Infection dynamics of marine *Eubothrium* sp. (Cestoda) in farmed Atlantic salmon

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Abstract

Infections with marine tapeworms have been reported as an increasing problem in aquaculture of Atlantic salmon (*Salmo salar* L.) in Norway over the last few years. These are caused by the cestode *Eubothrium* sp. Little is known about the infection dynamics of this species and there is a need for knowledge regarding when these infections occur and how they develop.

Four cohorts of Atlantic salmon were followed for their first half year at sea and examined for tapeworms. Two of these were launched in autumn 2017 and two in spring 2018. Sampling was planned to be carried out at one month, three months and six months after sea-launch, but some variations occurred due to practical reasons. A total of 531 salmon were examined during the study.

Eubothrium sp. was found to follow a seasonal pattern of infection, with the infection pressure being highest in summer-autumn. Cohorts launched to sea in spring were initially exposed to a lower infection pressure. Abundance of the parasite was negatively correlated with fish size at the time of infection, suggesting that smaller fish have a higher risk of infection. This was supported by early stages of the worms mostly being found in fish under the length 35 cm. Little evidence was seen of infections occurring in fish over this size, perhaps due to reduced feeding on the zooplankton that serve as intermediate hosts of *Eubothrium* sp. This suggests that effective treatment after the fish has passed this size could lower the chances of reinfection, suggesting that treatment would only be needed once.

Terms, abbreviations and definitions

Abundance – average number of parasites in a host regardless of whether the host is infected or not

DPT - Days post transfer, number of days the fish have been at sea

Gravid – Worms that have eggs in them

Incidence - Number of new hosts that become infected with a particular parasite during a time interval divided by the number of uninfected hosts present

Infrapopulation – all individuals of a parasite species in an individual host at a particular time

Intensity – Number of individuals of a particular parasite in a single infected host

MRL - Maximum residue limit

NS – Not significant

Paratenic host – An intermediate host that is not necessary for the parasite to complete the lifecycle, but can aid in spreading it

Plerocercoid – Larval stage of *Eubothrium* that is infective to the final host

Prevalence - Proportion of hosts infected with one or more individuals of a particular parasite

Proglottid – Segment of a tapeworm, self-sufficient reproducing unit

Scolex – The head of a tapeworm, contains suckers that help in attachment

Strobila – The part of a tapeworm body that is made up of several proglottids

WW – Mean worm weight, the average weight of individual worms in a sample

1. Introduction

Fish farming is a growing industry worldwide with total production representing 47 % of the global fish production in 2016 (FAO, 2018). In Norway, Atlantic salmon (*Salmo salar* L.) production dominates (94.5%), followed by rainbow trout (5.1%). (Statistics Norway [SSB], 2018). At present, growth has slowed down, and pathogens are the main limiting factor. Much focus has been given to the ectoparasitic salmon lice *Lepeophtheirus salmonis* which for many years has been widely seen as the biggest challenge faced by salmon farmers when it comes to managing diseases. Other metazoan parasites have generally received less focus when it comes to aquaculture diseases. However, in recent years cestodes have been observed to become an increasing problem (Hjeltnes B, Bang Jensen B, Bornø G, Haukaas A, 2019). Most cases are observed in western and central Norway, with few reports coming from north of the Trondheim fjord (Hansen & Bornø 2019).

Adult tapeworms infecting salmonids in seawater may have been acquired by the fish as parr in freshwater. The cestodes *Eubothrium crassum* and *Proteocephalus longicollis* are widespread in Atlantic salmon and trout, *Salmo trutta* in Norwegian freshwaters (Smith, 1983), and may occur in hatchery reared fish (Sundnes, 2003). Both cestodes can survive for at least 4 months after sea-transfer (E.K. unpubl. Obs.). However, salmon may also become infected with cestodes after sea-transfer. These infections are exclusively caused by *Eubothrium* sp., a marine cestode morphologically indistinguishable from *E. crassum.*

The specific identity of the marine *Eubothrium* sp. has been controversial. Kennedy (1978b) distinguished a marine race of *E. crassum*, while Bristow & Berland (1989) found genetic differences between the freshwater and marine form using enzyme electrophoresis, evidence suggesting the possible existence of two similar species. Based on morphology Scholz et al. (2003) recognized only a single species occurring in both fresh- and seawater. This problem should now be addressed using modern molecular tools. The marine *E. crassum* form or sibling species in Salmo spp. is here referred to as *Eubothrium* sp., following past studies (Glenn A. Bristow and Berland, 1991; Saksvik et al., 2001a, 2001b; Sevatdal, 2014; Sundnes, 2003).

1.1 Systematic placement

The genus *Eubothrium* was previously placed in the order Pseudophyllidea (R. Kuchta et al., 2008), which based on rDNA sequences were found to be paraphyletic. Pseudophyllidea has since been split into the monophyletic clades Diphyllobothriidea and Bothriocephalidea , with *Eubothrium* belonging to the latter in the family Triaenophoridae (Roman Kuchta et al., 2008). The order Bothriocephalidea is distributed globally, and adult individuals almost exclusively parasitize teleost fish (Scholz and Kuchta, 2017). The genus *Eubothrium* contains 9 species in addition to *Eubothrium* sp. that infect a wide array of marine and freshwater fish in the northern hemisphere (Brabec et al., 2015). Three of these appear in freshwater in Norway, *E. crassum* in *Salmo* spp.*, E. salvelini* in arctic char, *Salvelinus alpimus,* and *E. rugosum* in burbot, *Lota lota*. Three species occur in saltwater, *E. parvum* in capelin, *Mallotus villosus* and *E. fragile* in shad, *Alosa fallax* as well as *Eubothrium* sp. in Atlantic salmon and trout (Kennedy, 1978a).

1.2 Lifecycle

The lifecycle of *E. crassum* in freshwater has received more attention than that of *Eubothrium* sp. at sea, and will be shortly summarized here. Rosen (1919) considered the life cycle to involve a copepod first intermediate host and a fish (perch, *Perca fluviatilis*) secondary intermediate host. Vik (1963) found that perch was not necessary to the life cycle, as this could be completed in locations where perch was absent. Instead of serving as a secondary intermediate host, perch and various other small fish species such as ruffe, *Gymnocephalus cernua,* and sticklebacks, *Gasterosteus aculeatus,* appear to function as paratenic hosts instead. Trout has been experimentally infected with *E. crassum* from sticklebacks (Vik, 1963). Infections also occur under circumstances where the only possible source of infections are copepods, such as hatcheries where only copepods could have gotten in the water supply, and the infected fish are too small to have been piscivorous (Mulcahy and Kennedy, 1970).

Four species of marine copepods have been shown to become infected by ingesting *Eubothrium* sp. eggs and therefore seem likely to function as intermediate hosts: *Acartia tonsa* (Saksvik et al., 2001b)*, Acartia clausi, Temora longicornis* and to a certain degree

Pseudocalanus elongatus (Hodneland & Solberg, 1995). A fifth species of marine copepod tested, *Calanus finnmarchicus* does not appear to become infected. In addition to marine copepods, *Eubothrium* sp. eggs have been shown in lab experiments to be able to infect the freshwater copepods *Cyclops* spp. and *Eudiaptomus* sp. Similarly, *E. crassum* can successfully establish infection in marine *A. clausi* (Hodneland & Solberg, 1995) indicating that there might be some overlap between the two forms in the wild.

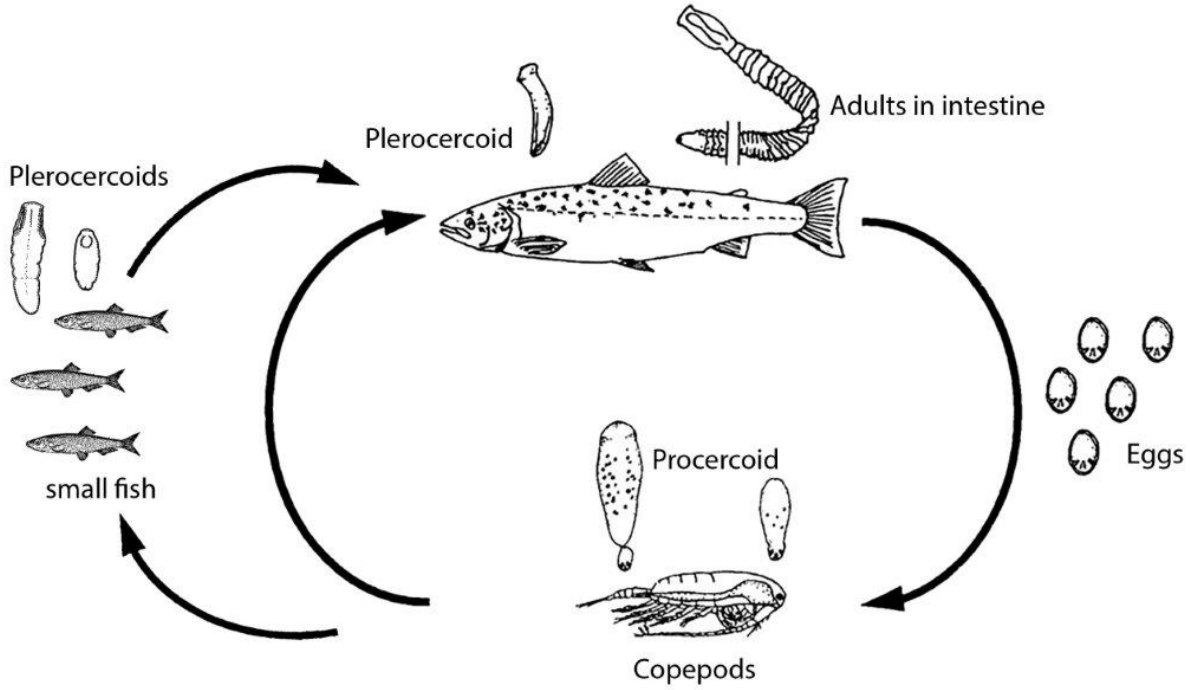

Figure 1 Illustration of the lifecycle of marine *Eubothrium* sp. Gravid adult worms in the guts of salmonid final hosts release eggs into the environment. These may be eaten by certain calanoid copepods, where the eggs hatch and a hexacanth-larva penetrate the gut and establish in the body cavity. There it develops as a procercoid larva. Copepods with infective procercoids may be eaten by smaller fish that act as transport hosts with free plerocercoids in the gut or are eaten by the final hosts directly where the cestode develop through the plerocercoid stage, attach, develop a strobila and mature. Based on a figure by Hodneland (1995).

Eggs ingested by copepods hatch in the gut, where the hexacanth larvae penetrates the intestinal wall. They then reside in the haemocoel where they develop into a proceroid larvae (Saksvik et al., 2001b). If this copepod is eaten by a final host, the larvae will be free in the intestine as a plerocercoid (Kennedy, 1996). Another route takes place when the copepod is eaten by a smaller fish that can serve as a paratenic host, where it can develop to an immature plerocercoid. In the final host, the worms will develop an apical disc and two bothria on the scolex and migrates to a caecum where it attaches with the scolex and starts

to grow and develop more strobila. There it can eventually reach a size of over one meter in length, and in some cases will fill up a substantial portion of the hosts gut.

While many have focused on the role of copepods as intermediate host, it has long been recognized that various small fish can fill the role of transport hosts. It seems likely that these may persist in the environment as a source of infection for a longer time than short-lived copepods, and so their role in the infection dynamics should not be overlooked. Observations of plerocercoid *Eubothrium* have been reported from cod, *Gadus morhua*, larvae halibut larvae, *Hippoglossus hippoglossus*, lumpfish , *Cyclopterus lumpus* (Rolbiecki and Rokicki, 2008) and Baltic sea herring, *Clupea harengus* (Schneider., 1902) indicating that these fish may be used as paratenic hosts.

To complete their life cycle in the wild, eggs should be released at a place and time that maximizes the likelihood of the eggs reaching and infecting the intermediary host. Copepods are most abundant in coastal areas in the spring around May-July (Deschutter et al., 2019). This coincides with adult salmon returning from sea before migrating up rivers to spawn, as well as with smolt migrating out to sea. This timing would be ideal for the parasite, as eggs released from mature parasites in adult salmon would have a comparatively high chance of reaching an intermediary host, which would again have a high chance of being eaten by smolt on their way out to sea.

1.3 Seasonality

Eubothrium crassum

There exists more information on the seasonality of freshwater *E. crassum* than its marine cousin. Zschokke (1884) reported gravid worms from February and onward. Rosen (1919) found gravid worms from the end of March to August. Nybelin (1922) found similar results, with gravid worms present from May to July. Wootten (1972) performed monthly samplings of trout and rainbow trout at a reservoir in the UK and found infections of newly stocked fish to take place during summer and autumn. Campbell (1974) found that numbers of plerocerciform *E. crassum* in trout were highest from July to September, however some plerocerciforms could be found throughout the year. In a natural population of brown trout *S. trutta* living in a small lake, Kennedy (1996) found that infections with *E. crassum*

commenced in spring and reached a peak in July. Hanzelova et al. (2002) studied *E. crassum* in alpine lakes and observed egg release to take place only during the summer months June-August.

Eubothrium sp.

