Parasite communities of two three-spined stickleback populations in subarctic Norway - effects of a small spatial-scale host introduction

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Abstract

We compared metazoan parasite communities of an introduced three-spined stickleback (*Gasterosteus aculeatus*) population with the nearby source population in northern Norway to study differences and clarify if factors controlling parasite dispersal act on a small spatial-scale. The two component communities were highly similar. All parasite taxa found in the source population also occurred in the introduced population illustrating high probability of successful parasite introduction on a small spatial scale. Among the parasites were the three-spined stickleback specialist *Schistocephalus solidus* and a massive occurrence in the eyes of non-lens-infecting trematodes found through genetic results to include *Diplostomum gasterostei*, *D. baeri* 2, and a non-encysted Strigeidae gen. sp.

On the infracommunity level, mean abundance differed significantly between lakes with regards to *Apatemon* sp. and the two autogenic three-spined stickleback specialists *Gyrodactylus arcuatus* and *Proteocephalus* sp. (assumedly *P. filicollis*). Mean dissimilarity among infracommunities within lakes was also significantly lower than mean dissimilarity among infracommunities between lakes, which was primarily accounted for by the allogenic cestode *Diphyllobothrium ditremum*, *G. arcuatus* and *Proteocephalus* sp.. We expect that the differences found between the two lakes were caused by dissimilar water temperatures, and stickleback and copepod intermediate host densities. Some inter-lake differences in abiotic and biotic factors were thus present, but caused only quantitative differences between the two parasite communities. Mechanisms contributing to qualitative differences were on the other hand absent or had low importance believed to be caused by similar ecosystems, exposure to the same parasite species pool and geographical proximity of the two lakes. We suggest that mechanisms influencing parasite dispersal are less important on a small spatial-scale causing high similarity between local parasite communities.

Introduction

Introduction of a new host species into an ecosystem can have important consequences for the native communities of organisms and may also change the structure of the parasite community of the involved host species (Williamson 1996). Host introduction is usually a deliberate or accidental human caused activity that occurs on both global and local scales (Kolar and Lodge 2002, Garcia-Berthou et al. 2005). Potentially, parasites can be brought along in this process, as introduced hosts can be infected with various parasites (Daszak 2000, Cleaveland et al. 2002). Many studies have focused on exotic species and host introduction on a large geographical scale (Poulin and Mouillot 2003, Kelly et al. 2009, Lymbery et al. 2010, Roche et al. 2010, Gendron et al. 2012), but few have addressed the effects of a small spatial-scale host introduction. In this paper we explore the mechanisms influencing parasite dispersal and community structure in relation to host introduction on a small spatial-scale by comparing parasite communities of an introduced three-spined stickleback (*Gasterosteus aculeatus*) population in subarctic Norway with that of its nearby source population.

Introduced fish species often have depauperate parasite communities as some parasites are either not brought along with the introduction, termed "missing the boat", or fail to establish in the new habitat, termed "drowning on arrival", sensu MacLeod et al. (2010). The introduction of a parasite species into a new ecosystem partly depend on its lifecycle strategy and dispersal capability. Parasites of freshwater fish are termed *autogenic* if the lifecycle is completed within the aquatic ecosystem using fish as final host (Esch et al. 1988). In contrast, a parasite is termed *allogenic* if it exploits a terrestrial mammalian or avian final host causing part of its lifecycle to occur outside its original aquatic ecosystem. As the final host of an allogenic parasite can disperse parasite eggs between lakes and across large distances, this lifecycle strategy has better dispersal capabilities compared to autogenic parasites where the final host is restricted to one water body (Esch et al. 1988). Also important for successful introduction of a parasite species is its host specificity. Host specificity is usually associated to a specific stage in the parasite lifecycle, but overall

parasites can be classified as being either *generalists* or *specialists*. Specialist parasites infect only one or a few host species, whereas generalist parasites are capable of infecting a much larger range (Bush and Kennedy 1994, Poulin and Morand 2004). The probability of encountering a suitable host in an introduced habitat is thus higher for generalist parasites, increasing their chance of successful introduction. In addition, successful introduction also depends on the lifecycle complexity of the parasite (Torchin et al. 2002). In an introduced ecosystem, a parasite with a highly complex lifecycle would thus be less likely to encounter all hosts necessary for it to complete its lifecycle as compared to parasites with lifecycles of lower complexity.

An introduced parasite species may depend on the presence of suitable abiotic and biotic factors to successfully colonize a new habitat. In this sense, abiotic factors such as salinity and pH have been shown to have important effects on parasite community similarity and component community diversity (Marcogliese and Cone 1996, Poulin et al. 2011). Abiotic factors would have a direct effect on ectoparasites and free living parasite developmental stages. They could however also have indirect effects by controlling biotic factors such as the presence of required intermediate hosts needed by some parasite species to complete their lifecycle. One important biotic factor in the new habitat might be the foraging behavior of the fish host population. As foraging behavior is directly linked to the host's acquisition of trophically transmitted parasites (Knudsen et al. 2004, Valtonen et al. 2010, Locke et al. 2014), this could be a potential factor controlling the abundance of trophically transmitted parasites. Furthermore, host population density could also be of importance since it can correlate positively with parasite abundance and richness through an increase in transmission rates (Dobson 1990, Takemoto et al. 2005). Overall, both abiotic and biotic factors are therefore important in facilitating successful parasite colonization and for the structuring of parasite communities (Poulin 2007, Kennedy 2009, Karvonen et al. 2013). Most likely related, numerous studies have found that parasite community similarity decreases with increasing geographical distance (Poulin and Morand 1999, Poulin 2003, Perez-del-Olmo et al. 2009, Thieltges et al. 2009). Accordingly, we expect that sites separated by small geographical distances would have similar biotic and abiotic factors of importance for the successful colonization of parasites thus leading to high similarity between parasite communities on a small spatial-scale.

