Adams, C. E., and Amundsen, P.-A. (2019) 'Causes and consequences of ontogenetic dietary shifts: a global synthesis using fish models', *Biological Reviews*, 94, 539–554.

Scholz, T. (1999) 'Life cycles of species of *Proteocephalus*, parasites of fishes in the Palearctic region: a review.', *Journal of Helminthology*, 73, 1–19.

Scholz, T., Kuchta, R., Shinn, A.P., Šnábel, V., and Hanzelová, V. (2003) 'Host specificity and geographical distribution of *Eubothrium* in European salmonid fish', *Journal of Helminthology*, 77, 255–262.

Shaw, D. J. and Dobson, A. P. (1995) 'Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review'. *Parasitology*, 111, 111-133.

Skovlund, E. and Fenstad, G. U. (2001) 'Should we always choose a nonparametric test when comparing two apparently nonnormal distributions?', *Journal of Clinical Epidemiology*, 54, 86–92.

Smith, H. D. (2015) 'Observations on the cestode *Eubothrium salvelini* in juvenile sockeye salmon (*Oncorhynchus nerka*) at Babine Lake, British Columbia', *Journal of the Fisheries Research Board of Canada*, 30, 947–964.

Soldánová, M., Georgieva, S., Roháčová, J., Knudsen, R., Kuhn, J. A., Henriksen, E. H., Siwertsson, A., Shaw, J. C., Kuris, A. M., Amundsen, P.-A., Scholz, T., Lafferty, K. D., and Kostadinova, A. (2017) 'Molecular analyses reveal high species diversity of trematodes in a sub-Arctic lake', *International Journal for Parasitology*, 47, 327–345.

Tedla, S. and Fernando, C. H. (2011) 'Observations on the seasonal changes of the parasite fauna of yellow perch (*Perca flavescens*) from the Bay of Quinte, Lake Ontario', *Journal of the Fisheries Research Board of Canada*, 26, 833–843.

Thieltges, D. W., Jensen, K. T. and Poulin, R. (2008) 'The role of biotic factors in the transmission of free-living endohelminth stages', *Parasitology*, 135, 407–426.

Thomas, J. D. (1958) 'Studies on *Crepidostomum metoecus* (Braun) and *C. farionis* (Müller), parasitic in *Salmo trutta* L. and *S. salar* L. in Britain', *Parasitology*, 48, 336–352.

Tuğran, E., Kocak, M., Mirtagioğlu, H., Yiğit, S. and Mendes, M. (2015) 'A simulation based comparison of correlation coefficients with regard to type I error rate and power', *Journal of Data Analysis and Information Processing*, 3, 87–101.

Vik, R. (1958) 'Studies of the helminth fauna of Norway. II. Distribution and life cycle of *Cyathocephalus truncatus* (Pallas, 1781) (Cestoda).', *Nytt Magasin for Zoologi*, 6, 97–110.

Vik, R. (1963) 'Studies of the helminth fauna of Norway. IV. Occurrence and distribution of *Eubothrium crassum* (Bloch, 1779) and *E. salvelini* (Schrank, 1790) (Cestoda) in Norway, with notes on their life cycles.', *Nytt Magasin for Zoologi*, 11, 47–73.

Vik, R. (1964) 'The genus *Diphyllobothrium*. An example of the interdependence of systematics and experimental biology', *Experimental Parasitology*, 15, 361–380.

von Bonsdorff, B. and Bylund, G. (1982) 'The ecology of *Diphyllobothrium latum*.', *Ecology of Disease*, 1, 21–6.

Waeschenbach, A., Brabec, J., Scholz, T., Littlewood, D. T. J., and Kuchta, R. (2017). 'The catholic taste of broad tapeworms-multiple routes to human infection. ', *International Journal for Parasitology*, 47, 831-843.

Welz, A., Ruxton, G. D. and Neuhäuser M. (2018) 'A non-parametric maximum test for the Behrens–Fisher problem', *Journal of Statistical Computation and Simulation*, 88, 1336–1347.

