Temporal changes and between-host variation in the intestinal parasite community of Arctic

charr in a subarctic lake

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Abstract

The occurrence of trophically transmitted intestinal parasites in Arctic charr was analyzed from data material collected over two decades from Lake Takvatn, northern Norway. The main objectives were to investigate i) between-year variation in parasite infracommunity composition, ii) between-host variation in infracommunity composition and iii) possible interspecific interaction between parasites. The trematode *Crepidostomum* spp. and the cestode *Eubothrium salvelini* were most prevalent and had the highest mean intensities whereas the cestodes *Proteocephalus* sp. and *Cyathocephalus truncatus* were uncommon taxa. No clear indication of interspecific parasite interactions was evident. By far the most frequent infracommunity composition was *E. salvelini* co-existing with only *Crepidostomum* spp. Despite some indications of individual dietary specialization in Arctic charr, this strong species co-occurrence suggests that the majority of fish had been preying on the very different intermediate hosts of both parasites. Overall, even though some sporadic between-year variation was present, no long-term or cyclical variation in infracommunity composition and mean intensity was revealed, suggesting that the host-parasite system is in a steady state. Minor between-year variation was likely the result of changes in habitat and dietary utilization by Arctic charr, possibly linked to documented changes in the fish community structure of the lake.

Keywords

Introduction

The structure of parasite communities can be influenced by several biotic and abiotic factors (Holmes, 1987; Holmes, 1990). If such factors vary with time, it is likely that a parasite community will do the same. Several parasite-host systems have been reported to show seasonal fluctuations in parasite occurrence (Scott & Smith, 1994). Despite such seasonal variation, parasite-host systems might return to an overall equilibrium providing long-term stability (Scott & Smith, 1994). Variation in the structure of parasite communities might also occur between hosts. Pre-transmission processes, such as between-host variation in parasite exposure, might contribute to a variable parasite community stucture. As parasite communities often involve the coexistence of several parasite species in the same host or microhabitat, like e.g. the host intestine (Dobson et al., 2008), post-transmission processes such as competitive interactions between parasites might contribute to between-host variation in parasite community composition. Parasite communities of freshwater fish are often considered to be unstable and unregulated (Kennedy, 2009; and references therein). However, most studies performed in this field have been on a short-term scale. Long-term studies are needed to search for temporal patterns and to understand how these complex communities may vary over an extended temporal scale (Kennedy, 2009).

In the present study, we performed a long-term analysis of trophically transmitted intestinal parasite infections in Arctic charr (*Salvelinus alpinus* (L.)) from Lake Takvatn, a subarctic lake in northern Norway. The purpose was to address temporal and compositional variation in the intestinal parasite community. These data have been collected in several years since a large-scale fish density manipulation was carried out in the lake in the 1980s (Klemetsen et al., 2002). In Takvatn, and other lake systems, changes in habitat utilization, population

density and dietary choices have been observed to occur over time following fish removal (e.g., Amundsen & Kristoffersen, 1990; Klemetsen & Dahl-Hansen, 1995; Persson et al., 2007). Changes like these may also influence the transmission processes of parasites to Arctic charr. Long-term studies provides a valuable opportunity to increase our knowledge of the temporal dynamics and between-host variation possibly occurring in the parasite community of such lake systems.

As a species, Arctic charr can be considered as an opportunistic forager (Johnson, 1980; Klemetsen et al., 2003), but strong individual feeding specializations can also occur (e.g., Amundsen, 1995; Klemetsen et al., 2003; Knudsen et al., 2010). A broad diet will expose each individual Arctic charr to a wide range of trophically transmitted parasites, likely resulting in little variation between individuals in the intestinal parasite community (the infracommunity) within the fish population. Individual feeding specializations will, on the other hand, presumably lead to a more diverse range of intestinal parasite communities. In northern Norway, Arctic charr has been reported to be infected with at least 10 different species of macroparasites (Kristoffersen, 1995). Four trophically transmitted parasites, including the three cestode taxa *Eubothrium salvelini*, *Cyathocephalus truncatus* and *Proteocephalus* sp., and one trematode taxa *Crepidostomum* spp., are commonly found and have their adult stage in the intestine of the fish (Knudsen, 1995; Sterud, 1999). *E. salvelini* and *Proteocephalus* sp. have copepods as the first and only intermediate host, whereas *C. truncatus* typically uses amphipods (*Gammarus lacustris*) (Vik, 1958, 1963; Boyce, 1974; Sysoev et al., 1994; Scholz, 1999). *Crepidostomum* spp. has molluscs as the first intermediate host, and amphipods or aquatic insect larvae as the second intermediate host (Thomas, 1958; Awachie, 1968; Hoffman, 1999). With such complex lifecycles, it is likely that several biotic and abiotic factors may constrain the occurrence of these parasites and thus to what degree

Arctic charr are exposed to infection. Pre-transmission factors, such as the density of intermediate hosts and host feeding behavior, might control how exposed a host is to a specific parasite taxa. Presumably, pre-transmission factors could be highly affected by the large-scale fish density manipulation of Arctic charr performed in Lake Takvatn. Furthermore, post-transmission factors such as individual host immune responses and inter-specific competition between parasites, might affect the intensity of host infection. As both cestodes and trematodes compete with their fish host for nutrients within the intestine, they might have deleterious effects on the host (Hoffmann et al., 1986; Williams & Jones, 1994). Inter-specific interactions between parasite taxa co-occurring in the intestine could also possibly result in between-host variation in the parasite infracommunity structure.