Only a few studies have investigated the seasonality of *Eubothrium* sp. infections. Zschokke (1891) reported a prevalence of 100 % in *S. salar* in the summer months of July-August in the upper Rhine. Nybelin (1922) noted juvenile cestodes of what was probably *Eubothrium* sp. in *S. salar* in Sweden in October, suggesting a recent infection. Kennedy (1969) found mature and gravid *Eubothrium* sp. in *S. salar* and *S. trutta* migrating from the sea to the river Exe in England. Fahy (1980b) found that post-smolts of S. trutta acquired heavy infections shortly after migrating to sea with the proportion of plerocercoid and juvenile cestodes increasing dramatically as the summer progressed. Chubb (1982) argued that although some gravid individuals were present throughout all year, peak egg production is probably during the warmer seasons. The seasonality of *Eubothrium* sp. in Norwegian seawater farmed Atlantic salmon is poorly known. Spring-stocked salmon in Varaldsøy, Hardanger acquired infections a few weeks after launch in May/June on. A high (85-100%) prevalence and peak mean abundance of 9-14 was then observed in August-September, 3-4 months after sea transfer (Berland & Bristow 1990, 1991).

Due to the seasonal availability of the copepods that function as intermediate hosts, marine cestodes have been assumed to follow seasonal patterns of maturation (Arme and Pappas, 1983). The availability of intermediate hosts has been implicated as the cause of seasonally varying infection pressures. When studying *E. crassum* and *E. salvelini* in North Norway, Spitsbergen and Jan Mayen, Kennedy (1978a) found that the levels of infection in the respective final hosts could be related to the abundance of zooplankton. For *E. salvelini* a seasonal cycle of incidence and maturation was found, where maximum egg production occurred when plankton was building up and maximum infection of fish coinciding with the densest level of plankton. *E. crassum,* while also employing copepods as intermediate hosts did not exhibit this seasonal variation.

1.4 Effects

Negative effects on fish caused by cestodes have been reported from several studies on species in the genus *Eubothrium*.

E. salvelini has been reported to give reduced growth in sockeye salmon *Oncorhynchus nerka* (Boyce, 1979), reduced tolerance to zinc (Boyce and Yamada, 1977) , reduced swimming performance and aberrant behavior (Smith, 1973) as well as impaired saltwater adaptation (Boyce and Clarke, 1983), although this might have been a secondary effect due to the reduced growth. Yet another effect reported is anemia in arctic char, *Salvelinus alpinus* (Hoffmann et al, 1986).

In addition to effects on the final fish host, it has also been observed that copepods infected with *E. salvelini* exhibit altered behavior (increased activity) that makes them more susceptible to predation by fish (Poulin et al., 1992).

Eubothrium sp., meanwhile, appears to have less dramatic effects on its host. Berland & Bristow (1990) inferred a reduced growth rate in salmon sampled from aquaculture from the fact that infected fish were shorter and weighed less than uninfected ones.
In a lab study by Saksvik, Nilsen, et al. (2001a), the effects found were reduced growth rate, reduced length-growth, and in one sample a lower hematocrit level. These effects were not seen until several months after infection.

Factors that may influence the chance of infestation are the size and age of the fish, maturity, size of gill rakers and the behavioral implications of these factors. It appears likely that smaller fish are more likely to feed on zooplankton, and therefore have a higher likelihood of being infected, than large fish who prefer other sources of food.

1.5 Treatment

As infections with *Eubothrium* sp. seem to have negative effects on host growth treatment is often carried out. In previous years tapeworm infections were treated with either praziquantel or fenbendazole, both anthelmintic drugs administered in the feed. Fenbendazole went out of use after 2006 due to negative side effects such as reduced

appetite and potential mortality at low temperatures (Sevatdal and Hellberg, 2005). From 2010 to 2015 the sale of praziquantel increased markedly (as seen in figure 2) due to increasing abundance of tapeworm-infections and subsequent treatment. Since then the sale has decreased, likely due to treatment failure from widespread praziquantel resistance, and not because of a reduced problem with tapeworms infections (Hjeltnes B, Bang Jensen B, Bornø G, Haukaas A, 2019). In a questionnaire by the Norwegian veterinary institute, 13 % of the fish farmers who treated for tapeworms reported that the treatment had failed (Hjeltnes B, Bang Jensen B, Bornø G, Haukaas A, 2019). A bioassay for determining the sensitivity of tapeworms to praziquantel has been developed to avoid treatment of resistant populations (Sevatdal, 2014, 2008; Sevatdal and Hellberg, 2005), which would be both costly and pointless. This may be used in resistance mapping, cancelling treatments if the prevalence of resistant worms is high. The situation with only one anthelminthic and increasing resistance is unfortunate, and there is a need for additional anthelmintics. Several alternative drugs with established maximum residue limits (MRL) for other production animals have been tried out, but no good replacements have been found. Oxibendazole has been shown to have an effect, but like the now disused fenbendazole causes a loss of appetite (Sevatdal, 2008).

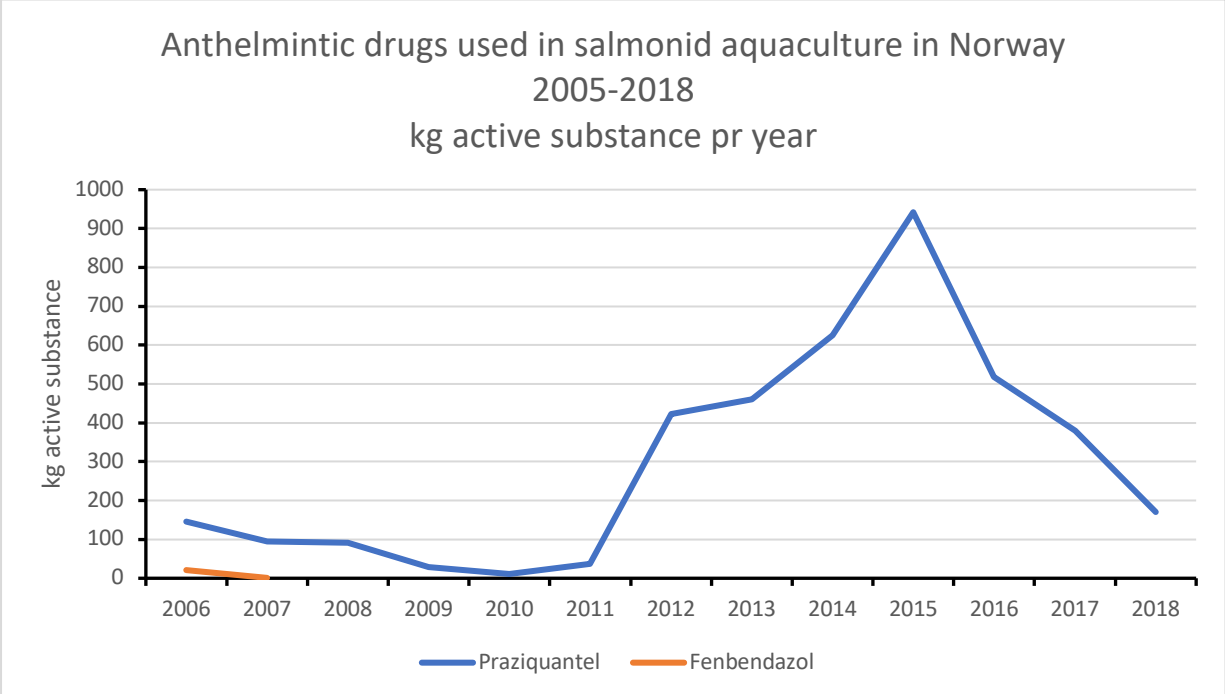

Figure 2 Anthelmintic drugs used in salmonid aquaculture in Norway 2005-2018. (Data sourced from Norwegian Institute of Public Health: https://www.fhi.no/nyheter/2019/2018-oppdrettsnaringen-bruker-stadig-mindre-legemidler-mot-lakselus/).

1.6 Aim

The aim of this study was to provide empirical data from Norwegian fish farms on the present dynamics of tapeworm infections in the field. Past studies could often not exclude the possible presence of the freshwater species *E. crassum*. Also, all studies from Norwegian aquaculture concerned spring stocked fish. These data could function as a base of knowledge for further research in the field, as well as aid in the decision-making process of fish farmers when selecting treatment strategies for areas with history of infection.
Of particular interest was the potential effect of the time of year the fish were put to sea on the infection dynamics, and the implications for potential seasonal variations and temporal patterns in the infection pressure. The effects of the parasite on its host under normal aquaculture conditions were also of interest and potential negative relationships between the cestode and host growth and was examined. The main goals were to:

1) Investigate the seasonality of *Eubothrium* sp. infections in farmed Atlantic salmon
2) Map the infection dynamics in salmon transferred to sea in spring
3) Map the infection dynamics in salmon transferred to sea in autumn
4) Examine the importance of salmon size in tapeworm recruitment
5) Examine the associations between fish growth and worm abundance

2. Materials and methods

2.1 Locations

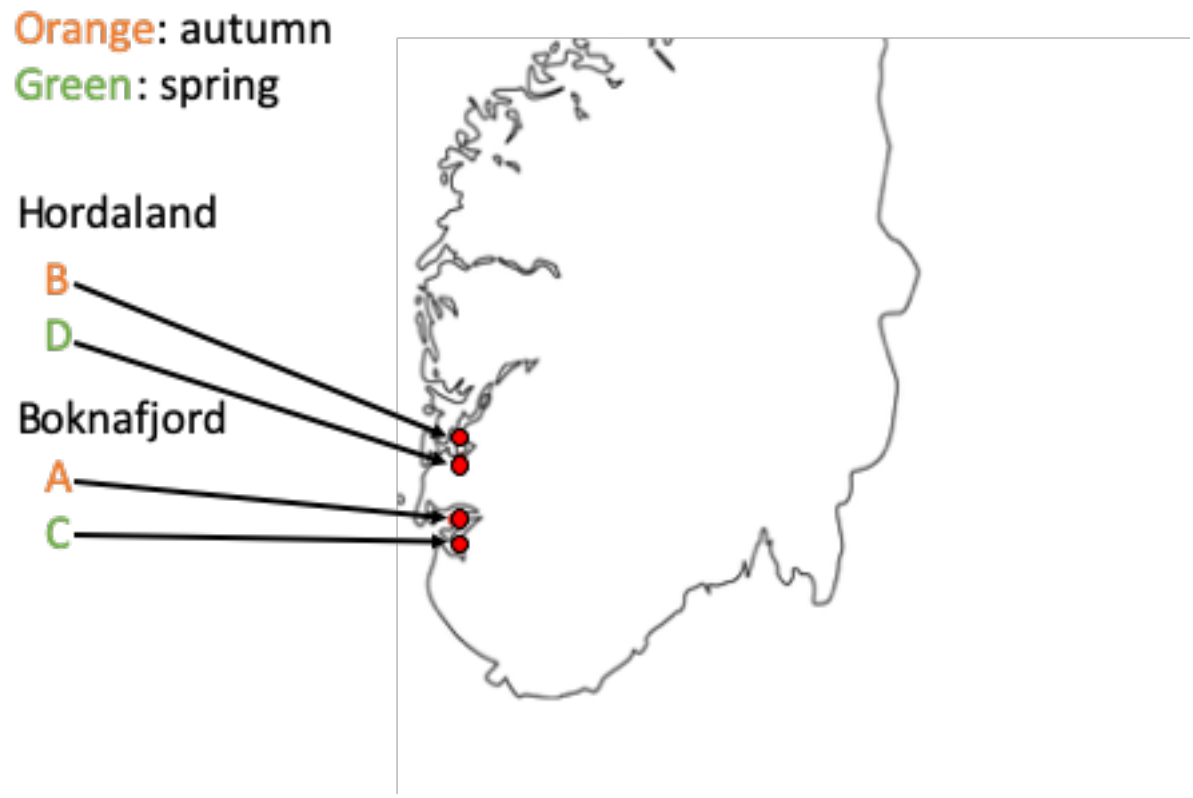

Figure 3 Map of locations surveyed in the project. Two locations are in a fjord in Hordaland county, the other two are in a fjord in Rogaland county.

As seen in figure 3, all four locations were placed in sheltered sites in the fjords on the western coast of Norway. The two southern locations were in the Boknafjord in Rogaland county and the two northern were in Hordaland county. Cohorts A and B were put to sea in the autumn om 2017, while cohorts C and D were put to sea during spring 2018 (exact dates given in table 1).

2.2 Material

The study sites were in regions know to have a history of infections with tapeworms. In order to examine temporal variation in infection, four cohorts of fish were followed during their first half year at sea (six to eight months). The two “Autumn” cohorts were put to sea in September and October, while the two “Spring” cohorts were sea-transferred in April (Table

2). Some information was also obtained from a fifth group, cohort X, that was put to sea at the same location and time as cohort A. From cohort X, only the freshwater and first seawater samples were examined, due to questionable labelling of the later samples. The sample size was 30 fish at each time point, and all fish were randomly sampled from a fixed pen at the fish farms by farm-employees. Growth data for the fish cohorts are given in appendix table 1. The preplanned sampling scheme was one month, three months and six months after sea-transfer, which was sometimes deviated from due to practical reasons. An overview of the actual sampling dates is provided in table 1. To ensure that the parasites observed in the seawater samples were *Eubothrium* sp. of marine origin, samples were also taken from the cohorts in the freshwater hatcheries at a time close to sea-transfer and examined for cestode infections. If they had been found positive for cestodes then these cohorts would be discarded.

Table 1 Sampling dates for the different cohorts. 30 salmon were sampled at each date.