Three-spined stickleback, here after referred to as *stickleback*, have been the subject of numerous parasitological studies (Barber 2013). In Norway, 19 parasite species have been reported from freshwater stickleback populations (Levsen 1992, Sterud 1999). In 1950, the decision was made to deliberately introduce sticklebacks to the subarctic lake Takvatn from the nearby lake Sagelvvatn (Jørgensen and Klemetsen 1995). We now know that the introduction altered the food web topology in Takvatn and introduced new parasite species (Amundsen et al. 2009, Amundsen et al. 2013). However, it remains unclear in what ways the parasite community of the introduced sticklebacks in Takvatn resembles the sticklebacks in the source lake. In the present study we compare the metazoan parasite communities of the two stickleback populations to address what effects this host introduction has had on the structure of the stickleback parasite communities. We hypothesized that on a component community level, the two parasite communities would in general be similar, caused by the small spatial-scale of the host introduction and the similar biotic and abiotic conditions in the two lakes. Secondly, we hypothesized that the same allogenic parasite species would be present in both lakes due to their superior dispersal capabilities, while potential inter-lake differences in species composition would be explained by autogenic parasite species. Thirdly, we hypothesized that the parasite community in the introduced population would be less species rich than in the source population, due to parasites potentially "missing the boat" or "drowning on arrival" (MacLeod et al. 2010). Lastly we hypothesized that the highest total number of parasites would be found in the lake with highest stickleback density.

Methods

The study lakes

Sagelvvatn (69°11'N, 19°05'E) and Takvatn (69°07'N, 19°05'E) are both oligotrophic lakes found in the northern county Troms of subarctic Norway (Figure 1). Takvatn is situated 214 m above sea level, has a surface area of 14.2 km^2 and a maximum depth of about 80 m. Sagelvvatn is situated 91 m above sea level, with a surface area of 5.1 km^2 and a maximum depth of ca. 70 m. The lakes are separated by a distance of 5 km and are both located uppermost in two separate watercourses. Both catchment areas contain minor agricultural activity but are otherwise dominated by birch forest (*Betula pubescens*). The degree of agricultural activity surrounding Sagelvvatn is however slightly more extensive causing this lake to have a larger nutrient input and higher productivity which is evident from an inter-lake difference in Secchi depth (Sagelvvatn = 8 m, Takvatn = 12-14 m). Both lakes now support fish communities comprised of Arctic charr (*Salvelinus alpinus*), brown trout (*Salmo trutta*) and stickleback. Expectedly, all three fish species had a postglacial invasion to Sagelvvatn whereas only brown trout inhabited Takvatn. In the early 1900's the brown trout was overexploited in Takvatn causing low fish catches which lead to the introduction of Arctic charr in the 1930's from the nearby Lake Fjellfroskvatn (Amundsen et al. 2007). By the 1940's, a stunted Arctic charr population had evolved to the point where it dominated the fish population in Takvatn. This drove the deliberate introduction of stickleback from the nearby Sagelvvatn in an attempt to provide a fish prey that could boost growth rates within the salmonid fish community (Jørgensen and Klemetsen 1995). Unfortunately the number of stickleback individuals that was introduced is unknown. In the 1980's, an intensive fishing program targeting the stunted Arctic charr population was carried out (Amundsen et al. 1993). The efforts not only decreased the density of old, small-sized Arctic charr, but also increased the abundance of brown trout and improved general fish growth (Amundsen et al. 2007, Persson et al. 2007).

Fish sampling and parasite screening

A total of 120 sticklebacks were collected from each lake during summer and fall of 2010. Fish were caught with bottom gillnets (mesh sizes 6 – 10 mm) in the littoral zone at depths

of 1 – 8 m. In the field-laboratory, fish were measured (fork-length to nearest mm) and freshly screened for parasites using a stereomicroscope. Firstly the skin and fins of each fish were examined for ectoparasites. The different organs and tissues, including intestine, stomach, eyes, body cavity, liver and swim bladder were then dissected and examined separately for endoparasites. All metazoan parasites were counted and assigned taxa in the field-laboratory, based on observable morphological features. As species differentiation was not possible in the field for some of the parasites, they were grouped as follows. Nematodes found in the swim bladder were grouped in Nematoda spp. Small diphyllobothrid plerocercoid larvae (length: 1-5 mm) were grouped into *Diphyllobothrium* spp. Free diplostomid metacercariae in the eye, all of which occurred outside the lens, were grouped in *Diplostomum* spp., whereas encysted metacercariae found in the eye and body cavity were grouped in *Apatemon* sp. In a subset of these metacercariae, identifications and species boundaries were analyzed using sequences from the barcode region of cytochrome c oxidase 1 (CO1). The extraction, amplification and sequencing of CO1 barcodes from this material was conducted as described by Moszczynska et al. (2009) and sequences were deposited on Genbank (accessions XXXX-XXXX) and BOLD (www.boldsystems.org). For CO1, specimens of *Diplostomum* were amplified and sequenced using the diplostomid-specific or degenerate primers, and degenerate primers were also used for the encysted metacercariae grouped in *Apatemon* sp. (Moszczynska et al. 2009). Sequences were compared to public data for *Diplostomum* and Strigeidae using the clustering algorithm of Ratnasingham and Hebert (2013) and in a separate alignment constructed with MEGA (Tamura et al. 2011).

Terms used and statistical analyses

Terms used to describe parasite communities and populations were applied as defined by Bush et al. (1997). Mean abundance is the number of individuals of a parasite taxon in a sample divided by the total number of hosts (including non-infected hosts). Mean intensity is the number of individuals of a parasite taxon found in a sample, divided by the number of hosts infected with this taxon. Prevalence is the percentage of hosts infected with a particular parasite taxon in a sample.

To identify the level of study in question we use four different terms. *Infrapopulation* includes all individuals of a parasite species found in a single stickleback host. *Infracommunity* is used for all individuals of all parasite species found in a single stickleback

host. *Component population* refers to all individuals of one parasite species found in all sticklebacks sampled in one lake. *Component community* denotes all individuals of all parasite species found in all sticklebacks sampled in one lake.