In this study we examined trophically transmitted intestinal parasites of Arctic charr sampled in August from Lake Takvatn over a 20 year period to see i) if there was long-term temporal variations in the composition of intestinal parasite infracommunities, ii) if there was variation in intestinal parasite infracommunity composition between individual hosts, and iii) if inter-specific interaction between intestinal parasite species could be detected. Firstly, we hypothesized that changes following the mass removal of the fish as a final host in Lake Takvatn could potentially cause long-term temporal variation in the infracommunity composition of trophically transmitted intestinal parasites in the Arctic charr population. Secondly, we hypothesized that expected dietary specializations in Arctic charr would result in between-host variation in intestinal parasite infracommunity composition. Lastly, we hypothesized that the presence of one intestinal parasite species would be affected by the intensity of a possible competing species present in the same microhabitat.

Methods

The study lake

Lake Takvatn (69°07'N, 19°05'E) is an oligotrophic and dimictic lake located 300 km north of the Arctic Circle in the county of Troms in subarctic Norway. The lake is situated 214 m above sea level uppermost in a watercourse, has a surface area of 14.2 km^2 and a maximum depth of about 80 m. The catchment area is dominated by birch forests (*Betula pubescens*) and occasional pine forests (*Pinus sylvestris*) as well minor agricultural activity. Average air temperature is 13 °C in July and -10 °C in January. The lake is ice free from June to November. Secchi depth is in the range 14 - 17 meters, and phosphorus concentrations are not above 5 µg L^{-1} (Primicerio & Klemetsen, 1999). Takvatn presently has a fish community comprised of Arctic charr, three-spined stickleback (*Gasterosteus aculeatus* L.) and brown trout (*Salmo trutta* L.). Originally, only brown trout occurred in Takvatn, but Arctic charr were introduced from Lake Fjellfrøsvatn in the 1930s (Amundsen et al., 2007) and three-spined sticklebacks were introduced in the late 1940s (Jørgensen & Klemetsen, 1995). From 1984 to 1989 an intensive fishing program, that targeted stunted Arctic charr, successfully removed 666.000 fish (Amundsen et al., 1993). The density of old and small Arctic charr decreased, the abundance of brown trout increased, and growth rates of the salmonids in the lake improved (Klemetsen et al., 2002; Amundsen et al., 2007; Persson et al., 2007).

Fish sampling and parasite screening

Takvatn has been sampled annually (Klemetsen et al., 2002), and data for Arctic charr and brown trout are available for the period 1984 to 2015. Similar sampling procedures were used

each year: Multi-mesh survey gillnets were deployed overnight in the littoral (< 15 meters depth), profundal (25 - 40 meters depth) and pelagic zones (0 - 6 meters depth from the surface) for approximately 12 hours. In the field laboratory, fork length and capture habitat were recorded for each individual fish. Fish were dissected, and the intestinal tract was frozen at -18 °C. Otoliths were removed for age determination as described by Klemetsen et al. (2002). Processing of the intestine involved thawing, cutting the intestine open, and scraping out the intestinal and pyloric caeca content. Intestinal content was then shaken vigorously in water and filtered through a 120 µm mesh net. The retained content was then examined for parasites by stereomicroscopy at 64x – 400x magnification. Parasites were counted using subsamples when intensities were high. In the case of cestodes, which have a tendency to break during processing, scolices were counted. The intestinal parasites encountered were distinguishable based on their external morphology, and all have previously been described from the area (Amundsen et al., 2013; Knudsen et al., 2014). *Proteocephalus* sp. was identified to genus, whereas *Crepidostomum* spp. likely includes both *Crepidostomum farionis* and *Crepidostomum metoecus*.

Fish selection

August has been the main sampling month throughout the fieldwork done in Takvatn, and in order to standardize the long-term study material, August samples are included here. To further minimize any biased variation among sampling years, we selected Arctic charr of 4-6 years of age to reduce differences in length- and age-distribution. Fish samples from 1995, 2001 to 2007, 2010 and 2014 were analyzed.

Terminology

Terms follow the definitions of Bush et al. (1997). Prevalence is the number of Arctic charr in a sampling year infected with a particular parasite taxon, divided by the number of Arctic charr examined that year, multiplied by 100. Mean intensity is the total number of parasites of a particular taxon infecting Arctic charr in a given year, divided by the total number of infected Arctic charr sampled that year. Infrapopulation includes all individuals of a parasite taxon found in a single Arctic charr. Infracommunity is used to describe all individuals of all parasite taxa found in a single Arctic charr.

Statistical analyses

Fish length differed among years (ANOVA ($F(9,325) = 12.07$, $p < 0.001$, Table 1) using 'fish length' as a continuous response variable and 'sampling year' as a 10-level categorical explanatory variable. Because of this, 'fish length' was included as an explanatory variable in the following statistical analyses.

Intensities of the four parasite taxa were modelled using generalized linear models (GLM) fitted with negative binomial errors to account for high overdispersion. For *C. truncatus* we used fourth root transformation as this created a model with the best fit to the data. Otherwise, log transformed parasite intensity was used as the response variable. As explanatory variables we used 'sampling year' (10-level categorical), 'fish length' (continuous), 'fish age' (3-level categorical; age-groups 4, 5 and 6) and 'habitat' (3-level categorical; littoral, pelagic and profundal). Model simplification was done while consulting the Akaike information criterion and residuals were checked for trends. The GLM's were then tested with chi-square tests to look for significant effects.