Season	Cohort	Sample 1 (FW)	SW transfer	Sample 2 (SW)	Sample 3 (SW)	Sample 4 (SW)
Autumn	A	07.09.2017	15.09.2017	09.11.2017	30.01.2018	15.05.2018
Autumn	X	07.09.2017	15.09.2017	09.11.2017	30.01.2018	15.05.2018
Autumn	B	12.10.2017	14.10.2017	05.12.2017	08.02.2018	11.05.2018
Spring	C	10.04.2018	08.04.2018	15.05.2018	15.08.2018	19.11.2018
Spring	D	18.04.2018	18.04.2018	15.06.2018	20.08.2018	15.10.2018

2.3 Methods

The fish sampled from the study pens were euthanized by administering an overdose of anesthetic (benzocaine/MS-222), weighed (g) and the fork-length measured (cm). The fish were then opened, and their gastrointestinal tracts dissected out and placed in individually

marked plastic bags so that infections could be correlated to the health condition of the fish. Some of the freshwater samples were sent as whole fish since they were so small. Any deformities, such as cataracts or scoliosis were noted. The samples were then deep-frozen (-20 °C). Later, these were sent in insulated Styrofoam containers by overnight mail to the University of Bergen, where they were received still frozen. They were kept at -20 °C prior to examination.

The frozen gut samples were thawed, and the different parts separated into different petri-dishes. The principal parts studied were the hindgut, midgut and caecal region. In a subset of fish, the caecal region was further split into three regions to determine if the worms had a preferred site within this region. This was a time-consuming process that was not always practical to do, as such valid results could not be gained from all individuals in a sample. As this was very time consuming, this was only done on cohort A. The dorsal caeca were one group, dubbed C2, while the ventral caeca were split into two groups of equal size: C3 closest to the pyloric sphincter and C1 further away. The caeca in groups C2 and C3 were longer than those found in C1. All regions of the guts are displayed in figure 4.

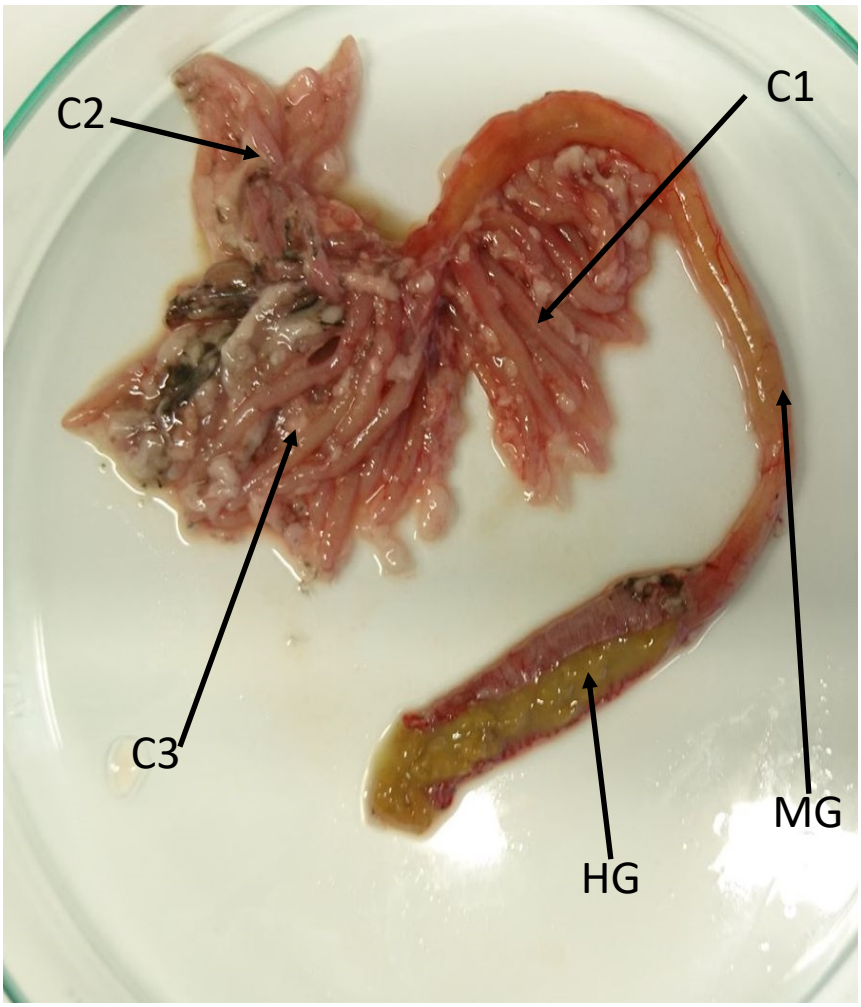

Figure 4 Gastrointestinal tract of an Atlantic salmon displayed in glass petri dish during dissection. HG = hindgut. MG = midgut. C1-3 = Caecal regions. The various regions of the gut are spread out in the picture to be more easily recognizable. The hind gut is cut open.

The stomach was removed, as this is not a known site for *Eubothrium* sp. The pyloric caeca were examined by squeezing them together between two glass petri dishes under a stereo microscope. Parasites were identified and later removed by opening the caeca with scissors, with care being taken as to not fragment larger individuals. Any parasites found were placed in a separate petri dish for counting and staging. Particularly the larger individuals were fragile, and the handling often caused them to break into several pieces. Therefore, the parasites were enumerated based on scolex counts, and in some cases sections of narrowing strobila ("neck region") that would obviously lead to a missing scolex. The location of the parasites was defined as where their scolex was found, large parasites would often occupy several caeca as well as stretching into the midgut.

The sections of gut were cut open lengthwise and contents were scraped to the side with tweezers. A few methods were tried to get the cestodes out of the caeca. Cutting open the caeca usually yielded good results but was a very time-consuming process. Flushing the cestodes out with water sometimes worked but often required a lot of water and ended up quite messy. The most consistently effective procedure turned out to be squeezing them out with a pair of tweezers, with one holding the end of the caeca in place and the other, preferably with a flat end, doing the squeezing.

For the small freshwater samples, the whole sample could be examined in one petri dish. For the larger samples where the fish had been to sea for six to eight months, the guts and their content had to be examined separately due to the sheer amount of material. The caecal region had to be cut into several pieces to be able to squeeze them enough to reveal any worms inside.

Worms were sometimes found lying free in the bag, these were assumed to be from punctured regions of the caeca. All worms were assigned to categories based on size and/or maturity as described in table 2 below. The staging system was practical to use as the worms were easily assigned to their correct category without using any time-consuming procedures, while also giving a basis for describing how the worms develop in the gut. Originally it was attempted to measure every individual down to the nearest millimeter, but this proved to be impractical both due to high abundances and the difficulty of extracting intact individuals. For even greater detail on how the worms develop in the gut, it was sought to register the length of the two shortest and two longest individuals in each sample. This could not always be done due to the cestodes breaking into pieces too easily.

Table 2 Cestode growth categories used in the study.

Categories	Description
I	Plerocercoid: no segments, thickest in the middle. Size: ca 0.3 - 0.6 mm
II	Juvenile 1: small, with scolex and strobila. Size: 0.6 - 10 mm
III	Juvenile 2: with scolex and strobila. Size: 1 - 5 cm
IV	Subadult: with scolex and strobila. No eggs. Size: > 5 cm
(V subset of IV)	Gravid: adult with egg production. Size: > 20 cm

For the first three categories, worm-length was measured using a millimeter-scale paper while in a glass petri dish filled with saline. The saline was made by diluting seawater to approximately 10 ‰ salt, (see Berland & Bristow 1990). Being suspended in saline prevented the worms from stretching when being laid out on a dry surface. For larger individuals a plastic tray about 40cm long filled with saline was used. Instead of a millimeter-scale paper a ruler was placed in the tray. Accurate measurements were hard to get for the large individuals, as they tended to be fragmented, and reconstructions have an innate margin of error. Variations in morphology of the strobila were observed, likely stemming from varying degrees of contraction of the worms at the time of death.

To ascertain whether or not the worms were gravid, sections were examined by squeezing between two glass petri dishes while under a stereomicroscope to reveal eggs in the strobila. In larger individuals the presence of eggs was often visible to the naked eye as the strobila, particularly the widest parts, had a yellowish color that stood out from the otherwise white coloration.

After these measurements, all worms from each fish were collected in a small glass petri dish prior to weighing. Before weighing, the worms were placed on a drying paper and rolled around for a standardized time of ten seconds to remove excess moisture. They were then transferred to small plastic dished and weighed using a Mettler Toledo AB204 weight to nearest 0.1 mg. Very small worms such as from the initial infections could not be weighed,

so these were ascribed a weight based on a standard curve of *Eubothrium* sp. length vs. weight. This was made by picking a number of entire worms with uncontroversial length (i.e. rounded posterior strobila) that were the weighed individually.

2.4 Statistics

Statistical analyses were performed in Statistica version 13.3, unless otherwise stated. Data were considered significant when $P < 0.05$.

Fischer´s exact test was used to determine significance of changes in prevalence between samples, as it is well suited for giving an exact value when comparing variables of this sample size.

Parasite distributions were usually found to be aggregated (variance-to-mean ratios >>1), with some individual hosts containing much more parasites than most others in their samples. Therefore, nonparametric statistical tests were used when analyzing abundance. Parasite abundance in different samples from a cohort were compared using Kruskal-Wallis (KW) ANOVA by ranks (H_{df}), with post-hoc multiple comparisons tests. The Mann-Whitney test was used to compare abundance in two consecutive samples (two sample hypotheses) and is indicated by asterisks on the abundance graphs.

Spearman´s rank correlation coefficient was used to measure correlations between various factors when investigation the possible effects of the parasite on the host.

The terms prevalence, abundance and infrapopulation are used in accordance with Bush *et. al.* (1997). Standard binomial 95% confidence intervals (Zar, 1984) for prevalence were calculated in Microsoft excel. 95% confidence intervals for abundance was obtained using bootstrapping, performed in the in Microsoft excel based platform “Resample” (Wood, 2003).

Condition factor was calculated using Fulton’s formula: $K=100\ wl^{-3}$, w=weight (g) and l=length (cm).

“Load” was calculated as w_{eub}/w_{fish}, where w_{eub}=total weight of cestodes in fish (g) and w_{fish}= weight of the fish (g). This was chosen as one of the parameters for correlation with fish condition as it is gives a measure of the relative strain of parasites on the fish, and has been used for this purpose in previous studies on cestodes in farmed Atlantic salmon (Sundnes, 2003).

3. Results

3.1 *Eubothrium* sp.

A total of 531 fish was examined for the presence of cestodes. Of these, 278 were infected with a total of 1989 worms. All fish were uninfected at time of sea launch according to results of the freshwater-tests. After sea launch all cohorts became infected with *Eubothrium* sp.

Eubothrium sp. (cf. *E. crassum*) was identified morphologically according to (Andersen & Kennedy 1983), based on the characteristic morphology with serrated strobila and scolex with distinct apical cap. No other cestode is known to infect Norwegian farmed salmon in the sea. Figure 5 below presents an assortment of the life stages of *Eubothrium* sp. found in this study.

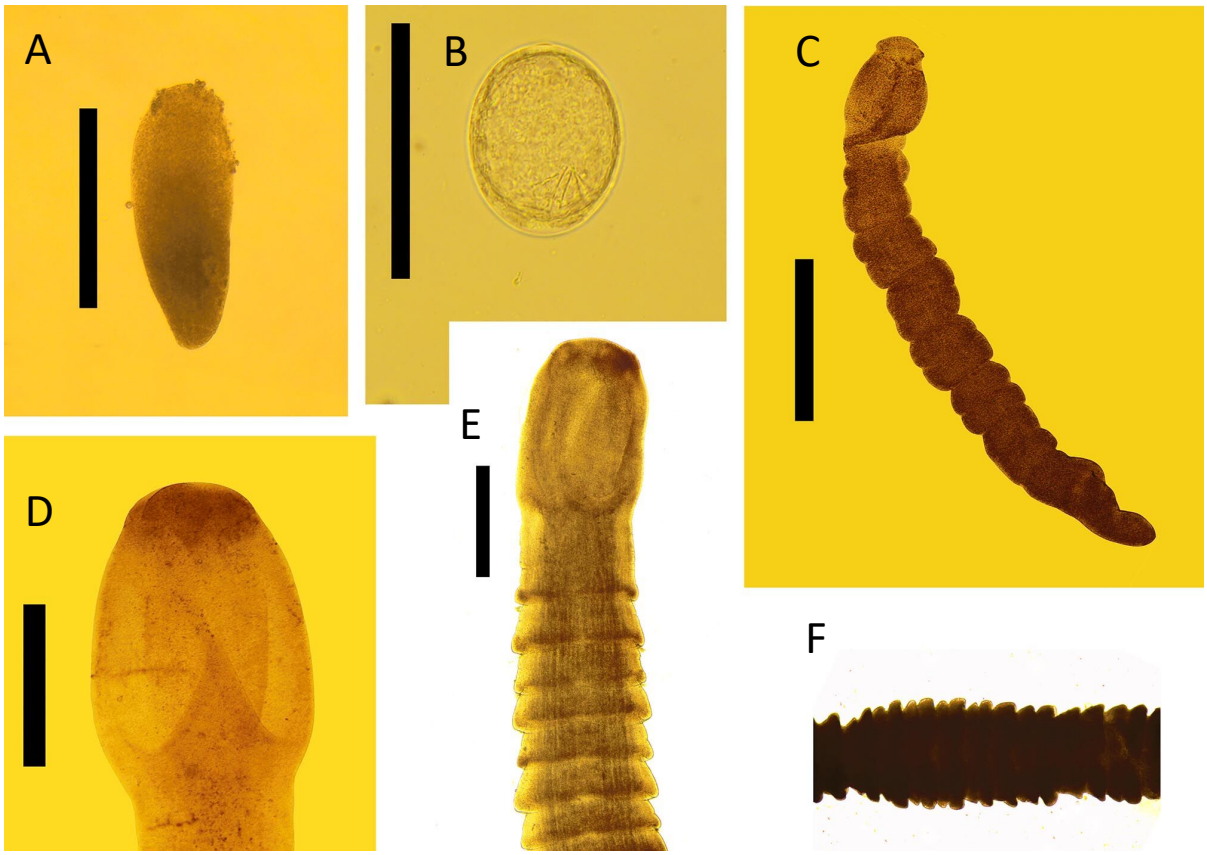

Figure 5 Various life stages of *Eubothrium* sp. found in this study. A: Plerocercoid, few features visible, no segmentation, B: hexacanth larvae still in the egg. Three pairs of hooks visible. C: Juvenile individual, scolex developed with apical disc and bothria, proglottids clearly visible. D: Close-up of the Scolex of a large adult individual. E: Scolex and neck-region of the largest individual found in this paper. F: Segments showing the serrated strobila characteristic of this cestode. Scale bars represent 1000 µm in picture B, 500 µm in picture A and 100 µm in pictures D and E. Picture F has the same scale as picture E.