To summarily describe the two component communities further, we used *total number of parasites*, and *total number of taxa* as well as *Simpson's Index of Diversity* (1-D), *Simpson's Measure of Evenness* ($E_{1/D}$) and Percentage Similarity (Krebs 1999). To eliminate the possibility that any differences between the two parasite communities were caused by differences in fish length, we used a Welch two sample t-test to test for significant difference in mean fish length between lakes (Ruxton 2006).

Nonmetric multidimentional scaling (NMDS) was used to illustrate tendencies and similarity between infracommunities in the two lakes. A stress-value illustrated how much of the dissimilarity in the data that was explained by higher dimensions than the two used in the plot (van der Gucht et al. 2005). To supplement the NMDS we used an analysis of similarity (ANOSIM) to test if mean dissimilarity among infracommunities within lakes was equal to mean dissimilarity among infracommunities between lakes (van der Gucht et al. 2005). Also to determine which parasite taxa contributed most to the dissimilarity between infracommunities in the two lakes we computed a Similarity Percentage Analysis (SIMPER) (Clarke 1993). All of these statistical analyses (NMDS, ANOSIM and SIMPER) were based on Bray-Curtis dissimilarity (Krebs 1999) calculated from log+1 transformed parasite abundances.

At the infrapopulation level, we tested if mean abundance for selected parasite taxa were significantly different between lakes. This was done by fitting generalized linear models to the abundance data of a taxa using the full data set (n=240) while at the same time using lake, length and sex as explanatory variables. Due to overdispersed distributions of all parasite taxa, we fitted the models with negative binomial errors. Model selections were based on the Akaike's Information Criterion (AIC), and simple models were chosen if $_\Delta$AIC < 2. Selected models were then analyzed with an analysis of deviance with chi-square tests, to test for significant effects. For statistical simplification, *Diphyllobothrium* spp. was grouped together with *D. ditremum* and *D. dendriticum*. All statistical analyses were done in R version 3.0.0 (R Core Team 2013)**Error! Hyperlink reference not valid.**.

Results

The relative density of sticklebacks in terms of catch per unit effort (CPUE; number of fish caught per gillnet placed for 24 hours) varied between lakes, being highest in Sagelvvatn with a CPUE of 8.0 as opposed to 2.7 in Takvatn (Table 1). No significant difference was found in mean length between the two stickleback samples (Welch two sample t-test, t(232.4) = 1.12, *p* = 0.27).

Out of a total of 12 parasite taxa recorded, nine were found in both lakes whereas three (*Eubothrium* spp., *Crepidostomum* spp. and Nematoda spp.) only occurred in the sticklebacks from Takvatn (Figure 2). The parasite taxon *Eubothrium* spp. was expected to be the species *E. crassum* or *E. salvelini*. As both are autogenic specialists, we treated the *Eubothrium* spp. taxon as such. The taxon *Crepidostomum* spp. was assumed to be either *Crepidostomum farionis* or *C. metoecus*. These are autogenic generalists, so we considered the taxon *Crepidostomum* spp. to be the same. Nematoda spp. was after sampling found to include two different species probably *Cystidicola farionis* and *Philonema oncorhynchi*. As both are autogenic specialists, we treated the taxon Nematoda spp. as such. The above assumptions are justified as *Crepidostomum* (unpublished) and the other four taxa occur in salmonids of Takvatn (Knudsen et al. 2002, Amundsen et al. 2013). In addition, the taxon *Proteocephalus* sp. was suspected to be the stickleback specialist *Proteocephalus filicollis*, which commonly infects stickleback (Scholz et al. 2007). While further sampling is necessary to confirm and refine the above identifications, they are sufficient for the purposes of testing our hypotheses. As for the metacercaria found in the eye and body cavity, the CO1 sequences obtained from 40 specimens formed four distinct clusters using the algorithm of Ratnasingham and Hebert (2013) for identifying operation taxonomic units, which takes into account all sequences on BOLD over 500 bp (n>3 million). The mean uncorrected variation in CO1 within the four putative species was less than 1%, and maximum intraspecific distances were 1 - 2 % (Table 2). Also, the sequences of CO1 in the four different species were at least 5 – 10 % different from those of any other species. Sequences obtained from four *Diplostomum* specimens from stickleback in Takvatn matched those of 16 isolates of *Diplostomum baeri* 2 sensu Georgieva et al. (2013) collected from the vitreous humour of

Perca fluviatilis in Germany by Behrmann-Godel (2013). In addition, a cluster from specimens found mainly in the retina of stickleback from both Takvatn and Sagelvvatn, was tentatively identified as *D. gasterostei*. The sequences of CO1 from the strigeid species found unencysted in the eye of sticklebacks in Takvatn were not clearly allied with any known genus (Figure 3). As it was not possible to retroactively split the *Diplostomum* spp. group into these three taxa and since they include both specialists and generalists, we have disregarded the host specificity of the *Diplostomum* spp. taxon. The CO1 sequences from encysted tetracotyles in the vitreous humour and body cavity of sticklebacks from Takvatn were similar to, but not conspecific with *Apatemon* species from North America. The four clusters (Figure 3), which we consider species, also formed reciprocally monophyletic clades in ML analysis, which had similar topology (not shown).

At the component community level, the total number of parasites counted from the 120 sampled fish, was nearly twice as high in the source lake Sagelvvatn (11218) as oppose to lake Takvatn (6807). Despite this, the recipient lake had a higher total number of taxa (Table 1). Parasite taxa found in this study included seven cestode taxa (*Eubothrium* spp., *Cyathocephalus truncatus*, *Schistocephalus solidus*, *Diphyllobothrium* spp., *Diphyllobothrium dendriticum*, *Diphyllobothrium ditremum*, *Proteocephalus* sp.), one nematode taxon (Nematoda spp.), one monogenean (*Gyrodactylus arcuatus*) and three digenean taxa (*Crepidostomum* spp., *Apatemon* sp., *Diplostomum* spp.). The number of autogenic versus allogenic and specialist versus generalist taxa were relatively equal (Figure 2). Three of the parasite taxa (*G. arcuatus*, *S. solidus* and *Proteocephalus* sp. (assumedly *P. filicollis*)) were stickleback specialists. Included also was the digenean *D. gasterostei.* This species was however included in the *Diplostomum* spp. taxon making it impossible to treat it separately. Three other specialists were also found, *Cystidicola farionis, P. oncorhynchi* (both expected to make up the Nematoda spp. taxon) and *Eubothrium* spp. These parasites are however specialists on salmonids.