To analyze if the intensity of *E. salvelini* influenced the presence of either *Proteocephalus* sp. or *C. truncatus*, we compared *E. salvelini* mean intensity between three groups of parasite infracommunity composition. The first group ($N = 213$) included *E. salvelini* and *E. salvelini* + *Crepidostomum* spp. infracommunities. The second group ($N = 56$) included *E. salvelini* + *Proteocephalus* sp. and *E. salvelini* + *Proteocephalus* sp. + *Crepidostomum* spp. infracommunities. The third group ($N = 26$) included *E. salvelini* + *C. truncatus* and *E. salvelini* + *C. truncatus* + *Crepidostomum* spp. infracommunities. Potential interspecific competition with *Crepidostomum* spp. was disregarded because this parasite taxon was primarily found to occur in the lower intestine and was therefore assumed to have little interaction with the cestodes in the pyloric caeca region. To test for significant differences between groups we used a GLM with log transformed *E. salvelini* intensity as the response variable in addition to the explanatory variables mentioned above. Also, to compare the observed frequency of pairwise parasite co-occurrence with what could be expected by chance, we performed a 1000 cycled randomization of the data.

Compositional differences in infracommunities between sample years were analyzed using a correspondence analysis (CA), a widely used ordination method applicable to count data (Greenacre & Primicerio, 2014). Infrapopulations were weighted relative to their respective infracommunity using chi-square distance on square root transformed parasite abundance. As there was extensive variation within infracommunities of each year, we analyzed the dataset by referring to the mean infracommunity of the individual years, illustrated as the centroids of each sampling year in the CA plot.

To analyze how variation in parasite abundance was explained by environmental variables, a canonical correspondence analysis (CCA) was used (Greenacre & Primicerio, 2014). Square root transformed parasite abundances was used as the response variable and 'sampling year' (10-level categorical), 'fish length' (continuous), 'fish age' (3-level categorical) and 'habitat' (3-level categorical) as explanatory variables. Monte Carlo permutations (999 cycles) were used to test which environmental variables explained a significant part of variation in parasite abundance. In addition, a similar CCA treating 'sampling year' as a continuous variable was performed in order to test for an overall temporal trend in parasite abundance.

Fish with no intestinal parasite infection were by default omitted from the CA and the CCA analyses. However, this only excluded 13 Arctic charr, spread evenly across sampling years.

Results

Mean intensity and prevalence

Regarding prevalence, *E. salvelini* and *Crepidostomum* spp. were highly prevalent with *E. salvelini* reaching values close to 100 % in most years. *Proteocephalus* sp. and *C. truncatus* were less common (Fig 1).

Overall, *E. salvelini* and *Crepidostomum* spp. showed the highest mean intensity across sampling years (Fig 1). According to GLMs performed on the intensity data, 'sampling year' and 'fish length' were significant explanatory variables for both *E. salvelini* (chi-square test; $p < 0.001$, respectively) and *Crepidostomum* spp. (chi-square test; $p < 0.001$, $p < 0.01$, for both). *Proteocephalus* sp. was found with intermediate mean intensity (Fig 1). Modelling its intensity, using 'sampling year' and 'fish length' as explanatory variables, only 'sampling year' was significant (chi-square test; $p < 0.001$, $p = 0.137$, respectively). *C. truncatus* had the lowest mean intensity across years and only 'sampling year' was used as an explanatory variable but was not significant (chi-square test; $p = 0.999$). Overall, GLM modelling identified significant between year temporal variation in the parasite intensity of *E. salvelini*, *Crepidostomum* spp. and *Proteocephalus* sp., but not for *C. truncatus*, as also indicated by the present or absent overlap of the 95 % confidence intervals in Fig 1.

Between-host variation in infracommunity composition

Co-infection with only *E. salvelini* and *Crepidostomum* spp. was by far the most frequent parasite species composition. Infections with only *E. salvelini* was the second most frequent

infracommunity pattern, whereas infections with all four parasite species were the least frequent and only observed in a single fish (Fig 2, Table 2). Out of a total of 335 Arctic charr, 301 were infected with *E. salvelini*, 253 with *Crepidostomum* spp., 62 with *Proteocephalus* sp. and 27 with *C. truncatus*. Of the Arctic charr infected with *Crepidostomum* spp., 92 % were co-infected with *E. salvelini*. Similarly, of the Arctic charr infected with *Proteocephalus* sp. or *C. truncatus*, 92 % and 100 % were co-infected with *E. salvelini*, respectively. Likewise 79 % infected with *Proteocephalus* sp. and 89 % infected with *C. truncatus* were co-infected with *Crepidostomum* spp., whereas only 4 % (one fish) infected with *C. truncatus* were co-infected with *Proteocephalus* sp. (Fig 2, Table 2).

Inter-specific interaction

The intensity of *E. salvelini*, when being the only cestode taxa present in the infracommunity, was not significantly different from when it co-occurred with either *Proteocephalus* sp. or *C. truncatus* (chi-square test; $p = 0.566$, $p = 0.151$, respectively). Also, the observed frequencies of pairwise parasite co-occurrences were similar to what could be expected by chance, except for *Proteocephalus* sp. co-occurring with *C. truncatus*. In this case, the observation of a single co-occurrence was lower than expected from the randomized frequency (Fig 3).