3.2 Infection pattern

Five to Eight weeks after sea launch, the prevalence of the spring cohorts was 0 % for C and 36-7 % for D, while for the autumn cohorts was 60 % for A and 20 % for B. By the end of the study all cohorts har reached a prevalence of roughly 90 % (range: 86.7-100 %). Abundance usually peaked before steadily decreasing for the rest of the study period. Mean cestode infrapopulation weight increased continually over the course of the study in three of the four cohorts; A, B and C. In cohort D, the cestode weight decreased slightly in the interval between the two last samplings.

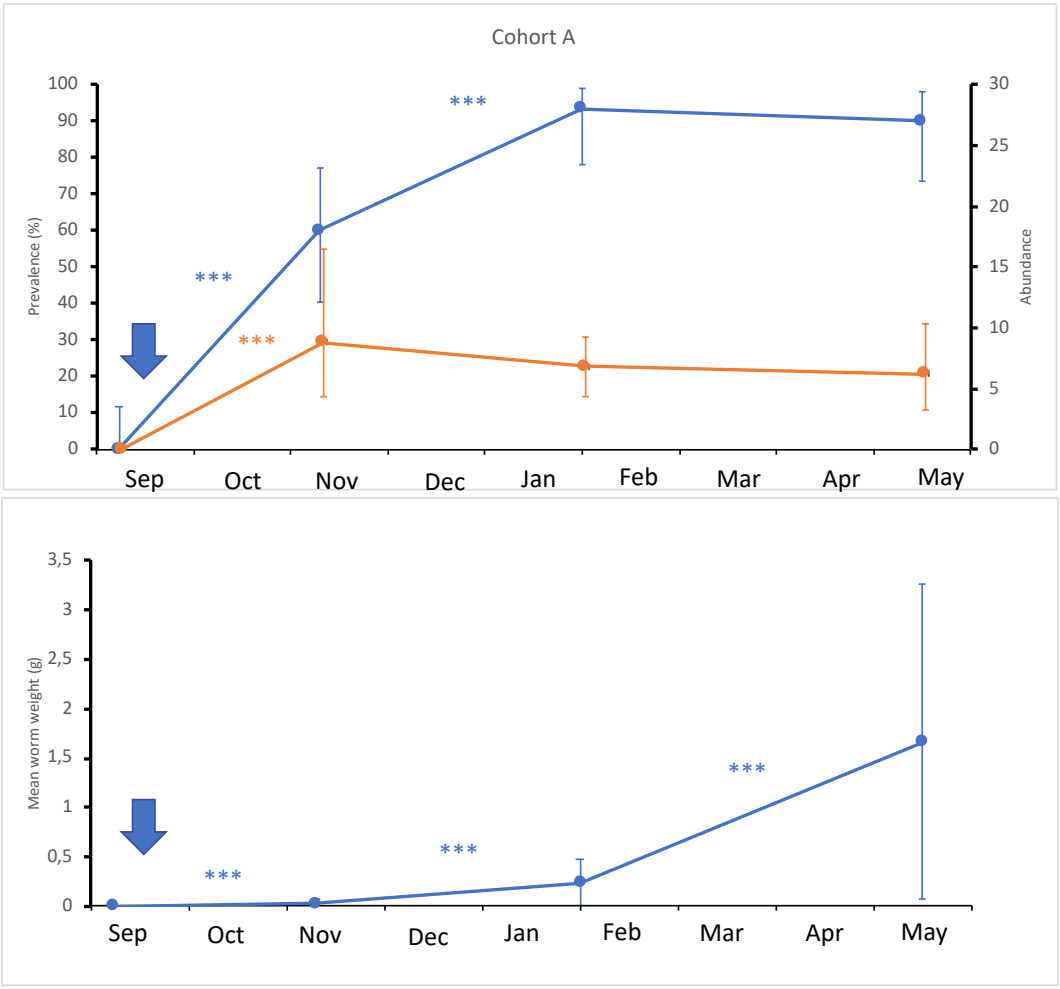

Figure 6 Infection pattern of *Eubothrium* sp. in salmon (S. salar) for cohort A during the study. Top: prevalence and mean abundance. Bottom: mean cestode infrapopulation weight. Time of sea launch marked with arrow. *P<0.05, *** P<0.001

In cohort A, there was a highly significant increase in prevalence from 15th September, when all were uninfected, to 60 % in the first seawater sample on 9th November. Prevalence increased further to 93 % in the 30th January sample, after which no further increase occurred to the last sample on 15th May (Figure 6).

Abundance also showed a highly significant increase from September to November (KW, H_3=51.8, P<0.001), when mean abundance peaked at 9 worms. Thereafter mean abundance decreased slowly to 6 worms in the last sampling on 15th May (not significant). Variance-to mean-ratio peaked in the first seawater sample in November before decreasing to February. This was followed by a rise to May (see figure 10). Mean worm weight (WW) increased significantly between each sample, reaching 1.7 g in May.

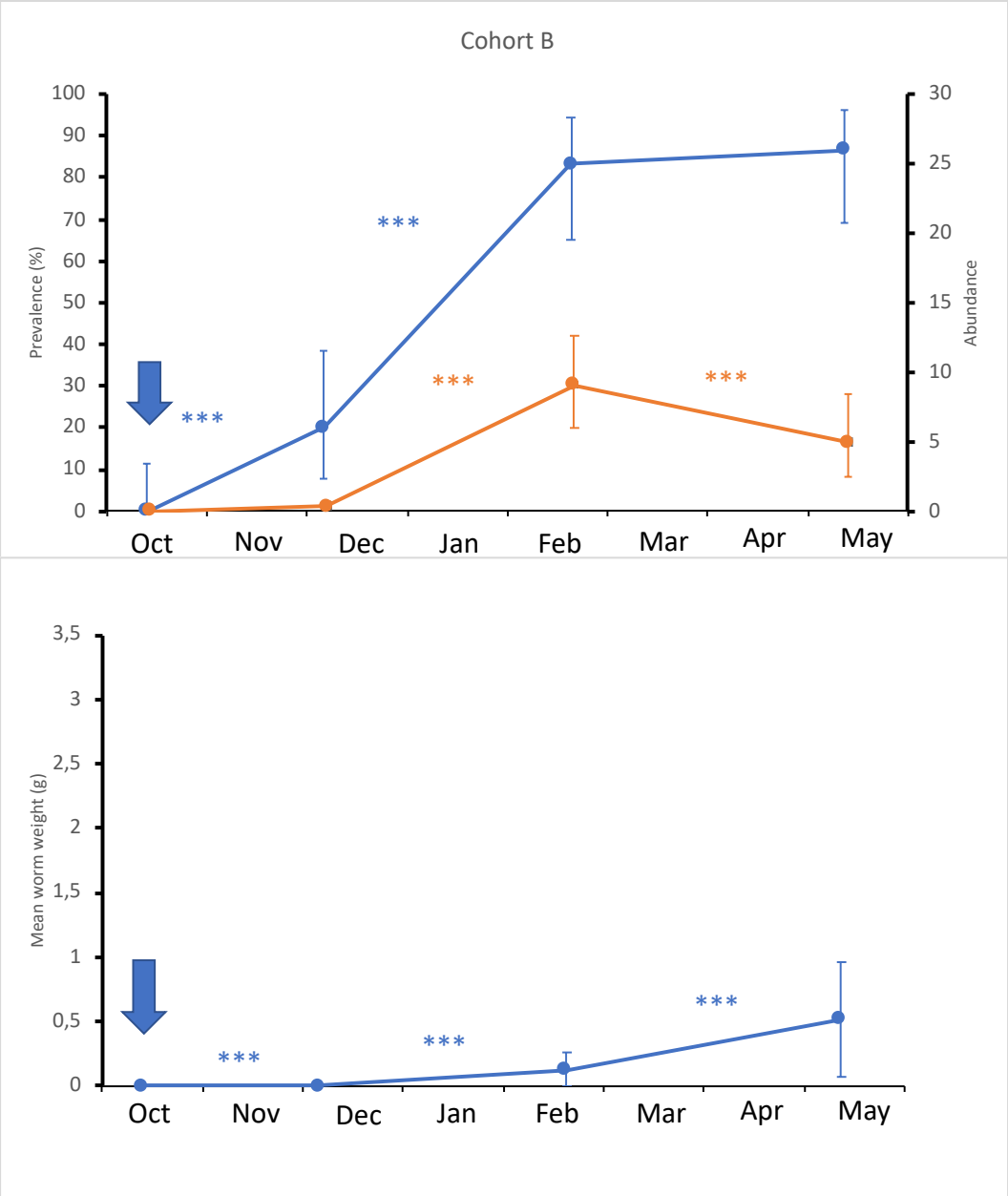

Figure 7 Infection pattern of *Eubothrium* sp. in salmon (S. salar) for cohort B during the study. Top: prevalence and mean abundance. Bottom: mean worm weight. Time of sea launch marked with arrow. *P<0.05, *** P<0.001

In cohort B, there was a highly significant increase in prevalence from 14th October, when all were uninfected, to 20 % in the first seawater sample on 5th December. Prevalence increased further to 83 % in the 19th February sample, after which no significant increase occurred to the last sample on 15th May (Figure 7).

The abundance increased from December to February (KW, H3=69.4, P<0.001), when mean abundance reached 9 worms, before apparently declining to 5 on 11th May (not significant). The variance-to-mean ratio of abundance increased throughout all samplings (see figure 10). Also, WW increased significantly between each sample, reaching 0.52 g in May.

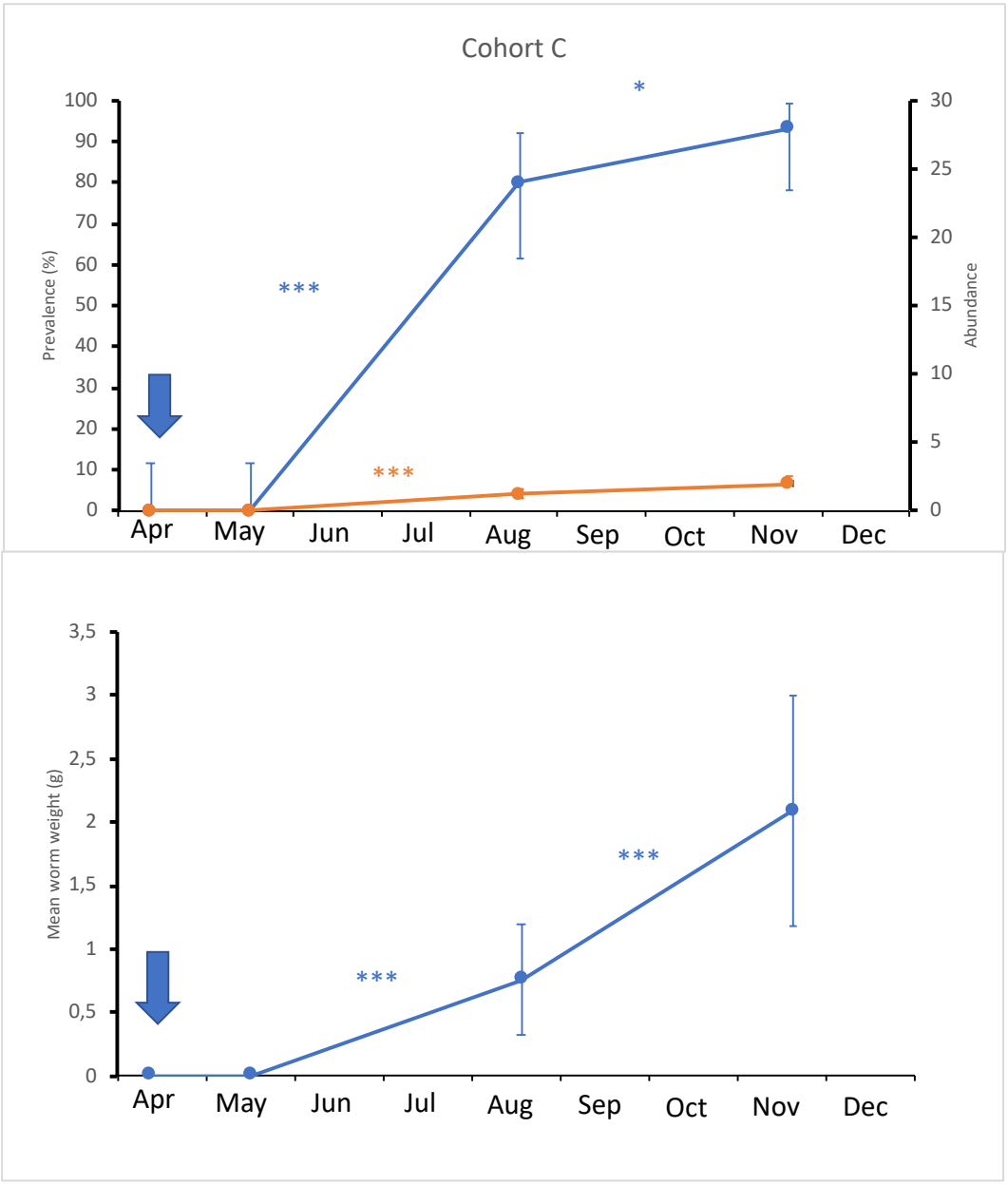

Figure 8 Infection pattern of *Eubothrium* sp. in salmon (S. salar) for cohort C during the study. Top: prevalence and mean abundance. Bottom: mean worm weight. Time of sea launch marked with arrow. *P<0.05, *** P<0.001

In cohort C, no infection was found to take place in the time from sea launch on 18th April to first seawater sampling on 15th May. In the following months there was a highly significant increase in prevalence, reaching 80 % by 17th August. A further, increase in prevalence took place August-November, reaching 93 % by 19th November (Figure 8).

Abundance also showed a significant increase from May to August (KW, H_3=86.4, P<0.001), when mean abundance reached a modest 1.2 worms. A peak mean abundance of 2 worms was seen in November (not significant increase).

The variance-to-mean ratio stayed low throughout the study period, reaching a top of 0.75 in the end (see figure 10). WW increased significantly from May to August and August to November, ending on 2.1 g, the highest WW seen in this study.

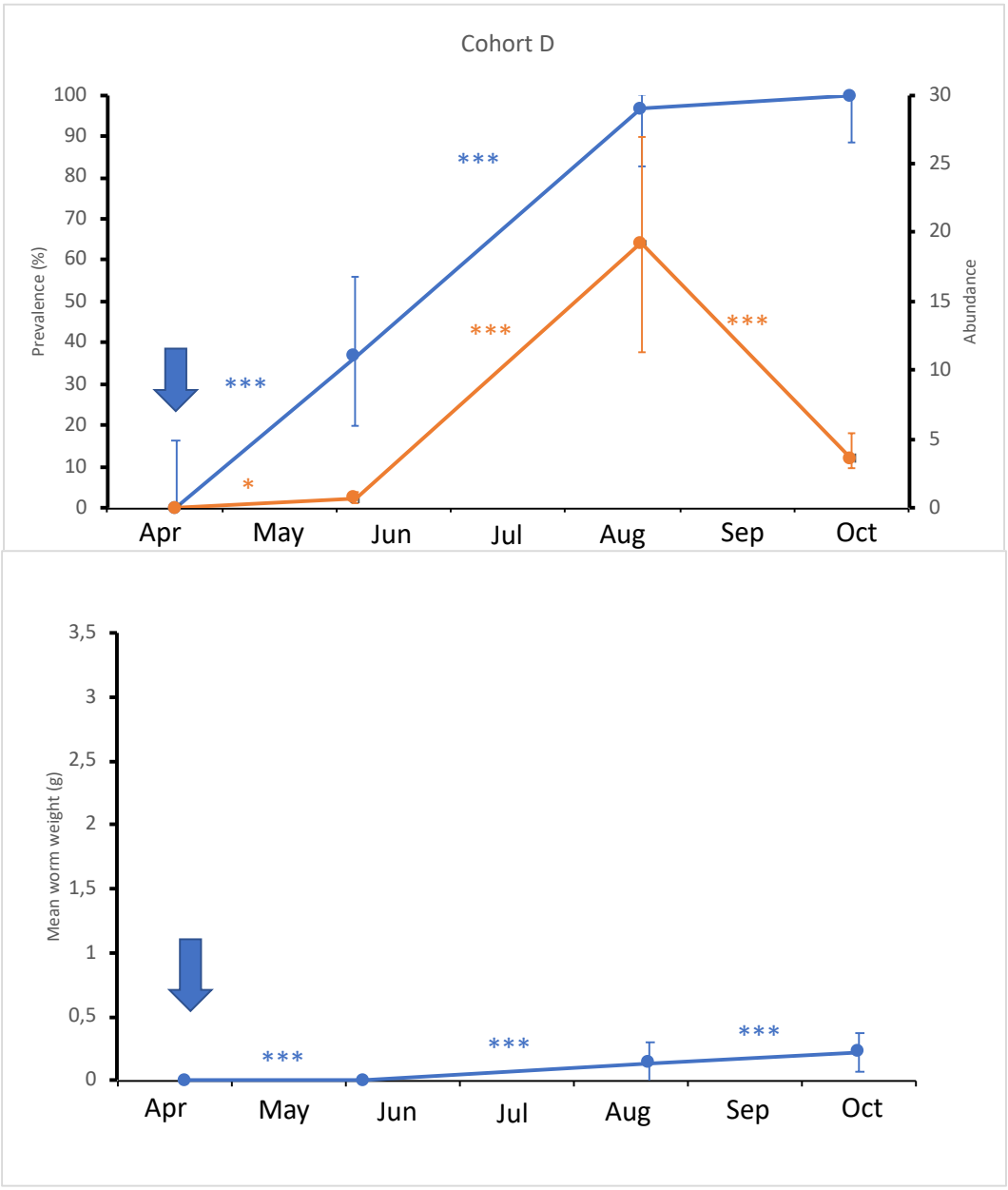

Figure 9 Infection pattern of *Eubothrium* sp. in salmon (S. salar) for cohort D during the study. Top: prevalence and mean abundance. Bottom: mean worm weight. Time of sea launch marked with arrow. *P<0.05, *** P<0.001

Cohort D showed highly significant increases in prevalence from sea launch on 18th April to 37 % on 15th June and later to 97 % on 20th August (figure 9).

Abundance varied significantly (KW, $H_{3(N=111)}$=77.8), with a highly significant increase June-August reaching 19 worms. A decline in abundance to 3.6 in October was seen but was not significant (P=0.22). A modest increase from 0 in May to 0.7 worms in June was also significant. The apparent fall in mean abundance August-October did not lead to a fall in WW, which increased slowly but significantly throughout the study period. This also coincided with a sharp decline in the variance-to-mean ratio of abundance (figure 10).

3.3 Variance-to-mean ratio

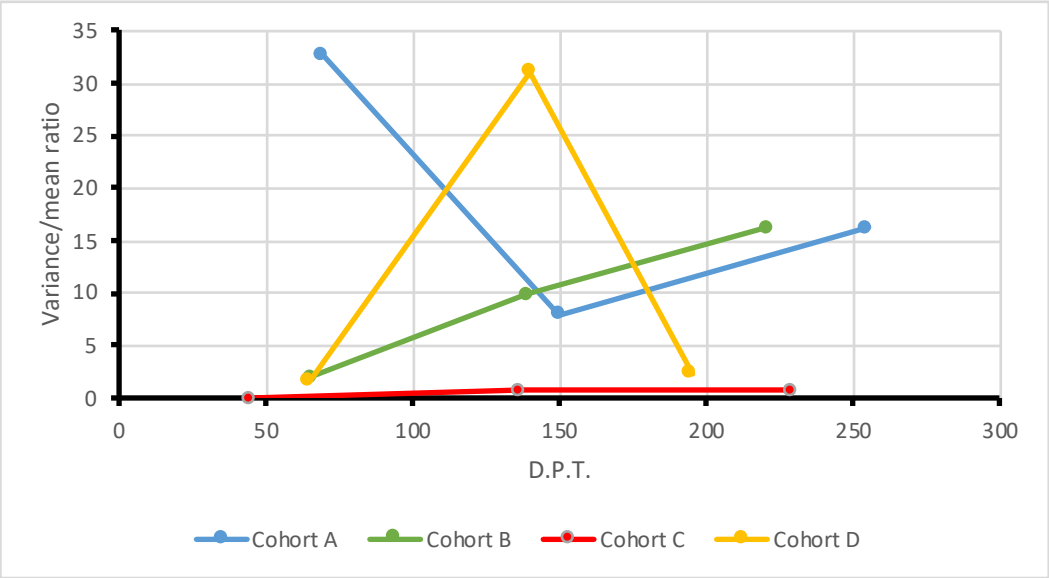

Figure 10 Variance-to-mean ratio of mean abundance for all cohorts, given at days post transfer.

No uniform pattern was observed in the variance-to-mean ratio of the different cohorts. A fall in variance-to-mean ratio was generally seen to coincide with sharp drops in mean abundance. Developments are described together with prevalence and abundance.

3.4 Relationship between *Eubothrium* sp. infection and host size

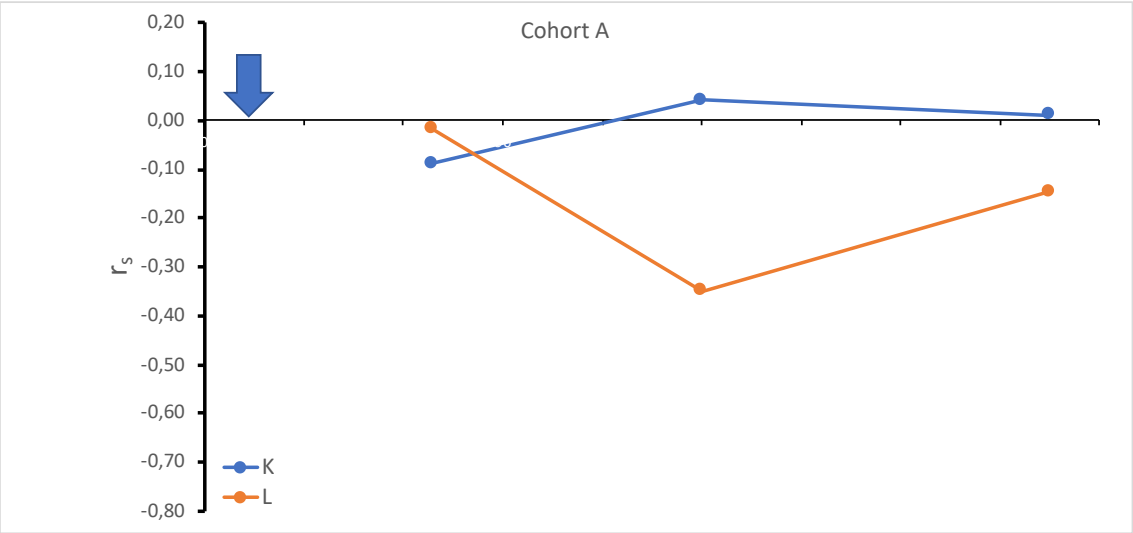

Figure 11 Spearman rank-order correlation coefficients between *Eubothrium* sp. mean abundance and fish condition factor (K) and fish length (L) for cohort A. Time of sea launch marked with arrow.

The spearman rank-order correlation coefficients for cohort A were not shown to be significant at any point in time. A negative coefficient between *Eubothrium* sp. abundance and fish length was seen, however this was not significant (figure 11).

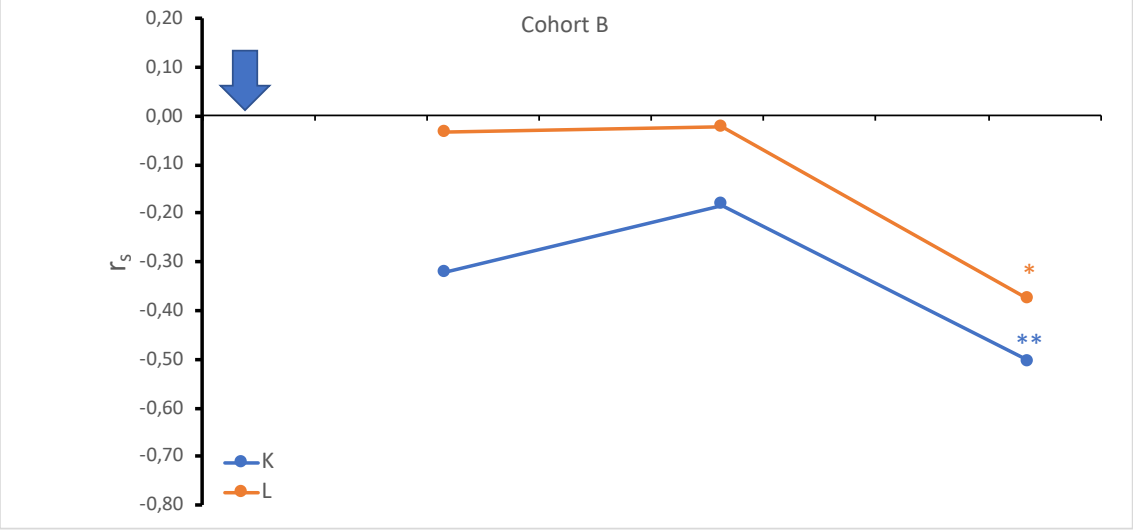

Figure 12 Spearman rank-order correlation coefficients between *Eubothrium* sp. mean abundance and fish condition factor (K) and fish length (L) for cohort B Time of sea launch marked with arrow. *P<0.05, ** P<0.01

The spearman rank-order correlation coefficients for cohort B were consistently negative, however only significant after 209 days at sea. At this time both length and condition factor were significantly negatively correlated with mean abundance (Figure 12).

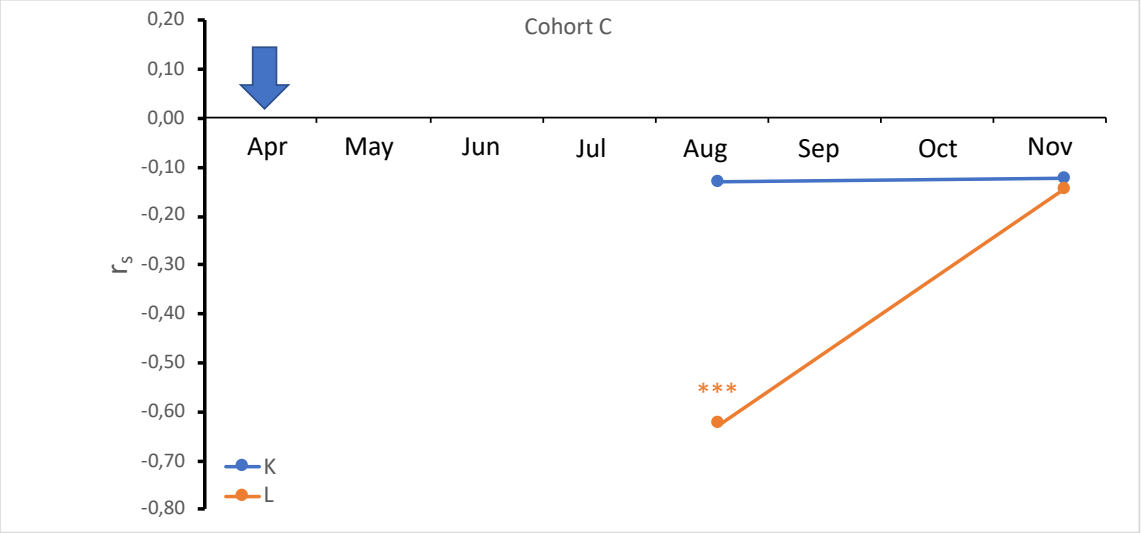

Figure 13 Spearman rank-order correlation coefficients between *Eubothrium* sp. mean abundance and fish condition factor (K) and fish length (L) for cohort C. Time of sea launch marked with arrow. *** P<0.001

Infections were first seen in cohort C in August, 4 months after sea launch. At this point, a highly significant negative correlation was seen between the abundance of *Eubothrium* sp. and length of the fish. This correlation was not significant three months later in November, though still negative. Condition factor and *Eubothrium* sp. abundance was negatively correlated, but not statistically significant (figure 13).

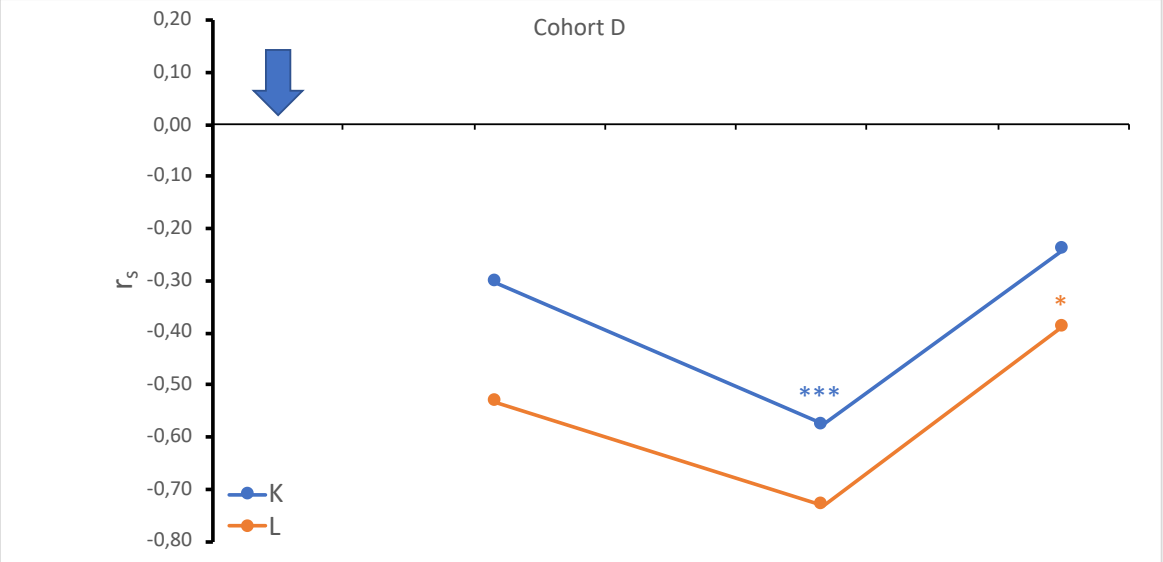

Figure 14 Spearman rank-order correlation coefficients between *Eubothrium* sp. mean abundance and fish condition factor (K) and fish length (L) for cohort D. Time of sea launch marked with arrow. *P<0.05, *** P<0.001

Eubothrium sp. abundance and fish length always showed consistent significant negative correlations, although the levels of significance varied. Condition factor was negatively correlated at all times but were only significant in August (figure 14).

3.5 Cestode growth and maturation

In order to make a standard curve of worm length/weigh, 44 individuals from this study that were in good condition were used. In addition to these, 25 individuals from a different study by Lena Geitung were used to further increase the accuracy of the graph.

A regression between cestode length (*EL*) and cestode weight (*EW*) yielded formula 1). This was used to give an estimated weight of cestode infrapopulations where worms could be enumerated and assigned a stage but were otherwise too small to weigh with the methods used.

1) $EW = 0{,}0339EL^{1{,}5964}$

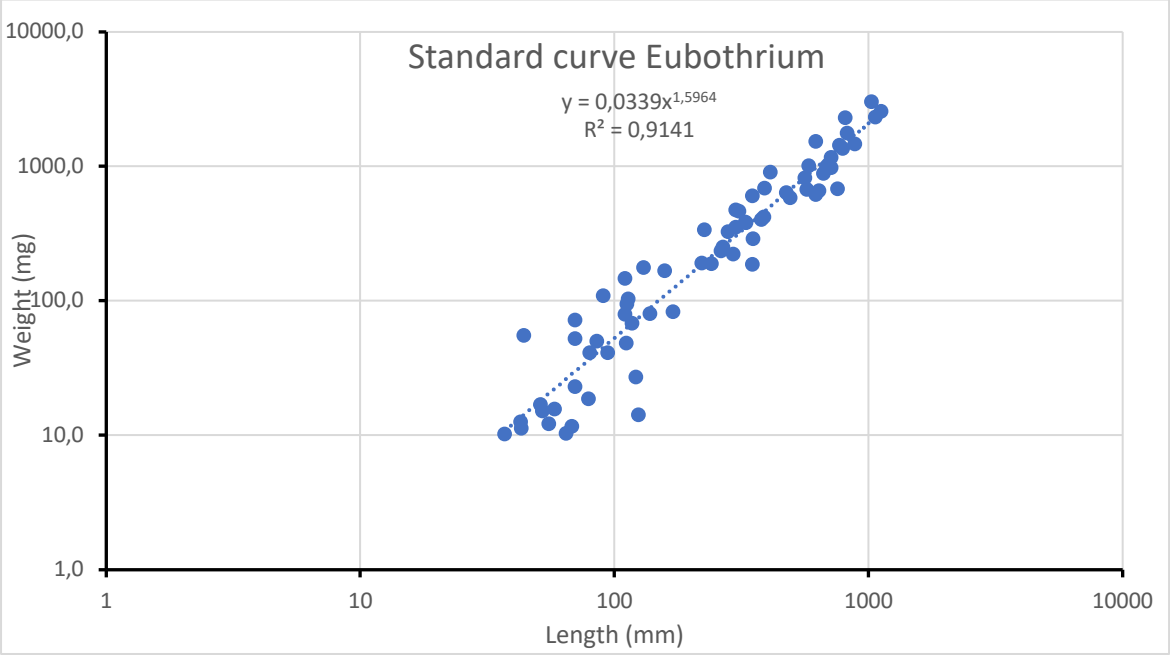

Figure 15 *Eubothrium* sp. length-weight relationship. Only intact worms were used, with a normal "relaxed" state after freezing.

Very few plerocercoid worms were found (N=5) These were all found in cohort D from the first and second seawater sampling, 15th June and 20th August respectively. The fish individuals harboring plerocercoids measured 23-29 cm in length and weighed 96-198 g. Juvenile I were found from 15th May to 5th December in fish ranging from 19.7 to 47.0 cm. Except for a few outliers, stages I-II were mostly found in fish with a length below 35 cm (see figure 16). Mature gravid cestodes were at the earliest observed 121 days after transfer to sea, 66 days after last sampling (figure 18).

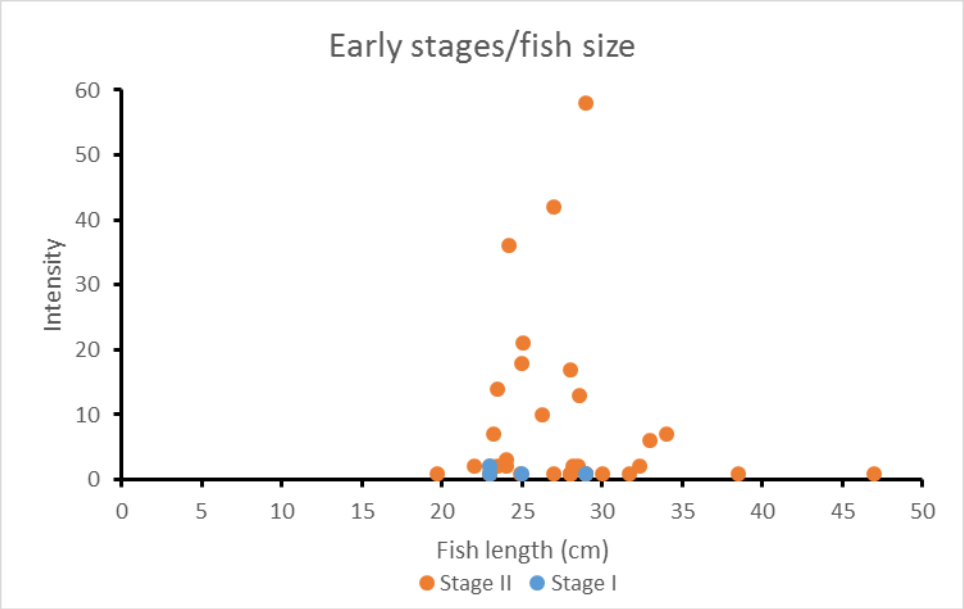

Figure 16 Occurrence of early stages of cestodes vs length of fish. Stages I (plerocercoids) and II (juveniles under 10 mm) could represent recent infections due to fish feeding on copepods.

The largest intact worm found had a length of 112 cm, maximum width of 4,6 mm and was gravid. The widest proglottids, close to the posterior end appeared to contain the most eggs. The highest abundance seen was 95 *Eubothrium* sp. in a single fish.

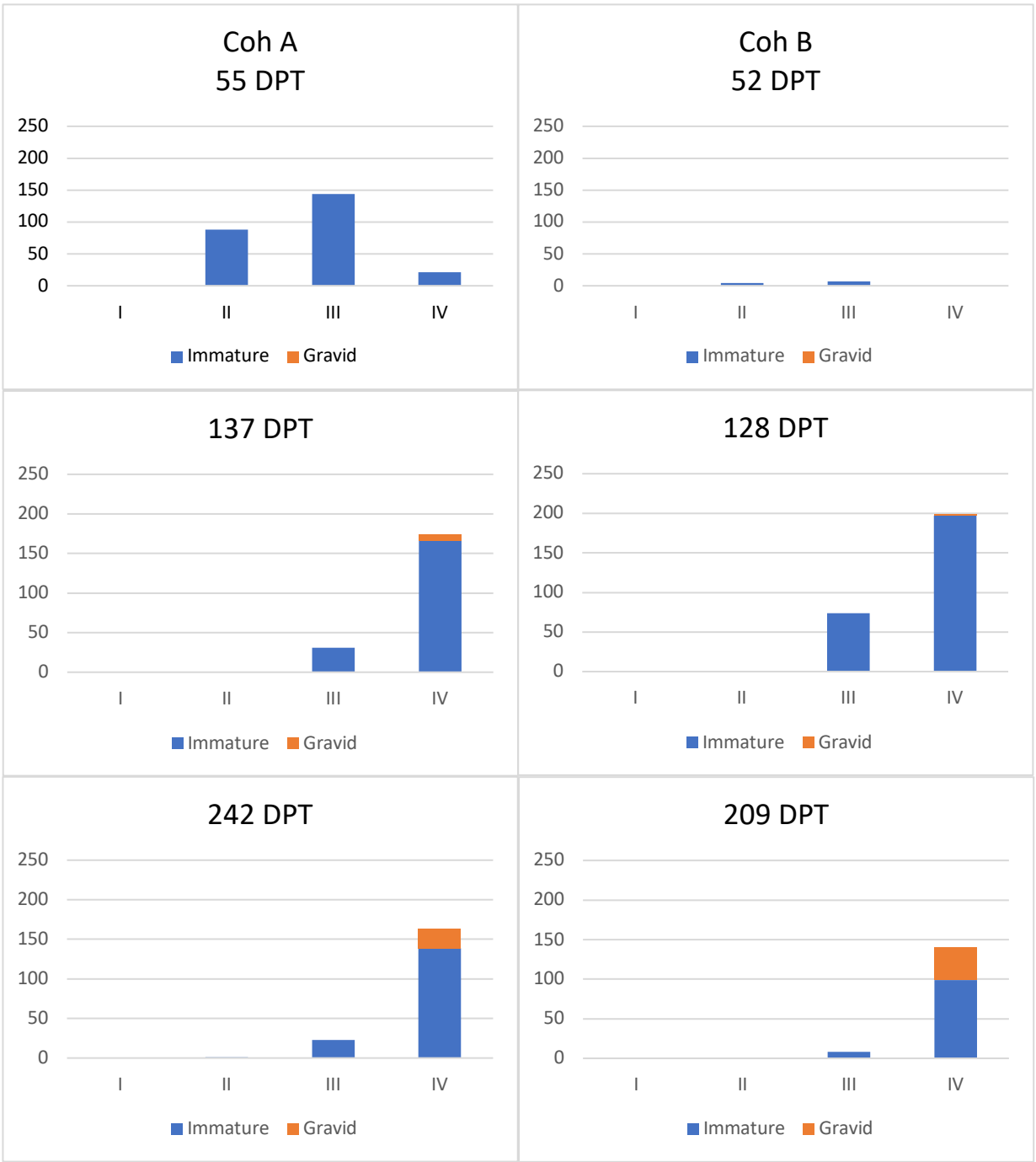

Figure 17 Overview of distribution of cestode developmental stages for the two autumn-cohorts. The number of individuals in each category is given at the point in time of sampling, given as days post transfer. Cohort A to the left, cohort B to the right.

In all samples most worms found belonged to one or two stages. Early stage worms (stage I-II) were mostly found in the first seawater sample, but some were found in cohort A at 242 DPT (figure 17).

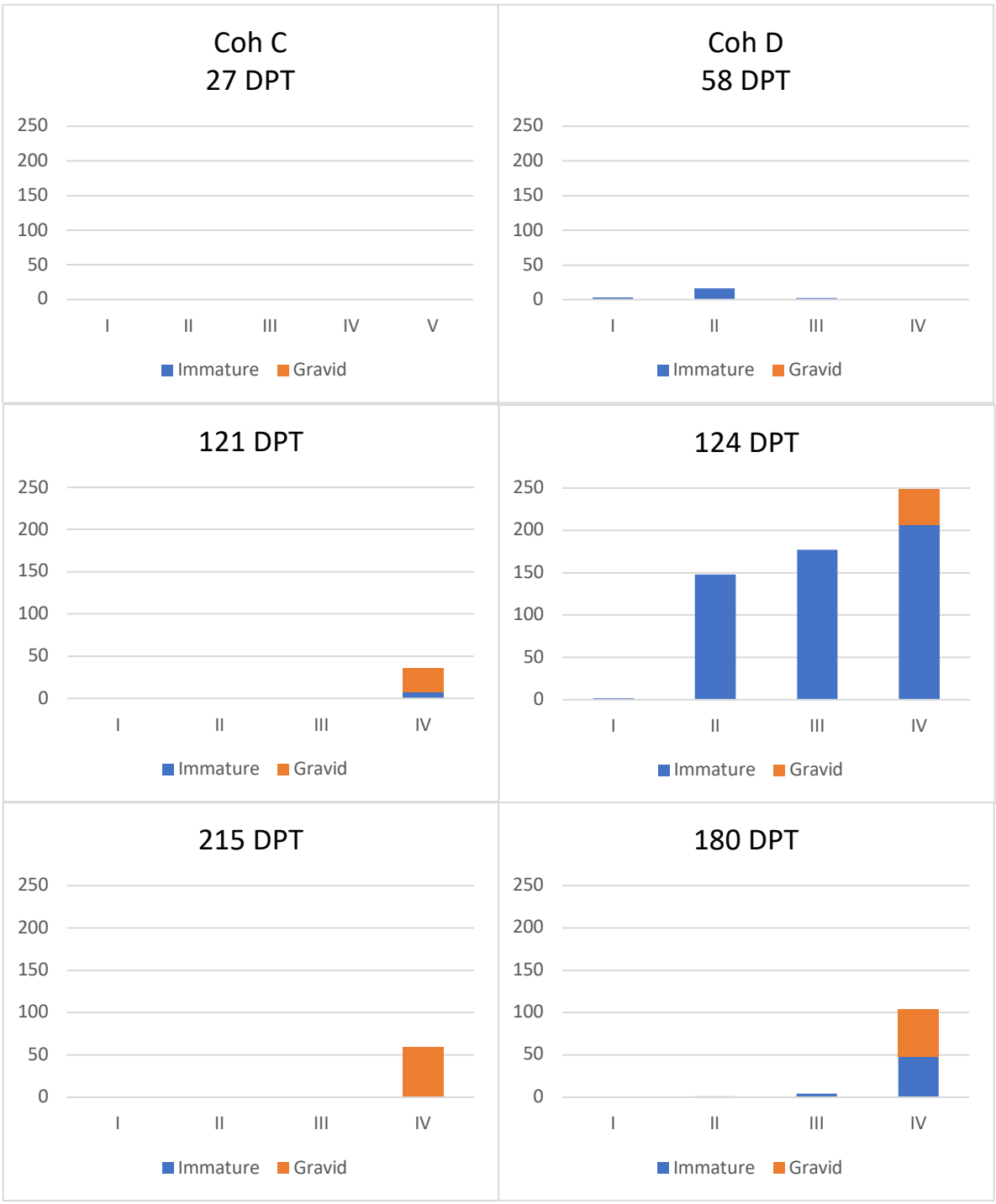

Figure 18 Overview of occurrence of cestode developmental stages for the two spring-cohorts. The number of individuals in each category is given at the point in time of sampling, given as days post transfer. Cohort C to the left, cohort D to the right.

In cohort C, no infection was seen until 121 days post transfer, at which point all worms were stage IV and often gravid. Cohort D differed from other cohorts in that it was not unusual to find worms of three different stages in the same sample, as opposed to two in the other cohorts. At the time of the second seawater sample at 124 D.P.T. all stages of *Eubothrium* sp. was found in cohort D (figure 18).

3.6 Stomach contents

Various remains crustaceans such as krill, skeleton shrimp, *Caprella* spp, and copepods were encountered in the intestines of the examined salmon. One such copepod could be identified as *T. longicornis*. Such findings were exclusive to the first seawater sample, with one sighting in cohort B, 6 in cohort D and one in cohort X.

3.7 Site

97.2% (N=2065) of all *Eubothrium* sp. found were placed in the caecal region, with the rest being evenly distributed between the midgut (N=29) and hindgut (N=30). In a few cases several worms could be found in parts of the gut other than the caecal region. This was observed exclusively when the intensity of the infection was relatively high (N>20). Plerocercoids were found in the caecal region (N=2) and in the hindgut (N=3).

In addition to whole worms, free strobila were on several occasions found in the mid and hind gut. These strobila sometimes contained large amounts of eggs. Whole worms and free segments found in the post-pyloric gut generally appeared to be more cadaverous than the others and could exhibit a slightly different white/transparent color.

The proportion of cestodes in the C1-region decreased noticeably over the course of the study, while the proportion in C2 increased. C3 remained more or less the same, with only a slight decrease (table 3).

Table 3 Detailed overview of cestode sites in Cohort 1 from first to last sampling. Only individuals where all worms could be accurately placed are included in this overview.

	n *Eubothrium* sp. in site (%)					
Sample	C1	C2	C3	Midgut	Hindgut	Total
1	0	0	0	0	0	0
2	69 (38)	51 (28)	63 (34)	0 (0)	1 (1)	184
3	6 (14)	21 (49)	14 (33)	0 (0)	2 (5)	43
4	2 (11)	10 (56)	5 (28)	0 (0)	1 (6)	18

3.8 Other parasites

In 11 fish, nematodes were found lying free inside the gastrointestinal tract. Except for one sample that contained two nematodes, these were all single infections.
Using microscopy, ten of these were identified as adult (or preadult) *Hysterothylacium aduncum* according to Berland (1961).
A single nematode encapsulated on the caeca, was morphologically identified as *Anisakis simplex* according to Berland (1961) and Longshaw (2012).
Five of the guts found to harbor nematodes also contained crustacean remains. Nematodes were only found in the first seawater sample at two location, A and D.

4. Discussion

4.1.1 Natural infection dynamics

The known natural final hosts of *Eubothrium* sp. in western Norway are wild Atlantic salmon and seatrout. In order to be able to infect outward migrating Atlantic salmon smolts, copepods containing tapeworm larvae should be present May-June (Ugedal et al., 2014). The salmon smolts migrate quickly from the coastal areas, and further infections in the oceanic feeding areas seems unlikely. Also, the known first intermediate copepod hosts are common only in the coastal areas (Gundersen, 1953; Matthews, 1967).
However, seatrout may have been important in the natural population dynamic of this tapeworm. Seatrout feed in the fjords throughout summer-autumn, and immature fish may overwinter in estuaries (Ugedal et al., 2014). Therefore, tapeworm maturation and egg release may lead to infected zooplankton during summer-autumn, perhaps coinciding with the autumn peak in *Acartia* spp. abundance (Gundersen 1953), including *A. clausi* which is known to act as first intermediate host. Among the copepods readily infected when exposed to *Eubothrium* sp. eggs, *A. clausi* and *T. longicornis* (Hodneland & Solberg 1995), adult *Acartia* spp. may peak in July and September, and tends to occur in the upper water masses. *Temora longicornis* seems more sporadic in occurrence, adults peaking spring-summer (Gundersen 1953; Matthews 1967). The source of eggs that infect the zooplankton during summer could, in addition to seatrout (Fahy 1980) be returning adult Atlantic salmon. Other

copepods could also play a role in the infection dynamics, as only a limited number of species have been tested. Prevalence of *Eubothrium* sp. in large wild salmon caught at the coast range from 36-54% (Kennedy 1978b; Bristow & Berland 1991). The relative importance of these two host species in the tapeworms life-cycle is unknown.
However, the population dynamics of *Eubothrium* sp. may have been changed during the last 40-50 years, due to the very high host density posed of farmed Atlantic salmon that also reside in the fjords throughout the year.

The autumn stocked fish was found to harbor gravid *Eubothrium* sp. in May, and the spring stocked salmon contained eggs in October-November. This could suggest egg-release at these times. However, the actual release of eggs may not coincide absolutely with presence of eggs *in-uteri* of the worms, as releasing eggs when copepod numbers are low would be a waste of energy for the parasite. Whether such a timing occurs is at present unknown. Susceptible species of copepods must also be present in the environment and be of appropriate size. It has previously been speculated that insufficient size of copepods during spring could make it impossible for *C. scutifer* to ingest eggs and become infected with *E. salvelini.* Therefore, a peak in infection occurs later in the summer when copepods have grown to a sufficient size (Smith 1973). This would make the presence of adult copepods the deciding factor for seasonality in this cestode species.

Within 15 days of infection of the copepod *Acartia tonsa*, the proceroid will have developed to at state where it is infective to fish (Saksvik et al., 2001b). Developmental stages found in the fish generally suggested a limited period of infection with most individuals belonging to one of the stages discerned, and fewer to the directly preceding stage. This was however not exclusively the case, and Saksvik et al. (2001) showed that cestodes acquired simultaneously in experimental infections can vary much in size and maturity.
The cestode has been reported to persist in the fish gut for two years, possibly even longer (Fahy, 1980b). If this is the case, then the infection dynamics might differ from that of *E. crassum* which has been reported to only survive for a maximum of one year (Kennedy, 1996).

As copepods are not the only source of infection, some consideration should be given to the alternate infection route that occurs when smaller fish functioning as paratenic hosts are eaten. This route might not be as seasonal as the assumed primary infection source due to the longer lifespan of fish, which may contribute to the diffuse seasonality of the infection pressure seen here.

4.1.2 Spring

Infections were first detected in the spring cohorts on 15th June in cohort D, at which point at third of the fish were infected. These infections must have taken place between sea-transfer on 18th April and this point in time. Interestingly, cohort C did not see any infection during the period from sea launch in April to first sampling in the middle of May. Therefore, it could be that the infective stages first appear in the period mid-May to mid-June. Berland & Bristow (1991) studied a cohort transferred to sea in late May at the outer coast of Hordaland, and found the first infection in late June, a single infected fish with very small worms. While the first infections may be acquired in May-June, most of the increase in both prevalence and mean abundance happened during summer from June to August, indicating that this is a period of high infection pressure. Cohort C saw a continued increase in abundance in the months from August to 19th November, a sign that infections occur in autumn as well.

However, in cohort D, there was a decrease in abundance in this period. The same was observed by Berland & Bristow (1991), a peak abundance in August followed by a decrease. This may have been due to worms being eliminated at a higher rate than they were acquired. Evidence for this is seen in the variance-to-mean of abundance, which also decreased markedly. This decrease suggests that worm loss is higher in the most intense infections (density-dependent mortality), a well-known phenomenon in cestode infections (E Bush & Lotz 2006; Esch & Fernández 2011).

4.1.3 Autumn

In the present study, significant increases in in prevalence and abundance was taken as evidence for infections being acquired, and hence that there was an infection pressure at

the time. Supporting evidence was also seen in the occurrence of plerocercoids and very small worms.

Infections were first detected in the autumn cohorts on 9th November in Cohort A Infections took place in the same time-frame for cohort B but rise in mean abundance was less pronounced. The highest rise in prevalence and mean abundance for this cohort took place between 5th December and 19th February. While cohort A also experienced new infections in this time as evidenced by the rise in prevalence, there was also a decrease in mean abundance. From this it appears that the main period of infection for the cohorts put to sea in autumn must have taken place somewhere between September and February.

The predetermined sampling design for the present study aimed at samples one month, three months and six months after sea launch. This allowed for more cohorts and locations to be investigated than would have been the case if the sampling was more frequent. In one case with cohort C, the long timespan between the second and third seawater sample (May-August) resulted in the fish going from having no infection to having mostly large gravid individuals. Clearly, more frequent sampling is needed in summer for a more accurate understanding of variation in infection pressure.

Taken together, the studied cohorts were observed to become infected in the period June-February. The absence of infection in the autumn-cohorts after February could be explained by the fish no longer being vulnerable to infections due to their size, it is however clear that the infection pressure must have decreased sometime between February and sea-launch in spring.

4.1.4 Fish size

Remains of crustaceans were only found in the gut of some fish from the first seawater sample. The identifiable remains represented copepods (including *T. longicornis*), krill and *Caprella* spp. The latter amphipods had likely been picked from the net walls where they may be common (AS, 2014), while the presence of zooplankton show that some fish (up to around in 30 cm in length) prey on them given the opportunity. These therefore could have a higher chance of acquiring copepod-transmitted parasitic infections. Copepod abundance

in the zooplankton varies regularly with season (Gundersen, 1953), so availability may vary. However, it seems likely that it is the smaller recently stocked salmon that is most prone to feed on small items such as copepods. While actual studies on this phenomenon seem to be lacking, the present observations of both crustacean remains and infections with the zooplankton transmitted nematode *H. aduncum* was found only in the first seawater samples. The occurrence of plerocercoids and other very small juveniles in the salmon were also biased toward the smaller fish. Hence, it seems likely that the smolts feed readily on available copepods the first weeks after sea-transfer, but that the importance of such prey decreases as the fish grow. Hence, the apparent decrease of infection pressure in winter could be an artefact of bigger fish not eating copepods. Another possibility is that the gill rakers of the bigger fish are too big to filter out copepods, leading to lower rates of ingestion and thereby fewer infections.

As the fish grew in size there was little proof of infection to be seen from changes in prevalence and abundance. Possibly, a low-grade recruitment of parasites in such fish could have been masked by mortality (i.e. loss) of cestodes, particularly from the largest infrapopulations. However, another line of evidence was the stages of the cestodes recovered from the fish. Stage I and II were practically never found in fish over 35 cm, suggesting that fish over this size have a lower chance of acquiring infection. Further supporting this line of reasoning is the fact that in many samples the abundance of worms was negatively correlated with fish length, suggesting that the individuals who acquire infection are the smaller ones. These may lose out in the competition for food and therefor be more likely to opportunistically feed on zooplankton.

4.2 Patterns of *Eubothrium* sp. infection and host size

The effects of *Eubothrium* sp. on Atlantic salmon hosts have previously been studied under lab conditions (Saksvik, Nilsen, et al. 2001). In that study, feeding was reduced to avoid masking any effects of the parasite. Still, only a modest negative effect was seen on the condition of the fish (but significantly). As the present study is a field study of normal aquaculture conditions feed availability was normal. Therefore, some potential effects of the

cestode infections, increased hunger and feeding, could counteract growth effects. Negative correlations between infection and fish growth could be explained by smaller fish being more likely to become infected, therefore the data from this study are not suited to uncover negative effects by *Eubothrium* sp. on its host.

Correlations between *Eubothrium* sp. abundance and fish condition were mostly negative, and when abundance was high generally significant. Three of the four cohorts showed a negative correlation between abundance of cestodes and length in at least one sample, with one of them (cohort D) showing this correlation consistently at all samplings. Overall, significant negative correlations between infections and fish size were seen during peaks in abundance, when the infection pressure could be assumed to be at its highest.

4.3 Processes in the cestode infrapopulation

Crowding is a phenomenon where, as the infrapopulation of cestodes increases in size, the individual worms are negatively affected due to there being too many of them. A classical manifestation of this is a negative correlation of worms size with infrapopulation size (Read 1950). Some possible explanations for this is competition over resources, interference between the worms themselves or due to the host immune system being triggered (Roberts, 2000).

After an initial phase of active infection during which mean abundance increased, the number of cestodes per fish generally went down in the following samples. This was the case in cohorts A, B and D, with D experiencing a maximum mean abundance of abundance of 19.2 during august before falling to 3.6 in October. In this case the majority of cestodes that entered the fish must have been eliminated. This fall in mean abundance was accompanied by a sharp decline in variance-to-mean ratio, indicating that density dependent mortality might have taken place (Anderson and M Gordon, 1982).

This pattern of initial heavy infection during summer followed by heavy mortality has been reported for *E. crassum* in brown trout in the wild (Campbell, 1974; Kennedy, 1996) as well as brown- and rainbow trout stocked in a reservoir (Wootten, 1972). The pattern was less

pronounced in cohorts with lower infection levels, in fact the cohort with the lowest abundance did not see a drop in cestode numbers at all during the study period. Mean worm weight was shown to increase continually throughout the study period, even in periods of mortality. This suggest that the worms being eliminated because of density dependent mortality were the smaller individuals, while the larger ones survived and continued to grow in size.

As infections progressed the proportion of worms in the various regions of the gut changed (table 3). The proportion of worms in the longer caeca of region C2 increased with every sampling while the other regions of the caeca decreased. This happened as the mean abundance went down, and seems to indicate that this is the preferred site of attachment for *Eubothrium* sp. In the earliest samples where mean abundance was greater, the cestodes had a more even distribution throughout the caecal region, indicating an extension of site where infection numbers are high. This has previously been shown for several cestode species (Kennedy, 1983), including *E. crassum* infecting trout (Kennedy, 1996). Pleroceroids were found in the hind gut. Possibly this is the site where a functional scolex is developed, by the procercoid-like juveniles obtained from feeding on zooplankton copepods. The plerocercoids may then migrate proximal towards the caeca where they may attach.

4.4 Prevention

The findings in this study indicate that smaller fish have a higher risk of becoming infected by *Eubothrium* sp. than larger ones. Presently no prophylactic measures exist to protect against tapeworm infections at sea, however several such measures are used against sea lice. These include skirts that acts as barriers, as well as snorkel-nets that forces the fish to not spend time in the part of the water column where lice are transmitted.
In the future such methods could potentially be used to guard fish from sea launch until they reach a size where they are unlikely to feed on infected zooplankton.

4.5 Nematodes

The fact that *H. aduncum* were found exclusively in the first seawater sample suggests that infections with this species disappeared after a short time. Parasitic nematodes have

previously been shown to be absent in harvest grade salmon, with few individuals being found in the viscera of runts (Levsen & Maage 2016).

5.Conclusion

The infection pressure of *Eubothrium* sp. was shown to vary seasonally. Farmed Atlantic salmon become infected throughout much of the year, but few infections occur late winter to May and an apparent peak in infection pressure is seen during summer-autumn. This could be explained by infected copepods that increase in abundance during summer and autumn.

Spring-cohorts initially experienced lower levels of infection than autumn-cohorts, which should give them more time to grow before acquiring infections. After half a year at sea, prevalence and abundance reached similar levels in all cohorts regardless of season.

Abundance of the parasite was negatively correlated with fish size at a point in time close to infection, indicating that smaller fish have a higher risk of becoming infected, a likely effect of increased plankton feeding.

Early life stages (I and II) of the cestode were practically never found in fish over 35 cm suggesting that fish over this size have a low chance of acquiring infection. If infection is acquired, treatment after the fish has passed this size could lower the chances of reinfection, suggesting that treatment would could be needed once.

6. References

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7. Appendix

Appendix table 1 Data for the fish used in this study. Fork length (cm), weight (g) and condition factor (k) is given.

Nine fish from the freshwater sample of cohort D could not be located. The mean weight of the fish from cohort A and X decreased from sample three to sample four. These cohorts were at the same location, and there appears to have been an error in the weighing procedure there.

	Cohort	Sample	Valid N	Mean	Minimum	Maximum	Std.Dev.
Fish L (cm)	A	1	30	23,9167	21,1000	26,7000	1,33780
Fish W. (g)	A	1	30	156,4567	122,7000	209,6000	24,29102
K	A	1	30	1,1416	0,9689	1,4350	0,11759
Fish L (cm)	A	2	30	29,4167	23,9000	39,9000	4,0887
Fish W. (g)	A	2	30	330,6667	122,0000	860,0000	190,2987
K	A	2	30	1,1954	0,7471	3,4650	0,4706
Fish L (cm)	A	3	30	42,067	33,000	45,500	2,5418
Fish W. (g)	A	3	30	1834,500	1240,000	2590,000	307,1991
K	A	3	30	2,453	2,160	3,450	0,2288
Fish L (cm)	A	4	30	48,817	38,5000	57,500	5,1167
Fish W. (g)	A	4	30	1444,600	534,0000	2488,000	540,6043
K	A	4	30	1,179	0,9122	1,506	0,1369
Fish L (cm)	B	1	30	23,8833	22,0000	27,0000	1,28441
Fish W. (g)	B	1	30	158,8000	123,0000	238,0000	26,13414
K	B	1	30	1,1588	1,0109	1,3242	0,07744
Fish L (cm)	B	2	30	28,3000	25,0000	32,0000	1,74494
Fish W. (g)	B	2	30	286,3333	190,0000	420,0000	57,86330
K	B	2	30	1,2543	1,0251	1,6640	0,14336
Fish L (cm)	B	3	30	36,2500	33,0000	39,5000	1,6905
Fish W. (g)	B	3	30	601,5000	410,0000	850,0000	109,9306
K	B	3	30	1,2500	1,0686	1,5491	0,0979
Fish L (cm)	B	4	30	42,1000	29,0000	48,000	3,4775
Fish W. (g)	B	4	30	939,2333	320,0000	1392,000	233,0167
K	B	4	30	1,2321	1,0424	1,399	0,0930
Fish L (cm)	C	1	30	25,5167	21,50000	28,0000	1,48256
Fish W. (g)	C	1	30	181,5333	97,00000	242,0000	32,26157
K	C	1	30	1,0820	0,97601	1,2784	0,06668
Fish L (cm)	C	2	30	33,6000	23,0000	36,5000	2,82049
Fish W. (g)	C	2	30	420,0000	230,0000	540,0000	74,50989
K	C	2	30	1,1293	0,7549	2,7944	0,33101
Fish L (cm)	C	3	30	49,667	42,000	55,000	2,9634

Fish W. (g)	C	3	30	1562,000	1000,000	2140,000	281,6993
K	C	3	30	1,273	0,995	2,241	0,2079
Fish L (cm)	C	4	30	63,267	58,000	68,000	2,9935
Fish W. (g)	C	4	30	3236,000	2460,000	4090,000	464,1834
K	C	4	30	1,271	1,128	1,461	0,0760
Fish L (cm)	D	1	21	19,3048	16,50000	22,2000	1,48171
Fish W. (g)	D	1	21	108,8571	73,00000	137,0000	15,35671
K	D	1	21	1,5236	1,21561	1,9060	0,20442
Fish L (cm)	D	2	30	26,3833	22,00000	30,0000	2,76581
Fish W. (g)	D	2	30	192,4667	96,00000	294,0000	66,64716
K	D	2	30	1,0021	0,78595	1,1297	0,08498
Fish L (cm)	D	3	30	37,5667	25,00000	43,0000	4,7900
Fish W. (g)	D	3	30	597,4000	96,00000	928,0000	228,1835
K	D	3	30	1,0553	0,61440	2,1644	0,2643
Fish L (cm)	D	4	30	47,667	42,000	56,000	3,5266
Fish W. (g)	D	4	30	1411,667	1080,000	2285,000	238,4878
K	D	4	30	1,321	0,698	1,535	0,2185
Fish L (cm)	X	1	30	23,6800	20,90000	26,1000	1,45825
Fish W. (g)	X	1	30	131,3667	96,00000	188,0000	25,57677
Fish L (cm)	X	2	30	33,7867	19,70000	40,5000	5,5481
Fish W. (g)	X	2	30	559,4000	56,00000	984,0000	233,5980

Appendix table 2 Raw data used for correlations between infection and fish size. Red color = P<0.05

Both load and mean abundance were correlated with fish size using spearmans rank order correlation. Load showed fewer correlations than mean abundance.

Coh(SW sample)	Fish K & Abun. N	Spear R	t(N-2)	P-value	Fish K & Load N	Spear R	t(N-2)	P-value
A(1)	30	-0,087519	-0,464893	0,645603	30	0,049663	0,263118	0,794386
A(2)	30	0,041323	0,21885	0,828358	30	0,145434	0,777836	0,443189
A(3)	30	0,011714	0,061989	0,951012	29	0,399014	2,261136	0,032017
B(1)	30	-0,322356	-1,80194	0,082332	30			
B(2)	30	-0,183679	-0,988762	0,331248	30	-0,107988	-0,574778	0,570031
B(3)	30	-0,505502	-3,10012	0,004378	30	-0,221381	-1,20124	0,239720
C(1)	30				30			
C(2)	30	-0,131247	-0,70055	0,489364	30	-0,151872	-0,81306	0,423043
C(3)	30	-0,123738	-0,659830	0,514754	30	-0,297285	-1,64757	0,110618
D(1)	30	-0,302350	-1,67844	0,104389	30			
D(2)	30	-0,575724	-3,72588	0,000872	30	0,239421	1,304848	0,202568
D(3)	30	-0,240036	-1,30840	0,201376	30	-0,002114	-0,01119	0,991154

Coh (SW sample)	Fish L & Abun. N	Spear R	t(N-2)	P-value	Fish L & Load N	Spear R	t(N-2)	P-value
A(1)	30	-0,017160	-0,090817	0,928284	30	-0,049669	-0,263147	0,794364
A(2)	30	-0,349461	-1,97361	0,058368	30	-0,108463	-0,577338	0,568325
A(3)	30	-0,146331	-0,782735	0,440353	29	0,172082	0,907703	0,372066
B(1)	30	-0,034173	-0,18093	0,857726	30			
B(2)	30	-0,023080	-0,122162	0,903643	30	0,139994	0,748146	0,460610
B(3)	30	-0,377821	-2,15929	0,039544	30	-0,327568	-1,83454	0,077220
C(1)	30				30			
C(2)	30	-0,624022	-4,22573	0,000229	30	-0,641266	-4,42223	0,000134
C(3)	30	-0,145732	-0,779461	0,442247	30	-0,076029	-0,40347	0,689664
D(1)	30	-0,532721	-3,33088	0,002440	30			
D(2)	30	-0,730690	-5,66339	0,000005	30	0,018813	0,099568	0,921397
D(3)	30	-0,388881	-2,23358	0,033679	30	0,027504	0,14560	0,885284