At the component population level, parasite taxa that occurred with low mean intensity were in general also found with low prevalence (Figure 4). Autogenic taxa had a tendency to be located closer to the unity line, indicating more similar mean intensity and prevalence in the two lakes as compared to allogenic parasite taxa (Figure 4). Of the different parasite

taxa, *Diplostomum* spp. stood out as it had 100 % prevalence and high mean intensity in both lakes. The two stickleback specialists, *G. arcuatus* and *Proteocephalus* sp., were also noteworthy as they were found with markedly higher mean intensity and prevalence in the source lake (Figure 4). The inter-lake difference in the occurrence of stickleback specialists was likely also part of the reason for Sagelvvatn scoring higher on the Simpson's Index of Diversity and Simpson's Measure of Evenness (Table 1). However, as indicated by the Percentage Similarity, the proportional abundances of taxa in the two component communities occurred with relatively high similarity (Table 1).

Most of the parasite taxa found in this study transmit trophically to their fish host typically by using copepods as intermediate host. Only *G. arcuatus*, *Apatemon* sp. and *Diplostomum* spp. transmit to their fish host directly. *Diplostomum* spp. were by far the most abundant in both populations with 6 508 individuals in the sticklebacks sampled from Sagelvvatn and 4 723 from the sticklebacks sampled in Takvatn. The remaining parasite taxa varied from one to 1 516, many being rare (Figure 2). The largest inter-lake differences observed were accounted for by the two autogenic stickleback specialists *G. arcuatus* (22 fold) and *Proteocephalus filicollis* (180 fold) (Figure 2). Both species had significantly higher mean abundance in Sagelvvatn ($p < 0.001$ for both). With regards to the diphyllobothrid cestodes (*Diphyllobothrium* spp., *D. ditremum* and *D. dendriticum*), inter-lake differences were not as pronounced (Figure 2), and pooled together, the diphyllobothrids showed no significant inter-lake difference in mean abundance ($p = 0.202$). The group *Diphyllobothrium* spp. was most likely comprised of *D. ditremum* as this species was much more abundant than *D. dendriticum*. In contrast to being more abundant in the source lake, as found for most of the parasite taxa, *Apatemon* sp. had a significantly higher mean abundance in Takvatn ($p < 0.001$). Overall, large variations in parasite intensities occurred (Figure 2) causing the variance to mean ratios for all parasite taxa to be higher than unity indicating overdispersed distributions. The parasite taxa that only occurred in Takvatn (*Eubothrium* spp., *Crepidostomum* spp. and Nematoda spp.) were all found with a low number of parasites, mean intensity and prevalence (Figure 2, Figure 4).

Sticklebacks sampled in the two lakes were in general highly infected also at the infracommunity level. Mean abundance was 93 in Sagelvvatn as compared to 56 in Takvatn

(Table 1). Even though parasite abundance showed a considerable range in the two lakes (Table 1), mean abundance was still significantly higher in Sagelvvatn (Wilcoxon rank sum test, W = 9586.5, p < 0.001). Despite this, there was no significant inter-lake difference in mean number of taxa (Wilcoxon rank sum test, W = 7787.5, p = 0.08) as the parasite taxa exclusive to Takvatn were found with low prevalence (Table 1, Figure 4).

In the NMDS plot, the ellipses covering 95 % of the data from each population had a substantial degree of overlap (Figure 5). Mean dissimilarity among infracommunities within lakes was however significantly different from mean dissimilarity between lakes (ANOSIM, R = 0.27, *p* = 0.001) (Figure 5). Contributing on average to more than 10 % of the dissimilarity was the parasite taxa *D. ditremum* (17.6 %), *Diphyllobothrium* spp. (16.9 %), *G. arcuatus* (16.5 %), *Apatemon* sp. (14.1 %), *Proteocephalus* sp. (13.7 %) and *Diplostomum* spp. (10.1 %) (Figure 5). The non-overlapping notches of the boxplot also indicated that mean dissimilarity within Takvatn was significantly lower than mean dissimilarity within Sagelvvatn (Figure 5). There was however a substantial variation in dissimilarity between infracommunities in both lakes causing the ANOSIM to have low R-value (Figure 5). As *Eubothrium* spp., *Crepidostomum* spp. and Nematoda spp. were only found in Takvatn and *Proteocephalus* sp. and *G. arcuatus* both had a markedly higher prevalence and mean intensity in Sagelvvatn, these taxa were found in the perimeter of the NMDS plot. The eye fluke group *Diplostomum* spp. was on the other hand located close to the center reflecting a similar occurrence in the two lakes (Figure 5).

Discussion

As expected, the parasite communities from sticklebacks in Sagelvvatn and Takvatn were highly similar at the component community level. Most parasite species occurred in both lakes with similar number of parasites, as illustrated by the extensive overlap of the 95 % ellipses in the NMDS plot and high Percentage Similarity. While supporting our first hypothesis, this also suggests that the two lakes are susceptible to colonization by the same regional pool of parasite species, likely caused by the close geographical distance between the two lakes. Some differences between the two component communities were however evident as the Simpson's Index of Diversity and Simpson´s Measure of Evenness scored higher for the source lake Sagelvvatn. This was likely caused by a higher number of stickleback specialists and fewer rare parasite taxa in this lake.

As more than 30 % of the inter-lake dissimilarity between infracommunities in the two lakes was explained by *Diphyllobothrium*, this genus was important in explaining the small but significant inter-lake difference in parasite communities at the infracommunity level. *Diphyllbothrium* is transmitted to its fish host via copepods (Hoffman 1999) and since planktonic surveys performed in the summer 2010 (Skoglund et al. 2013) found higher density in Sagelvvatn as compared to Takvatn (unpublished data), inter-lake differences in intermediate copepod host density might explain the importance of *Diphyllbothrium*. It is thus plausible that inter-lake differences between infracommunities are partly related to biotic dissimilarities of the two lakes.

