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The distribution of *Eubothrium crassum* (Cestoda: Pseudophyllidea) in brown trout, *Salmo trutta*, in allopatric and sympatric populations

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Summary

Sticklebacks in particular have been suspected to have a role in transmission of *E. crassum* to its final host, the brown trout. In this study, prevalence and mean abundance of *E. crassum* in trout have been examined in allopatric and sympatric populations to address the suspected role of sticklebacks in the life cycle of *E. crassum*.

1106 trout from 22 lakes in the northern parts of Norway were examined for *E. crassum* infections. The lakes represented four different compositions of fish communities: 1) allopatric trout, 2) trout in sympatry with charr, 3) trout in sympatry with charr and sticklebacks and, 4) trout in sympatry with sticklebacks. 605 of sampled trout, from 13 lakes were examined regarding piscivory. All four fish communities were represented.

19.6 % (217) of examined trout were infected with *E. crassum*. The prevalence and mean abundance of *E. crassum* in trout were significantly higher in sympatric populations. In the allopatric populations none of the trout were infected with *E. crassum*. Trout in sympatry with charr had low prevalence (< 7 %) and mean abundance (< 0.1) of *E. crassum* in some of the lakes, but the majority of lakes had no infected trout. The most significant prevalence and mean abundance were recorded in lakes where sticklebacks were present. In lakes with a fish community of trout, charr and stickleback, the prevalence and mean abundance of *E. crassum* varied among the lakes (prevalence: 0 - 90 %, mean abundance (0 - 13.8) but were overall significantly higher than the fish communities without sticklebacks. The lakes with a fish community of trout and sticklebacks had significantly higher prevalence (57 - 84 %) and mean abundance (4.1 - 39.4) than any of the other fish communities.

Trout was found to be more frequently piscivorous in sympatric populations with sticklebacks. Intensity of *E. crassum* in trout was found to have a strong, positive correlation with degree of piscivory. Accordingly, length of trout had a slight, positive correlation with intensity of *E. crassum*.

Sticklebacks were documented to have a crucial role in transmission of *E. crassum* to trout. From the data in this study, copepods seems to be of low importance in transmission of *E. crassum* to trout.

Introduction

Eubothrium (Nybelin, 1922) is a holarctic and circumpolar distributed genus of cestodes known to infect several species of salmonids (Kennedy 1978, Andersen & Kennedy 1983). The species of this genus typically reside in the final host intestinal tract, where they absorb nutrients, which may reduce the growth of the host (Kennedy 1978, Andersen & Kennedy 1983, Saksvik et al. 2001b, Hanzelová et al. 2005). *E. crassum* (Bloch, 1779) and *E. salvelini* (Linnaeus, 1758) are two species in this genus, commonly found in freshwater salmonids in Norway (Vik 1963, Kennedy 1978). These species are known to have a high specificity towards their final hosts: *E. crassum* utilizes species of *Salmo* (Linnaeus, 1758), and *E. salvelini* utilizes species of *Salvelinus* (Richardson, 1863) as final hosts (Scholz et al. 2003).

E. crassum is a trophically transmitted cestode commonly found in Norwegian brown trout, *Salmo trutta* (Linnaeus, 1758), populations (Vik 1963, Kennedy 1978). The brown trout (hereafter referred to as trout) gets infected by *E. crassum* by ingesting prey carrying the larval stage of the parasite (Vik 1963, Kennedy 1978). *E. crassum* has an expected lifetime of one to two years in the final host (Hanzelová et al. 2002, Prati et al. 2020). Copepods are the only intermediate host for this parasite (Vik 1963, Kennedy 1978). However, there is growing evidence that prey fish may also act either as second intermediate hosts, paratenic hosts or as accidental hosts for *E. crassum* (Vik 1963, Kennedy 1978, Williams and Jones 1994, Saksvik et al. 2001a, Hanzelová et al. 2002).

Trout favour the littoral zone of lakes (Klemetsen et al. 2003, Knudsen et al. 2008, Eloranta et al. 2013). However, which habitat the trout inhabit may vary with size and age, available resources, co-occurrence with other fish species and predation risk (Jonsson & Gravem 1985, Sánchez-Hernándes & Amundsen 2015). Consequently, larger trout have been found to shift periodically or more permanently to the pelagic habitat (Jonsson 1989, Klemetsen et al. 2003). The composition of the fish community can also influence the habitat choice of the trout (Sánchez-Hernándes & Amundsen 2015). In sympatric populations with three-spined sticklebacks, *Gasterosteus aculeatus* (Linnaeus, 1758) (hereafter referred to as stickleback), trout have been shown to partly stay in the pelagic, whereas the sticklebacks dominated in the littoral (Sánchez-Hernándes & Amundsen 2015). In sympatry with arctic charr, *Salvelinus alpinus* (Linnaeus, 1758), (hereafter referred to as charr), trout dominated in the littoral, while charr were driven towards the pelagic and the profundal (Sánchez-Hernándes & Amundsen 2015).

Trout are opportunistic feeders that includes a variety of prey in their diet, ranging from small zooplankton and other invertebrates to relatively large fish (Jonsson 1989, Klemetsen et al. 2003). With increasing length, they undergo ontogenetic dietary shifts with some individuals becoming piscivorous (L´Abée-Lund et al. 1992, Klemetsen et al. 2003, Prati et al. 2020). Hence, trout are regarded secondary piscivores and often undertake a piscivorous diet when reaching a body length of 20 – 25 cm (L´Abée-Lund et al. 1992, Damsgård 1995, Jensen et al. 2004). However, small prey fish like sticklebacks can be feed upon by even smaller sizes of trout, from ~ 15 cm (L´Abée-Lund et al. 1992). Trout are typically more frequently piscivorous in sympatry than in allopatry (L´Abée-Lund et al. 1992, Sánchez-Hernández & Amundsen 2015). In sympatry, L´Abée-Lund et al. (1992) found that trout of all lengths preferred sticklebacks as prey fish over small salmonids. These ontogenetic dietary shifts might limit the infection rate of *E. crassum* in bigger trout because they do not feed upon the small intermediate hosts of the parasite, copepods. Still, high *E. crassum* intensities have been observed in large piscivorous individuals, indicating that prey fish such as sticklebacks might act as either second intermediate hosts, paratenic hosts or accidental hosts for this parasite (Vik 1963, Kennedy 1978, Williams & Jones 1994, Prati et al. 2020).

Sticklebacks are common in Norwegian lakes and constitute an important food source for piscivorous trout (L'Abée-Lund et al. 1992, Amundsen 1994, Knudsen et al. 2008, Eloranta et al. 2013, Knudsen et al. 2016). Sticklebacks feed on zooplankton, hence they are susceptible to infections by copepod-transmitted parasites (Langeland 1982, Jørgensen & Klemetsen 1995). In examining the food web of a sub-arctic lake stickleback has been found to be a key species in transmission of several parasites to predators (Amundsen et al. 2009, 2013). The species is an intermediate or paratenic host for other parasitic cestodes like *Schistocephalus solidus* (Müller, 1776), *Diphyllobothrium ditremum* (Creplin, 1825) and *D. dendriticum* (Nitzsch, 1824) (Halvorsen 1970, Giles 1983, Kuhn et al. 2015, 2016). Vik (1963) suggested that sticklebacks may also have a role in transmission of *E. crassum* to trout, due to findings of plerocercoids embedded in the liver, encysted on the surface of the viscera and free larvae in the abdominal cavity.

Several other studies also have indicated that small prey fish have a role in the transmission of *E. crassum* to (piscivorous) trout. For instance charr, trout and fish of the genus *Coregonus* (Linnaeus, 1758) and *Perca* (Linnaeus, 1758) have been suspected to act as either second intermediate hosts, paratenic hosts or accidental hosts for *E. crassum* (Vik 1963, Kennedy 1978, Williams and Jones 1994, Saksvik et al. 2001a, Hanzelová et al. 2002). If so piscivorous trout can get infected without feeding upon infected copepods.

In allopatric populations of trout, *E. crassum* obviously has no other smaller fish species to exploit, and infections in trout must therefore originate from predation upon infected copepods. In sympatric populations, however, the chances of the parasite completing its lifecycle may increase if suitable intermediate, paratenic or accidental fish hosts are available and predated upon by trout. Hence, the fish community composition can affect the rate of infections in large piscivorous trout.

This study addresses whether sticklebacks have a significant role in the transmission of *E. crassum* to brown trout. To my knowledge, no similar studies to date have been performed to investigate this suggestion presented in several papers (e.g. Vik 1963, Kennedy 1978, Williams and Jones 1994, Saksvik et al. 2001a, Hanzelová et al. 2002). The prevalence and abundance of *E. crassum* in brown trout from subarctic lakes in Norway with four different fish communities were analysed: trout in 1) allopatry, 2) sympatry with charr, 3) sympatry with charr and sticklebacks and, 4) sympatry with sticklebacks. The aim of this study was to investigate if sticklebacks have a role in transmission of *E. crassum* to trout. On basis of this, the following hypothesis has been examined:

The prevalence and mean abundance of *E. crassum* in brown trout are higher when trout lives in sympatry with other species of fish than in allopatric populations.

Based on this hypothesis the following expectations have been examined: (1) Infections of *E. crassum* in brown trout are expected to be higher in lakes where three-spined sticklebacks are present, and (2) infections are expected to be positively correlated with the degree of piscivory.