CA and CCA

Of the total variation in parasite abundance, dimension 1 and 2 of the CA plot explained 44.6 % and 31.8 % respectively (Fig 4). Dimension 3 explained the remaining 23.6 % (not shown). The non-overlap of the 95 % confidence intervals indicated significant difference in average parasite infracommunity composition between certain sampling years. No clear separation

between sampling years was present relative to dimension 1, the dimension mostly associated with separating infracommunities high in *Proteocephalus* sp. abundance from the rest of the dataset. The largest separation of sampling years was observed relative to dimension 2. This dimension separated infracommunities with high abundance of *E. salvelini* from infracommunities with high abundance of *C. truncatus* and *Crepidostomum* spp. According to the CA, the infracommunities in 2005 had on average a higher abundance of *Crepidostomum* spp. and *C. truncatus* compared to 2004, 2010 and 2014, which in turn had a higher abundance of *E. salvelini* (Fig 4). Generally, however, no overall trend or cyclic tendencies were observed in the long-term temporal changes in average parasite infracommunity composition across the 19-year sampling period.

According to the CCA, the environmental variables 'fish length', 'fish age', 'year of sampling' and 'habitat' were together significantly associated with the variation in parasite abundance (permutation test; $p < 0.001$). Together the three dimensions of the CCA accounted for 14 % of the total variation. Dimension 1, mostly correlated with the explanatory variable 'sampling year', accounted for 8 % of the total variation in the parasite abundance data (Fig 5). Similarly to the CA, this dimension also separated 2005 from 2004, 2010 and 2014. Dimension 2, mostly correlated with 'fish length' and 'fish age', accounted for 4 % of the total variation in the parasite abundance data (Fig 5). Dimension 3 accounted for 2 % (not shown). All three dimensions accounted for a significant part of the variation in the dataset (permutation test; $p < 0.001$, $p < 0.001$ and $p < 0.01$, respectively). Of the explanatory variables used in the CCA, 'sampling year', 'fish age' and 'fish length' were significantly associated with variation in the parasite abundance data whereas 'habitat' was not significant (permutation test; $p < 0.001$, $p < 0.05$, $p < 0.05$ and $p = 0.253$, respectively). By running the same analysis but treating 'sampling year' as a continuous variable, the CCA indicated no

overall temporal trends in parasite abundance as 'sampling year' was not significantly associated with variation in the parasite abundance data both when testing it separately or together with the other variables (permutation test; $p = 0.172$, $p = 0.241$, respectively).

Discussion

Contrary to our hypotheses, no overall long-term temporal variation in the intestinal parasite infracommunity composition of Arctic charr in Lake Takvatn was revealed. Instead, the system appeared overall stable with no long-term or cyclic tendencies. Relatively low between-host variation in intestinal parasite infracommunity composition was found as a few specific infracommunity structures dominated. Lastly, no clear signs of inter-specific competition was detected between cestode parasite species co-existing in the same microhabitat.

Overall infection

As *E. salvelini* and *Crepidostomum* spp. were both common and they appeared as typical 'core' species following the terminology of Hanski (1982). The intermediate host of *E. salvelini* (copepods) usually has extremely low frequency of infection (Marcogliese, 1995). The observed infection levels of *E. salvelini* in the present study therefore suggest a high level of foraging on copepods by Arctic charr and possibly selective feeding on infected individuals (Poulin et al., 1992). The high occurrence of this parasite species might however also be reinforced by an accumulation over time as *E. salvelini* are believed survive for several years in the final host (Smith, 1973; Hoffmann et al., 1986). Between the two *Crepidostomum* species that are known from Arctic charr in Lake Takvatn, *C. farionis* is known to infect the lower part of the intestine, whereas *C. metoecus* usually infects the pyloric caeca and the upper part of the intestine (Thomas, 1958; Crompton, 1973). Unpublished intestinal parasite data from Arctic charr in Takvatn sampled in 1995 found more than 80 % of the *Crepidostomum* spp. in the lower part of the intestine (data not shown). This suggests that the

majority of *Crepidostomum* spp. sampled in the present study was likely comprised of *C. farionis* residing in the lower intestine. Thus, *Crepidostomum* spp. and *E. salvelini*, which are commonly attached in the pyloric caeca (Vik, 1963; Smith, 1973), should occur in different microhabitats of the host intestine.

Proteocephalus sp. were in general less common than the two core species, and infected the pyloric caeca niche usually along with *E. salvelini*, making it appear as a 'satellite' species sensu Hanski (1982). *Proteocephalus* sp. and *E. salvelini* both use copepods as their only intermediate host. Arctic charr feeding on copepods should therefore be exposed to both parasite species and a positive correlation would be expected. In contrast, these two parasite taxa appeared negatively correlated according to the second dimension of the CA and the first dimension of the CCA. *Proteocephalus* sp. infection also had a tendency to be positively associated with the profundal habitat and correlated negatively with 'fish length' (i.e. more common in small Arctic charr). In Takvatn, juvenile Arctic charr commonly occur in deep water where they feed on zooplankton (Klemetsen et al., 1992). Several copepod species are potential intermediate hosts for *Proteocephalus* sp. (Scholz, 1999). Knudsen et al. (1997) speculated that *Proteocephalus* sp. might use a deep-water copepod species such as *Acanthocyclops gigas* as an important intermediate host, because profundal Arctic charr, heavily infected with *Proteocephalus* sp., commonly utilized this prey item throughout all seasons in Lake Fjellfrøsvatn (a lake from the same watercourse as Takvatn). Different copepod species as intermediate hosts may explain the observed negative correlations between these two copepod-transmitted parasite species mentioned above. The overall low occurrence of *Proteocephalus* sp. in the present study could be because the majority of the fish were sampled in the littoral zone of the lake.

In addition, possible seasonal variation in parasite infection might also have an effect on the correlation of the parasite taxa found in this study. *Proteocephalus* sp. in brown trout have been found to be low in August - September (Lien & Borgstrøm, 1973). If we assume similar seasonal variation in *Proteocephalus* sp. in Arctic charr in Lake Takvatn, this could certainly contribute to explain the low occurrence of this parasite species as the material included in the current study was collected in August.

The least common of the four intestinal parasites was *C. truncatus*. The only intermediate host for this species is *Gammarus lacustris* (Vik, 1958) that typically resides in the littoral habitat where most of the fish in this study were caught. This benthic crustacean may also transmit *Crepidostomum* spp. (Thomas, 1958; Awachie, 1968; Hoffman, 1999), a possible explanation to why these parasites were positively associated with each other according to the correspondence analyses. Still, *C. truncatus* occurred with low infection levels while C*repidostomum* spp. were common. A possible explanation could be that *Crepidostomum* spp. also utilizes aquatic insect larvae as their potential intermediate host (Hoffman, 1999) and hence might attain higher infection rates. Furthermore, the two parasite taxa have different life expectancies in the final host. *Crepidostomum* spp. might survive about one year, whereas *C. truncatus* is considered to spend only about two months in its final host (Thomas, 1958; Vik, 1958; Awachie, 1968). In addition, Amundsen et al. (2003) found low infections of *C. truncatus* in Arctic charr from the nearby lake Fjellfrøsvatn across all summer months (June to August) which coincided with low predation on *G. lacustris*. Changes in host feeding behavior might in this way create strong seasonality in infection rates, and are likely a part of the explanation to why *C. truncatus* appeared as a satellite species in the present study (see also Amundsen et al. 2003).

Having data available only from August restricts us from drawing general conclusions on the overall parasite infection of the entire year, as sampling during other months would potentially have produced different results. However, our long-term August datasets are highly suitable for addressing long-term temporal variations in the intestinal parasite community, which was the main approach of this study.

Between-host variation in infracommunity composition

Individual hosts supporting infracommunities containing a single parasite species did occur in the dataset indicating possible dietary specialization in Arctic charr from Lake Takvatn, which is in accordance with our hypothesis and the findings of previous studies (e.g. Amundsen, 1995; Klemetsen et al., 2003). However, the far most frequent intestinal parasite infracommunity composition was *E. salvelini* co-occurring with only *Crepidostomum* spp., indicating favorable transmission of these parasite taxa in Lake Takvatn. Even though we observed some signs of dietary specialization by using parasites as ecological markers of host behavior, low between-host variation in intestinal parasite infracommunity composition of Arctic charr is a contrast to the documented dietary specialization in this fish species (Knudsen et al., 2011). Most likely, this arises from the fact that these parasite species utilize the two main prey groups of Arctic charr, zooplankton (copepods) and benthic prey (*G. lacustris*, and insect larvae), as intermediate hosts (Knudsen, 1995; Klemetsen et al., 2003). In addition, the low occurrences of *C. truncatus* and the expected profundal utilization by *Proteocephalus* sp. certainly adds to the stability of the dataset, but our results still indicate that Arctic charr in the littoral, with assumedly strong feeding specialization, utilize the main prey groups in Lake Takvatn. Other studies performed in the same region also found *E. salvelini* and *Crepidostomum* spp. to be the most common intestinal parasite species infecting

Arctic charr (Skarstein et al., 2005; Knudsen et al., 2008; Knudsen et al., 2011). Their presence therefore seems to be relatively unaffected by potential specialized host feeding behavior.

No co-infections of *Proteocephalus* sp. and *C. truncatus* were observed except in a single fish. According to our randomization analysis, this observed frequency was clearly lower than expected by chance. As both taxa have a preference for the anterior parts of the intestine (Vik, 1958; Scholz, 1999; and references therein), the low co-infection could potentially be due to competitive interactions between these two parasite species. However, this pattern could also be explained by inter-individual diet and habitat specialization by the final host, as *Proteocephalus* sp. is acquired through a zooplanktivorous diet, possibly in the profundal zone (Knudsen et al., 1997), while *C. truncatus* is solely transmitted via a benthivorous feeding strategy in the littoral zone. Thus, the most likely explanation for the low co-occurrence of the two parasite taxa is a partly differential habitat and diet segregation within the charr population.

Inter-specific interactions

Shortage of space is considered an important limiting factor regarding competition between intestinal parasites (Crompton, 1973). As *E. salvelini* infects primarily the pyloric caeca region (Vik, 1963; Smith, 1973), this parasite species is separated from *Crepidostomum* spp. in their respective microhabitats, making inter-specific competition between the two species unlikely. Still, *E. salvelini* and *Crepidostomum* spp. were negatively correlated in the CA and the CCA, indicating a tendency for infracommunities with high abundance of *E. salvelini* on average to have a low abundance of *Crepidostomum* spp. This was less likely a result of

segregation in foraging habitat between the hosts, as most fish (74%) were caught in the littoral, but more likely caused by individual host feeding preferences within the littoral zone (Knudsen et al., 2011).

As no significant differences in the intensity of *E. salvelini* was observed when *Proteocephalus* sp. was present or not in the infracommunities, low inter-specific interactions or crowding effects are suggested. This indicates that *Proteocephalus* sp. is not outcompeted when the intensity of *E. salvelini* increases. Neither was the mean intensity of *E. salvelini* significantly lower when *C. truncatus* was present in the infracommunity as compared to infracommunities where *E. salvelini* was the only cestode species occurring. This also indicates that also *C. truncatus* has low interaction with *E. salvelini*, which is in accordance with other parasitological studies done on salmonids (Halvorsen & MacDonald, 1972; Knudsen et al., 2008). In general, the intestinal helminth community of freshwater fish are considered isolationist in nature as interactions between parasites are rarely seen, indicating that pre-transmission factors, such as density of potential intermediate hosts and host feeding behavior, are likely the most important for the structuring of the intestinal parasite community of freshwater fish (Kennedy, 1990).

Temporal variation

According to the CA, the temporal separation in average intestinal parasite infracommunity composition was associated with a differentiation between infracommunities high in *E. salvelini* from infracommunitites high in *Crepidostomum* spp. and *C. truncatus*. This may indicate that the observed temporal variation was caused by a switch from a copepod dominated diet to a more benthic invertebrate based diet. Temporal variation in the

zooplankton community of Lake Takvatn has been observed (Dahl-Hansen, 1995). Changes such as these could potentially explain the observed temporal variation in intestinal parasite composition, as Arctic charr may change their rate of copepod feeding accordingly.

Also, changes in fish densities could be a potential explanation. Heavy reduction in fish density has been reported to bring along a dietary shift from a more copepod dominated diet to a more benthic prey dominated diet as fish move to the littoral to feed (Amundsen & Kristoffersen, 1990; Klemetsen & Dahl-Hansen, 1995). Furthermore, changes in brown trout density in Lake Takvatn (Persson et al., 2007) could also affect the degree of littoral utilization by Arctic charr, and thus the proportion of benthic prey in their diet potentially explaining the temporal variation we observed in the average infracommunity composition.

Overall, mean intensity, prevalence and average intestinal parasites infracommunity composition was relatively temporally stable as just minor sporadic changes and no cyclic tendencies were revealed. This indicates that if we assume that the environmental and biological changes, following the removal of Arctic charr in the 1980s, had an effect on the infections of trophically transmitted parasites in Arctic charr, the system now appears in steady state or at least without significant disturbances. Other similar studies investigating long-term variation in parasite communities of freshwater fish neither observed any long-term variation or cyclical oscillations (Kennedy & Rumpus, 1977; Knudsen et al., 1999; Knudsen et al., 2002). In particular, Kennedy & Rumpus (1977) designated the recorded long-term consistency in the abundance of parasites solely to a sustained stability in the ecosystem since density dependent controls were apparently lacking.

In conclusion, this study identified relatively low between-host variation in the intestinal infracommunities of Arctic charr sampled in August over a two-decade period in Lake

Takvatn, as a few specific infracommunity structures dominated. This may indicate favorable transmission of certain taxa, regardless of the tendency for feeding specialization seen commonly to occur in Arctic charr. Interspecific interactions between the intestinal parasites seemed to be low, and the observed between-host variation was most likely due to habitat and dietary choice of individual Arctic charr. The sporadic temporal variation observed among some years, was assumed to be caused by temporal changes in the copepod intermediate host community. Overall, long-term changes or cyclic tendencies in the parasite community structure were however lacking, indicating that the composition of the intestinal parasite infracommunities of Arctic charr in Lake Takvatn is in a relative steady state following the extensive Arctic charr removal in the 1980s.

Acknowledgements

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Tables

Table 1 Number of Arctic charr caught and analyzed during each sampling year in Takvatn, including mean length and age of fish. CI indicates 95 % confidence intervals

Year	nr fish	Mean length (mm)±CI	Mean age±CI
1995	40	230±6	4.7±0.3
2001	28	194±15	5.0±0.3
2002	17	189±14	4.8±0.4
2003	31	201±12	4.7±0.2
2004	30	208±12	4.8±0.3
2005	30	226±18	5.0±0.3
2006	31	200±14	5.0±0.3
2007	27	194±14	5.0±0.3
2010	72	221±8	4.5±0.2
2014	29	269±17	5.2±0.2

Table 2 Frequency of different parasite species compositions in the infracommunities and total number of fish infected with a specific parasite species observed from 335 Artic charr sampled across a 19 year period in Lake Takvatn

	Parasite species compositions											No. of infected fish
Eubothrium salvelini	1	1	1	1	0	0	1	0	1	0	1	301
Crepidostomum spp.	1	0	1	1	1	0	0	1	0	0	1	253
Proteocephalus sp.	0	0	1	0	0	0	1	1	0	1	1	62
Cyathocephalus truncatus	0	0	0	1	0	0	0	0	1	0	1	27
Frequency	165	53	45	23	16	13	11	3	3	2	1	

Figure captions

Fig. 1 Mean intensity and prevalence of the intestinal parasite species identified from Arctic

charr in Takvatn sampled in August across a time period of 20 years. Error bars indicate 95 %

confidence intervals of the mean.

Fig. 2 Venn diagram illustrating the frequency of the different parasite infracommunity

compositions. As uninfected fish (13 charr) are not incorporated in this illustration, the total

dataset is 322 Arctic charr. Eu *Eubothrium salvilini*, Pr *Proteocephalus* sp., Cr

Crepidostomum spp., Cy *Cyathocephalus truncatus*.

Fig. 3 Frequency distribution of pairwise parasite co-occurrence based on a 1000-cycled

randomization using the data from table 2. Eu *Eubothrium salvilini*, Pr *Proteocephalus* sp., Cr

Crepidostomum spp., Cy *Cyathocephalus truncatus*. Observed frequencies are illustrated with

grey crosses. Randomized frequency distributions are illustrated with boxplots including

median (bold lines), upper and lower quartiles (top and bottom borders of the boxes),

minimum and maximum (top and bottom endings of the vertical lines) as well as outliers

(small circles).

Fig. 4 Correspondence analysis (CA) based on chi-square distances calculated on square root

transformed intestinal parasite abundances. Eu *Eubothrium salvilini*, Pr *Proteocephalus* sp.,

Cr *Crepidostomum* spp., Cy *Cyathocephalus truncatus*. (upper left) CA plot zoomed in to

show variation in average infracommunity structure among years. The location of a specific

year indicates the centroid of all infracommunitites in that year, including 95 % confidence

intervals. Points aggregated in the straight line are parasite infracommunities with only *E.*

salvelini and *Crepidostomum* spp. (upper right) Same CA plot but with isolines for *Eubothrium salvelini* (dark grey) and *Cyathocephalus truncatus* (light grey). (lower left) Same CA plot but with isolines for *Crepidostomum* spp. (dark grey) and *Proteocephalus* sp. (light grey). (lower right) Same CA plot but zoomed out to show all 322 included infracommunities (13 uninfected fish were excluded). Noise was added to the data points so that identical infracommunities are more visible.

Fig. 5 Canonical correspondence analysis (CCA) performed on square root transformed parasite abundances as a function of the explanatory variables 'sampling year', 'fish length', 'fish age' and 'habitat'. Eu *Eubothrium salvilini*, Pr *Proteocephalus* sp., Cr *Crepidostomum* spp., Cy *Cyathocephalus truncatus*. A age, H habitat, Y year.
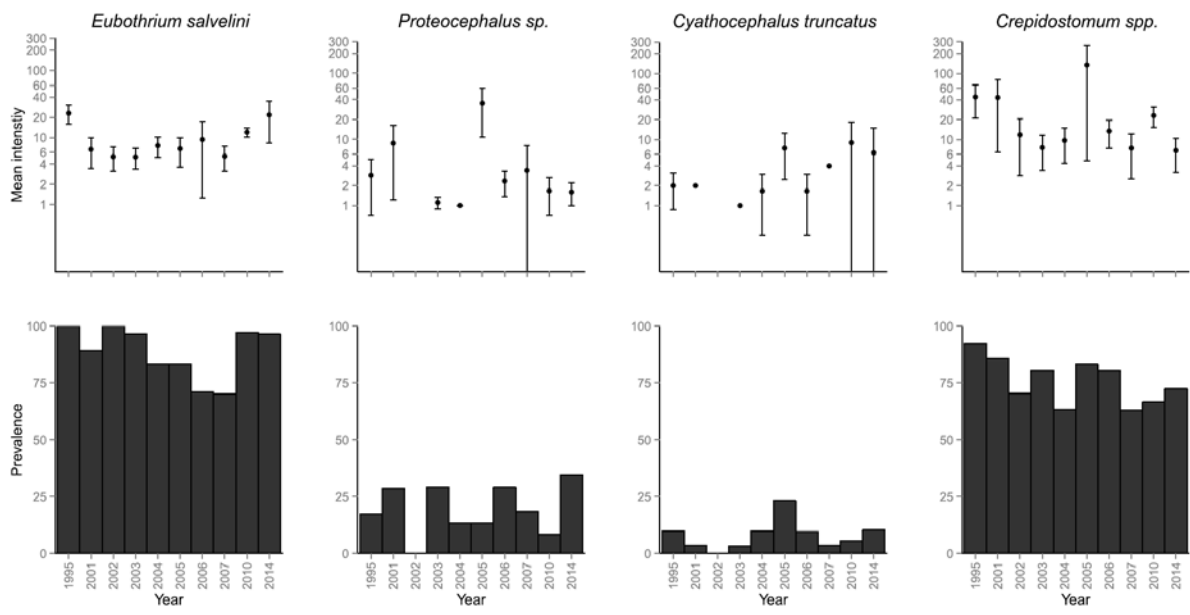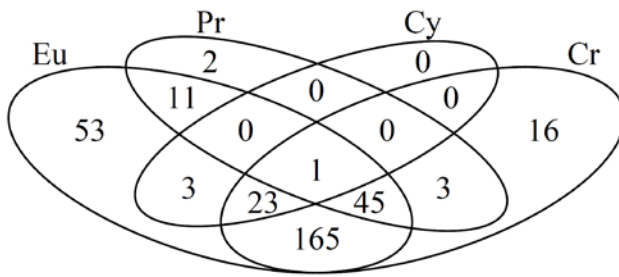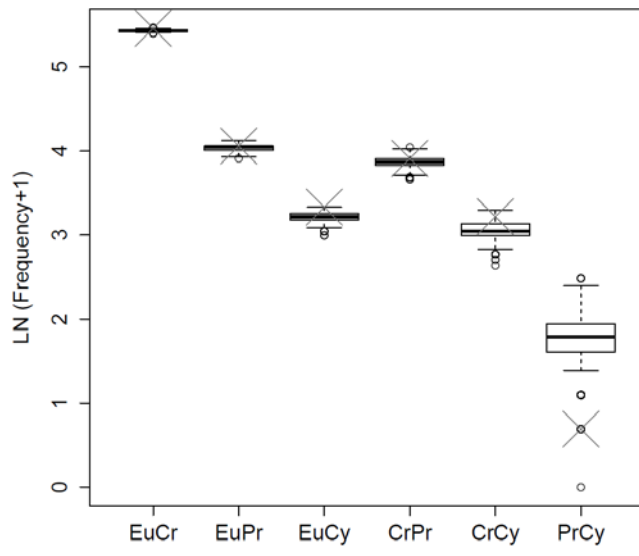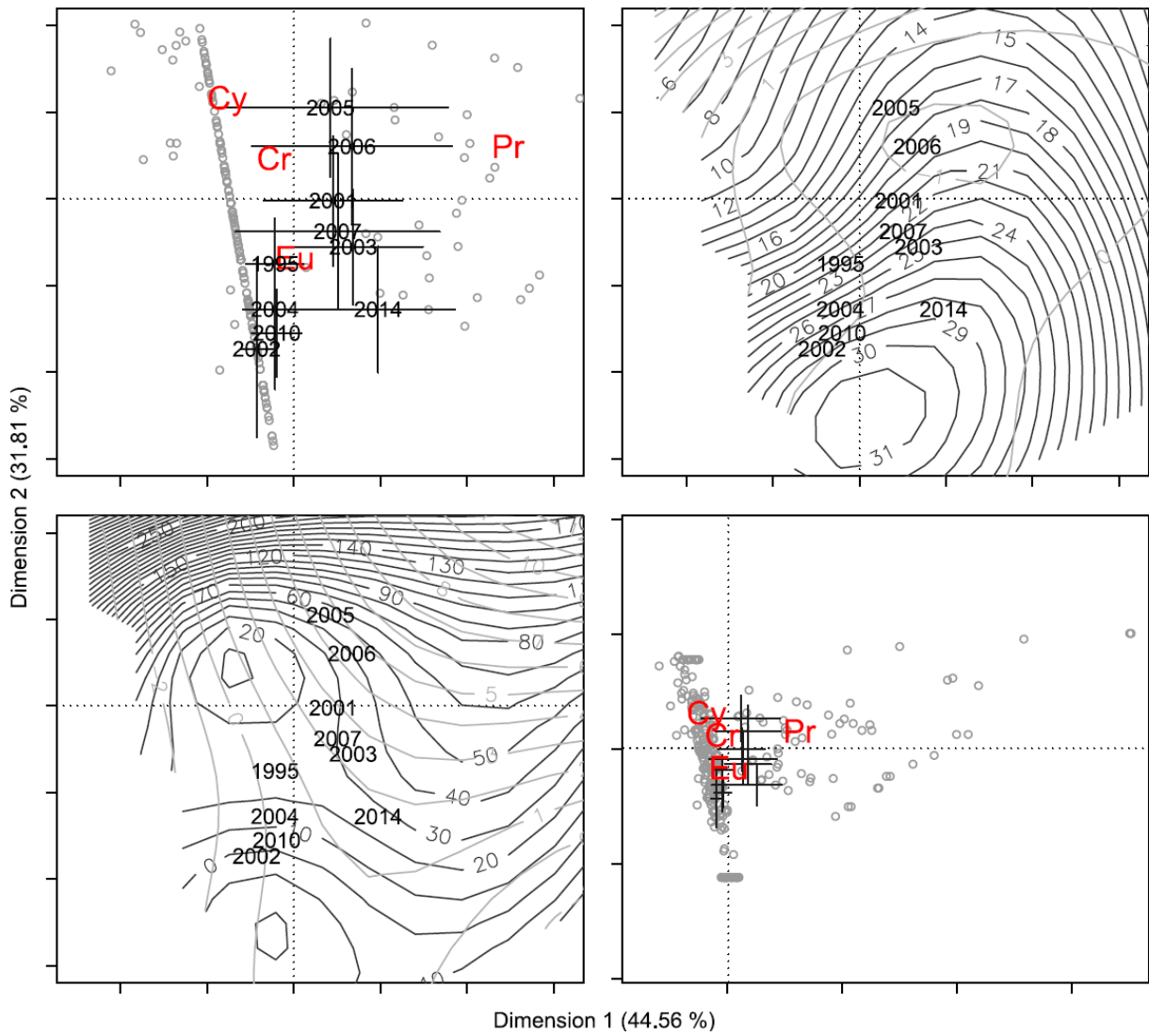
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5