

Effects of fish species composition on *Diphyllbothrium* spp. infections in brown trout - is three-spined stickleback a key species?

Jesper A. Kuhn^{1*}, André Frainer^{1,2}, Rune Knudsen¹, Roar Kristoffersen¹, Per-Arne Amundsen¹

¹Department of Arctic and Marine Biology, Faculty of Bioscience, Fisheries and Economics, UiT The Arctic University of Norway, N-9037 Tromsø, Norway

²Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden

Correspondence

J Kuhn, Department of Arctic and Marine Biology, Faculty of Bioscience, Fisheries and Economics, UiT The Arctic University of Norway, N-9037 Tromsø, Norway

(e-mail: jesper.a.kuhn@uit.no)

Short running title

Diphyllbothrium spp. infections in brown trout

Published in: **Journal of Fish Diseases** 2016, **39**, 1313-1323. doi:10.1111/jfd.12467

Abstract

Subarctic populations of brown trout (*Salmo trutta*) are often heavily infected with cestodes of the genus *Diphyllobothrium*, assumedly because of their piscivorous behavior. This study explores possible associations between availability of fish prey and *Diphyllobothrium* spp. infections in lacustrine trout populations. Trout in i) allopatry (group T); ii) sympatry with Arctic charr (*Salvelinus alpinus*) (group TC); and iii) sympatry with charr and three-spined stickleback (*Gasterosteus aculeatus*) (group TCS) were contrasted. Mean abundance and intensity of *Diphyllobothrium* spp. were higher in group TCS compared to group TC and group T. Prevalence however was similarly higher in group TCS and group TC compared to group T. Zero-altered negative binomial modelling identified lowest probability of infection in group T and similar probabilities of infection in group TC and group TCS, whereas the highest intensity was predicted in group TCS. Evidently, the most infected trout were from the group co-occurring with stickleback (TCS), assumedly due to a high availability of suitable prey fishes. In conclusion, our study demonstrates elevated *Diphyllobothrium* spp. infections in lacustrine trout populations where fish prey are available, and suggests that highly available and easily caught stickleback prey may play a key role in the transmission of *Diphyllobothrium* spp. parasite larvae.

Keywords: trophically transmitted parasites, piscivory, salmonids, *Salmo trutta*, *Gasterosteus aculeatus*

Introduction

Piscivory is an effective feeding strategy that can be undertaken by certain fish species as a way to substantially increase their energy intake (Keast 1985; Mittelbach & Persson 1998; Elliott & Hurley 2000). However, preying on fish may activate alternative parasite transmission routes as some parasite species are able to reestablish in piscivore predators (Williams & Jones 1994; Valtonen & Julkunen 1995). Some of these parasites may have a longevity of many years in a piscivorous host and may thus accumulate in high numbers over time. High parasite infection in piscivorous fish may possibly affect the fitness of the host, hamper their recreational value as well as increase the possibility of human infections (Torres et al. 2002). A piscivorous feeding strategy is more likely expressed when suitable prey fish are available (L'Abée-Lund et al. 1992; L'Abée-Lund et al. 2002), and the fish species composition of a habitat may therefore influence the level of parasite infections in these hosts.

Larger-sized individuals of salmonids in lakes of northern Europe are commonly observed to be piscivorous (L'Abée-Lund et al. 1992; Amundsen 1994; Kahilainen & Lehtonen 2002; L'Abée-Lund et al. 2002; Jensen et al. 2004; Hyvärinen & Huusko 2006). Salmonids like brown trout (*Salmo trutta* L.) and Arctic charr (*Salvelinus alpinus* (L.)), hereafter referred to as trout and charr respectively, are regarded as secondary piscivores as fish prey are included in their diet relatively late in life (Keast 1985). At onset, piscivory is usually a small part of the diet, but may account for the majority of the food intake in large individuals (L'Abée-Lund et al. 1992; Amundsen 1994; L'Abée-Lund et al. 2002).

According to several studies, three-spined stickleback (*Gasterosteus aculeatus* L.), hereafter referred to as stickleback, is a common fish prey for subarctic salmonid species (L'Abée-Lund et al. 1992; Amundsen 1994; Knudsen et al. 2008; Eloranta et al. 2013). Sticklebacks have been shown to be a key species with extensive linkages to the parasite community in the food web of a subarctic lake (Amundsen et al. 2009; Amundsen et al. 2013). As a result, sticklebacks can be infected with a wide variety of different parasite species, including endoparasitic cestode larvae of the genus *Diphyllbothrium* (Kuhn et al. 2015). Targeting sticklebacks as prey might therefore be the reason for elevated parasite infections in piscivorous salmonids (Knudsen et al. 1996; Knudsen et al. 2008). Compared to charr, trout is however known to feed more frequently and extensively on sticklebacks with an earlier size-dependent onset of piscivory (L'Abée-Lund et al. 1992; Knudsen et al. 2008; Eloranta et al. 2013). In addition, the frequency of piscivorous trout preying upon sympatric charr has been observed to increase with trout length (L'Abée-Lund et al. 1992; Damsgård & Langeland 1994; Knudsen et al. 2008). In this study, we investigate how the presence of stickleback and charr may affect larval infections of *Diphyllbothrium* spp. in lacustrine trout populations.

The genus *Diphyllbothrium* has a cosmopolitan distribution and includes the most important fish-borne zoonosis among the cestode parasites (Dick et al. 2001; Chai et al. 2005; Scholz et al. 2009). Two parasite species, *Diphyllbothrium ditremum* and *D. dendriticum*, commonly infect brown trout, Arctic charr and three-spined stickleback in northern Europe (Halvorsen 1970; Henricson 1977; Andersen & Valtonen 1992; Rahkonen & Koski 1997; Knudsen et al. 2008; Kuhn et al. 2015). Both parasite species utilize copepods as first intermediate host and fish as second intermediate host, but they differ in terms of their

final hosts. Whereas *D. ditremum* infects diving ducks such as mergansers and loons, *D. dendriticum* infects gulls and several other birds, as well as some mammal species (Vik 1964; Halvorsen 1970; Bakke 1985). Both parasites can reestablish in piscivorous predators (Vik 1964; Halvorsen & Wissler 1973; Curtis 1984). The encysted parasite larvae have a longevity of many years and may accumulate in large piscivorous individuals. In this way, intensities of more than one thousand parasites in a single fish can be found (Curtis 1984; Knudsen & Klemetsen 1994; Knudsen et al. 1996).