Following our second hypothesis, all allogenic parasite taxa recorded were present in both component communities. Most of them occurred with relatively similar number of parasites, mean intensity and prevalence in the two lakes. This indirectly confirms a high ecosystem similarity between lakes as we expected, and also illustrates the effective dispersal abilities coupled to the life-history strategy of allogenic parasites (Fellis and Esch 2005). As avian final hosts have the ability to move from one lake to another, allogenic parasite taxa could have been dispersed from Sagelvvatn to Takvatn independent of the stickleback introduction. Allogenic generalists might even have colonized Takvatn prior to the stickleback introduction as they have the ability to infect Arctic charr and brown trout. However, if we consider the

parasite abundances and prevalences in stickleback from Sagelvvatn in the 1950's to be as high as we have observed in this study, the probability of any parasite taxa "missing the boat" seems low. In addition, the two allogenic stickleback specialists *S. solidus* and *D. gasterostei* were directly dependent on the stickleback host introduction in order to colonize the recipient lake. Also partly in accordance with our second hypothesis, the inter-lake difference in total number of taxa was explained by autogenic parasite taxa that to some extent illustrates the limited dispersal abilities of this lifecycle strategy. Contrary to our third hypothesis, however, these parasite taxa (*Eubothrium* spp., *Crepidostomum* spp. and Nematoda spp.) were found in the introduced stickleback population. As *Crepidostomum farionis* and *C. metoecus* (unpublished data) as well as *Eubothrium salvelini*, *E. crassum*, *Cystidicola farionis* and *Philonema oncorhynchi* infect sympatric salmonids in Takvatn (Knudsen et al. 1996, Amundsen et al. 2013), we strongly expect that their infections in the sticklebacks from Takvatn are accidental. Adding to this assumption is the fact that they were found with low number of parasites, mean intensity and prevalence. Preliminary studies indicates however that *Crepidostomum* and *Eubothrium* are present in the salmonids of Sagelvvatn but *Cystidicola farionis* and *P. oncorhynchi* are absent (unpublished data). It is therefore likely that *Cystidicola farionis* and *P. oncorhynchi* were present in Takvatn prior to the introduction and infected the stickleback in Takvatn following their introduction there. This could also be the case for *Eubothrium* spp. and *Crepidostomum* spp., but as very few individuals were found in Takvatn, the difference between the two lakes with regards to these two parasite taxa may be a sampling artifact. In the same way, the autogenic generalist *C. truncatus* may have been introduced with the sticklebacks, but was probably present in Takvatn before the stickleback introduction as it also infects salmonids (Knudsen et al. 2004). However, with regards to the autogenic stickleback specialists *Gyrodactylus arcuatus* and possibly *Proteocephalus* sp. (i.e., assumedly *P. filicollis*), these must have been directly introduced with the stickleback introduction. Overall, the colonization and establishment of the stickleback parasite community in Takvatn may have been related to a combination of many different factors, potentially including both autogenic and allogenic parasites being introduced along with the stickleback introduction, allogenic parasites being dispersed by avian hosts from Sagelvvatn or other sources, and generalist parasites that were already present in Takvatn prior to the stickleback introduction.

Our findings of a more species-rich parasite community in an introduced population contradicts our expectations and previous findings of studies on host introductions (Torchin et al. 2002, Torchin et al. 2003, MacLeod et al. 2010, Roche et al. 2010). It is however in line with studies on round goby (*Neogobius melanostomus*), which concluded that local-scale effects had led to high similarity between parasite communities, and that differences were best explained by ecological differences between habitats (Ondrackova et al. 2010, Francova et al. 2011). Ecological factors such as fish host density (Takemoto et al. 2005), lake productivity (Poulin et al. 2003) and availability of potential intermediate hosts (Marcogliese and Cone 1991), can result in differences in fish-parasite species richness among lakes. Even though Sagelvvatn had higher stickleback density, higher productivity and higher copepod density, the lake still had a lower total number of taxa as compared to Lake Takvatn. We therefore suggest that, in our study, the effects of these ecological factors were reduced by high ecosystem similarity, shared parasite species pool and geographical proximity of the two lakes, causing there to be no distinct inter-lake differences in total number of stickleback parasite taxa. The difference that we did observe was assigned to accidental infections by parasites of sympatric salmonids.

In comparison with other studies on sticklebacks (Chappell 1969, Pennycuick 1971, Kalbe et al. 2002), the total number of taxa and in particular the number of parasites were very high in the present study. In line with our final hypothesis, Sagelvvatn had the highest total number of parasites while also exhibiting higher stickleback density. Host density is positively related to parasite transmission rates (Anderson and May 1978, Dobson 1990, Arneberg et al. 1998) and could therefore explain the overall higher number of parasites in the source lake. In addition, parasite abundance has been shown to be positively correlated with lake productivity (Esch 1971). The higher agricultural activity in the catchment area of Sagelvvatn causing the lake to have increased productivity could therefore present an additional explanation to why parasites occur more abundant in Sagelvvatn. Inter-lake difference in stickleback foraging behavior could be a third explanation as sticklebacks feed preferentially on prey items with higher density (Visser 1982). The higher copepod density in Sagelvvatn is thus expected to cause sticklebacks from this lake to feed more on this particular prey item leaving them more heavily infected with copepod transmitted cestodes. This is to some extent evident from our data as most of the copepod transmitted cestodes

showed higher number of parasites, mean intensity and prevalence in the sticklebacks sampled in Sagelvvatn.