Materials and methods

Study lakes

The brown trout were collected from 22 lakes in the central and northern part of Norway (fig. 1, tab. 1). The sampling lakes differ in composition of fish community (tab. 1): three lakes have allopatric populations of trout, whereas trout live in sympatry with other species of fish in the remaining. Nine of the lakes are inhabited by two fish species. Two lakes hold trout and sticklebacks, and seven lakes are inhabited by trout and char. The remaining 10 lakes have a fish community consisting of trout, charr and sticklebacks.

Two lakes are located in Trøndelag region (fig. 1, tab.1), while the remaining are situated in northern Norway, south of the former border between Troms region and Finnmark region with 17 lakes being above the Arctic Circle.

The lakes are oligotrophic, relatively deep and dimictic (Knudsen et al. 2008, Eloranta et al. 2013, Hans-Henrik Grøn 2014, Sánchez-Hernández et al. 2017, Paterson et al. 2018, Paterson et al. 2019). Among the studied lakes, Buttelvatn is the shallowest with a maximum depth of 21 m, and Lille Rostavatn is the deepest with a maximum depth of 92 m (Knudsen et al. 2008, Paterson et al. 2018, Paterson et al. 2019, Birkeland et al. 2020, NVE 2021). The lakes Gangåsvatn, Våvatn, Rekvatn, Forsanvatn, Storvatn (Hamarøy), Jernvatn and Sirkelvatn are regulated (tab. 1) (NVE 2021).

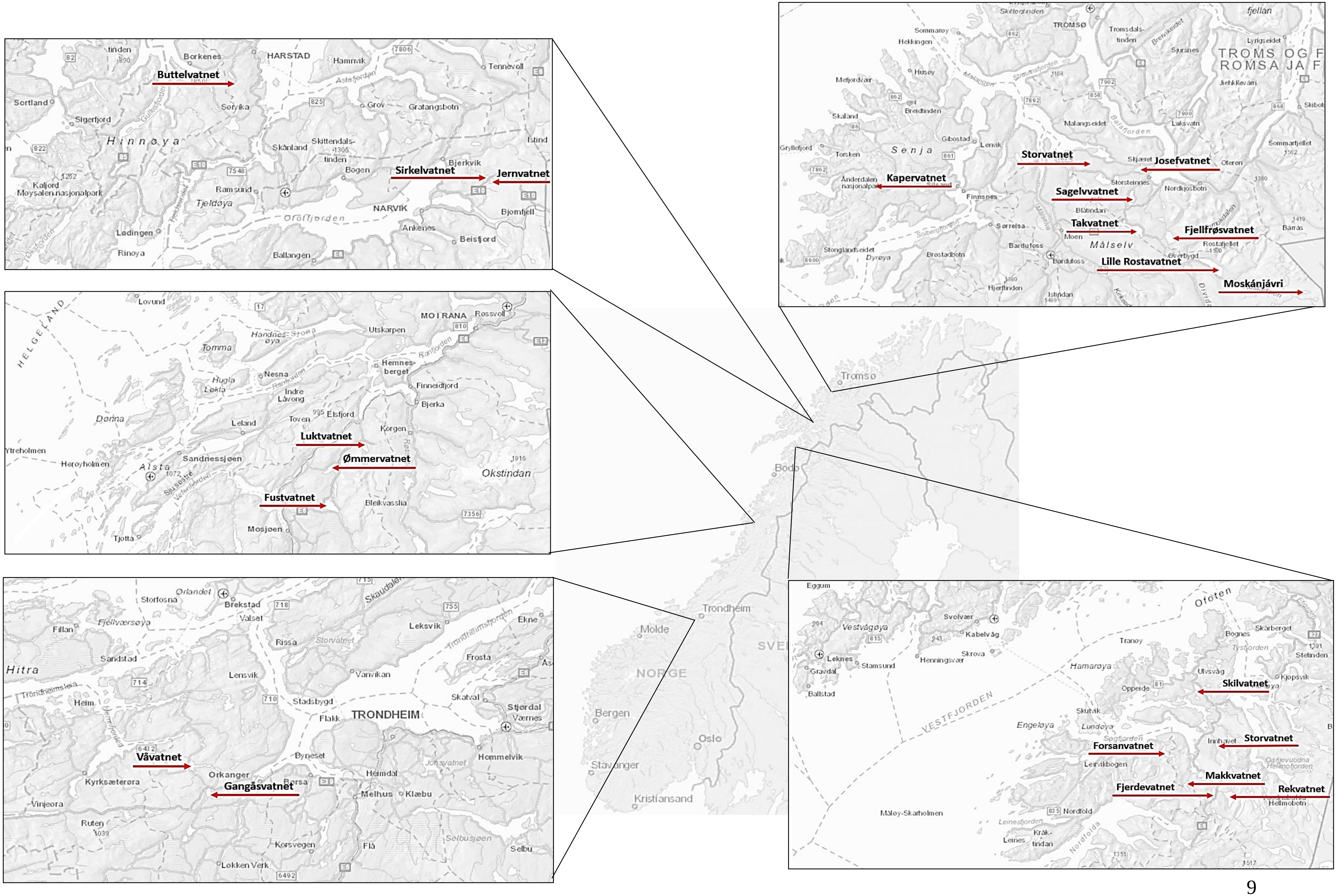

Fig. 1. Map over Norway showing approximate location of study lakes. Made by Anne Hofstad Lian based on The Norwegian Water Resources and Energy Directorates map database 2021.

Tab. 1. Information on studied trout lakes: geographical coordinates, fish community, meters above sea level (MSL), size and regulation of lake. (Knudsen et al. 2008, Hans-Henrik Grøn 2014, Eloranta et al. 2013, Sánchez-Hernández et al. 2017, Paterson et al. 2018, Paterson et al. 2019, NVE 2021).

LAKE	**LOCATION (COORDINATES, DMS)**	**SPECIES IN LAKE**		**MSL**	**SIZE (KM^2)**	**REGULATED LAKE**
		Salvelinus alpinus	*Gasterosteus aculeatus L.*			
GANGÅSVATN	63°16´12.6´´N, 09°38´38.0´´E	x	x	153	5.50	x
VÅVATN	63°19´39.0´´N, 09°32´40.3´´E	x		300	4.94	x
FUSTVATN	65°54´22.2´´N, 13°23´55.6´´E	x	x	38	10.71	
ØMMERVATN	65°59´35.2´´N, 13°25´09.8´´E	x	x	42	5.47	
LUKTVATN	66°02´30.4´´N, 13°34´26.1´´E	x	x	137	3.74	
REKVATN	67°48´36.3´´N, 16°04´57.2´´E	x		284	7.39	x
SKILGÁJÁVRRE (FJERDEVATN)	67°49´18.3´´N, 15°58´51.9´´E		x	72	2.27	
MAKKVATN	67°50´16.4´´N, 15°49´30.1´´E	x	x	114	3.03	
FORSANVATN	67°55´07.8´´N, 15°41´57.8´´E			259	4.81	x
STORVATN (HAMARØY)	67°56´21.8´´N, 16°00´29.7´´E			149	2.63	x
SKILVATN	68°04´21.6´´N, 15°53´45.1´´E	x	x	35	3.29	
JERNVATN	68°29´56.9´´N, 17°51´00.7´´E	x		299	3.65	x
SIRKELVATN	68°30´17.7´´N, 17°47´22.9´´E	x		273	1.23	x
BUTTELVATN	68°44´00.8´´N, 16°24´34.9´´E	x	x	174	0.29	
MOSKÁNJÁVRI*	68°55´15.2´´N, 20°11´48.4´´E	x		592	1.78	
LILLE ROSTAVATN**	69°00´24.4´´N, 19°36´08.8´´E	x		102	13.27	
FJELLFRØSVATN (GEATKEJÁVRI)	69°04´57.7´´N, 19°20´18.0´´E	x		125	6.74	
TAKVATN	69°06´50.5´´N, 19°04´55.3´´E	x	x	214	15.27	
SAGELVVATN	69°11´27.6´´N, 19°06´13.0´´E	x	x	92	5.11	
KAPERVATN (GAHPERJÁVRI)	69°14´53.0´´N, 17°24´32.4´´E			168	1.32	
JOSEFVATN	69°15´46.8´´N, 19°09´47.3´´E	x	x	91	3.40	
STORVATN (BALSFJORD)	69°16´42.6´´N, 18°49´52.2´´E		x	222	0.39	

Additional fish species: «*» burbot (*Lota lota* L.) (Linnaeus, 1758), «**» burbot (*Lota lota* L.), grayling (*Thymallus thymallus* L.) (Linnaeus, 1758), Atlantic salmon parr (*Salmo salar* L.) (Linnaeus, 1758) and Eurasian minnow (*Phoxinus phoxinus*) (Linnaeus, 1758).