Trout, charr and stickleback are widespread freshwater fish species (Wootton 1984; Klemetsen et al. 2003). In northern Scandinavia, allopatric populations of trout and charr are often found to prefer the littoral habitat along with a benthivore diet niche (Klemetsen et al. 2003; Knudsen et al. 2008). In sympatry, the two salmonid species differ more distinctly. Trout typically dominates the littoral zone with a diet comprised of benthic macroinvertebrates, surface insects and fish (Jansen et al. 2002; Sánchez-Hernández & Amundsen 2015), while charr are more commonly found in the pelagic habitat feeding extensively on zooplankton (Langeland et al. 1991; Knudsen et al. 2008; Eloranta et al. 2013; Skoglund et al. 2013). Sticklebacks are frequently found in the littoral zone, but may also occur in the pelagic (Langeland 1982; Wootton 1984). In both habitats, stickleback include zooplankton in their diet (Langeland 1982; Jørgensen & Klemetsen 1995). Feeding on zooplankton makes charr and sticklebacks highly susceptible to infection by copepod-transmitted *Diphyllbothrium* spp. larvae. Trout, on the other hand, may alternatively become exposed from their piscivorous behavior.

In this study, we analyzed *Diphyllbothrium* spp. infections in trout from eight subarctic lakes representing three different groups of fish species composition: i) trout in allopatry, ii) trout in sympatry with only charr, and iii) trout in sympatry with charr and stickleback. Firstly, we hypothesized that the *Diphyllbothrium* spp. infections in trout from the three groups would vary according to the differences in fish species composition. More precisely, we predicted that trout in allopatry would show low infections of *Diphyllbothrium* spp. as suitable fish prey were absent. Secondly, we predicted that trout in sympatry with only charr would show intermediate infections of *Diphyllbothrium* spp. as charr is considered harder to locate and catch than stickleback (L'Abée-Lund et al. 1992). Thus, thirdly, we expected that trout in sympatry also with stickleback would show high *Diphyllbothrium* spp. infections. Finally, we hypothesized that independent of fish species composition, the infection of *Diphyllbothrium* spp. in trout would increase in intensity as a function of fish length due to the accumulation of parasite larvae in the fish host over time.

Material and methods

The study lakes

Samples were taken from eight subarctic lakes of northern Norway located in Nordland county, including Fjerdevatn (67°46'N, 15°58'E), Forsanvatn (67°54'N, 15°42'E), Makkvatn (67°50'N, 15°49'E), Rekvatn (67°56'N, 16°04'E), Skilvatn (68°04'N, 15°53'E) and Storvatn (67°56'N, 16°00'E), and the neighboring Troms county, including Fjellfrøsvatn (69°05'N, 19°20'E) and Sagelvatn (69°11'N, 19°06'E) (Fig. 1). All lakes are oligotrophic and dimictic with well-developed littoral, profundal and pelagic habitats. They are normally ice covered from November to June. The catchment areas are situated in mountainous landscapes covered by downy birch (*Betula pubescens*) and occasional Scots pine (*Pinus sylvestris*) forests. For additional physical parameters of the lakes and their surroundings, see Knudsen et al. (2008), Sánchez-Hernández & Amundsen (2015) and Kuhn et al. (2015). Rekvatn and Forsanvatn are regulated for hydroelectric purposes.

Three different fish species compositions are represented across the eight lakes. Trout is allopatric in Storvatn and Forsanvatn, lives in sympatry with only charr in Rekvatn and Fjellfrøsvatn, and occurs in sympatry with charr and stickleback in Makkvatn, Skilvatn, Sagelvatn and Fjerdevatn. These pre-assessments of fish species composition were based on local knowledge and databases from the Norwegian Institute for Nature Research (NINA). Ultimately, they were confirmed by our own fishing efforts.

Fish sampling and parasite screening

The six lakes in Nordland county were sampled in August 2013 whereas the two lakes in Troms county were sampled in August 2010 (Sagelvvatn) and August 1992 (Fjellfrøsvatn; Knudsen (1995)). *Diphyllbothrium* spp. infections in salmonids appear to be relatively stable over time in sub-Arctic lakes (Knudsen et al. 2011). The temporal sampling gap between a few of the lakes in the present study is therefore not likely to affect the validity of our comparisons and findings.

In each lake, multi-mesh survey gillnets (10–45 mm mesh size from knot to knot) were deployed overnight in the littoral (1–10 meters depth), profundal (> 20 meters depth) and pelagic zones (0–6 meters) for approximately 12 hours. To confirm the presence or absence of stickleback, 6–8 mm mesh-sized gill nets and minnow traps were also deployed in the littoral zone of all lakes. After retrieval, trout body length was measured (fork length to nearest mm) and their body cavity was carefully examined for the presence of encysted *Diphyllbothrium* spp. Special attention was given to the outer surface of the stomach, the viscera and the surrounding muscles tissue. If cysts were noted, the entire stomach wall and infected tissue were placed in a petri dish and covered in digestive fluid (2% HCL with 5 gr/L pepsin and 9 gr/L NaCl) in order to extract the encysted parasites. Samples were kept at room temperature and excysted plerocercoid larvae were collected after approximately 12 and 24 hours after which they were preserved in 4 % formaldehyde. The preserved plerocercoids were later identified as *Diphyllbothrium ditremum* or *D. dendriticum* under 64x–400x magnification using the morphological descriptions by Andersen et al. (1987) and Andersen & Gibson (1989). Sampled sticklebacks were also examined for the presence of *Diphyllbothrium* spp.

The sampled trout were assigned to three different groups depending on the fish species composition of the lake in which they were caught, including i) lakes with allopatric trout (group T), ii) lakes where trout occurs in sympatry with only charr (group TC), and iii) lakes where trout occurs in sympatry with charr and stickleback (group TCS).

Terminology

Ecological terms used in this study follow the definitions of Bush et al. (1997). Mean abundance is the total number of *Diphyllbothrium* spp. individuals counted in a group divided by the total number of examined hosts in that group including non-infected individuals. Prevalence is the number of hosts in a group infected with *Diphyllbothrium* spp. divided by the total number of hosts examined in that group. Mean intensity is the total number of *Diphyllbothrium* spp. individuals counted in a group divided by the total number of infected hosts in that group.

Statistical analyses

Trout length

Differences in trout length between the three groups of fish species composition (T,TC and TCS) were tested using a one-way analysis of variance (ANOVA). In this analysis, log-transformed fish length (mm) was used as a continuous response variable and fish species composition was implemented as a three-level categorical explanatory variable.

The abundance, prevalence and mean intensity of *D. ditremum* and *D. dendriticum* showed similar trends for the three different groups of fish species compositions (see appendix, Fig. 6, Table 2). The two parasite species were therefore pooled prior to statistical analyzing and are referred to as *Diphyllbothrium* spp. in the following.

Diphyllbothrium spp. infection in trout

We tested if the three groups of fish species composition (T, TC and TCS) differed with regards to mean abundance, mean intensity and prevalence of *Diphyllbothrium* spp. in trout. As the data in all three groups of fish species composition was not normally distributed even after log-transformation, we used 10000 cycled permutation tests (Greenacre & Primicerio 2014). This analysis disregarded potential between-group differences driven by changes in mean trout length.

To take into account between-group differences in mean trout length, we analyzed how variation in trout length, fish species composition, and the interaction between them affected the infection of *Diphyllbothrium* spp. in trout using parasite abundance and trout length data from each fish individual. As the overall data set based on parasite abundance from each trout individual was highly skewed towards zero (i.e., many fish had no parasites), we used generalized linear models that can handle non-normal data distributions. There are currently two options more commonly applied for this type of analysis, the zero-altered and the zero-inflated models (Zuur et al. 2009). The two models differ in the sense that the latter assumes the presence of both true and false zeros (Martin et al. 2005). However, separating between zeros brought unnecessary complexity to the model, and did not affect our

conclusions. We therefore chose the zero-altered model approach. Nonetheless, we compared model fit of the zero-altered negative binomial model (ZANB) with an alternative zero-altered Poisson model (ZAP) and a zero-inflated negative binomial model (ZINB) using likelihood ratio tests and Akaike's Information Criteria (AIC) (see appendix, Table 3).

The ZANB model uses a binomial distribution to analyze and calculate the probability of infection. A truncated negative binomial distribution is used to analyze and predict the intensity of infection. The ZANB model was simplified by systematically dropping non-significant interaction terms and comparing the competing models using AIC. To validate the fit of the ZANB model, we plotted Pearson's residuals against the fitted values. Our analyses indicated potential auto-correlation patterns in all the ZANB, ZAP and ZINB models. To identify causing factors for the spatial auto-correlation, and to properly address differences among the three groups of fish species composition, we performed a supplementary mixed-effect model analysis excluding all parasite abundance data equal to zero, and with lake as the random effect. Residuals from this model did not show auto-correlation, indicating that the observed patterns in the residuals were caused by spatial autocorrelation of fish with zero parasite infection. Nonetheless, this supplementary model identified the same significant explanatory variables and reached the same overall conclusions as the ZANB model.

All statistical analyses and modelling performed in this study were done in R, version 3.1.1 (R Core Team 2015).

Results

Fish length

Trout body-length differed between the three groups of fish species composition ($F_{2,592} = 7.465$, $p < .001$; Table 1). A post-hoc Tukey test indicated that trout in group TCS were significantly longer (14%) than in group TC (mean length \pm SD: 215 ± 73 mm and 185 ± 58 mm, respectively; Tukey test: $p < 0.001$), whereas none of these differed significantly from group T (201 ± 66 mm; all Tukey test: $p > 0.07$).

Diphyllobothrium spp. abundance, intensity and prevalence

The overall parasite infections of *Diphyllobothrium* spp. in trout differed between the three groups of fish species composition (Fig. 2, Fig. 3, Table 1). Mean abundance was significantly higher in group TCS (mean abundance = 17.9) compared to group TC (2.6) and group T (0.2), while mean abundance in trout from group TC was significantly higher than in group T (all permutation tests₍₁₀₀₀₀₎, $p < 0.01$) (Fig. 2, Table 1).

Prevalence of *Diphyllobothrium* spp. in trout from group TCS (prevalence = 45.5 %) was significantly higher than in group T (10.2 %; permutation test₍₁₀₀₀₀₎, $p < 0.001$), but did not differ from group TC (46.0 %; permutation test₍₁₀₀₀₀₎, $p = 0.912$). The prevalence in group TC was also significantly higher than in group T (permutation test₍₁₀₀₀₀₎, $p < 0.001$; Table 1).

Mean intensity in group TCS (mean intensity = 39.4) was significantly higher than in group TC (5.7; permutation test₍₁₀₀₀₀₎, $p < 0.001$) and group T (2.2; permutation test₍₁₀₀₀₀₎, $p = 0.048$)

and was furthermore significantly higher in group TC compared to group T (permutation test₍₁₀₀₀₀₎, $p = 0.025$; Table 1).

Modelled *Diphyllbothrium* spp. infection

The probability of *Diphyllbothrium* spp. infection was significantly dependent on trout body length (binomial generalized linear part of the ZANB model, $X^2 = 115.6$, $df = 1$, $p < 0.001$). A 50 % probability of infection was obtained in trout of length 35 cm in group T, 19 cm in group TC and 22 cm in group TCS (Fig. 4). Fish species composition also significantly affected the probability of infection ($X^2 = 103.9$, $df = 2$, $p < 0.001$). Trout from group T had lower probability of becoming infected with *Diphyllbothrium* spp. compared to trout from group TC and group TCS, whereas trout from group TC had a similar probability of infection as those in group TCS (Fig 4).

The predicted intensity of *Diphyllbothrium* spp. infection varied both with trout length and fish species composition (truncated negative binomial generalized linear part of the ZANB model, trout length x species composition interaction term: $X^2 = 14.3$, $df = 2$, $p < 0.001$).

Predicted intensity of *Diphyllbothrium* spp. was always less than one in trout from group T, but increased distinctly with trout length in trout from group TC and group TCS (Fig. 5). In addition, *Diphyllbothrium* spp. intensity was predicted to increase faster in trout from group TCS compared to trout from group TC as a function of fish length. In fact, the intensity of *Diphyllbothrium* spp. in trout more than 40 cm in length was predicted from the model to be 10 times higher in group TCS than in group TC (Fig. 5).

In the above statistical modelling, inter-lake variation within each of the three groups of fish species composition were disregarded due to complexities regarding implementation of mixed effect models with zero-inflated data. However, similar statistical results were obtained when using lake as an eight-level categorical explanatory variable or as a random variable, instead of species composition.

Discussion

According to our hypothesis, the current study documented significant differences in *Diphyllbothrium* spp. infections of brown trout depending on lake fish species composition. Trout in allopatry had significantly lower infections of *Diphyllbothrium* spp. than trout from the other two groups, assumedly as a consequence of the absence of suitable fish prey species. Trout in sympatry with only charr had intermediate infections while trout in sympatry with stickleback showed the highest infections of *Diphyllbothrium* spp. Also in agreement with our expectations, parasite intensity increased significantly with trout length regardless of fish species composition, which indicated an accumulation of the parasite in the fish hosts.

Trout in allopatry (group T) were found to have low *Diphyllbothrium* spp. infections. In Norwegian lacustrine trout populations, cannibalism is considered uncommon (L'Abée-Lund et al. 1992; Knudsen et al. 2008), mainly as a result of spatial segregation in brooks versus lakes of small- and large-sized trout, respectively (Klemetsen et al. 2003). Accordingly, no fish remains were found in the stomachs of allopatric trout from the present study lakes

(Sánchez-Hernández & Amundsen 2015). Instead, allopatric trout (group T) were found occasionally to feed on copepods, the intermediate hosts of *Diphyllbothrium* spp. (Sánchez-Hernández & Amundsen 2015). Although copepods are not considered a common prey for trout, they are occasionally included in their diet (Langeland et al. 1991; Knudsen et al. 2008; Jonsson & Jonsson 2011; Eloranta et al. 2013). It is therefore likely that the few *Diphyllbothrium* spp. infections observed in allopatric trout were caused by copepod predation. Hence, the absence of suitable fish prey and an infrequent copepod predation likely explain the low *Diphyllbothrium* spp. infection levels observed in allopatric trout, which is in accordance with our first hypothesis.

When trout occur in sympatry with charr and/or sticklebacks, they have a greater opportunity to turn piscivorous. In general, lacustrine salmonids start to include fish in their diet at a length of about 15 cm (Keeley & Grant 2001), but the onset of piscivory in trout of Norwegian lakes may occur at an even smaller size if sticklebacks are present (L'Abée-Lund et al. 1992; Eloranta et al. 2013). This was not apparent from our findings as the probability of infection was similar for trout in group TC and TCS as indicated by the model output. The probability of trout turning piscivorous thus seemed to be independent of whether charr or stickleback was available as prey. Piscivory is suggested to be the predominant feeding strategy in large (> 30 cm) salmonid individuals (Keeley & Grant 2001; Hyvärinen & Huusko 2006). As *Diphyllbothrium* spp. is capable of re-infecting piscivorous fish (Hammar 2000; Gallagher & Dick 2010), trout preying on *Diphyllbothrium* spp. infected fish, such as charr or stickleback, are highly exposed to infections. The availability of suitable fish prey is therefore a likely explanation to why trout in group TC and TCS exhibited significantly higher infections than allopatric trout, which is in agreement with our hypotheses.

Many factors seem to advocate that sticklebacks are favorable prey compared to charr for piscivorous trout. Whereas charr is considered an excellent swimmer (Johnson 1980), sticklebacks are poor swimmers and hence exposed to elevated predation risk (Lindsey 1978; FitzGerald & Wootton 1986). Trout has been shown to prey mostly upon fish less than 10 cm in length (L'Abée-Lund et al. 1992; Hyvärinen & Huusko 2006; Knudsen et al. 2008; Eloranta et al. 2013), and as sticklebacks obtain a maximum length of 8 cm (Wootton 1984), they are within the trout preferred prey-size range during their entire life span. Charr, on the other hand, eventually outgrow their potential as prey for trout. In the present study, sticklebacks were heavily infected with *Diphyllbothrium* spp. (prevalence = 75.4%, mean intensity = 15.5, data not shown), similar to observations from another subarctic lake (Kuhn et al. 2015), which suggest that sticklebacks in general may have a high *Diphyllbothrium* spp. infection. In comparison, no diphyllbothriids were found in small charr (< 10 cm) in the present study ($N_{\text{charr}} = 14$, data not shown), suggesting that charr of this size have a low prevalence of infection. Hence, stickleback appears to be the most likely candidate as an important paratenic host for *Diphyllbothrium* spp. transmission to piscivorous trout.

Sticklebacks also seem to be the preferred fish prey for several avian and mammalian species (Reimchen 1990). This probably also applies to the potential final bird hosts of *Diphyllbothrium* spp. that commonly occur in northern Norwegian lakes (Klemetsen & Knudsen 2013). The occurrence and abundance of sticklebacks may suggestively influence the presence of fish-eating birds at a lake (Klemetsen & Knudsen 2013). Fish-foraging by birds will therefore assumedly take place more frequently in lakes where sticklebacks are present, which may cause an overall increase in the occurrence of *Diphyllbothrium* spp. in

such lakes as parasite eggs are released into the water by the infected final hosts.

Accordingly, it is likely that trout living in sympatry with sticklebacks will become more heavily infected with *Diphyllbothrium* spp. than conspecifics residing in sympatry with charr only. Our results support this reasoning and thus also support our third hypothesis as both mean abundance and mean intensity were found to be significantly higher in trout if stickleback were present (group TCS) compared to when trout only occurred in sympatry with charr (group TC). In support of this, the ZANB model also predicted a faster increase in parasite intensity as a function of trout length in group TCS as compared to group TC.

The total prey consumption of a piscivorous trout (length > 25 cm) during the summer season in the Pasvik river in northern Norway has been estimated as roughly 200 fish (Jensen et al. 2006). Trout of this size, preying on sticklebacks with infections as indicated above would thus be exposed to more than 2000 diphyllbothriids in a single summer season. This estimate, although rough, indicates how exposed to *Diphyllbothrium* spp. a trout potentially may become as it turns piscivorous. At intensities above 1500–2000, *Diphyllbothrium* spp. plerocercoids may be fatal to their salmonid hosts (Henricson 1977; Rahkonen et al. 1996; Hammar 2000). Studies regarding how trophic interactions between sympatric fish species affect their parasite infections are therefore relevant for management purposes and also offer insight to the potential benefits of controlling fish species introductions (see also Amundsen et al. 2013). Furthermore, at high levels of *Diphyllbothrium* spp. intensities, the parasites start to become encysted in the flesh of the fish host. This reduces the quality of the fish concerning both commercial and recreational fisheries but also increase the possibility of human infections. Among the species in the genus *Diphyllbothrium*, *D. dendriticum* can cause diphyllbothriasis and reported cases

might be underdiagnosed (Kuchta et al. 2013). Human infections are mostly related to traditions of eating raw or undercooked fish (Wicht et al. 2008). As fish preparation techniques such as marinating and smoking have long traditions in the north, the diphyllbothriid infections in subarctic fish populations should be carefully considered. Introduction of both sticklebacks and charr to allopatric trout lakes are known to have occurred deliberately (e.g. Amundsen et al. 2013). Based on the results of the present study, this is a management practice that should be strongly discouraged.

The occurrence of brown trout, Arctic charr and three-spined sticklebacks in lakes in subarctic Norway varies due to constraints on natural dispersion and the extent of anthropogenic fish translocations. Fish species composition can influence predator-prey interactions, habitat choice and feeding behavior, all of which are important factors in structuring the parasite community of a fish population (Wootton 1998; Knudsen et al. 2004; Knudsen et al. 2008; Fernández et al. 2010). Lakes that vary in species composition are therefore also likely to vary with regards to trophically transmitted parasites such as *Diphyllbothrium* spp. The present study demonstrates that *Diphyllbothrium* spp. infections in trout are in fact higher in lakes where trout live in sympatry with potential prey species such as charr and stickleback as compared to allopatric trout populations. Furthermore, the presence of sticklebacks in particular seems to result in elevated *Diphyllbothrium* spp. levels in brown trout, probably as a result of a possible extensive parasite transmission from the fish prey to the piscivorous fish.

Acknowledgements

We are grateful to the technicians and students of the Freshwater Ecology group for their contribution to the field and laboratory work. This study was financially supported by the Norwegian Research Council (NFR213610/F20) and UiT The Arctic University of Norway.

References

- Amundsen P-A. (1994) Piscivory and cannibalism in Arctic charr. *Journal of Fish Biology* **45**, 181-189.
- Amundsen P-A., Lafferty K.D., Knudsen R., Primicerio R., Klemetsen A. & Kuris A.M. (2009) Food web topology and parasites in the pelagic zone of a subarctic lake. *Journal of Animal Ecology* **78**, 563-572.
- Amundsen P-A., Lafferty K.D., Knudsen R., Primicerio R., Kristoffersen R., Klemetsen A. & Kuris A.M. (2013) New parasites and predators follow the introduction of two fish species to a subarctic lake: implications for food-web structure and functioning. *Oecologia* **171**, 993-1002.
- Andersen K., Ching H.L. & Vik R. (1987) A review of freshwater species of *Diphyllobothrium* with redescrptions and the distribution of *D. dendriticum* (Nitzsch, 1824) and *D. ditremum* (Creplin, 1825) from North America. *Canadian Journal of Zoology* **65**, 2216-2228.
- Andersen K.I. & Gibson D.I. (1989) A key to three species of larval *Diphyllobothrium* Cobbold, 1858 (Cestoda: Pseudophyllidea) occurring in European and North American freshwater fishes. *Systematic Parasitology* **13**, 3-9.
- Andersen K.I. & Valtonen E.T. (1992) Segregation and co-occurrence of larval cestodes in freshwater fishes in the Bothnian Bay, Finland. *Parasitology* **104**, 161-168.
- Bakke T.A. (1985) Studies of the helminth fauna of Norway XL: the common gull, *Larus canus* L., as final host for Cestoda (Platyhelminthes). *Fauna Norvegica, Series A* **6**, 42-54.
- Bush A.O., Lafferty K.D., Lotz J.M. & Shostak A.W. (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* **83**, 575-583.
- Chai J-Y., Darwin Murrell K. & Lymbery A.J. (2005) Fish-borne parasitic zoonoses: status and issues. *International Journal for Parasitology* **35**, 1233-1254.
- Curtis M.A. (1984) *Diphyllobothrium* spp. and the Arctic charr: parasite acquisition and its effects on a lake-resident population. In: *Biology of the Arctic charr: Proceedings of the International Symposium on Arctic Charr, Winnipeg, Manitoba, May 1981* (ed. by L. Johnson & B. L. Burns), pp. 395-411. University of Manitoba Press, Winnipeg.

- Damsgård B. & Langeland A. (1994) Effects of stocking of piscivorous brown trout, *Salmo trutta* L., on stunted Arctic charr, *Salvelinus alpinus* (L.). *Ecology of Freshwater Fish* **3**, 59-66.
- Dick T.A., Nelson P.A. & Choudhury A. (2001) Diphyllbothriasis: update on human cases, foci, patterns and sources of human infections and future considerations. *Southeast Asian Journal of Tropical Medicine and Public Health* **32**, 59-76.
- Elliott J.M. & Hurley M.A. (2000) Daily energy intake and growth of piscivorous brown trout, *Salmo trutta*. *Freshwater Biology* **44**, 237-245.
- Eloranta A.P., Knudsen R. & Amundsen P-A. (2013) Niche segregation of coexisting Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) constrains food web coupling in subarctic lakes. *Freshwater Biology* **58**, 207-221.
- Fernández M.V., Brugni N.L., Viozzi G.P. & 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.
- FitzGerald G.J. & Wootton R.J. (1986) Behavioural ecology of sticklebacks. In: *The Behaviour of Teleost Fishes* (ed. by T. J. Pitcher), pp. 409-432. Croom Helm, London.
- Gallagher C.P. & Dick T.A. (2010) Trophic structure of a landlocked Arctic char *Salvelinus alpinus* population from southern Baffin Island, Canada. *Ecology of Freshwater Fish* **19**, 39-50.
- Greenacre M. & Primicerio R. (2014) Multivariate analysis of ecological data. Fundacion BBVA, Bilbao.
- Halvorsen O. (1970) Studies of the helminth fauna of Norway XV: on the taxonomy and biology of plerocercoids of *Diphyllbothrium* Cobbold, 1858 (Cestoda, Pseudophyllidea) from north-western Europe. *Nytt Magasin for Zoologi* **18**, 113-174.
- Halvorsen O. & Wissler K. (1973) Studies of the helminth fauna of Norway XXVIII: an experimental study of the ability of *Diphyllbothrium latum* (L.), *D. dendriticum* (Nitzsch), and *D. ditremum* (Creplin) (Cestoda, Pseudophyllidea) to infect paratenic hosts. *Norwegian Journal of Zoology* **21**, 201-210.

- Hammar J. (2000) Cannibals and parasites: conflicting regulators of bimodality in high latitude Arctic char, *Salvelinus alpinus*. *Oikos* **88**, 33-47.
- Henricson J. (1977) The abundance and distribution of *Diphyllbothrium dendriticum* (Nitzsch) and *D. ditremum* (Creplin) in the char *Salvelinus alpinus* (L.) in Sweden. *Journal of Fish Biology* **11**, 231-248.
- Hyvärinen P. & Huusko A. (2006) Diet of brown trout in relation to variation in abundance and size of pelagic fish prey. *Journal of Fish Biology* **68**, 87-98.
- Jansen P.A., Slettvoll H., Finstad A.G. & Langeland A. (2002) Niche segregation between Arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta*): an experimental study of mechanisms. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 6-11.
- Jensen H., Amundsen P-A., Elliott J.M., Bøhn T. & Aspholm P.E. (2006) Prey consumption rates and growth of piscivorous brown trout in a subarctic watercourse. *Journal of Fish Biology* **68**, 838-848.
- Jensen H., Bohn T., Amundsen P-A. & Aspholm P.E. (2004) Feeding ecology of piscivorous brown trout (*Salmo trutta* L.) in a subarctic watercourse. *Annales Zoologici Fennici* **41**, 319-328.
- Johnson L. (1980) The Arctic charr, *Savelinus alpinus*. In: *Charrs, Salmonid fishes of the genus Salvelinus* (ed. by E. K. Balon), pp. 15-98. Dr W. Junk Publishers, The Hague.
- Jonsson B. & Jonsson N. (2011) Ecology of Atlantic salmon and brown trout: habitat as a template for life histories. Fish & Fisheries Series, vol 33. Springer, Dordrecht.
- Jørgensen L. & 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.
- Kahilainen K. & Lehtonen H. (2002) Brown trout (*Salmo trutta* L.) and Arctic charr (*Salvelinus alpinus* (L.)) as predators on three sympatric whitefish (*Coregonus lavaretus* (L.)) forms in the subarctic Lake Muddusjärvi. *Ecology of Freshwater Fish* **11**, 158-167.

- Keast A. (1985) The piscivore feeding guild of fishes in small freshwater ecosystems. *Environmental Biology of Fishes* **12**, 119-129.
- Keeley E.R. & Grant J.W.A. (2001) Prey size of salmonid fishes in streams, lakes, and oceans. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1122-1132.
- Klemetsen A., Amundsen P-A., Dempson J.B., Jonsson B., Jonsson N., O'Connell M.F. & 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. & Knudsen R. (2013) Diversity and abundance of water birds in a subarctic lake during three decades. *Fauna Norvegica* **33**, 21-27.
- Knudsen R. (1995) Relationships between habitat, prey selection and parasite infection in Arctic charr (*Salvelinus alpinus*). *Nordic Journal of Freshwater Research* **71**, 333-344.
- Knudsen R. & Klemetsen A. (1994) Infections of *Diphyllbothrium dendriticum*, *D. ditremum* (Cestoda), and *Cystidicola farionis* (Nematoda) in a north Norwegian population of Arctic charr (*Salvelinus alpinus*) during winter. *Canadian Journal of Zoology* **72**, 1922-1930.
- Knudsen R., Klemetsen A. & 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., Curtis M.A. & Kristoffersen R. (2004) Aggregation of helminths: the role of feeding behavior of fish hosts. *Journal of Parasitology* **90**, 1-7.
- Knudsen R., Amundsen P-A., Nilsen R., Kristoffersen R. & Klemetsen A. (2008) Food borne parasites as indicators of trophic segregation between Arctic charr and brown trout. *Environmental Biology of Fishes* **83**, 107-116.
- Knudsen R., Siwertsson A., Adams C.E., Garduño-Paz M., Newton J. & Amundsen P-A. (2011) Temporal stability of niche use exposes sympatric Arctic charr to alternative selection pressures. *Evolutionary Ecology* **25**, 589-604.

- Kuchta R., Brabec J., Kubáčková P. & Scholz T. (2013) Tapeworm *Diphyllobothrium dendriticum* (Cestoda)-neglected or emerging human parasite? *PLoS Neglected Tropical Diseases* **7**, 1-8.
- Kuhn J.A., Kristoffersen R., Knudsen R., Jakobsen J., Marcogliese D.J., Locke S.A., Primicerio R. & Amundsen P-A. (2015) Parasite communities of two three-spined stickleback populations in subarctic Norway—effects of a small spatial-scale host introduction. *Parasitology Research* **114**, 1327-1339.
- L'Abée-Lund J.H., Aass P. & Sægvog H. (2002) Long-term variation in piscivory in a brown trout population: effect of changes in available prey organisms. *Ecology of Freshwater Fish* **11**, 260-269.
- L'Abée-Lund J.H., Langeland A. & Sægvog H. (1992) Piscivory by brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.) in Norwegian lakes. *Journal of Fish Biology* **41**, 91-101.
- Langeland A. (1982) Interactions between zooplankton and fish in a fertilized Lake. *Holarctic Ecology* **5**, 273-310.
- Langeland A., L'Abée-Lund J.H., Jonsson B. & Jonsson N. (1991) Resource partitioning and niche shift in Arctic charr *Salvelinus alpinus* and brown trout *Salmo trutta*. *Journal of Animal Ecology* **60**, 895-912.
- Lindsey C.C. (1978) Form, function and locomotory habits in fish. In: *Fish Physiology vol. 7* (ed. by W. S. Hoar & D. J. Randall), pp. 1-100. Academic Press, London.
- Martin T.G., Wintle B.A., Rhodes J.R., Kuhnert P.M., Field S.A., Low-Choy S.J., Tyre A.J. & Possingham H.P. (2005) Zero tolerance ecology: improving ecological inference by modelling the source of zero observations. *Ecology Letters* **8**, 1235-1246.
- Mittelbach G.G. & Persson L. (1998) The ontogeny of piscivory and its ecological consequences. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 1454-1465.
- R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.

- Rahkonen R., Aalto J., Koski P., Sarkka J. & Juntunen K. (1996) Cestode larvae *Diphyllbothrium dendriticum* as a cause of heart disease leading to mortality in hatchery-reared sea trout and brown trout. *Diseases of Aquatic Organisms* **25**, 15-22.
- Rahkonen R. & Koski P. (1997) Occurrence of cestode larvae in brown trout after stocking in a large regulated lake in northern Finland. *Diseases of Aquatic Organisms* **31**, 55-63.
- Reimchen T.E. (1990) Size-structured mortality in a threespine stickleback (*Gasterosteus aculeatus*) - cutthroat trout (*Oncorhynchus clarki*) community. *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 1194-1205.
- Sánchez-Hernández J. & Amundsen P-A. (2015) Trophic ecology of brown trout (*Salmo trutta* L.) in subarctic lakes. *Ecology of Freshwater Fish* **24**, 148-161.
- Scholz T., Garcia H.H., Kuchta R. & Wicht B. (2009) Update on the human broad tapeworm (genus *Diphyllbothrium*), including clinical relevance. *Clinical Microbiology Reviews* **22**, 146-160.
- Skoglund S., Knudsen R. & Amundsen P-A. (2013) Selective predation on zooplankton by pelagic Arctic charr, *Salvelinus alpinus*, in six subarctic lakes. *Journal of Ichthyology* **53**, 849-855.
- Torres P., Lopez J.C., Cubillos V., Lobos C. & Silva R. (2002) Visceral diphyllbothriosis in a cultured rainbow trout, *Oncorhynchus mykiss* (Walbaum), in Chile. *Journal of Fish Diseases* **25**, 375-379.
- Valtonen E.T. & Julkunen M. (1995) Influence of the transmission of parasites from prey fishes on the composition of the parasite community of a predatory fish. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 233-245.
- Vik R. (1964) The genus *Diphyllbothrium*: an example of the interdependence of systematics and experimental biology. *Experimental Parasitology* **15**, 361-380.
- Wicht B., de Marval F., Gottstein B. & Peduzzi R. (2008) Imported diphyllbothriasis in Switzerland: molecular evidence of *Diphyllbothrium dendriticum* (Nitsch, 1824). *Parasitology Research* **102**, 201-204.
- Williams H. & Jones A. (1994) Parasitic worms of fish. Taylor & Frances Ltd., London.

Wootton R.J. (1984) A functional biology of sticklebacks. Croom Helm, London.

Wootton R.J. (1998) Ecology of teleost fishes. 2nd edn. Kluwer Academic Publishing, Dordrecht.

Zuur A.F., Ieno E.N., Walker N.J., Saveliev A.A. & Smith G.M. (2009) Mixed effects models and extensions in ecology with R. In: *Statistics for Biology and Health* (ed. by M. Gail, K. Krickeberg, J. M. Samet, A. Tsiatis & W. Wong). Springer, New York.

Table 1 Catch data and summary statistics on the *Diphyllbothrium* spp. infections recorded in trout from the three groups of fish species composition.

Group	T	TC	TCS
Fish species composition	Brown trout	Brown trout Arctic charr	Brown trout Arctic charr Three-spined stickleback
Number of trout	214	113	268
Mean trout length, mm \pm SD	201 \pm 66 ^{1,2}	186 \pm 58 ^{1,3}	215 \pm 74 ^{2,3}
<i>Diphyllbothrium</i> spp.			
Mean abundance	0.2 ^{a,b}	2.6 ^{a,c}	17.9 ^{b,c}
Prevalence	10.2 ^{d,e}	45.7 ^{d,f}	45.6 ^{e,f}
Mean intensity	2.2 ^{g,h}	5.7 ^{g,i}	39.4 ^{h,i}

Significant difference in means: One-way analysis of variance (ANOVA): ³ 0.001 level, ^{1,2} no significant difference. Permutation test: ^{g,h} 0.05 level, ^c 0.01 level, ^{a,b,d,e,i} 0.001 level, ^f no significant difference.

Legends

Figure 1 Locations of the eight lakes sampled in northern Norway. Six of the lakes are found in the Nordland county (lower insert) while two are found in the Troms county (upper insert).

Figure 2 Mean abundance of *Diphyllbothrium* spp. in brown trout as a function of fish species composition. Error bars indicate standard error.

Figure 3 Abundance ($\log x + 1$) of *Diphyllbothrium* spp. as a function of fish length in brown trout from the three groups of fish species composition. Linear regressions are included to indicate the overall pattern in the data. Group T = brown trout in allopatry. Group TC = brown trout in sympatry with only Arctic charr. Group TCS = brown trout in sympatry with Arctic charr and three-spined stickleback.

Figure 4 Probability of *Diphyllbothrium* spp. infection in brown trout as a function of fish length according to the binomial generalized linear part of the ZANB model. Lines indicate the three groups of fish species composition. Solid line = brown trout in allopatry. Dashed line = brown trout in sympatry with only Arctic charr. Dotted line = brown trout in sympatry with Arctic charr and three-spined stickleback.

Figure 5 Intensity of *Diphyllbothrium* spp. in brown trout as a function of fish length predicted by the truncated negative binomial generalized linear part of the ZANB model. Lines indicate the three groups of fish species composition. Solid line = brown trout in

allopatry. Dashed line = brown trout in sympatry with only Arctic charr. Dotted line = brown trout in sympatry with Arctic charr and three-spined stickleback. Note the exponential y-axis.

Figure 1

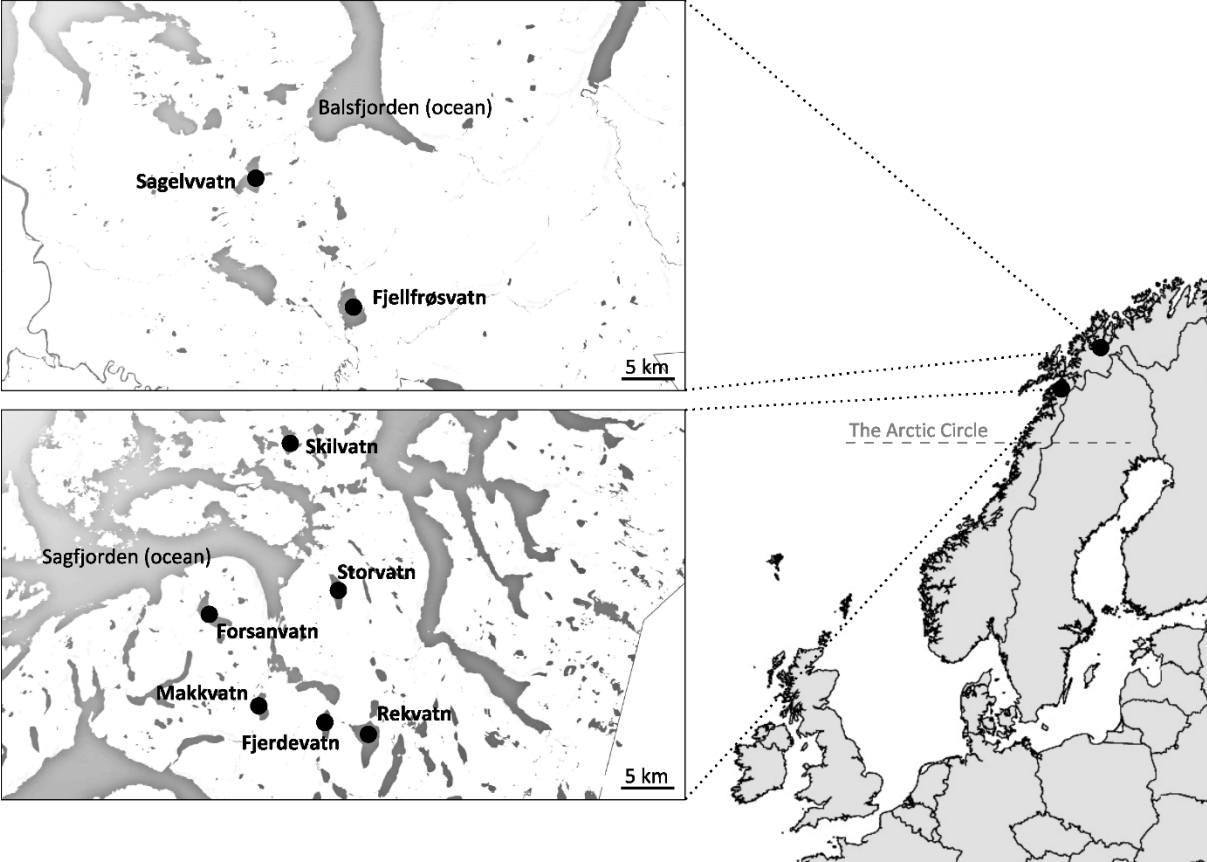


Figure 2

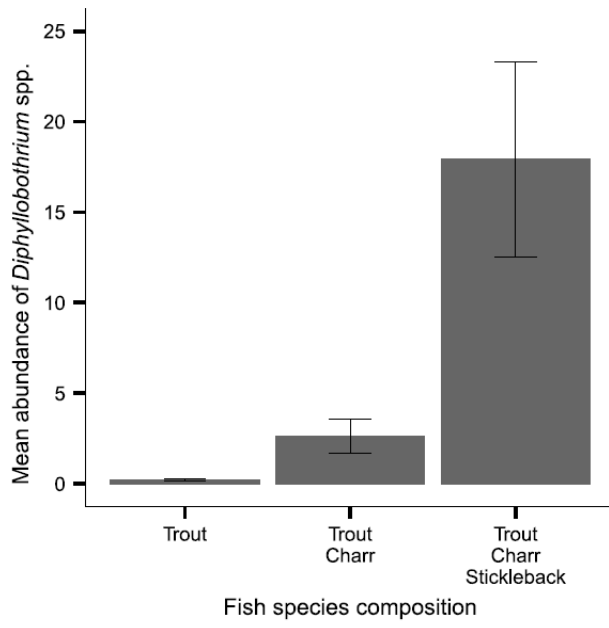


Figure 3

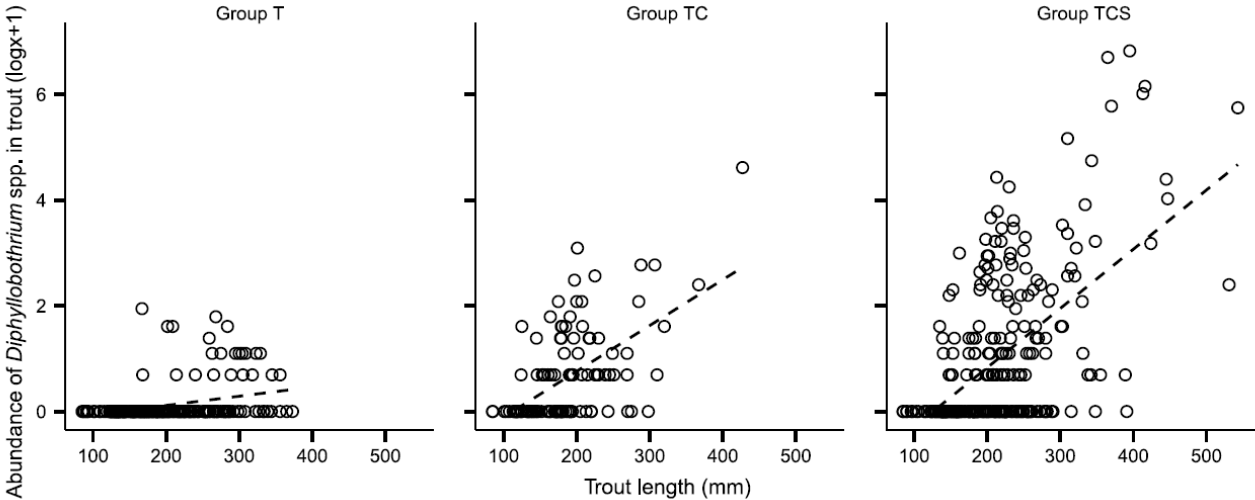


Figure 4

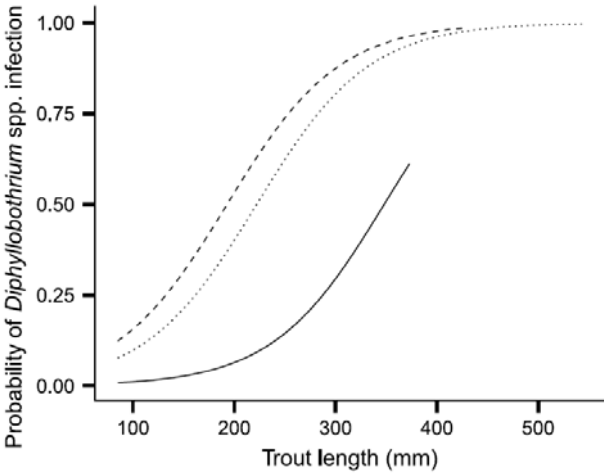


Figure 5