Most striking among the different parasite taxa recorded in this study was the obvious inter-lake differences with regards to the stickleback specialists *G. arcuatus* and the assumed *Proteocephalus filicollis*. With regards to *Proteocephalus*, this genus uses copepods as intermediate host and its higher occurrence in Sagelvvatn might therefore be related to the increased density of copepods in the source lake. But also the diphyllobothrids use copepods as intermediate host, and it is therefore peculiar that they did not show similar results. This may however be a result of their generalist life cycle strategy, which in addition makes them dependent on the sympatric salmonid populations in the two lakes. The differences in occurrence between these two copepod transmitted cestodes taxa could therefore indicate that *Proteocephalus* sp. is not a generalist which adds to our assumption that it might in fact be the stickleback specialist *P. filicollis*.

As for the directly transmitted ectoparasite *G. arcuatus*, its transmission rate is thought to be related to host density (Arneberg et al. 1998). The three-fold higher stickleback density in Sagelvvatn could therefore explain why this parasite species was more abundant in the source lake. Additionally, the mass removal of Arctic charr performed in Takvatn in the 1980's caused high annual variation in stickleback density (Klemetsen et al. 2002). This could impair the transmission rate of a directly transmitted parasite like *G. arcuatus*, possibly contributing to explain the low infection rate in Takvatn. Further, abiotic factors such as temperature have been known to directly affect the reproduction rate of *Gyrodactylus* (Sereno-Uribe et al. 2012). As lower water temperature generally occurs in Takvatn caused by a higher altitude (unpublished data), the reproduction cycle of *G. arcuatus* in Takvatn might be prolonged providing an additional explanation to the inter-lake difference in the occurrence of this parasite.

As an exception to the general pattern of a higher number of parasites in the source stickleback population, *Apatemon* sp. was more abundant in the sticklebacks sampled from the introduced population in Takvatn. This trend is difficult to explain but it is unlikely to have been caused by inter-lake differences in intermediate host density. If this had been the

case, the same trend would have been expected for *Diplostomum* spp. as the two trematode genera utilizes the same Lymnaeid snail species as first intermediate host (Blair 1976, Chappell 1995, Hoffman 1999). Spatial distribution of trematode snail infections can however be parasite species specific (Jokela and Lively 1995) and might be part of an explanation to why *Apatemon* sp. and *Diplostomum* spp. are showing opposite trends.

Molecular data can clarify species boundaries and facilitate the analysis of fish parasite communities (Rellstab et al. 2011, Behrmann-Godel 2013, Desilets et al. 2013, Locke et al. 2013) and life cycles (Locke et al. 2011). Unfortunately we were unable to map most molecular identifications onto the ecological data, but it is unlikely to have affected our conclusions to a great extent. Concerning the encysted metacercariae in the eye and body cavity, no sequences were obtained from specimens from Sagelvvatn. It is however reasonable to assume that these were the same *Apatemon* species as found in Takvatn. Also, the detection of two species of *Diplostomum* among non-lens-infecting metacercariae in a single host species was somewhat surprising given the relatively small number of specimens sequenced and the limited spatial scale. In a number of previous studies of larger scope (Galazzo et al. 2002, Locke et al. 2010a, Locke et al. 2010b, Rellstab et al. 2011, Georgieva et al. 2013), molecular data revealed relatively low diversity among metacercariae inhabiting non-lens sites. Our findings (see also Chibwana et al. (2013)) thus indicate that it is still too early to generalize about diplostomid diversity based on patterns observed from existing studies.

Overall this study revealed high similarity between the component community of the source and the introduced stickleback population. Evidently, all parasite species present in the source population in Sagelvvatn had successfully colonized and established themselves in Takvatn. Some parasite species, especially autogenic ones, were likely infecting the introduced stickleback specimens. Some species were probably already present in Takvatn as parasites of Arctic charr and/or brown trout, whereas the transfer and establishment of others, in particular allogenic species, have been facilitated by the close geographical proximity and the relatively similar biotic and abiotic conditions in the two lakes. Some inter-lake differences did however occur. Parasite taxa that contributed most to the dissimilarity between infracommunities in the two lakes were copepod transmitted cestodes and

autogenic stickleback specialists. This emphasizes the possible importance of inter-lake differences in copepod intermediate host density and parasite lifecycle strategies. The observed difference in total number of taxa between the two component communities were most likely caused by accidental infections by parasites from sympatric salmonids. On the other hand, differences in number of parasites and mean abundances might have been related to inter-lake difference in stickleback density, but also to minor inter-lake differences in abiotic and biotic factors such as temperature, productivity and copepod density. Overall we conclude that, on a small spatial-scale, moderate abiotic and biotic differences between lakes are likely the main cause for the quantitative differences between the parasite communities. Mechanisms that contribute to qualitative differences (i.e., the species composition of the parasite communities) are on the other hand absent, or of minor importance, due to high ecosystem similarity between lakes on a small spatial scale.

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Table text

Table 1. Catch data and summary statistics of parasite communities in two three-spined stickleback populations (*Gasterosteus aculeatus*) sampled in 2010 from Lake Sagelvvatn and Lake Takvatn in northern Norway.

Table 2. Variation in sequences of cytochrome oxidase 1 obtained from diplostomoid metacercariae from three-spined stickleback (*Gasterosteus aculeatus*) sampled from Lake Sagelvvatn and Lake Takvatn in northern Norway 2010. As comparison, sequences published in other studies of closely related diplostomoids are included. Sequences were only obtained from material from Takvatn except for *D. gasterostei* where material was available from both lakes.

Figure text

Figure 1. Location of Lake Sagelvvatn and Lake Takvatn where 120 three-spined stickleback (*Gasterosteus aculeatus*) were sampled from each lake in 2010.

Figure 2. (upper) Barplot illustrating number of each parasite taxon found in the 240 three-spined sticklebacks (*Gasterosteus aculeatus*) sampled in Lake Sagelvvatn (▪, source lake) and Lake Takvatn (▫, recipient lake). Included are parasites life strategies where (AU) = autogenic, (AL) = allogenic, (G) = generalist and (S) = specialist (Levsen 1992, Sterud 1999). (lower) Boxplot illustrating the distribution of parasite intensities for each parasite taxon including median, upper and lower quartiles, minimum and maximum as well as outliers for the two sampled stickleback populations. Note the x-axis is on a log scale.