Fish sampling

In total, 1106 trout collected during 25 samplings conducted in the months of July to October from 1992 to 2020 were examined (tab. 2). These include multiple samplings for Lille-Rostavatn (two), and Takvatn (three). Sampling was carried out using multi-mesh survey gill nets. Most of the fish was caught in the littoral habitat with bottom nets placed between 1.5 and 15 m depths. In ten lakes (Gangåsvatn, Fjerdevatn, Forsanvatn, Makkvatn, Skilvatn, Jernvatn, Sirkelvatn, Josefvatn, Sagelvvatn and Takvatn) fish were also sampled in the pelagic habitat using floating nets. These nets were 6 m deep and placed from the surface down towards the profundal (above 20 m depth). The samplings were conducted over a period of one to four days with the nets set overnight for 10-13 hours. Further details about the samplings in the different lakes are given by Knudsen et al. (2008), Hans-Henrik Grøn (2014), Eloranta et al. (2013), Sánchez-Hernández et al. (2017), Paterson et al. (2018) and Paterson et al. (2019). In the field, fork length (mm), weight (g), and sex of the trout were recorded. Stomachs were opened, and the total fullness was visually determined on a percentage scale ranging from empty (0 %) to full (100 %). The stomach contents were preserved in 96 % ethanol, and the intestines were frozen at – 20 ℃ for parasitological and dietary analyses at a later time in the laboratory.

Analyses of fish

Parasite examinations

The intestinal parasites were sampled by cutting the intestines open, including the pyloric caecae, and filtering the contents under running water with a 50-micron mesh size nylon net. The clean matter was then transferred to a Petri dish and examined under a stereomicroscope using 10-40x objective lenses. The number of scolexes matching characteristics of *E. crassum* specified by Andersen & Kennedy (1983) and Chubb et al. (1987) were identified and counted in each trout (tab. 2). The scolex was recognised as *E. crassum* when two deep indentations and a flat surface on the smooth apical disc was observed (fig. 2).

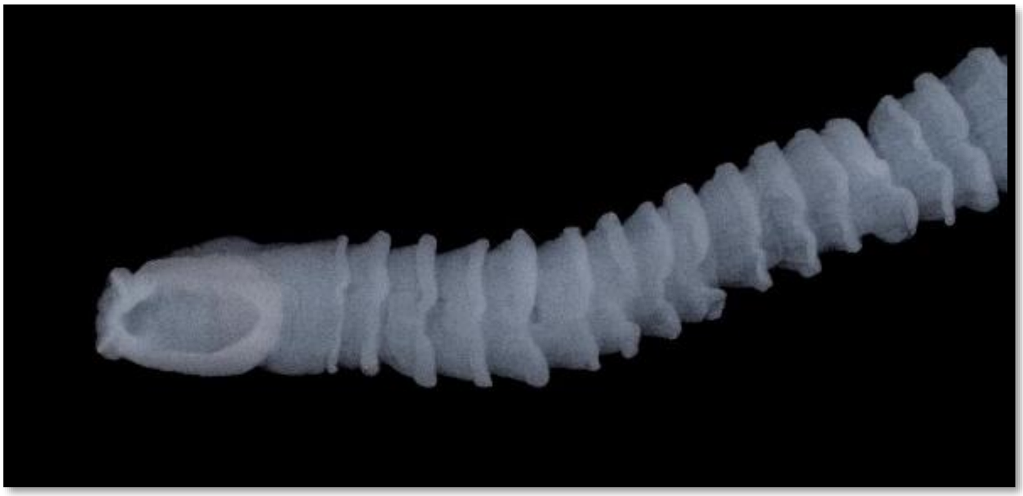

Fig. 2. *E. crassum* found in brown trout from Lake Takvatn 2020. (Photo: Sebastian Prati).

Diet examinations

In the laboratory, the gastrointestinal content of each trout was analysed by identifying prey items to the species, genus, or family level and estimating their relative contribution to the total stomach contents according to the method described by Amundsen (1995). Stomach content was examined in the lakes Fjellfrøsvatn, Lille-Rostavatn, Buttelvvatn, Ømmervatn and Luktvatn. For the lakes Rekvatn, Fjerdevatn, Makkvatn, Forsanvatn, Skilvatn, Jernvatn and Sirkelvatn, however, only the intestinal prey content was examined. From Takvatn both stomach and intestinal food contents were examined. Fish that had empty stomachs were excluded from further analyses. In total, the gastrointestinal tract of 605 trout individuals between 8,9 cm and 44,7 cm of length, were used for subsequent analyses (tab. 2, appendix tab. 2). For this study purpose, the trout were categorized according to visual traces of prey fish in the gastrointestinal tract, hence either as piscivorous or without prey fish. Each of the four fish community compositions (allopatric trout, trout in sympatry with charr, trout in sympatry with charr and sticklebacks, and trout in sympatry with sticklebacks) were represented among the 13 lakes (appendix tab. 2).

Tab. 2. Information on sampled trout: time of sampling, average length of trout, age, and diet analysis.

LAKE	TIME OF SAMPLING		CAUGHT *SALMO TRUTTA*		DIET ANALYSIS	
	YEAR	MONTH	N caught	AVERAGE LENGTH (mm) ± SD	N FISH	N PISCIVOROUS
GANGÅSVATN	2017	August	29	236 ± 28.9	0	-
VÅVATN	2017	August	27	226 ± 30.0	0	-
FUSTVATN	2012	October	30	303 ± 36.9	0	-
ØMMERVATN	2012	October	30	285 ± 84.9	19	6
LUKTVATN	2013	October	30	262 ± 36.2	30	0
REKVATN	2013	August	43	167 ± 38.2	38	0
SKILGÁJÁVRRE (FJERDEVATN)	2013	August	47	244 ± 93.5	45	8
MAKKVATN	2013	August	63	198 ± 44.8	51	9
FORSANVATN	2013	August	80	219 ± 64.3	79	0
STORVATN (HAMARØY)	2013	July	80	182 ± 52.0	0	-
SKILVATN	2013	August	33	195 ± 38.2	31	9
JERNVATN	2014	August	20	252 ± 45.4	16	1
SIRKELVATN	2014	August	18	189 ± 40.5	16	0
BUTTELVATN	2000	October	33	217 ± 21.0	33	12
MOSKÁNJÁVRI	2016	August	9	302 ± 76.5	0	-
LILLE ROSTAVATN	2010	August	19	236 ± 51.1	0	-
LILLE ROSTAVATN	2018	August and October	43	179 ± 34.7	43	1
FJELLFRØSVATN (GEATKEJÁVRI)	1992	August-October	154	193 ± 56.8	154	1
TAKVATN	2010	August	76	213 ± 65.9	0	-
TAKVATN	2017	August	50	205 ± 42.0	50	6
TAKVATN	2020	August	51	222 ± 87.1	0	-
SAGELVVATN	2010	August	60	240 ± 89.0	0	-
KAPERVATN (GAHPERJÁVRI)	2019	August	20	226 ± 54.8	0	-
JOSEFVATN	2010	August	36	225 ± 71.9	0	-
STORVATN (BALSFJORD)	2019	September	25	247 ± 63.8	0	-

Statistical analyses

Statistical and descriptive analyses were carried out with use of the software R, version 4.0.3 (R Development Core Team, 2020), and Microsoft Office Excel 365.

Prevalence and mean abundance of *E. crassum* infecting brown trout

The quantitative parameters prevalence and mean abundance were calculated according to Bush et al. (1997) for the overall sampled trout, the individual lakes, and for each of the four fish community compositions. A 95 % confidence interval for the mean abundance of *E. crassum* was calculated using Bootstrap BCA with 5000 replications for all four fish community compositions. For visualization of prevalence and mean abundance in each lake, bar charts were made using Excel, whereas R was used to illustrate prevalence and mean abundance (with 95 % confidence interval) in relation to the four different fish communities.

The variance of prevalence and mean abundance of *E. crassum* were calculated to assess the distribution for each of the four fish community compositions. Due to overdispersion ($S^2/\bar{x} > 1$) (appendix tab. 1), unequal variances and independent observations the Chi square test and the Kruskal-Wallis test was further employed. This was done to assess significant differences in prevalence (Chi square) and mean abundance (Kruskal-Wallis) of *E. crassum* between the four different fish community compositions. A significant difference between the fish community compositions was concluded with a p-value < 0.05 (corresponding confidence level of 95 %).

Length as a factor on prevalence and intensity of *E. crassum*

Distribution of the data for trout length, intensity of *E. crassum* and prevalence of *E. crassum* was assessed employing the Shapiro-Wilk test in R. The data was concluded to have a non-normal distribution with a p- value < 0.05 (appendix tab. 5).

Infected trout was categorized in to three groups according to length of trout: < 15 cm, 15 – 20 cm and > 20 cm. For visualization of the prevalence of *E. crassum* among the different length groups a box chart was made in Excel. The Chi square test was employed to assess significance of the difference in prevalence of *E. crassum* between the length groups. The differences were determined significant with a p- value < 0.05.

Due to overdispersion of the data the Spearman rank correlation test was employed in R to assess correlation between intensity of *E. crassum* and length of trout. For illustration of the correlation a scatterplot was made. The correlation was concluded significant with a p-value < 0.05.

To assess length of trout and fish community as factors affecting prevalence and intensity of *E. crassum*, separate General linear models (GLM) was employed in R. The prevalence (presence-absence) and intensity were set as the response variables and length of the trout and fish community as predictors. As the presence-absence data were not equally distributed (p-value < 0.05), a binomial GLM with cloglog link was used for prevalence, while a negative binomial GLM was used for intensity to account for overdispersion of the data (appendix tab. 5). An analysis of variance (ANOVA) was then performed to compare the predictors affecting prevalence and intensity of *E. crassum*. The predictors were concluded to significantly affect the variables with a p- value < 0.05.