Werner, E. E. and Gilliam, J. F. (1984) 'The ontogenetic niche and species interactions in size-structured populations', *Annual Review of Ecology and Systematics*, 15, 393–425.

Wilcox, R. R. (2001) 'Measures of Association', in *Fundamentals of modern statistical methods*. Springer New York, 179–203.

Williams, H. and Jones, A. (1994) 'Parasitic worms of fish', *Revista do Instituto de Medicina Tropical de São Paulo*, 36, 530–530.

Wilson, K. and Grenfell, B. T. (1997) 'Generalized linear modelling for parasitologists', *Parasitology Today*, 13, 33–38.

Zelmer, D. A. and Arai, H. P. (2006) 'The contributions of host age and size to the aggregated distribution of parasites in yellow perch, *Perca flavescens*, from Garner Lake, Alberta, Canada', *Journal of Parasitology*, 84, 24-28.

6 Appendix

Appendix Tab.1. Littoral water temperature and ice cover throughout the seasons

Sampling period	Season	Water temperature in °C	Ice thickness
June 2017	Summer	2.26	-
August 2018	Summer	11.03	-
September 2018	Autumn	8.60	-
October 2018	Autumn	6.41	-
November 2018	Early winter	2.93	Ice formation week 48
January 2018	Early winter	1.15	45 cm
March 2018	Late winter	1.21	100 cm
May 2018	Late winter	2.21	60 cm - Ice melting week 20

Appendix Tab.2. One-way ANOVA output for differences in length between seasons

	Length differences between seasons
Arctic charr	**0.010**
Brown trout	**<0.001**

Appendix Tab.3. GLM negative binomial regression, ANOVA output for main effects in parasites intensity

Species	Effects	Parasite species					
		E. salvelini	*E. crassum*	*C. truncatus*	*Proteocephalus* sp.	*Crepidostomum* spp.	*Dibothriocephalus* spp.
Arctic charr	Season	**0.031**	-	**<0.001**	**<0.001**	**<0.001**	0.291
	Length	**<0.001**	-	**<0.001**	**0.004**	**<0.001**	**0.006**
Brown trout	Season	-	**<0.001**	**<0.001**	0.058	0.516	0.525
	Length	-	0.096	**0.021**	**<0.001**	0.771	**<0.001**

Appendix Tab.4. GLM logistic regression, ANOVA output for main effects in prey occurrence

Species	Effects	Prey					
		Amphipods	Mollusks	Surface insects	Insects larvae	Zooplankton	Fish
Arctic charr	Season	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.252
	Length	**<0.001**	**<0.001**	0.149	0.915	**<0.001**	0.839
Brown trout	Season	**0.015**	**<0.001**	**<0.001**	0.177	0.149	0.148
	Length	0.851	0.129	0.286	**<0.001**	0.675	**<0.001**

Appendix tab.5. Summary chart with statistical analysis ([a] Infected/sample (prevalence percentage); Fisher's exact test for seasonal and monthly variations, and χ^2 test with Yates correction for between-host comparisons. [b] Mean intensity ± SE; max test for between-host comparisons. [c] Mean abundance ± SE (max value); Kruskal-Wallis test for seasonal variation and max test for between-host comparisons.

	Eubothrium		C. truncatus		Proteocephalus sp.		Crepidostomum spp.		Dibothriocephalus spp.	
	Arctic charr (*E. salvelini*)	Brown trout (*E. crassum*)	Arctic charr	Brown trout	Arctic charr	Brown trout	Arctic charr	Brown trout	Arctic charr	Brown trout
Jun. 2017	90.00% (45/50)[a] 4.40±0.55[b] 3.96±0.48 (15)[c]	-[a] -[b] -[c]	16.00% (8/50)[a] 16.63±2.28[b] 2.66±0.30 (39)[c]	-[a] -[b] -[c]	10.00% (5/50)[a] 1±0.00[b] 0.1±0.04 (1)[c]	-[a] -[b] -[c]	56.00% (28/50)[a] 12.21±1.66[b] 6.48±0.89 (124)[c]	-[a] -[b] -[c]	12.00% (6/50)[a] 1.67±0.15[b] 0.20±0.06 (2)[c]	-[a] -[b] -[c]
Aug. 2017	86.00% (43/50)[a] 7.70±1.02[b] 6.62±0.86 (54)[c]	18.00% (9/50)[a] 3.56±0.43[b] 0.64±0.02(12)[c]	14.00% (7/50)[a] 4.86±0.61[b] 0.68±0.07 (21)[c]	22.00% (11/50)[a] 1.64±0.14[b] 0.36±0.07 (4)[c]	38.00% (19/50)[a] 18.32±2.52[b] 6.96±0.91 (243)[c]	16.00% (8/50)[a] 3.25±0.38[b] 0.52±0.07 (6)[c]	54.00% (27/50)[a] 63.89±8.96[b] 34.50±4.81 (621)[c]	70.00% (35/50)[a] 5.51±0.71[b] 3.86±0.47 (21)[c]	8.00% (4/50)[a] 1.00±0.00[b] 0.08±0.04 (1)[c]	18.00% (9/50)[a] 1.33±0.09[b] 0.24±0.06 (2)[c]
Summer	88.00% (88/100)[a] 6.01±0.55[b] 5.29±0.48 (54)[c]	18.00% (9/50)[a] 3.56±0.43 [b] 0.64±0.07 (12)[c]	15.00% (15/100)[a] 11.13±1.06[b] 1.67±0.11 (39)[c]	22.00% (11/50)[a] 1.64±0.14[b] 0.36±0.07 (4) [c]	24.00% (24/100)[a] 14.71±1.42[b] 3.53±0.30 (243)[c]	16.00% (8/50)[a] 3.25±0.38[b] 0.52±0.07 (6)[c]	55.00% (55/100)[a] 37.58±3.71[b] 20.67±2.02 (621)[c]	70.00% (35/50)[a] 5.51±0.71[b] 3.86±0.47 (21)[c]	10.00% (10/100)[a] 1.40±0.07[b] 0.14±0.03 (2)[c]	18.00% (9/50)[a] 1.33±0.09[b] 0.24±0.06 (2)[c]
Sep. 2017	95.83% (23/24)[a] 5.96±1.11[b] 5.71±1.06 (15)[c]	0.00% (0/36)[a] -[b] -[c]	16.67% (4/24)[a] 5.00±0.91[b] 0.83±0.08 (8)[c]	22.22% (8/36)[a] 1.13±0.06[b] 0.25±0.07 (2)[c]	62.50% (15/24)[a] 15.13±2.99[b] 9.46±1.83 (47)[c]	2.78% (1/36)[a] 1.00±0.00[b] 0.03±0.03(1)[c]	83.33% (20/24)[a] 35.15±7.07[b] 29.29±5.88 (109)[c]	63.89% (23/36)[a] 5.43±0.82[b] 3.47±0.49(24)[c]	4.17% (1/24)[a] 1.00±0.00[b] 0.04±0.04 (1)[c]	16.67% (6/36)[a] 1.83±0.21[b] 0.31±0.08 (3)[c]
Oct. 2017	91.67% (33/36)[a] 11.73±1.87[b] 10.75±1.71 (61)[c]	2.08% (1/48)[a] 2 .00±0.20[b] 0.04±0.03 (2)[c]	36.11% (13/36)[a] 4.08±0.59[b] 1.47±0.14 (14)[c]	12.50% (6/48)[a] 1.67±0.15[b] 0.21±0.06 (3)[c]	52.78% (19/36)[a] 21.37±3.48[b] 11.28±1.79 (129)[c]	22.92% (11/48)[a] 2.55±0.29[b] 0.58±0.07 (7)[c]	80.56% (29/36)[a] 36.52±6.00[b] 29.4±4.82(254)[c]	75.00% (36/48)[a] 7.08±0.95[b] 5.31±0.69 (59)[c]	8.33% (3/36)[a] 1.33±0.11[b] 0.11±0.05 (2)[c]	27.08% (13/48)[a] 5.62±0.73[b] 1.52±0.13 (41)[c]
Autumn	93.33% (56/60)[a] 9.36±1.14[b] 8.73±1.06 (61)[c]	1.19% (1/84)[a] 2.00±0.15[b] 0.02±0.02 (2)[c]	28.33% (17/60)[a] 4.29±0.49[b] 1.22±0.07 (14)[c]	16.67% (14/84)[a] 1.36±0.08[b] 0.23±0.05 (3)[c]	56.67% (34/60)[a] 18.62±2.34[b] 10.55±1.30 (129)[c]	14.29% (12/84)[a] 2.42±0.20[b] 0.35±0.05 (7)[c]	81.67% (49/60)[a] 35.96±4.58[b] 29.37±3.73 (254)[c]	70.24% (59/84)[a] 6.44±0.65[b] 4.52±0.44 (59)[c]	6.67% (4/60)[a] 1.25±0.07[b] 0.08±0.04 (2)[c]	22.62% (19/84)[a] 4.42±0.42[b] 1.00±0.00 (41)[c]
Nov. 2017	90.00% (45/50)[a] 8.64±1.15[b] 7.78±1.03 (58)[c]	7.32 (3/41)[a] 12.33±1.85[b] 0.90±0.05 (24)[c]	62.00% (31/50)[a] 9.06±1.21[b] 5.62±0.72 (42)[c]	39.02% (16/41)[a] 2.75±0.34[b] 1.07±0.04 (9)[c]	20.00% (10/50)[a] 2.60±0.29[b] 0.52±0.07 (7)[c]	17.07% (7/41)[a] 2.00±0.22[b] 0.34±0.07 (3)[c]	88.00% (44/50)[a] 52.98±7.42[b] 46.62±6.52 (385)[c]	80.49% (33/41)[a] 7.94±1.16[b] 6.39±0.92 (34)[c]	2.00% (1/50)[a] 1.00±0.00[b] 0.02±0.02 (1)[c]	19.51% (8/41)[a] 2.63±0.32[b] 0.51±0.08 (5)[c]
Jan. 2018	96.00% (48/50)[a] 7.56±1.00[b] 7.26±0.95 (59)[c]	0.00% (0/11)[a] -[b] -[c]	64.00% (32/50)[a] 13.50±1.84[b] 8.64±1.15 (61)[c]	45.45% (5/11)[a] 3.00±0.35[b] 1.36±0.21 (8)[c]	4.00% (2/50)[a] 3.00±0.35[b] 0.12±0.05 (5)[c]	45.45% (5/11)[a] 5.20±1.41[b] 2.36±0.54 (12)[c]	82.00% (41/50)[a] 98.46±13.85[b] 80.74±11.35 (737)[c]	63.63% (7/11)[a] 3.29±0.83[b] 2.09±0.46 (7)[c]	14.00% (7/50)[a] 1.29±0.09[b] 0.18±0.05 (2)[c]	18.18% (2/11)[a] 2.50±0.58[b] 0.45±0.15 (3)[c]
Early winter	93.00% (93/100)[a] 8.09±0.76[b] 7.52±0.70 (59)[c]	5.77% (3/52)[a] 12.33±1.64[b] 0.71±0.06 (24)[c]	63.00% (63/100)[a] 11.32±1.08[b] 7.13±0.66 (61)[c]	40.38% (21/52)[a] 2.81±0.31[b] 1.13±0.05 (9)[c]	12.00% (12/100)[a] 2.67±0.21[b] 0.32±0.05 (7)[c]	23.08% (12/52)[a] 3.33±0.39[b] 0.77±0.06 (12)[c]	85.00% (85/100)[a] 74.92±7.44[b] 63.68±6.32 (737)[c]	76.92% (40/52)[a] 7.13±0.92[b] 5.48±0.69 (34)[c]	8.00% (8/100)[a] 1.25±0.00[b] 0.10±0.03 (2)[c]	19.23% (10/52)[a] 2.60±0.28[b] 0.50±0.07 (5)[c]
Mar. 2018	98.00% (49/50)[a] 7.33±0.96[b] 7.18±0.94 (38)[c]	0.00% (0/7)[a] -[b] -[c]	34.00% (17/50)[a] 10.63±1.43[b] 3.62±0.44 (76)[c]	14.29% (1/7)[a] 26.00±9.64[b] 3.71±1.20 (26)[c]	6.00% (3/50)[a] 10.33±1.39[b] 0.62±0.07 (29)[c]	14.29% (1/7)[a] 2.00±0.53[b] 0.29±0.17 (2)[c]	66.00% (33/50)[a] 68.85±9.67[b] 45.44±6.36 (471)[c]	71.43% (5/7)[a] 5.00±1.69[b] 3.57±1.15 (15)[c]	10.00% (5/50)[a] 1.40±0.11[b] 0.14±0.05 (3)[c]	100.00% (7/7)[a] 4.43±1.47[b] 4.43±1.47 (8)[c]
May 2018	90.91% (40/44)[a] 7.75±1.09[b] 7.05±0.98 (32)[c]	60.00% (6/10)[a] 2.67±0.67[b] 1.60±0.31 (5)[c]	25.00% (11/44)[a] 7.36±1.03[b] 1.84±0.19(26)[c]	20.00% (2/10)[a] 1.50±0.27[b] 0.30±0.14 (2)[c]	9.09% (4/44)[a] 2.50±0.29[b] 0.23±0.06 (6)[c]	20% (2/10)[a] 2.50±0.61[b] 0.50±0.16 (3)[c]	68.18% (30/44)[a] 29.17±4.32[b] 19.89±2.92 (293)[c]	50.00% (5/10)[a] 6.20±1.80[b] 3.10±0.81 (12)[c]	13.64% (6/44)[a] 1.67±0.16[b] 0.23±0.06 (3)[c]	60.00% (6/10)[a] 1.17±0.14[b] 0.70±0.14 (2)[c]
Late winter	94.68% (89/94)[a] 7.52±0.72[b] 7.12±0.68 (38)[c]	35.29% (6/17)[a] 2.67±0.51[b] 0.94±0.06 (5)[c]	29.79% (28/94)[a] 9.36±0.91[b] 2.79±0.23 (76)[c]	17.65% (3/17)[a] 9.67±2.22[b] 1.71±0.27 (26)[c]	7.45% (7/94)[a] 5.86±0.55[b] 0.44±0.05 (29)[c]	17.65% (3/17)[a] 2.33±0.43[b] 0.41±0.12 (3)[c]	67.02% (63/94)[a] 49.95±5.10[b] 33.48±3.40 (471)[c]	58.82% (10/17)[a] 5.60±1.23[b] 3.29±0.67 (15)[c]	11.70% (11/94)[a] 1.55±0.09[b] 0.18±0.04 (3)[c]	76.47% (13/17)[a] 2.92±0.58[b] 2.24±0.40 (8)[c]
Entire year	92.09% (326/354)[a] 7.59±0.38[b] 6.99±0.34 (61)[c]	9.36% (19/203)[a] 4.58±0.28[b] 0.43±0.03 (24)[c]	34,75% (123/354)[a] 9.88±0.50[b] 3.43± 0.15 (76)[c]	24.14% (49/203)[a] 2.55±0.14[b] 0.62±0.03(26)[c]	21.75% (77/354)[a] 13.75±0.70[b] 2.99±0.13 (243)[c]	17.24% (35/203)[a] 2.91±0.17[b] 0.50±0.04 (12)[c]	71.19% (252/354)[a] 52.95±2.79[b] 37.69± 1.98 (737)[c]	70.94% (144/203)[a] 6.35±0.41[b] 4.50±0.28 (59)[c]	9.32% (33/354)[a] 1.39±0.04[b] 0.13±0.02 (3)[c]	25.12% (51/203)[a] 3.14±0.18[b] 0.79±0.03 (41)[c]
Monthly variation:	P=0.396[a]	P=<**0.001****[a]	P=<**0.001****[a]	P=0.062[a]	P=<**0.001****[a]	P=**0.029***[a]	P=<**0.001****[a]	P=0.401[a]	P=0.452[a]	P=<**0.001****[a]
Seasonal variation	P=0.381[a] P=0.419[c]	P=<**0.001****[a] P=**0.009****[c]	P=<**0.001****[a] P=0.162[c]	P=**0.018***[a] P=0.209[c]	P=<**0.001****[a] P=0.328[c]	P=0.607[a] P=0.761[c]	P=<**0.001****[a] P=0.267[c]	P=0.528[a] P=0.809[c]	P=0.741[a] P=0.600[c]	P=<**0.001****[a] P=0.191[c]
Monthly between hosts comparison (jun. excl.)			P=**0.006***[a] P=**0.037***[b] P=**0.041***[c]		P=<**0.001****[a] P=**0.018***[b] P=0.271[c]		P=0.222[a] P=<**0.001****[b] P=<**0.001****[c]		P=<**0.001****[a] P=**0.015***[b] P=<**0.001****[c]	
Seasonal between hosts comparison			P=0.117[a] P=0.114[b] P=0.114[c]		P=<**0.001****[a] P=0.114[b] P=0.6b85[c]		P=0.346[a] P=**0.029***[b] P=**0.029***[c]		P=**0.04***[a] P=0.114[b] P=**0.029***[c]	
Parasites richness hosts comparison	P= **<0.001**** (Mann–Whitney U-test)									

Appendix tab.6. Variance-to-mean-ratios of parasite distributions in Arctic charr and brown trout throughout seasons. Uninfected and infected fish included.

Species	Season	Parasite species					
		E. salvelini	*E. crassum*	*C. truncatus*	*Proteocephalus* sp.	*Crepidostomum* spp.	*Dibothriocephalus* spp.
Arctic charr	Summer	11.05	-	21.76	169.22	262.7	1.45
	Autumn	16.21	-	5.94	47.43	78.83	1.34
	Early winter	15.99	-	21.11	4.1	230.5	1.31
	Late winter	8.55	-	31.5	21.38	181.27	1.9
Brown trout	Summer	-	6.55	1.79	3.71	6.33	1.29
	Autumn	-	2	1.42	3.73	19.4	21.64
	Early winter	-	17.82	3.49	5.54	10.37	2.71
	Late winter	-	2.98	23.14	2.14	6.48	2.66
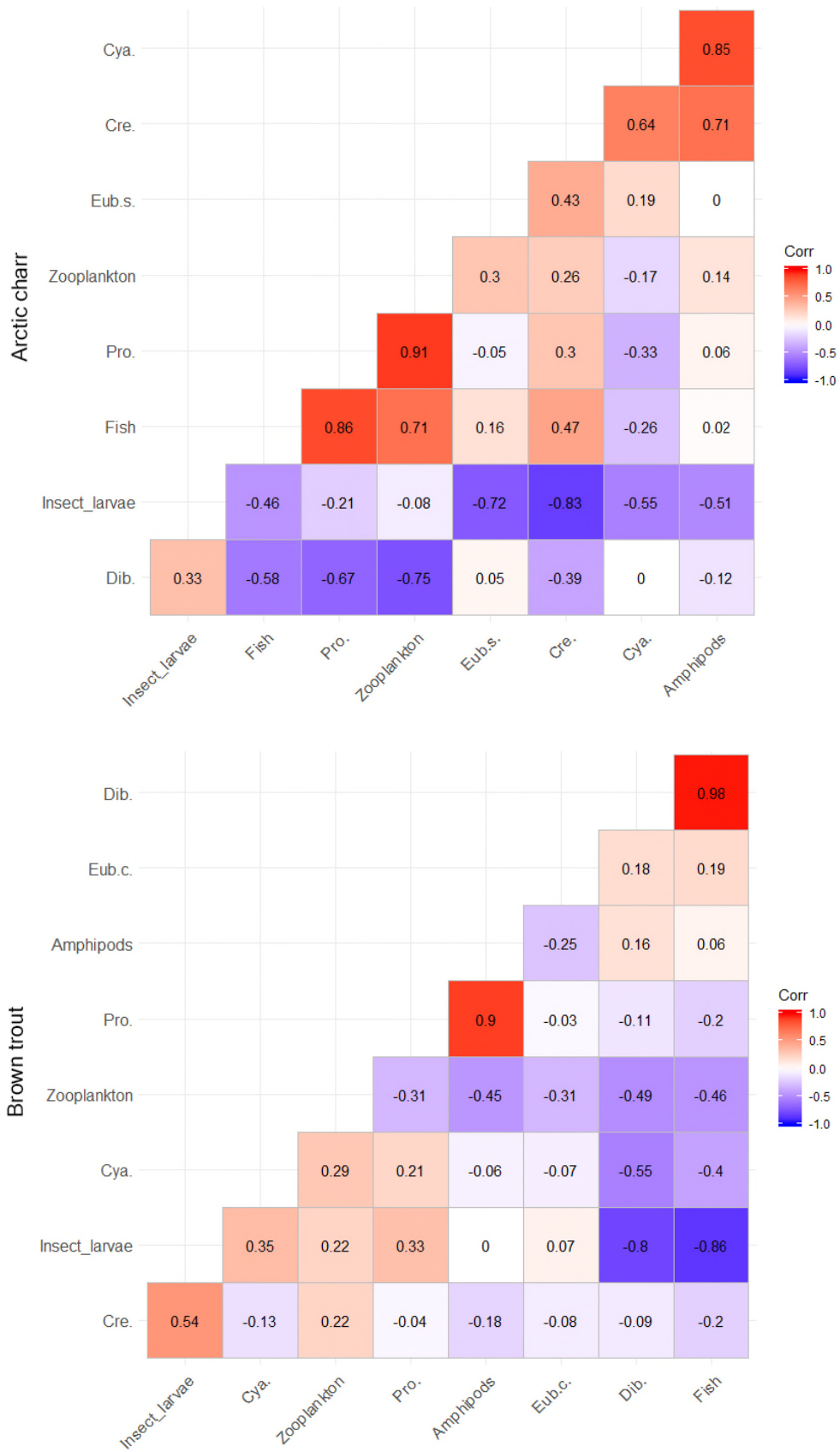
Appendix Fig.1. Winsorized correlation coefficient between parasite prevalence and frequency of occurrence of potential intermediate host in the diet of Arctic charr and brown trout
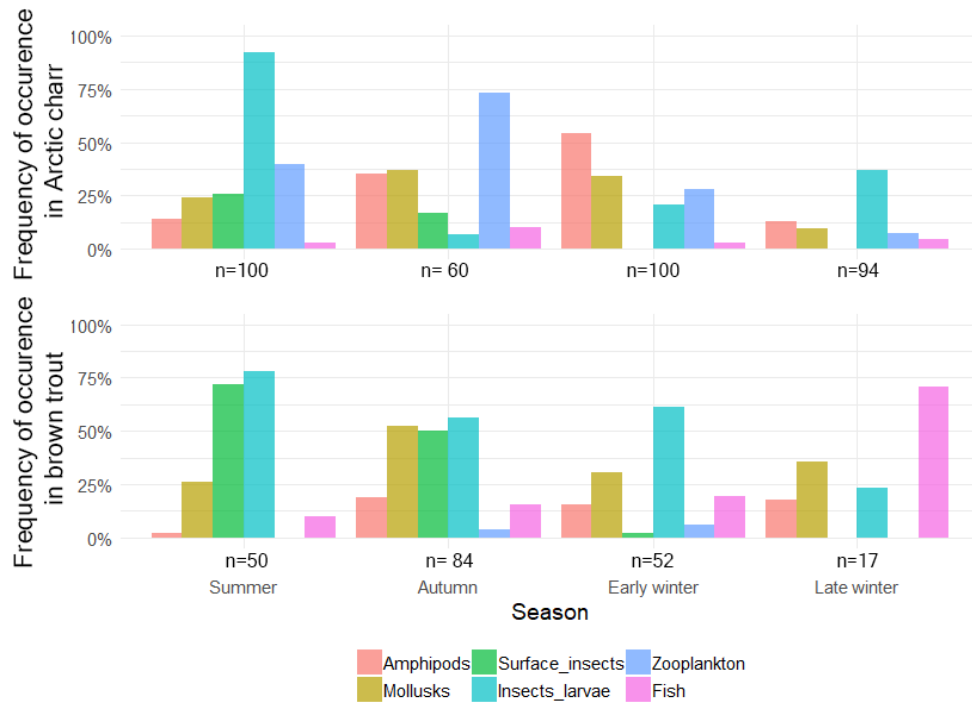
Appendix Fig.2. Seasonal variation in the frequency of occurrence of all six main prey categories in the diet of Arctic charr and brown trout
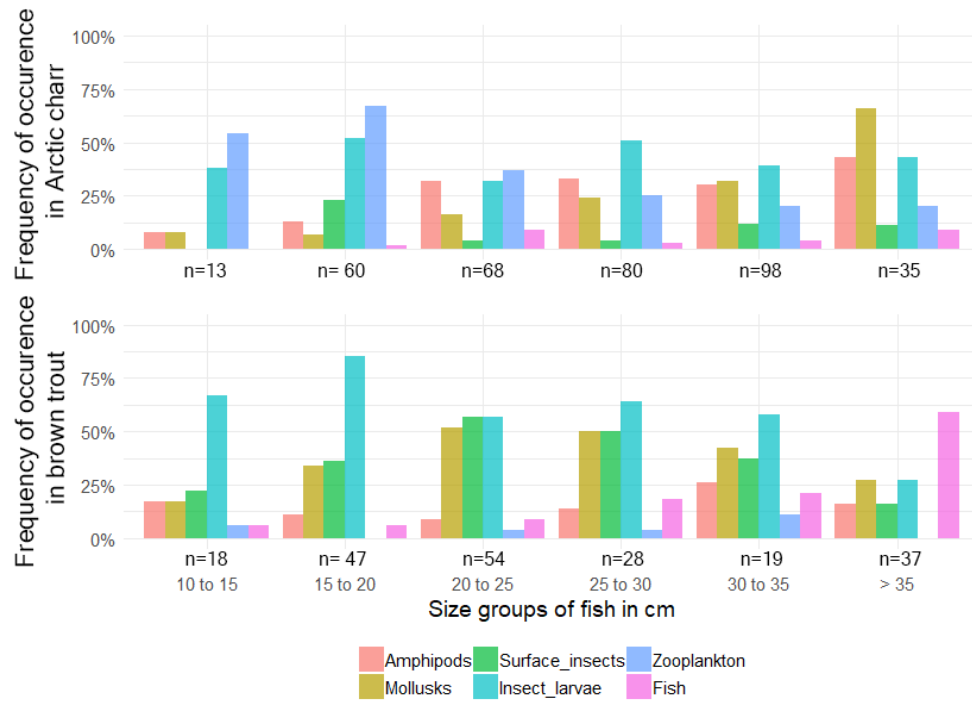
Appendix Fig.3. Ontogenetic variation in the frequency of occurrence of all six main prey categories in the diet of Arctic charr and brown trout