Figure 3. Neighbour-joining tree of uncorrected percent distances among 302 sequences from the barcode region of cytochrome c oxidase 1. Collapsed grey clusters are groups of similar sequences corresponding to species according to original publications (Moszczynska et al. 2009, Locke et al. 2010a, Locke et al. 2010b, Locke et al. 2011, Behrmann-Godel 2013, Chibwana et al. 2013, Georgieva et al. 2013). Collapsed black clusters contain data (47 sequences, see Table 2) from the present study. Genbank accessions are XXXX-XXX [present

study] and FJ477183-6; FJ477188-9; FJ477194-200; FJ477204-6; GQ292475-502; HM064610-47; HM064660-732; JF769450-2; JQ639170-95; JX986859-907; KC685359-60.

Figure 4. Mean intensity and prevalence of parasite taxa found in the 240 sampled three-spined sticklebacks from Lake Sagelvvatn (source lake) and Lake Takvatn (recipient lake). Parasite taxa are color coded depending on their life cycle strategy (allogenic (◉), autogenic (●)). Mean intensity for *Eubothrium* spp. (1), *Crepidostomum* spp. (1.4) and Nematoda spp. (1.6) are absent from the plot as these were only found in Takvatn.

Figure 5. (Left) Nonmetric multidimensional scaling plot of parasite infracommunities in 240 three-spined sticklebacks from Lake Sagelvvatn (●) and Lake Takvatn (◉) based on Bray-Curtis dissimilarity of log+1 transformed parasite abundance (stress = 0.2). Ellipses contain 95 % of the infracommunities sampled in each lake. Parasite taxa abbreviation and results from the Similarity Percentage Analysis (SIMPER): Di = *Diphyllobothrium ditremum* (17.6 %), D = *Diphyllobothrium* spp. (16.9 %), Ga = *Gyrodactylus arcuatus* (16.5 %), Ap = *Apatemon* sp. (14.1 %), Pr = *Proteocephalus* sp. (13.7 %), Dip = *Diplostomum* spp. (10.1 %), Ss = *Schistocephalus solidus* (4.6 %), Ne = Nematoda spp. (3.0 %), Ct = *Cyathocephalus truncatus* (2.3 %), De = *Diphyllobothrium dendriticum* (0.7%), Cr = *Crepidostomum* spp. (0.5 %), Eu = *Eubothrium* spp. (0.1 %). (Right) Boxplot of ranked infracommunity dissimilarities between lakes, within Sagelvvatn and within Takvatn. Medians, upper and lower quartiles as well as minimum and maximum values are shown.

Tables

Table 1

Lake	Sagelvvatn (source lake)	Takvatn (recipient lake)
Fish (N)	120	120
CPUE	8.0	2.7
Fish length mm, mean (±SD)[-]	57.7 (±4.6)	57.0 (±5.3)
Component community level		
Total number of taxa	9	12
Total number of parasites	11218	6807
Simpson's Index of Diversity (1-D)	0.620	0.482
Simpson's Measure of Evenness ($E_{1/D}$)	0.293	0.161
Percentage Similarity (%)	76.26	
Infracommunity level		
Abundance, mean (range)*	93 (9-598)	56 (19-206)
Number of taxa, mean (range)[-]	4.67 (1-8)	4.08 (2-8)

* = $P<0.001$, [-] = $P>0.05$

Table 2

N	Mean (maximum) intraspecific distance (%)	Minimum distance (%) to nearest heterospecific	Host(s), tissue and notes
Diplostomum baeri 2*			
4[I] (16**)	0.73 (1.52)	5.37	Free in non-lens eye tissues (humour, retina) of *G. aculeatus*, *Perca fluviatilis***
Diplostomum gasterostei			
21[I,II]	0.74 (2.04)	9.67	Free in non-lens eye tissues, mainly retina, of *G. aculeatus*
Apatemon sp.			
3[I]	0.11 (0.16)	7.57	Encysted in non-lens eye tissues and in body cavity of *G. aculeatus*
Strigeidae gen. sp.			
12[I]	0.50 (1.25)	9.95	Free in non-lens eye tissues of *G. aculeatus*