Correlation between piscivory and prevalence of *E. crassum*

The Shapiro-Wilk test was employed to assess distribution of piscivorous trout and the prevalence of *E. crassum* for the dietary dataset. Due to an overdispersion (p- value <0.05) (appendix tab. 5) the Spearman Rank correlation test was employed to assess correlation between the proportion of piscivorous trout and the prevalence of *E. crassum*. The correlation was determined significant with a p- value < 0.05. For visualization a scatterplot was made in R. The plot was made to illustrate prevalence of *E. crassum* in relation to piscivory in each lake and for the different fish community compositions.

Factors varying with intensity of *E. crassum* in brown trout

To assess variation of *E. crassum* infections between lakes with different fish community composition, a Principal Component Analysis (PCA) was employed in R. Intensity of *E. crassum* was used as the response variable whereas the different fish communities, fish length, lake elevation and surface area of lake were used as predictor variables. The lakes with allopatric trout were excluded from the analysis due to no infections of *E. crassum* amongst this group.

To assess correlation between predictor variables and the response variable from the PCA, distribution of lake elevation and surface area of lake data was tested using the Shapiro-Wilk test in R. Due to overdispersion (p- value < 0.05) (appendix tab. 5) the Spearman Rank correlation test was employed in R. Correlation was tested between intensity of *E. crassum* and lake elevation, and between intensity of *E. crassum* and surface area of lake. The correlation was concluded significant with a p- value output from the Spearman rank correlation test < 0.05.

Results

Prevalence and mean abundance of *E. crassum* infecting brown trout

Overall, 19.6 % (217 trout) of the 1106 examined trout were infected by *E. crassum*. The parasite infected trout in 14 of the 22 lakes (fig. 3, appendix tab. 1). In eight of the lakes there were no infected trout: None of the three allopatric trout populations, four of the seven lakes with a fish community of trout and charr and one of the ten lakes with trout, charr and sticklebacks (Josefsvatn). The prevalence of *E. crassum* varied greatly among the different lakes (0 - 90 %). In the trout populations in sympatry with charr there was a slight occurrence of the parasite in three of the seven lakes, but it varied from 3 % to 7 %. The lakes where trout lives in sympatry with charr and sticklebacks had great variations in prevalence of *E. crassum*, ranging from 0 % to 90 %. Six of the ten lakes with all three fish species present, had a prevalence > 20 %. The two lakes with trout in sympatry with sticklebacks had a high prevalence of *E. crassum*, > 50 %. One lake had a prevalence of 57 % and the other a prevalence of 84 %.

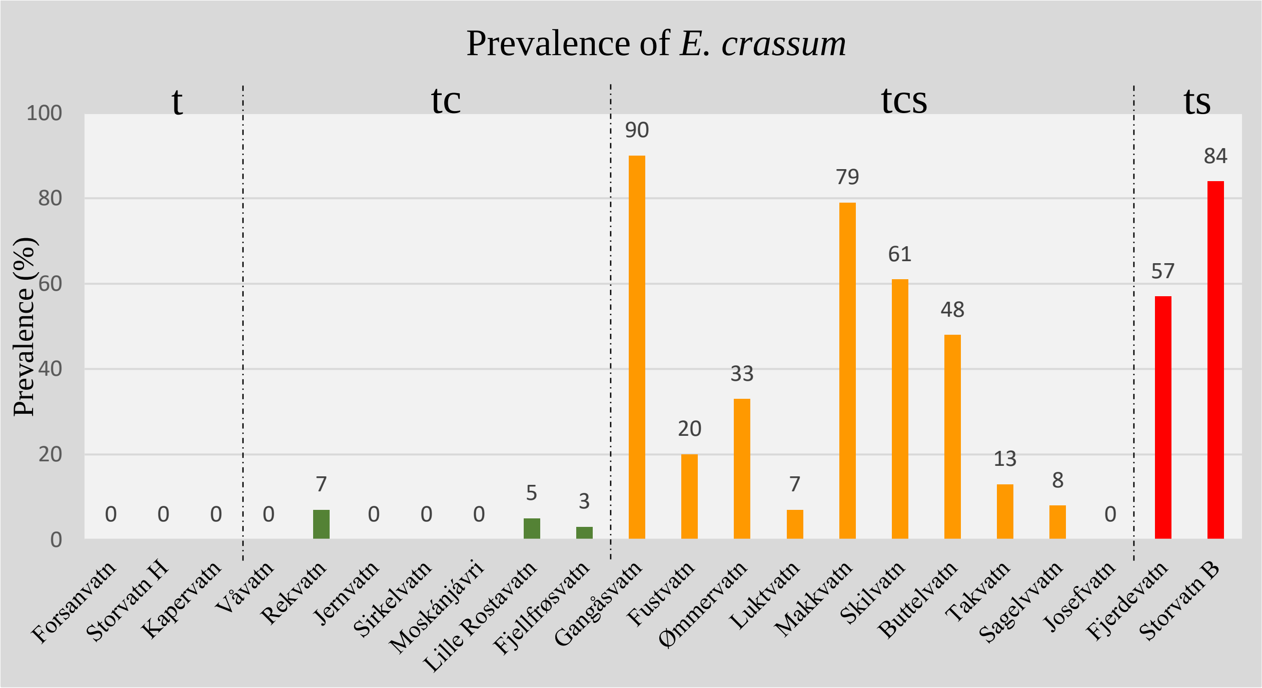

Fig. 3. Prevalence of *E. crassum* in brown trout from each sample lake. Lakes are sorted in relation to composition of the fish community. From left: three lakes with allopatric trout (t), seven lakes with trout and charr (tc), ten lakes with trout, charr and sticklebacks (tcs), and the two lakes with trout and sticklebacks (ts).

The mean abundance for the 1106 sampled trout was estimated to 2.41. However, the mean abundance varied greatly among the lakes, ranging from 0 to 39.4 (fig. 4, appendix tab. 1). The mean abundance was very low in the fish communities consisting of trout and charr, 0 - 0.1. In the lakes with trout, charr and sticklebacks however the mean abundance was higher, although it varied between the lakes, 0 - 13.9. The lakes with a fish community of trout and sticklebacks had very different mean abundances. One of these lakes, Storvatn in Balsfjord, had by far the highest mean abundance of *E. crassum* (39.4) than any of the other lakes in this study. The other lake with a fish community of trout and sticklebacks had a mean abundance of 4.1.

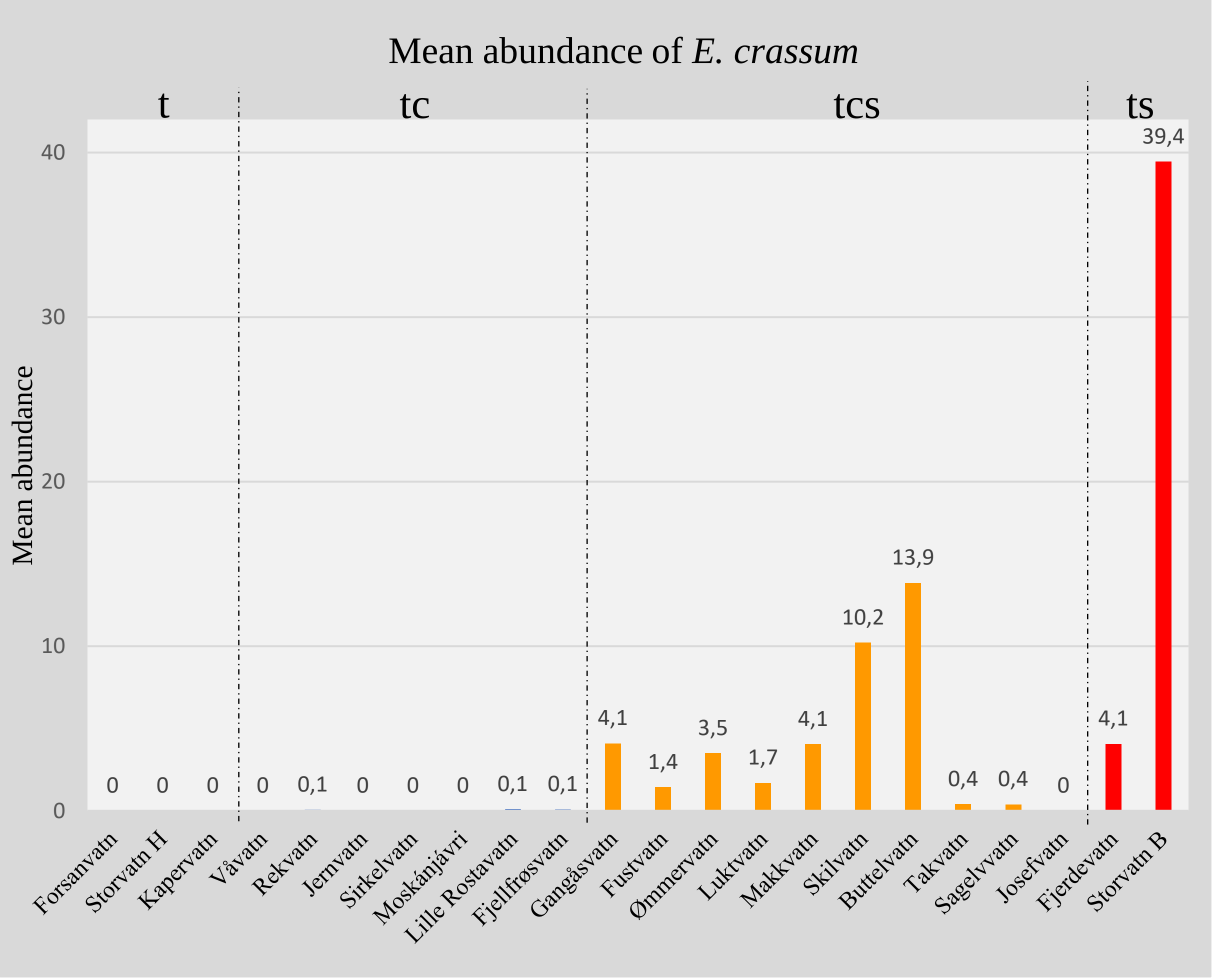

Fig.4. Mean abundance of *E. crassum* in brown trout from each sample lake. Lakes are sorted in relation to composition of the fish community. From left: three lakes with allopatric trout (t), seven lakes with trout and charr (tc), ten lakes with trout, charr and sticklebacks (tcs), and the two lakes with trout and sticklebacks (ts).

Overall, both prevalence and mean abundance of *E. crassum* in trout varied greatly among the different compositions of fish communities (fig. 5, appendix tab. 1). Trout in sympatry with charr had an overall low prevalence (3 %) and mean abundance (0.07) of *E. crassum*. Both prevalence and mean abundance was significantly different from the allopatric fish community ($p < 0.05$) (appendix tab. 3 and 4). The fish community of trout, charr and sticklebacks had significantly higher prevalence (30 %) ($p < 0.01$) and mean abundance (2.80) ($p < 0.01$) than allopatric trout and trout in sympatry with charr (appendix tab. 3 and 4). Trout in sympatry with sticklebacks had the greatest prevalence (67 %) and mean abundance (16.30) of *E. crassum* in this study. Both prevalence ($p < 0.01$) and mean abundance ($p < 0.01$) varied significantly from the other compositions of fish communities (appendix tab. 3 and 4). The confidence intervals of mean abundance between the fish communities did not overlap.

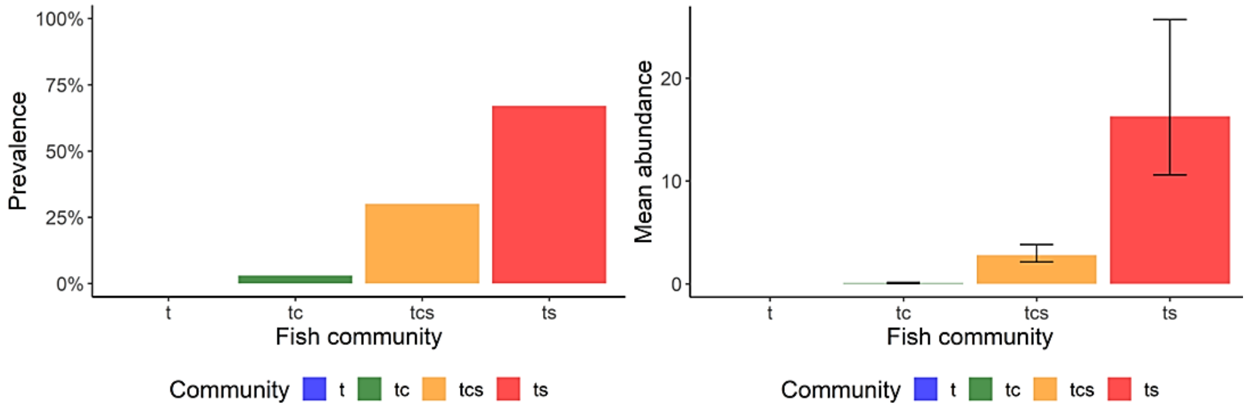

Fig. 5. Prevalence (left panel) and mean abundance (right panel) of *E. crassum* in trout sorted by lakes with different fish communities compositions: t (trout) (three lakes, 180 trout), tc (trout and charr) (seven lakes, 333 trout), tcs (trout, charr and sticklebacks) (ten lakes, 521 trout), and ts (trout and sticklebacks) (two lakes, 72 trout). 95 % confidence interval is given for the mean abundance.

Length of trout as a factor on prevalence and intensity of *E. crassum*

The majority of the 217 infected trout, 73.7 %, had a length > 20 cm (fig. 6). 21.2 % of examined trout with *E. crassum* infections had a length between 15 and 20 cm. 5.1 % of infected trout had a length < 15 cm. The smallest infected trout had a length of 10.8 cm. The difference in infection among the three length groups was significant ($p < 0.05$) (appendix tab. 6). Testing for correlation between length of trout and intensity of *E. crassum*, however, did not show a strong positive correlation (fig. 7, appendix tab. 8). The test included all 1106 of the sampled trout and the data was overdispersed: 889 of the trout were not infected and 849 of the trout had a length between 15 and 30 cm (appendix tab. 5). Length of trout and intensity of *E. crassum* were found to be slightly positively correlated (R = 0.19) (fig. 7). The correlation was significant ($p < 0.01$).

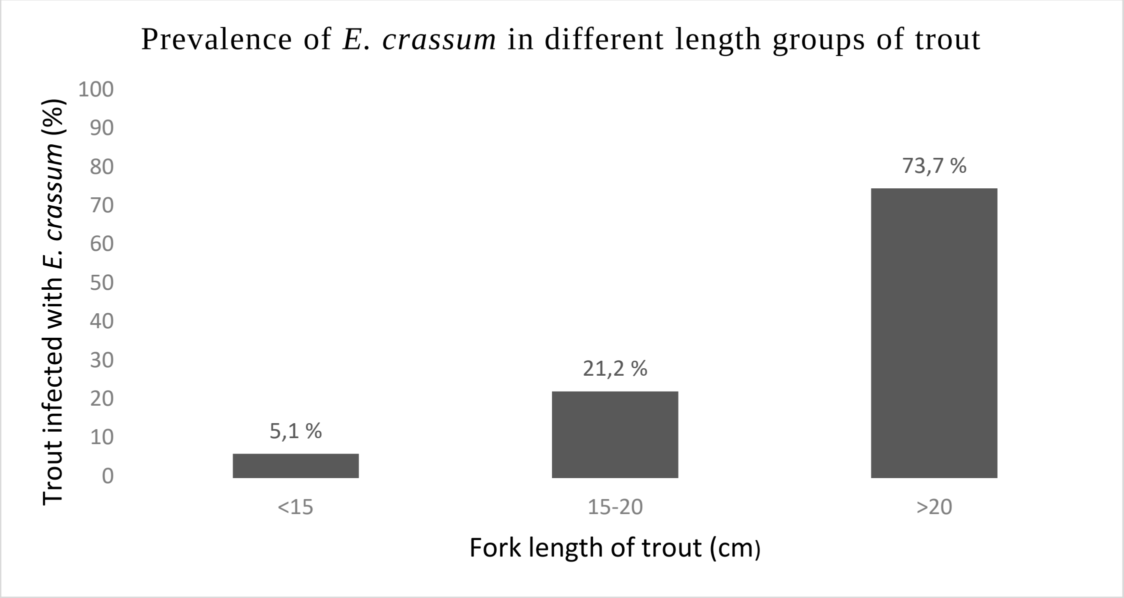

Fig. 6. Trout infected (N = 217) with *E. crassum* categorized in to three length groups of trout: < 15 cm, between 15 and 20 cm, and > 20 cm fork length. The difference in *E. crassum* infections amongst the different length groups was significant ($p < 0.05$) (Appendix tab. 6).

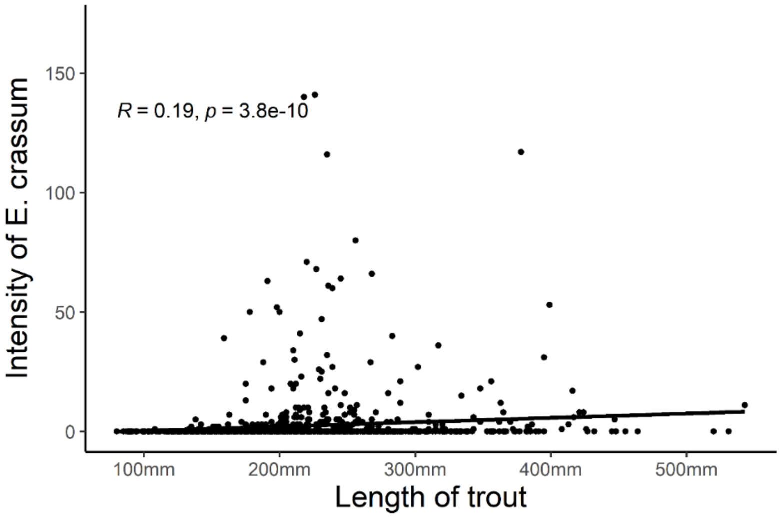

Fig. 7. The correlation between length of trout and intensity of *E. crassum* among 1106 sampled trout. The line illustrates the best fitted linear correlation, and R denotes the correlation coefficient. The correlation was significant ($p < 0.01$).

Both length of trout and composition of fish community were found to vary with prevalence and intensity of *E. crassum* infections. The variations were statistically significant (p < 0.01) (appendix tab. 7). Hence, length of trout and composition of fish community affects prevalence and intensity of *E. crassum*. However, composition of fish community was found to be an even stronger factor than the length of the host (appendix tab. 7).

Correlation between piscivory and prevalence of *E. crassum*

Nine of the thirteen lakes had piscivorous trout in the sample. The proportion of piscivorous individuals was never higher than 40 % (appendix tab. 2). Piscivory increased with an increased number of species in the fish community (fig. 8). More trout were found to be piscivorous in sympatry with sticklebacks (trout, charr and stickleback: 19.6 %) (trout and stickleback: 17.9 %) than in sympatry with solely charr (1,1 %) (appendix tab. 2). Piscivory in trout populations was found to be strongly positive correlated with prevalence of *E. crassum* (R = 0.73) (fig. 8). The correlation was significant (p < 0.01).

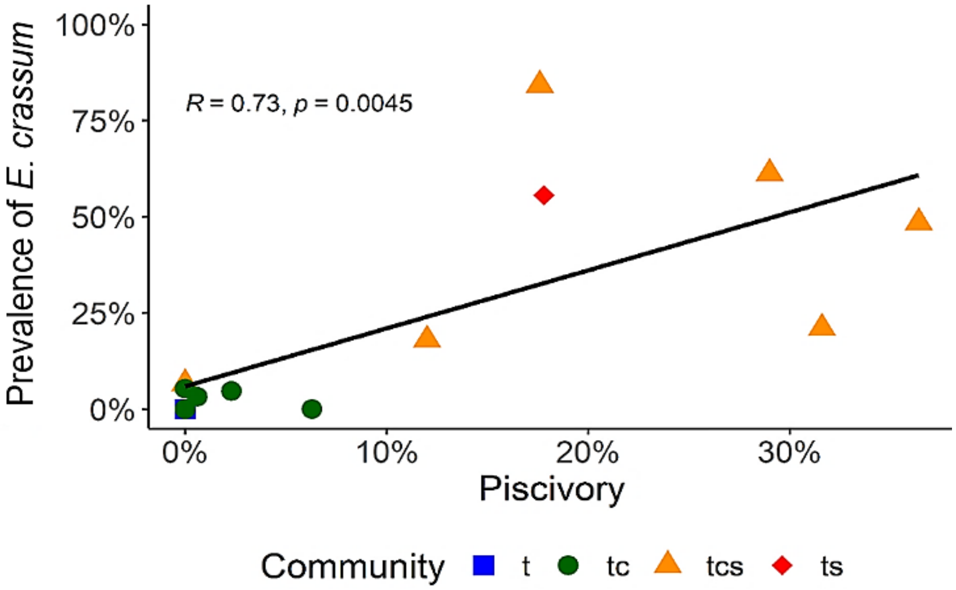

Fig. 8. The correlations between the prevalence of *E. crassum* in brown trout, fish community, and proportion of piscivorous trout. Fish community compositions: t (trout) (one lake, 79 trout), tc (trout and charr) (five lakes, 267 trout), tcs (trout, charr and sticklebacks) (six lakes, 214 trout), and ts (trout and sticklebacks) (one lake, 45 trout). The line illustrates the best fitted linear correlation, and R denotes the correlation coefficient. The correlation was significant (p < 0.01).

Intensity of *E. crassum* and variables in the dataset

A PCA plot for between-lakes variation of *E. crassum* intensities revealed that these are strongly associated with lakes in which the fish community is composed solely by trout and sticklebacks (fig. 9). Accordingly, the first dimension, which represents 32.7 % of the variance, was driven mainly by the variability of *E. crassum* intensities and the fish community of trout and sticklebacks. The second dimension, which represents 20.9 % of the variance, was driven mainly by lakes with the fish community composed of trout and charr, lake elevation and size of lake. Globally the two axes explained 53.6 % of the variance.

The size (R= -0.25) and altitude (R= -0.18) of the lake were found to be slightly negatively correlated with intensity of *E. crassum*. Both correlations were statistically significant (p < 0.01) (appendix tab. 8, appendix fig. 2 and 3).

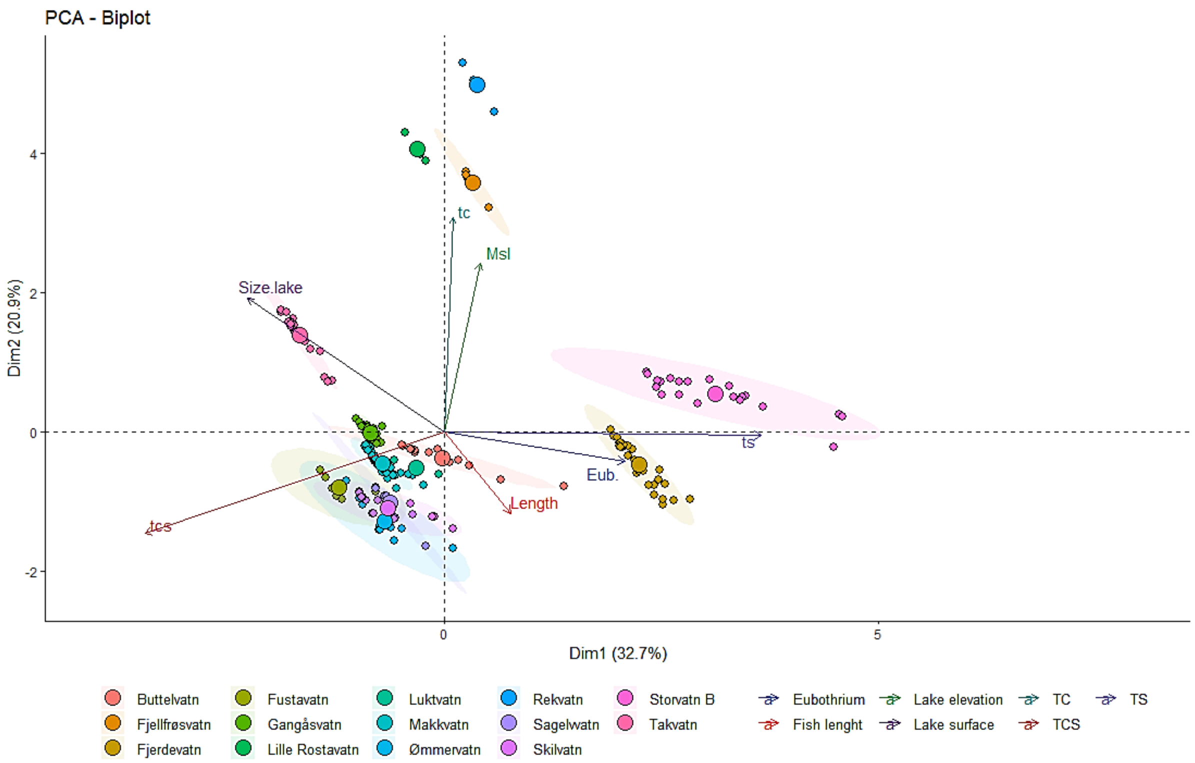

Fig. 9. PCA biplot on factors influencing intensity of *E. crassum* in brown trout with 95% confidence intervals. Dimension 1 and 2. Fish communities are represented as tc (trout and charr), tcs (trout, charr and sticklebacks), and ts (trout and sticklebacks).

Discussion

In accordance with the hypothesis, this study documented significant differences in *E. crassum* infections in brown trout between lakes in relation to the fish community composition. As expected, both prevalence and mean abundance of *E. crassum* were significantly higher in lakes with sticklebacks as part of the fish community. Examining piscivory in trout populations revealed higher frequencies in communities with sticklebacks than those without. Furthermore, in agreement with the expectations, the prevalence of *E. crassum* and piscivory in the different trout populations were positively correlated.

In allopatric populations infections of *E. crassum* in trout would have to be acquired through trout predating upon infected copepods (Vik 1963, Kennedy 1978). The lifetime is one to two years for *E. crassum* in final host, hence the trout needs to be reinfected within this time to have a continuous infection (Hanzelová et al. 2002, Prati et al. 2020). However, copepods are not considered a common prey for trout (Langeland et al. 1991, Knudsen et al. 2008, Eloranta et al. 2013, Kuhn et al. 2016). *Cyclops* spp., a common first intermediate host for *E. crassum,* are small copepods that typically lives in stagnant or slow-flowing water of the littoral or pelagic zones of lakes (Kennedy 1978, Rozendaal & World Health Organization 1997, Hansen & Santer 2003, Holynska & Wyngaard 2019). These copepods are generally not found in the hatching areas of trout which are typically in running water with strong currents (Borgstrøm & Hansen 1987). The young trout also typically stay in this area the first years of their life (Kraabøl & Museth 2019). Regarding this, including an ontogenetic shift in diet, how large the trout have grown when migrating to lake from the hatching area may affect the transmission of *E. crassum.* If the trout are resident in the river until they reach a body size where small zooplankton are an even more sporadic or undesired prey, the chance of *E. crassum* infection decreases. The diet was not analysed to confirm if the trout were feeding on zooplankton. Since the majority of sampled trout were >15 cm (81.7 % of the allopatric trout) it is discussed whether this fish could have been infected earlier in life, but not re-infected when growing larger. However, none of the allopatric trout < 15 cm (18.3 %) were infected either. Cannibalism is considered uncommon in Norwegian freshwater populations of trout, primarily due to a spatial size segregation between small and larger trout (L´Abée-Lund et al. 1992, Klemetsen et al. 2003, Knudsen et al. 2008). Hence, even if some of the smaller trout in the lake were infected by *E. crassum*, but not included in the sampling, the likelihood of infecting bigger cannibalistic trout is small. None of the allopatric trout were found with remains of prey fish in stomachs. Vik (1963) also addressed the subject of trout acting as an intermediate host for *E. crassum*, however, concluded that it is unlikely. No infections of *E. crassum* in the allopatric populations of trout may also be due to the absence of this autogenic parasite in these lakes. If the ancestors of the trout populations, either immigrants to the lake or introduced by people, were not infected by *E. crassum*, it is possible that there never were *E. crassum* in the lake (Hesthagen & Østborg 2004). However, the lakes with allopatric trout might have a possible migration route from the sea, so it is possible that sea migrating trout could enter these lakes and therefore introduce the parasite (Dalen 2011). In conclusion, the absence of the trophically transmitted parasite, *E. crassum*, in allopatric trout populations suggests that copepods are an inefficient transmission route for the parasite. Even if the parasite was introduced by sea migrating trout it does not seem like the parasite is able to establish without small prey fish, like sticklebacks, present.

Three lakes with a fish community of trout and charr had very low prevalence and mean abundance of the parasite. Only eleven out of the 333 trout were infected with *E. crassum* in this fish community. Six of the infected trout were < 20 cm and are therefore most likely infected through predation upon infected copepods. This means that *E. crassum* is present in the three lakes, Rekvatn, Lille-Rostavatn and Fjellfrøsvatn, but prevalence and mean abundance are very low. This documents that there is some transmission through predation upon copepods, but from the data is seems to be of low importance in the transmission of *E. crassum* to trout. Five of the infected trout in sympatry with charr were > 20 cm. Small charr can be predated by large (> 20 cm) trout (L´Abée-Lund et al. 1992). Typically, trout are known to inhabit the littoral habitat of the lake, while charr typically inhabit the pelagic habitat (Sánchez-Hernándes & Amundsen 2015). However, bigger trout have been found to shift periodically or permanently to the pelagic habitat and could therefor encounter and predate more frequently on small charr (Klemetsen et al. 2003, Jonsson 1989). Charr is known to predate on zooplankton and could therefore ingest *Eubothrium* sp. infected copepods (Eloranta et al. 2013). If charr was a suitable intermediate host for *E. crassum* higher prevalence and mean abundance of the parasite in trout would have been expected. Vik (1963) concluded that charr was not likely to be a suitable intermediate host for *E. crassum*, and Scholz et al. (2003) concluded that *E. crassum* have never been found in species of *Salvelinus* and that the parasite was very specific towards species of *Salmo*. Regarding an ontogenetic shift in diet and the low prevalence and mean abundance of the parasite in the trout > 20 cm, it is likely that these infections are due to accidental infections of *E. salvelini.* If trout have predated upon *E. salvelini* infected charr, the parasite may survive in the intestine of the trout for a short period of time. Hence, if sampled trout had *E. salvelini* in the intestine it might wrongly have been identified as *E. crassum* due to method of identification. In conclusion, there is some transmission of *E. crassum* to trout through predation upon copepods, but possibly not enough to ensure an efficient life-cycle turnover. Infections in large trout in sympatry with charr are probably a result of accidental infections of *E. salvelini* due to recent predation upon charr.

The prevalence and mean abundance of *E. crassum* in trout were significantly higher in the fish community of trout, charr and sticklebacks than in those without sticklebacks. Trout and sticklebacks typically inhabit the littoral habitat of lakes and therefore they encounter each other more often than trout and charr (Sánchez-Hernández & Amundsen 2015). When sticklebacks are present it has been found to be favoured as prey fish by the trout (L´Abée-Lund et al. 1992). Sticklebacks can be predated upon by trout of smaller sizes (15 cm) than those who predate upon charr (20 cm) (L´Abée-Lund et al. 1992). Sticklebacks are known to predate on zooplankton and can therefore ingest *Eubothrium* sp. infected copepods (Langeland 1982, Jørgensen & Klemetsen 1995). Accordingly, species of *Eubothrium* sp. have been found in sticklebacks (Vik 1963, Kuhn et al. 2015). However, there were large differences in *E. crassum* infections among the lakes with trout, charr and sticklebacks. Low prevalence and mean abundance in some lakes is likely due to lower predation upon sticklebacks than those lakes with higher prevalence and mean abundance. Firstly, this could be due to more frequent predation upon charr than sticklebacks in some lakes than others (L´Abée-Lund et al. 1992). Secondly, lesser density of the stickleback population, or a larger littoral habitat, may lead to a more scattered distribution of the sticklebacks and trout, and therefore less encounters between the two species. And lastly, competition from piscivorous charr on stickleback predation may also led to fewer sticklebacks predated upon by trout (Amundsen 1994, Sánchez-Hernández & Amundsen 2015). In conclusion, the significantly higher prevalence and mean abundance of *E. crassum* in the fish community of trout, charr and sticklebacks strongly suggests that sticklebacks have a crucial role in transmission of *E. crassum* to trout. The great differences in *E. crassum* infections in trout within this fish community are likely due to unequal frequencies of stickleback predation. Vik (1963) also concluded that it is likely that sticklebacks have a role in the transmission of *E. crassum* to brown trout.

The fish community of trout and sticklebacks had significantly higher prevalence and mean abundance of *E. crassum* than the rest of the fish communities. In these lakes, trout are the single predatory fish and sticklebacks are the only other species available as prey. The highest prevalence and mean abundance of *E. crassum* in trout here, is likely due to a higher frequency of stickleback predation by trout than in the other fish communities. However, the prevalence and mean abundance of *E. crassum* were different between the two lakes. Storvatn in Balsfjord had by far the highest estimate of mean abundance among all the sampling lakes. This led to a very high average of mean abundance compared to the other fish communities. Storvatn in Balsfjord is a very small lake compared to Fjerdevatn. The higher mean abundance of *E. crassum* in Storvatn may therefore be a consequence of the trout and stickleback living together in a more confined space, and consequently increased frequency of stickleback predation by trout. In conclusion, sticklebacks as part of the fish community points to a greater transmission rate for *E. crassum* to trout, hence a higher life-cycle turnover for *E. crassum*.

The findings in this study are similar to Kuhn et al (2016). Kuhn et al. (2016) documented higher mean abundance and intensity of *Diphyllobothrium* sp. in trout in lakes inhabited by sticklebacks. Kuhn et al. (2016) concluded it most likely that sticklebacks can act as important paratenic hosts for *Diphyllobothrium* spp. *Diphyllobothrium* spp. creates a more efficient transmission route to piscivorous host by utilizing suitable prey fish as paratenic hosts, and this is likely the case for *E. crassum* as well (Kuhn et al. 2016).

Regarding an ontogenetic shift in diet and trout being a secondary piscivorous species, higher prevalence and mean abundance of *E. crassum* were expected in larger, piscivorous trout. Trout that were infected with *E. crassum* were mainly of the larger size group (> 20 cm). However, the correlation between intensity of *E. crassum* and length were weakly positive. The correlation was expected to be stronger based on the apparent crucial role of sticklebacks in transmission of *E. crassum* to trout. However, sampled trout do not have an even representation of different length groups, nor do they include a broad spectrum of different lengths. Only 9.9 % of the trout were over 30 cm, and 13.7 % were under 15 cm. Hence, a more evenly representation of the length groups might have shown a stronger correlation. Prevalence of *E. crassum* was strongly positively correlated with frequency of piscivory in trout populations. This strengthens the expectation that prey fish, like sticklebacks, have a role in transmission of *E. crassum* to larger, piscivorous trout.

There were different intensities of *E. crassum* in lakes of different sizes and altitudes. Both lake size and altitude were found to be weakly negatively correlated with *E. crassum* intensities. The weak negative correlations may be associated with sticklebacks. Sticklebacks are typically not found in high altitude lakes due to no migration route from sea, and seldom introduction to highland lakes by people (Berger et al. 1999). Hence, absence of sticklebacks when they are crucial for transmission of *E. crassum* to trout (as shown in this study) may lead to a negative correlation between intensity of *E. crassum* and lake altitude. A small size of a lake can, as discussed previously, lead to more encounters between sticklebacks and trout and therefore more frequent predation upon sticklebacks. Hence, size of lake and intensity of *E. crassum* might be negatively correlated due to higher intake of prey fish housing *E. crassum*. Both lake size and altitude were unevenly represented between the four fish community compositions. Hence, a weak negative correlation between lake size, lake altitude and intensity of *E. crassum* can not be confirmed.

No relation was exposed between regulation of lake and prevalence or mean abundance of *E. crassum* in trout. Only seven of the 22 lakes were regulated, and they were not evenly distributed among the four compositions of fish communities.

In conclusion, very few trout were infected with a likely transmission route through copepods. This suggests that it is a transmission route of low importance in infecting trout. Some trout in sympatry with charr were likely accidentally infected by *E. salvelini*, however genetical analysis are required to conclude this. Prevalence of *E. crassum* in trout were strongly, positively correlated with degree of piscivory, and the majority of infected fish were of longer lengths. Trout infected with *E. crassum* with a likely transmission route through sticklebacks had high prevalence and mean abundance. In this study, sticklebacks are documented to have a crucial role in the transmission of *E. crassum* to trout.

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Appendix

Appendix tab. 1. Prevalence and mean abundance of *Eubothrium* sp., including Bootstrap BCA confidence interval for the mean abundance. Dispersion was calculated for each of the four fish communities and was concluded overdispersed when $S^2/\bar{x} > 1$. Lakes sorted by fish community.

Lake sorted by fish community	**Prevalence (%)**	**Mean abundance with confidence interval of fish communities**	**Dispersion $S^2/\bar{x}$**
TROUT	0	0 [-, -]	0
FORSANVATN	0	0	
STORVATN (HAMARØY)	0	0	
KAPERVATN	0	0	
TROUT AND CHARR	3	0,1 [0,03, 0,14]	3.1
VÅVATN	0	0	
REKVATN	7	0,1	
JERNVATN	0	0	
SIRKELVATN	0	0	
MOSKÁNJÁVRI	0	0	
LILLE ROSTAVATN	5	0,1	
FJELLFRØSVATN	3	0,1	
TROUT, CHARR AND STICKLEBACK	30	2,8 [2,14, 3,82]	31.5
GANGÅSVATN	90	4,1	
FUSTVATN	20	1,4	
ØMMERVATN	33	3,5	
LUKTVATN	7	1,7	
MAKKVATN	79	4,1	
SKILVATN	61	10,2	
BUTTELVATN	48	13,9	
TAKVATN	13	0,4	
SAGELVVATN	8	0,4	
JOSEFVATN	0	0	
TROUT AND STICKLEBACK	67	16,3 [10,6, 25,7]	60.2
FJERDEVATN	57	4,1	
STORVATN (BALSFJORD)	84	39,4	

Appendix tab. 2. Diet results sorted by fish community of lake.

Lake sorted by fish community	**N trout**	**Length of trout (mm) ±SD**	**N piscivorious trout**	**% piscivory**	**Prevalence (%) of *E. crassum***
TROUT	79		0	0	0
FORSANVATN	79	220 ± 63.6	0	0	0
TROUT AND CHARR	267		3	1,1	3,4
REKVATN	38	173 ± 36.5	0	0	5,3
JERNVATN	16	255 ± 49.0	1	6,3	0
SIRKELVATN	16	192 ± 41.5	0	0	0
LILLE ROSTAVATN	43	179 ± 34.7	1	2,3	4,7
FJELLFRØSVATN	154	193 ± 56.8	1	0,7	3,2
TROUT, CHARR AND STICKLEBACK	214		42	19,6	43,5
ØMMERVATN	19	277 ± 84.7	6	31,6	21,1
LUKTVATN	30	262 ± 36.2	0	0	6,7
MAKKVATN	51	205 ± 38.5	9	17,7	84,3
SKILVATN	31	190 ± 31.9	9	29,0	61,3
BUTTELVATN	33	217 ± 21.0	12	36,4	48,5
TAKVATN	50	205 ± 42.0	6	12,0	18,0
TROUT AND STICKLEBACK	45		8	17,8	55,6
FJERDEVATN	45	241 ± 94.2	8	17,8	55,6

Appendix tab. 3. Output of the Chi square test. A significant difference in prevalence among the fish communities was concluded with $p < 0.05$. Fish communities referred to as: T (trout), Tc (trout and charr), Tcs (trout, charr and sticklebacks) and Ts (trout and sticklebacks).

	Prevalence of *E. crassum*
	p- value
T – Tc	0.014
T – Tcs	< 0.01
T – Ts	< 0.01
Tc – Tcs	< 0.01
Tc– Ts	< 0.01
Tcs– Ts	< 0.01

Appendix tab. 4. Significance output of the Krukal-Wallis test. A significant difference in mean abundance among the fish communities was concluded with $p < 0.05$. Fish communities referred to as: T (trout), Tc (trout and charr), Tcs (trout, charr and sticklebacks) and Ts (trout and sticklebacks).

	Mean abundance of *E. crassum* p-value (Kruskal-wallis test)
T – Tc	0.014
T – Tcs	< 0.01
T – Ts	< 0.01
Tc – Tcs	< 0.01
Tc– Ts	< 0.01
Tcs– Ts	< 0.01

Appendix tab. 5. Shapiro-Wilk test output to test normality of distribution in dataset. Distribution was concluded non-normal when $p < 0.05$.

	Shapiro-Wilk test output	
	p-value	w-value
Total dataset		
Intensity of *E. crassum*	< 0.01	0.225
Presence/Absence of *E. crassum*	< 0.01	0.486
Length of trout	< 0.01	0.948
Altitude (Msl) of lake	< 0.01	0.902
Surface area of lake	< 0.01	0.850
Piscivory dataset		
Prevalence of *E. crassum*	< 0.01	0.807
Piscivory in trout populations	0.017	0.832

Appendix tab. 6. Output of Chi square test. A significant difference in length groups of infected trout was concluded with $p < 0.05$.

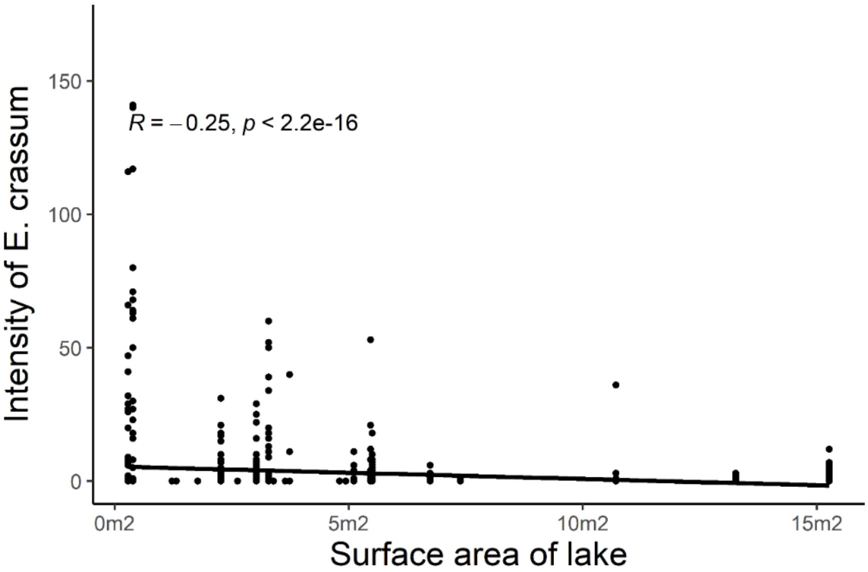
	Chi-square length groups p- value
< 15 cm – 15 – 20 cm	0.04
< 15 cm -- > 20 cm	< 0.01
15 – 20 cm -- > 20 cm	< 0.01

Appendix tab. 7. Statistical result on variables associated with *E. crassum* prevalence (ANOVA from GLM binomial regression) and intensity (ANOVA from GLM negative binomial regression) in brown trout. The variations were concluded significant when $p < 0.05$. Higher χ^2 for one predictor than the other concluded bigger effect on the response variable.

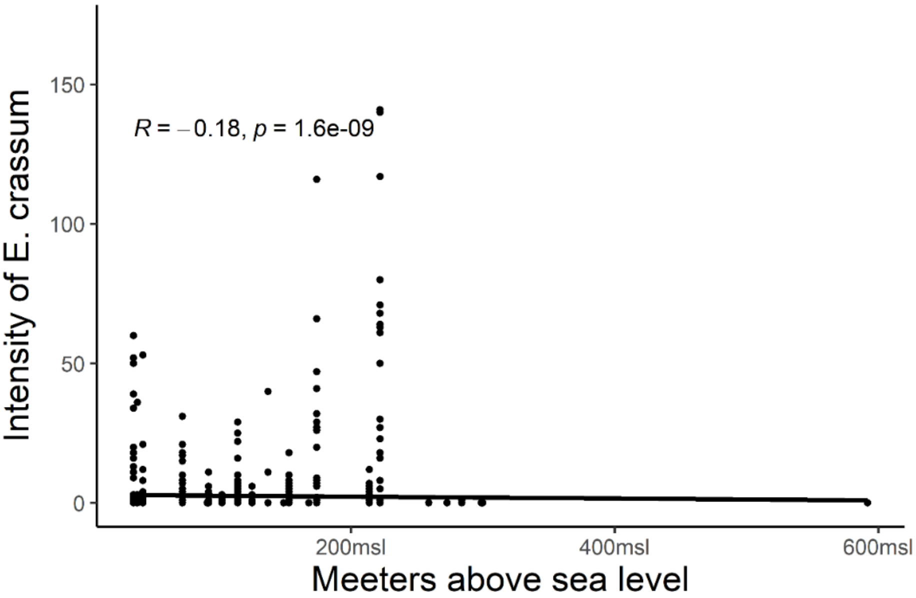
	Prevalence			**Intensity**		
	χ^2	df	p	χ^2	df	p
Fish community	487,95	21	< 0,01	349,72	21	< 0,01
Trout fork length	28,78	1	< 0,01	16,96	1	< 0,01

Appendix tab. 8. Spearman rank correlation output. Correlation coefficient (R_s) reflects degree of correlation: 1 strong positive, 0 no correlation and -1 strong negative. P- value < 0.05 concludes a significant correlation.

Correlation test between intensity of *E. crassum* and:	**Coefficient (R_s)**	**p-value**
Length of trout	0.19	<0.01
Size lake	-0.25	<0.01
Msl	-0.18	<0.01



Appendix fig. 1. Correlation between surface area of lake and intensity of *E. crassum* among the 1106 sampled trout. The line illustrates the best fitted linear correlation, and R denotes the correlation coefficient. The correlation was significant ($p < 0.01$).



Appendix fig. 2. Correlation between lake elevation and intensity of *E. crassum* among the 1106 sampled trout. The line illustrates the best fitted linear correlation, and R denotes the correlation coefficient. The correlation was significant ($p < 0.01$).