* Name follows Georgieva et al. (2013) , ** 16 sequences from *P. fluviatilis* in Lake Constance, Germany (Behrmann-Godel 2013). Origin: [I] Takvatn, [II] Sagelvvatn.
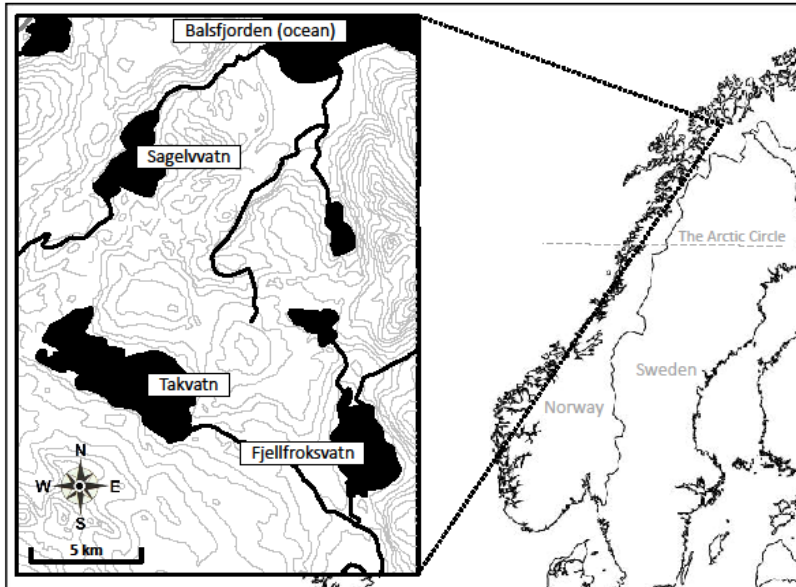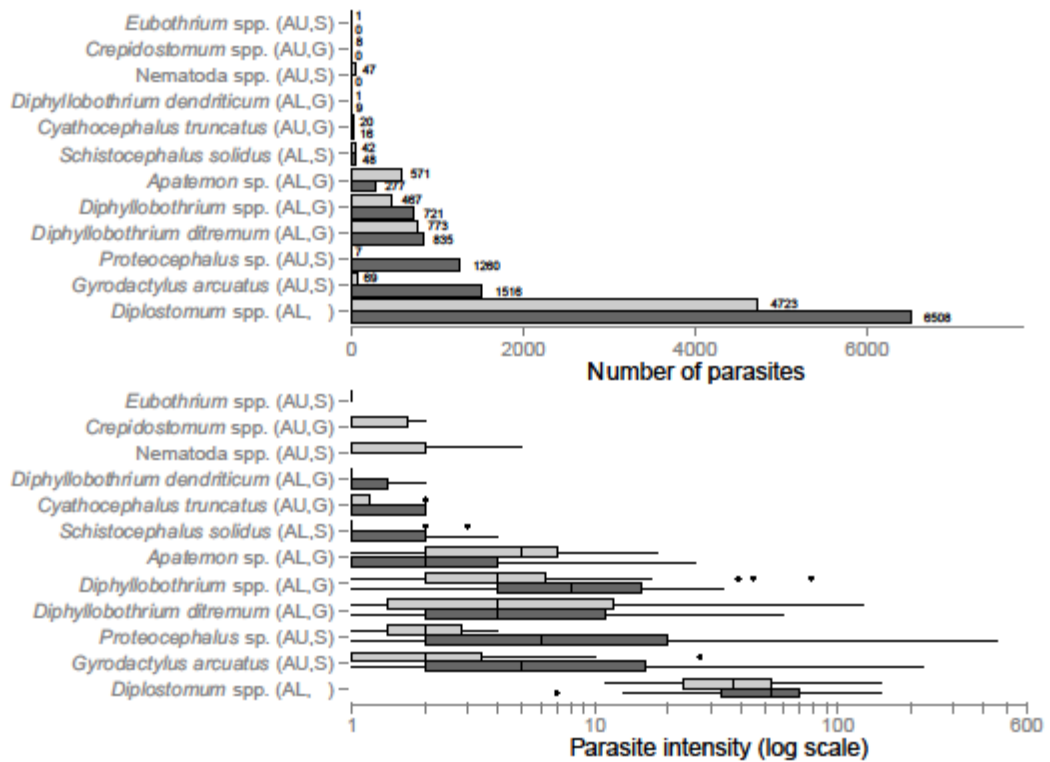
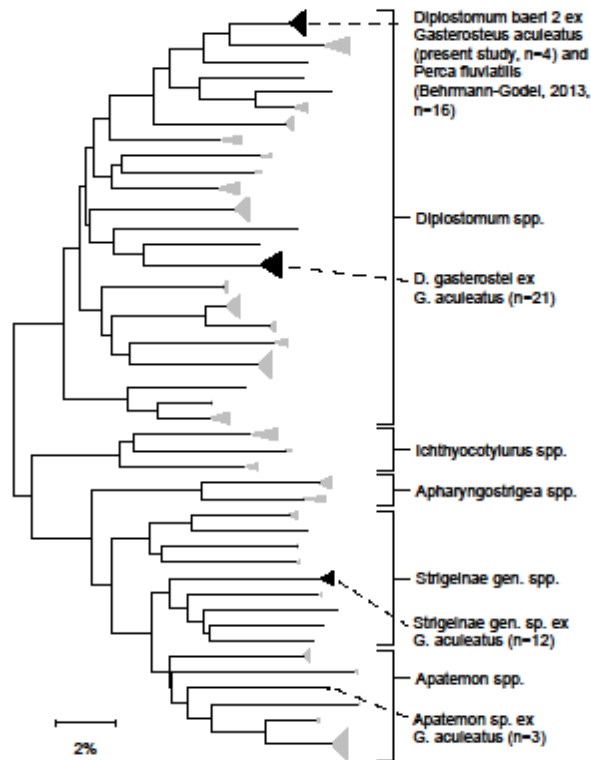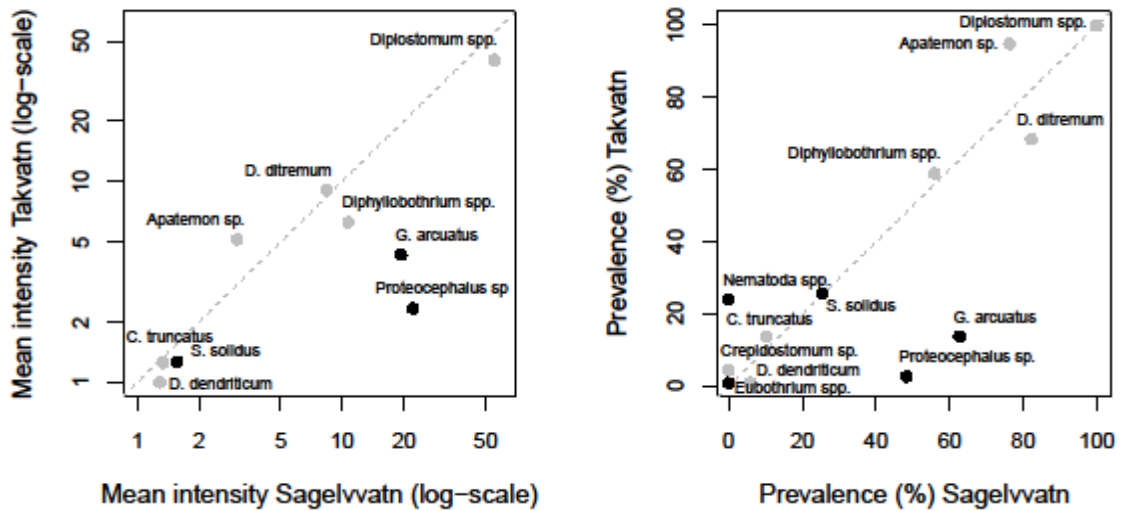# Figures

Figure 1:

Figure 2:

Figure 3

Figure 4

Figure 5: