Cestodes in Atlantic salmon (*Salmo salar* L.) at a W Norwegian hatchery: Infection dynamics, aspects of development and pathology

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Table of contents

Abstract

Many cestodes use copepods as first intermediate hosts, and some may infect juvenile salmonids in hatcheries that use water from sources that are inhabited by native salmonids. Little is known about their infection dynamics and development under these artificial conditions.

The cestode fauna of juvenile Atlantic salmon (*Salmo salar* L.) was examined from February (fry) to the end of July (parr) at a hatchery in Hordaland, W Norway. Two fish groups, which followed standard rearing conditions, were studied. These groups received inlet water through filter systems with differing efficiency. Thus the fish groups were infected at different times in spring. Sampling was carried out at bi-weekly intervals, and a total of 1709 juvenile salmon were examined during the study. In addition, a total of 43 wild salmonids (salmon, rainbow trout, charr and brown trout) from the supplying watercourse were examined for the presence of cestodes.

Two adult tapeworms, *Eubothrium crassum* (Bloch, 1779) and *Proteocephalus longicollis* (Zeder, 1800), and a single *Diphyllobothrium dendriticum* (Nitzsch, 1824) plerocercoid were found in the juvenile salmon.

Eubothrium crassum and *P. longicollis* were first found in the juvenile salmon in late March. The principal infection with *E. crassum* occurred from late March to mid May, while infections with *P. longicollis* mainly took place in two pulses, one in March – April and one in May – June. Prevalence of *E. crassum* and *P. longicollis* reached a maximum of 42% and 41%, respectively. A few tapeworms were usually found in each infected salmon.

The development time from larva to the gravid state was estimated to 50 days (750 day-degrees) and 33 days (460 day-degrees) for *E. crassum* and *P. longicollis*, respectively, suggesting that these cestodes may complete their life cycle in less than a year.

Small fish showed higher abundance of *E. crassum* or *P. longicollis* than larger fish.

Abundance and load (weight worm/weight fish) of *E. crassum* were negatively correlated with host condition, suggesting that *E. crassum* may have a negative effect on salmon growth. *Proteocephalus longicollis* infections appeared to be less harmful.

The study highlights the importance of effective filtering of the inlet water in hatcheries, since the evidence suggest that *E. crassum* interfere with the production of smolts.

Introduction

Fish farming, especially Atlantic salmon (*Salmo salar* L.) production, is a growing industry worldwide. Important limiting factors for the industry are a wide range of pathogens, including cestode parasites. The natural reservoirs for most of these are wild fish populations interacting with farmed fish (Bristow & Berland 1991b, Bristow 1993).

The large production of Atlantic salmon has created a demand for smolts throughout the year, i.e. underyearlings (0+) and yearlings (1+), and this has resulted in a very intensive production in hatcheries. Such hatcheries control and manipulate several factors important for the development and smoltification of salmon (e.g. food, water temperature and photoperiod). Hatcheries often utilise a filter to avoid organic fouling in the tanks, and this may also prevent the entrance of infective stages of some parasites.

Production of salmonids in freshwater is often associated with a range of parasites, originating from native fish living in the water sources. The parasites may enter directly or indirectly by intermediate hosts (Wootten 1972, Ingham & Arme 1973, Hare & Frantsi 1974, Wootten & Smith 1980, Engelhardt & Mirle 1993, Platten *et al*. 1994, Valtonen & Kosivaara 1994, Buchmann *et al*. 1995, McGeorge *et al*. 1996, Uldal & Buchmann 1996, Buchmann & Bresciani 1997, Muzzall 2000). Some cestodes infecting salmonids use copepods as intermediate hosts, which may be carried into the hatchery's fish tanks. Production of smolts is mainly conducted in western Norway, where three different species of wild salmonids (*Salmo trutta* L., *S. salar* and *Salvelinus alpinus* L.) are common in freshwater systems (Bristow 1993). Ten different freshwater cestode species are recorded from these salmonids in Norway (Bristow 1993, Sterud 1999), and at least three of these, *Eubothrium crassum* (Bloch, 1779), *Diphyllobothrium dendriticum* (Nitzsch, 1824) (Pseudophyllidea) and *Proteocephalus longicollis* (Zeder, 1800) (Proteocephalidea)*,* may cause problems to hatchery reared salmon (Berland 1987, Bristow 1993).

The simplest pseudophyllidean and proteocephalidean life cycle, exhibited by *Eubothrium crassum* and *Proteocephalus longicollis*, respectively, consists of two hosts, a copepod and a fish definitive host (cf. Kennedy 1978a, Scholz 1999a). *Diphyllobothrium dendriticum* has a copepod first intermediate host, a piscine second intermediate host, and an avian definitive host (cf. Henrickson 1977). Infection by *Eubothrium* spp., *Diphyllobothrium* spp. and/or *P. longicollis* (syn. *P. neglectus* and *P. exiguus* cf. Scholz & Hanzelova 1998) in salmonids may reach high prevalence and intensity in nature (Vik 1957, Lien & Borgstrøm 1973, Henrickson 1978, Kennedy & Burrough 1978, Chubb 1982, Alexandersen 1993, Dorucu *et al*. 1995,

Rahkonen & Koski 1997). Ample evidence suggests that these cestodes may harm their fish hosts. *Eubothrium* spp. infections in salmonids may reduce growth (Dombroski 1955, Smith 1973, Boyce 1979, Bristow & Berland 1991a, Saksvik *et al*. 2001a), condition (Hoffmann *et al*. 1986), orientation capability (Garnick & Margolis 1990), swimming performance (Smith 1973, Boyce 1979), ability to adapt to seawater (Boyce & Clarke 1983) and increase susceptibility to environmental stressors (zinc toxicity) (Boyce & Yamada 1977) and pathogens (Bacterial Kidney Disease) (Boyce 1979). There is also a report on direct fish mortality caused by *Eubothrium salvelini* (Gerdeaux *et al*. 1995). Infections by *P. longicollis* in rainbow trout have been associated with reduced growth (Priemer & Goltz 1986, Engelhardt *et al*. 1988), and heavy infections have also been associated with stress related fish death (Engelhardt *et al*. 1988). Plerocercoids of *D. dendriticum* are usually found encapsulated on the viscera of salmonids, but have been reported to kill salmonids by penetrating vital organs (Berland 1987, Rahkonen *et al*. 1996).

Knowledge of the parasite fauna in cultured salmonids has been accumulated through more than a dozen studies (cf. Buchmann 1997). However, only a few of these deal with the infection pattern of cestodes through an extended time period. Intensive production of smolt is carried out under artificial conditions that may alter the natural infection dynamics of cestodes, and Berland (1987) considered it unlikely, due to the rearing conditions (i.e. tanks, food pellets, filtered water), that hatchery reared smolt should become infected by cestodes to any greater extent. Still, despite the intensive production of salmon in Norway little is known about the extent of infections by these parasites in hatchery reared fish under normal rearing conditions.

The aim of the present study was to examine the infection pattern of cestodes in a salmon hatchery in western Norway, and the objectives were to: 1) identify and quantify cestode infections under standard rearing conditions, 2) detect infection periods, 3) describe the course of infection, and 4) examine which subsets of fish become infected and 5) the relationship between cestode infections and host condition.

Materials and Methods

Location

The study was carried out at a salmon and rainbow trout hatchery, located in Sævareid (60° 11' 11N, 5° 46' 28E), western Norway (Figure 1). This hatchery produces approximately 4 million smolts a year. It receives surface water from a nearby lake (Henangervatnet), which is a part of the Sævareid watercourse. The watercourse contains 3 lakes, Henangervatnet, Skogseidvatnet and Gjønnavatnet, which are inhabited by Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), charr (*Salvelinus alpinus*), eel (*Anguilla anguilla*) and three-spine stickleback (*Gasterosteus aculeatus*). There is a waterfall (dam) between the sea and Henangervatnet, preventing anadromous salmonids from entering the lakes. Hence salmon and rainbow trout are escapees from rearing pens that are situated in the watercourse.
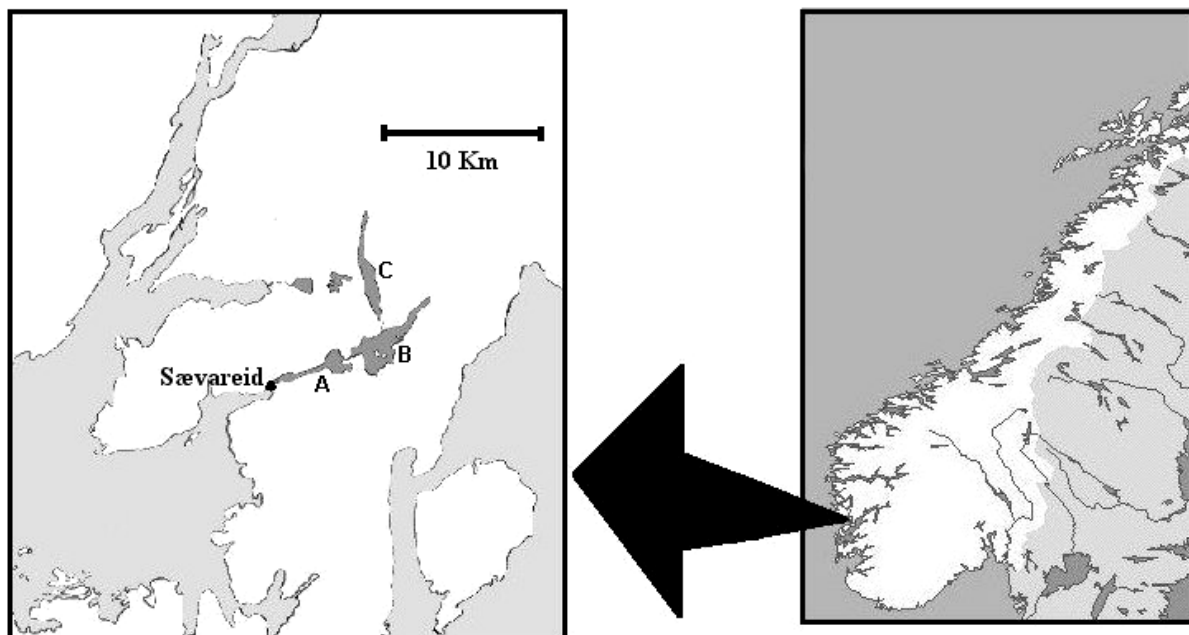

Figure 1 The hatchery is located in Sævareid (left), western Norway (right). Sævareid watercourse consists of three lakes, Henangervatnet (A), Skogseidvatnet (B) and Gjønnavatnet (C), which supply the hatchery with water for the production of smolt.

Facilities

The hatchery (Sævareid Fiskeanlegg A/S) kindly provided fish for this study. The fish were kept in green glass-fibre tanks that were round unless otherwise stated. The employees at the farm reared the fish in the study-tanks in the same way as other fish at the farm regarding handling and feeding. They measured and logged temperatures, oxygen level and pH in the tanks as a daily routine.

The hatchery provided an equipped laboratory with a dissecting microscope (Leitz, 50x) and a standard light microscope that were used for examination of fresh fish. At the University of Bergen, Department of Fisheries and Marine Biology, a fully equipped laboratory for the study of fish diseases was available, and this was used for examination of frozen fish samples. Examination of the wild fish and processing of preserved parasites were also carried out at this laboratory.

Material

Farmed fish

The fish studied belonged to a cohort with initially 60 000 Atlantic salmon (*Salmo salar*) eyed eggs. The study period started in February 2000 at first feeding (fry) and ended in July (parr). A total of 1709 salmon were examined in this period. An outbreak of furunculosis (caused by *Aeromonas salmonicida* subsp. *salmonicida*) occurred in August and the farmed fish were stamped out.

Wild fish

Four species of wild fish, a total of 59 individuals, were caught in Henangervatnet during spring 2000 (gillnets) and summer 2001 (angling) (Table 1). Alexandersen (1993) studied the parasites of charr (N=238) in Lake Henangervatnet, so efforts were concentrated on other salmonids. Most of the fish that were caught in spring 2000 were salmon and rainbow trout escapees from lake pens. The fish were transported on ice to the University laboratory and immediately examined, except 25 fish from the April 8th sample, which had to be deep frozen and examined later. The fish caught in 2000 was examined for the presence of cestode species in the manner used for large farmed fish. Fish from 2001 was only used to retrieve specimens of *Eubothrium*.

Table 1 Number of wild fish caught in Henangervatnet in the years 2000 and 2001, with fish length as mean (range) in cm.

Fish species	Catching date			
	26th March 2000	8th April 2000	1st June 2001	12th June 2001
Oncorhynchus mykiss	7 35.8 (26.7-42.2)	9 23.4 (20.9-27.2)	1 26.8	0
Salvelinus alpinus	0	3 28.5 (27.2-29.6)	0	0
Salmo salar	0	18 27.1 (24.5-32.3)	0	0
Salmo trutta	0	6 26.3 (25.0-27.4)	10 27.4 (21.3-35.4)	5 27.9 (24.9-30.9)

Environmental conditions

During winter and early spring the inlet water to the hatchery was heated with heat pumps. In late spring, the lake-water temperature sufficed for a good production. The water flow in the tanks was regulated to keep the oxygen level at ca. 85%, and oxygen (g) was added if necessary. The inlet water was filtered until the water requirements in the tanks exceeded the filtering capacity. All fish were kept indoors and were reared under continuous (24h) artificial light unless otherwise stated. Automatic feeders with commercial dry pellets (Nor Aqua) fed the fish to satiety and the employees checked this daily. The tanks were also inspected daily for dead fish, which were gathered and counted.

Study set up

Group A was made up of the cohort with 60 000 eyed eggs, and after some mortality it comprised 52 000 fry in late February. From the initial group (A), approximately 1200 fish were randomly selected on March 9th to form a new smaller group (B). This group was established as a control group with no chemical treatment. An effort was made to keep a similar fish density in the different groups. Still, these groups experienced different handling (i.e. mixing, thinning, sorting) and rearing (i.e. tank size and shape, temperature, water filtering). The sampling was done at biweekly intervals and the layout of the study is illustrated in figure 2.
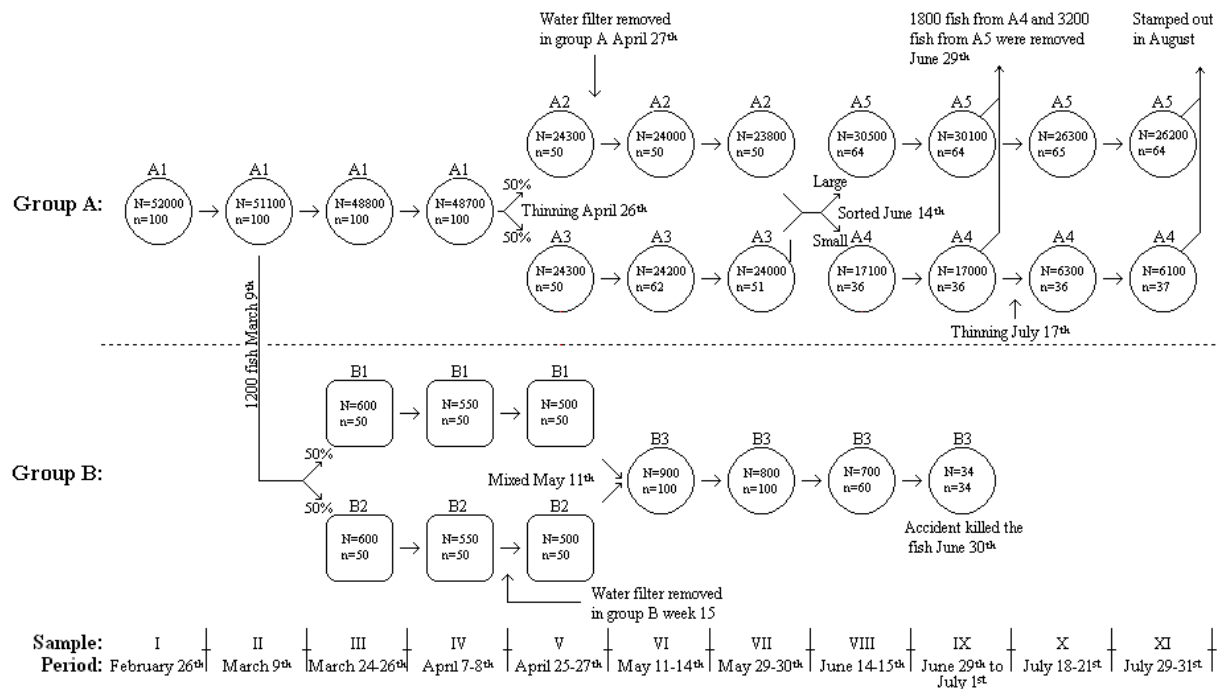
Figure 2 One cohort of salmon (*Salmo salar*) was followed from first feeding in February till August in the year 2000. Group B was kept in another section of the hatchery, and thus these groups went through different handling and rearing regimes. Each sample (I-XI) consisted of a number of fish from the fish tanks that were active at that moment (i.e. every tank vertically above the sample number) and was sampled during a time period of 1 to 4 days. On each tank, the estimated total number of fish is denoted N and sample size is denoted n.

A total of 11 samples (I-XI) were examined from group A, and 7 samples (III-IX) from group B. Sample I and II from group A were common for both groups.

Each sample is, for simplicity, referred to by which third of the month they belong to (early, mid and late) (e.g. sample IV=early April, V=late April and VI=mid May).

Examination of fish in each sample (group A and B) was carried out as fast as possible and took from 1 to a maximum of 4 days. Still, in a few cases (see below) this was not practicable and some fish had to be deep frozen for later examination. All the fish were examined fresh, except 76 fish from tank B3 in sample VII, and 28 from tank A4, 51 from A5 and 54 from B3 in sample VIII, which were frozen and brought to the University.

Group A

The eyed eggs and alevins were first reared in hatching troughs, and as the alevins' activity increased they were transferred to tank A1. First feeding started on February 23rd and sampling commenced on February 26th. The inlet water was filtered (Hydrotec drum-filter, 40 μm) until April 27th. The fish were kept in a tank (A1) with a diameter (ø) of 4 m (5000 l) until April 25th. On the next day the fish were divided into two equal groups (A2 & A3) (ø 4 m, 5000 l) to avoid overcrowding (i.e. thinning). On June 14th the fish in these two tanks (A2

10

& A3) were mixed, and sorted according to size. The largest fish, 64% of the total, were placed in a new tank (A5) (ø 5 m, 25000 l) and henceforth received unheated ("natural") water from the water source. This fish was light manipulated from July 3rd in order to produce underyearling (0+) smolts. The light (L)/dark (D) regime was changed from LD24:0 to LD12:12 followed by a gradual increase in day length over a period of approximately 4 weeks to LD24:0. The remaining small fish (36%) were transferred to a 4 m (ø) tank (5000 l) (A4) that henceforth received LD24:0 to produce yearling (1+) smolts. On June 29th 1800 fish from A4 and 3200 from A5 were removed to participate in another (unrelated) study. On the 17th of July approximately half of the fish in A4 were transferred to a new tank to avoid overcrowding (thinning).

One of the tanks in group A were treated with formalin (1:4000) on 10th March (A1), 16th June (A4) and 21st July (A4) when protozoan infections were suspected, beyond that no other chemical treatment was used.

Furunculosis was diagnosed in the farm on August 18th and all fish had to be killed. The studied tanks did not show elevated mortality prior to the stamping out.

Group B

Approximately 1200 fish were taken from group A on March 9th to form group B. These fish were kept in two quadrangular tanks (B1 & B2) (width 1 m, 200 l), with ca. 600 fish each. Group B received filtered (UNIK disc-filter, 60 μm,) water until week 15. On May 11th the fish from these two tanks had to be moved to a larger tank (B3) (ø2 m, 1200 l). On June 30th the water in B3 was accidentally supersaturated with oxygen (500%), thus killing ca. 600 fish. The remaining 34 fish were examined, and the cestode data are shown in this study. However, since the survivors may represent a potential non-random sample, data are not included in statistical analysis.

Sampling

Farmed salmon were collected randomly from each tank with a landing net and carried to the hatchery laboratory in a bucket with water. Few fish were gathered each time and they were kept live in a small tank until examination. Fish were anaesthetized individually with benzocain (10%w/v in 96% ethanol) in a concentration of 3‰, and weight (g) and total length (cm) were measured.

Blood samples from fish in sample VI to IX in group B and from sample X and XI in group A were examined. Blood was either collected from the caudal vessels with a syringe or directly into the Hct tubes by cutting off the fish tail (smallest fish). To determine hematocrit (Hct) levels, blood was transferred to heparinized microhematocrit tubes (20 or 50 μl). These were centrifuged at 12 000 rpm for five minutes (Sigma 201M). In addition to haematocrits, leucocrit (Lct) values were read for all fish except sample VI (B3).

Dissection

The small fry in sample I was examined for cestodes by squeezing the whole fish between two small glass plates and screened with a dissecting microscope. In all other samples the fish was opened, viscera removed and the abdominal cavity examined. The gastrointestinal tract was dissected out and the stomach was removed, but not examined for lumenal parasites. The pylorus region (i.e. caeca + adjoining section of the intestine) and the intestine were separated, placed in Petri dishes with physiological salt water (1.0% NaCl) and examined separately. In the smallest fish (sample II-III) the pylorus region and intestine could be squeezed and examined directly. In the larger fish these parts were cut open before squeezing, and the mucosa scraped off with a scalpel. The respective gut sections were then squeezed between two glass Petri dishes and screened under a binocular dissecting microscope for cestodes.

Processing of cestodes

Cestodes from farmed fish

As a measure of cestode size, both length and weight of each worm was sought. Since an accurate weight (μg) was not available during fieldwork, this was accomplished by conserving the worms in ethanol for later weighing at the University laboratory.

The cestodes were killed by being dropped into hot (near boiling) 70% ethanol, labelled and kept in 70% ethanol for later examination. Cestodes from frozen fish were put directly into cold 70% ethanol.

At the University the cestodes were identified to species level. Worm length was measured on a millimetre-scale paper and, if possible, the *Eubothrium* specimens were wiped lightly on a paper till dryness (to get rid of excess ethanol) and weighed (μg) (Mettler Toledo AB204). Specimens of *Proteocephalus* were very fragile after some time in ethanol and could not be weighed.

A staging system was designed to describe worm maturation. The stages are based on the examination of live, ethanol conserved and stained and mounted specimens (see below). The specimens of *Eubothrium* were assigned to five stages: 1) very small plerocerciform with no proglottids, 2) small immature worms with 1 to 20 proglottids, 3) immature worms (>20 proglottids and no vitellaria), 4) "mature worms" (vitellaria present but no eggs) and 5) gravid worms (with eggs). The specimens of *Proteocephalus* were assigned into four stages: 1) very small plerocercoids with no proglottids, 2) small immature worms, 3) mature worms (with testes/ovaries), and 4) gravid worms (with eggs). When there was doubt to which stage a worm belonged it was cleared in lactic acid to reveal internal structure.

To develop the staging system cestodes from sample III and IV were stained in paracarmine, destained in HCl-alcohol, dehydrated in an ethanol series, cleared in metyl-salicylate and mounted in Canada balsam. These cestodes were not weighed, only their length was measured.

A few worms broke into several parts in ethanol. These could be reconstructed by assembling strobila segments. Also, in a few tubes with worms the ethanol had evaporated so only length could be measured.

Standard curves

Wild fish were caught in 2001 to obtain live specimens of *Eubothrium* of varying size to establish a standard curve for the relationship between live and ethanol weight of the worms. For this purpose, *Eubothrium* specimens were weighed (µg) live, fixed in hot (near boiling) 70% ethanol, and stored in 70% ethanol for some weeks, and subsequently weighed and length (mm) measured. From these data the relationship between "live weight" and "ethanol weight" was calculated.

To estimate the "live" weight of *Eubothrium* recovered from frozen and thawed fish, worms collected from wild fish were weighed live, and then put back into fish intestines and deep frozen (-20°C) for some days. The intestines were later thawed, and the cestodes were dissected out. These cestodes were then preserved in 70% ethanol for several weeks before being weighed again.

All cestode weights from ethanol samples (*EW*) were transformed to live weights (*LW*) using these standard curves. The smallest worms could not be accurately weighed, so these were assigned weights from the length-weight regressions.

Cestode ethanol weight to live weight in freshly examined fish

1) $LW = 1.7893 \times EW$ $R^2 = 0.9955$ n=31   fresh fish

Cestode ethanol weight to live weight from frozen fish

2) $LW = 1.0814 \times EW$ $R^2 = 0.9946$ n=11   frozen fish

Cestode length to live weight

A polynomial regression between "cestode length" (EL) and "ethanol weight" was calculated from undamaged cestodes from the freshly examined hatchery fish. This gave formula 3) and yielded estimates of the ethanol weight of worms with only length measures This procedure was used on cestodes that were too small to be weighed, cestodes that had been fragmented, desiccated tapeworms and the Canada balsam mounted worms.

3) $WE = 3\text{E-}06(EL)^2 + 7\text{E-}0.5EL$ $R^2 = 0.9649$ n=128   fresh fish

Definitions

The ecological terms prevalence, intensity, abundance, incidence and infrapopulations are used in accordance with Bush *et al.* (1997). As a measure of fish condition, Fultons condition factor (CF) was calculated $CF = 100 \, wl^{-3}$, w=weight (g) and l=length (cm). Its use was restricted to the parr stage (i.e. period of isometric growth).

Statistical methods

Statistical tests and other data management (i.e. figures, tables) were done using StatSoft, Inc. STATISTICA, version 6.1 and Microsoft® Excel 2000. Data were considered significant when $p < 0.05$.

All measurements of length and weight of fish, cestodes and cestode stages, and temperatures are shown as range followed by mean±S.D in parenthesis unless otherwise stated. S.D. is the standard deviation.

Parasite counts were non-normal and overdispersed so nonparametric statistics were used (i.e. following the initial infection). Two-sample comparisons were done with Mann-Whitney U Test. When the variance of the abundance did not violate the requirement for homoscedasticity, the Kruskall-Wallis test was applied.

Correlations were examined with Spearman rank order correlation coefficients (r_s). However, when an additional variable could potentially be associated with the considered variables, causing spurious correlations, the Kendall Tau and Kendall partial rank-order correlation was

applied (Siegel & Castellan 1988). A Pearson product-moment correlation was used when the data showed normal distributions. Pearson's Chi-square (χ^2_{df}) tests were applied to compare prevalences (i.e. following the initial infection) and to examine associations between the cestode species. When prevalence appeared relatively constant, contigency tables (χ^2_{df}) were calculated to examine H_0: "prevalence does not vary". Bonferoni corrections were applied in cases of multiple testing.

Results

Fish and rearing conditions

The fish were healthy during the study period with no epizootics, disregarding the oxygen accident and the later bacterial disease. While almost no fish mortality was observed in group B, maximum mortality (4%) in group A occurred in the first feeding period, i.e. late February to late March (see Figure 2).

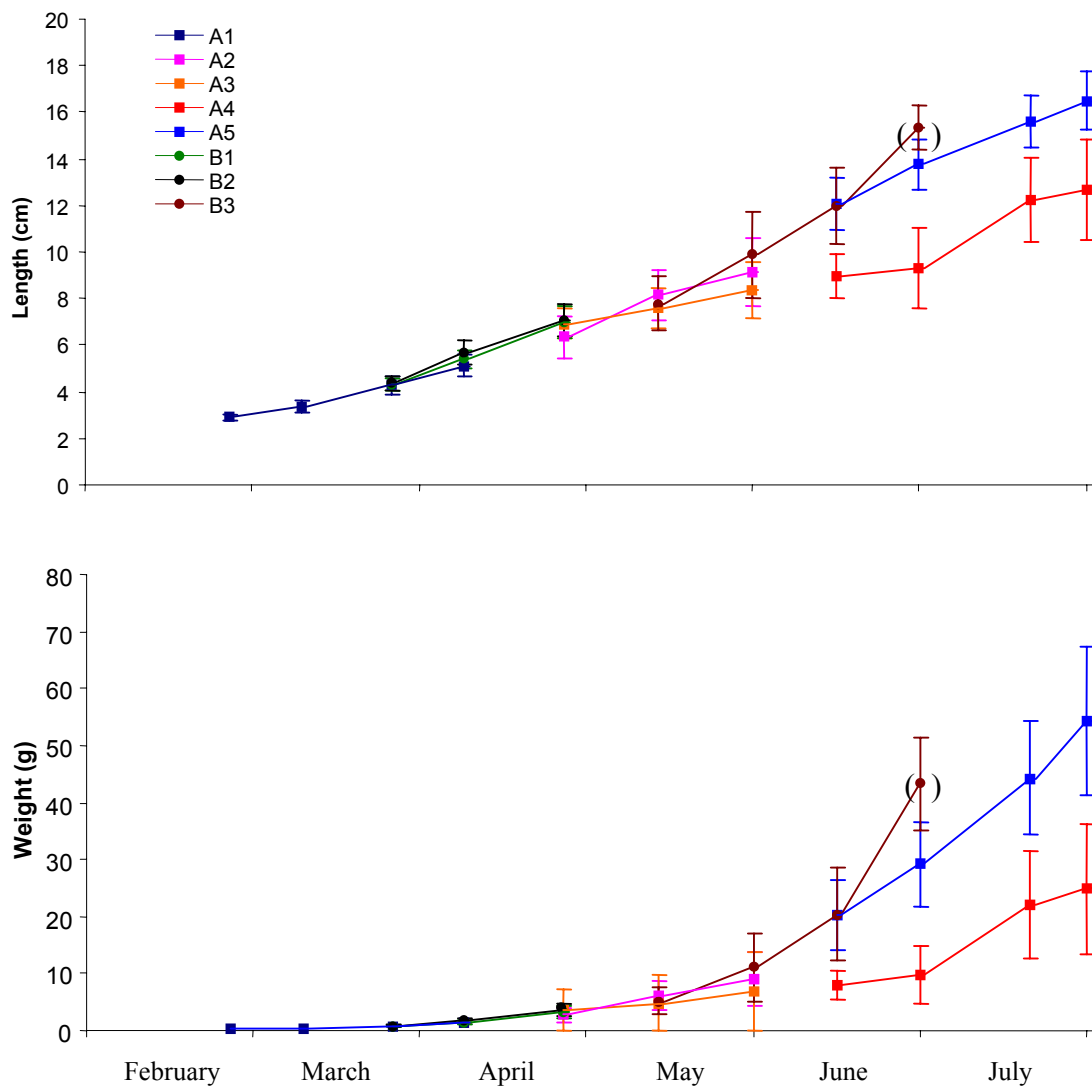**Figure 3** Length (mean ±S.D.) and weight (mean ±S.D.) of salmon (*Salmo salar*) from each tank (A1-A5 and B1-B3) during the study period (February - July 2000). The salmon in A2 and A3 were mixed on 14th June and sorted into new tanks with small (A4) and large (A5) sized fish. S.D. =standard deviation of the mean. Data points in brackets: survivors after hyper-oxygenation in tank B3 (95% mortality).

Sorting of the fish (June 14th) into the new groups A4 (small fish) and A5 (large fish) resulted in significantly shorter and lighter fish in A4 than in A5. A4 fish was also significantly smaller than B3 fish and this difference persisted as long as there were fish in tank B3 (Figure 3).

The water temperatures in the tanks are shown in figure 4. The mean water temperatures were fairly stable in both groups from late March until mid June, with 15.1±1.5 and 15.0±0.8 °C in group A and B, respectively. Fish in tank A4 (14.2-18.4 (16.9±0.9) °C) experienced a higher mean temperature than fish in A5 (9.6-17.8 (13.9±2.2) °C) after the sorting.
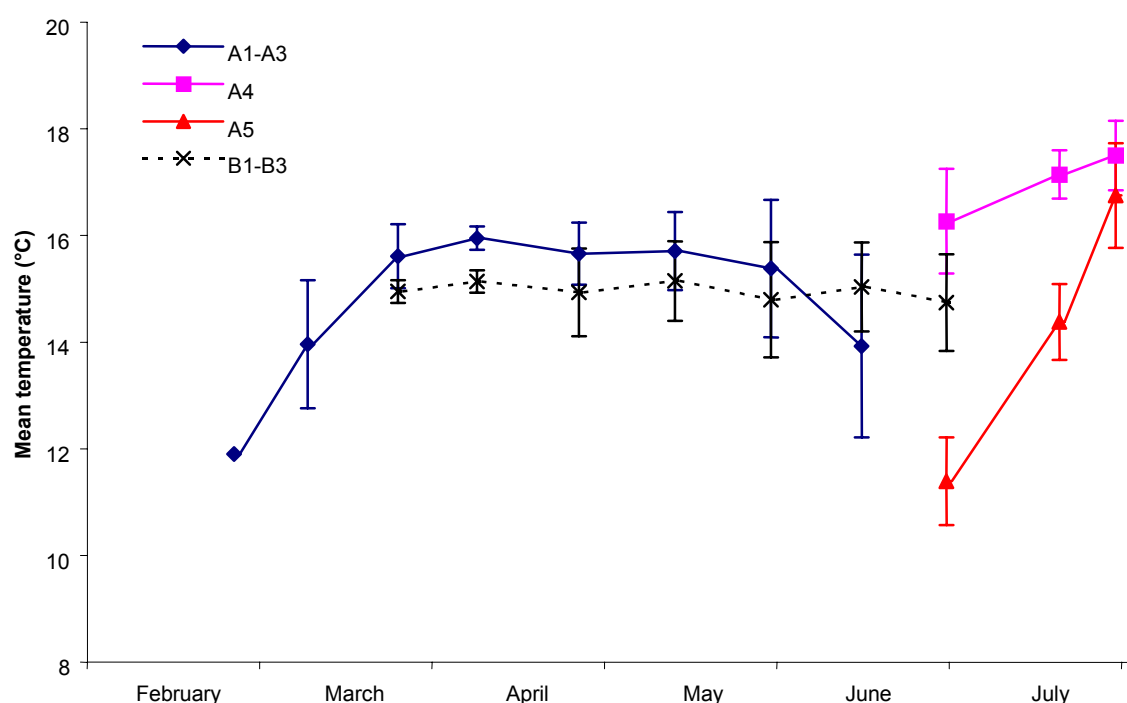

Figure 4 The water temperatures (mean ±S.D.) in the tanks from the respective groups, A and B, during the study period (February - July 2000. Within the groups the parallel tanks with identical temperatures, i.e. A2 and A3, and B1 and B2, are pooled. The fish in group A was sorted June 14th. While A4 (small fish) still received heated water, the fish in A5 (large fish) thereafter received "natural" (unheated) water. S.D. =standard deviation of the mean

Cestode species

Two species of adult cestodes and a plerocercoid larva were identified in the hatchery-reared salmon. *Eubothrium crassum* (Bloch, 1779) was identified accordingly to Andersen (1979). *Proteocephalus longicollis* (Zeder, 1800) (syn. *P. neglectus* and *P. exiguus*) was identified according to Scholz & Hanzelova (1998). At the end of May, a single encysted

Diphyllobothrium dendriticum (Nitzsch, 1824) plerocercoid was found in the abdominal cavity attached to the pyloric caeca. It was identified following Andersen & Gibson (1989).

All three cestode species were also found in wild fish from the water source (Henangervatnet) in the spring of 2000. Brown trout harboured all three species of cestodes. Rainbow trout and salmon were found infected by *E. crassum* and *P. longicollis*, while *P. longicollis* and *D. dendriticum* were detected in three charrs (Table 2).

Table 2 Cestodes identified from wild fish caught in the water source (Henangervatnet) in the spring (i.e. 26th March and 8th April) of 2000.

Hosts	N infected/ N examined (%)		
	Eubothrium crassum	*Proteocephalus longicollis*	*Diphyllobothrium dendriticum*
Oncorhynchus mykiss	10/16 (63)	9/16 (56)	0/9 (0)
Salvelinus alpinus	0/3 (0)	1/3 (33)	2/3 (67)
Salmo salar	10/18 (56)	6/18 (33)	0/18 (0)
Salmo trutta	5/6 (83)	1/6 (17)	2/6 (33)

Eubothrium crassum

Site

Location of worms in the gut was recorded as the position of the scolex. *Eubothrium crassum* was mainly (93.4%, N=412) found in the pyloric region and most of them were attached to the end of a caecum. A small proportion (6.6%) was found free in the remaining intestine, these were exclusively small immature worms (stage 1-3) (Table 3). Larger cestodes, especially gravid ones, were often folded into several caeca and could have the posterior end free in the post-caecal intestine. Some gravid and mature cestodes were fragmented, with parts both in the pyloric region and intestine. On two occasions (tank A4, mid July and late July) a single large scolex (ca. 1.2mm long) of a tapeworm that had apparently destrobilated was found attached to the end of a caecum. One of these fish had part of a gravid strobila (ripe eggs) in the intestine. These cestodes were considered to have been gravid, but were excluded from analyses involving cestode length and weight.

Table 3 Occurrence of *Eubothrium crassum* developmental stages in the two principal sections of the intestinal tract. Frequency refers to the allocation of the stages within these sections. Pyloric region=caeca+adjoining intestine, intestine=remaining intestine, N=number of observations

Stages	Pyloric region			Intestine		
	N	Frequency (%)	Length range (mm)	N	Frequency (%)	Length range (mm)
1: Plerocerciform	21	5.5	0.3-1.8	12	44.4	0.4-1.4
2: 1-20 proglottids	65	17.0	0.5-21	7	25.9	1.3-5.0
3: Immature	242	63.2	3.8-134	8	29.6	6-30
4: Mature	9	2.3	49-90	0		
5: Gravid	46	12.0	37-198	0		
Total at site	385*	93.4		27	6.6	

* Includes two single scolices from destrobilated *E. crassum*

Infection pattern

Cestodes were not detected at start feeding (February 26th) or at sampling two weeks later (March 9th). At that time group B was started with fish from group A, in two parallel tanks that received water passed through another filter.

These fish (group B) were found infected with *Eubothrium crassum* in the late March sample, two weeks after transfer. The infected fish then measured 3.2-4.7 (4.0±0.6) cm (N=6). The prevalence of *E. crassum* increased significantly between late March and early April (χ^2_1=70.92, p<0.001) and from early April to mid May (χ^2_1=13.31, p<0.001), reaching 42% (Figure 5). There was no further significant change in prevalence in group B (χ^2_2=1.44, p=0.49). The abundance of *E. crassum* increased significantly from late March to early April (Mann-Whitney U Test, p<0.001) and between early April and mid May (Mann-Whitney U Test, p=0.02). Abundance was thereafter relatively stable (Kruskal-Wallis, H (2, N=260) =1.70, p=0.43). The intensity of *E. crassum* increased gradually to a peak in mid June (Figure 5).

Fish in group A remained uninfected in the period with filtered water (until April 27th), and infection with *E. crassum* was first observed in late May, one month after filter removal. The prevalence of *E. crassum* was ca. 11% at that time (Figure 6), and further change in prevalence was not observed (χ^2_4=1.69, p=0.79). The abundance of *E. crassum* also did not change significantly in samples from late May to late July (Kruskal-Wallis, H (4, N=503) =1.74, p=0.78). Intensity of *E. crassum* in group A remained low during the whole study period (Figure 6). After sorting, the smallest fish (7.5-10.6 (9.0±0.9) cm, N=36) had a significantly higher abundance of *E. crassum* than the largest fish (9.9-15.4 (12.1±1.1) cm, N=64) (Mann-Whitney U Test, p=0.016) (see also Figure 6).

Group B

Figure 5 Infection pattern of *Eubothrium crassum* in hatchery reared salmon (*Salmo salar*) during the study period (February – June 2000) of group B. Sample prevalence, mean intensity and mean abundance of *E. crassum* for each tank and for group B total. Fish in tank B1 and B2 was moved to tank B3 on May 11th. *=Survivors from the hyper oxygenation (95% mortality).
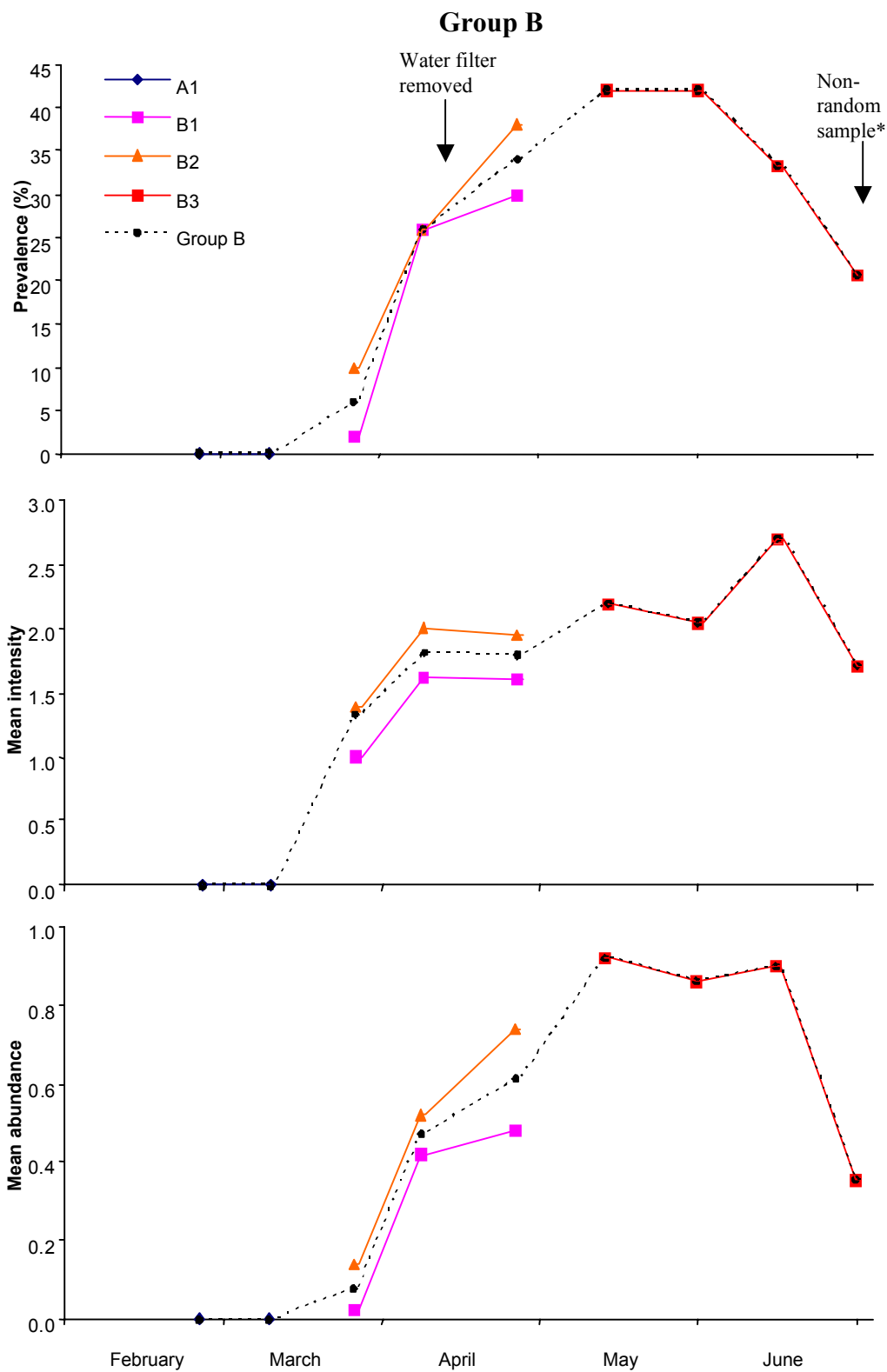
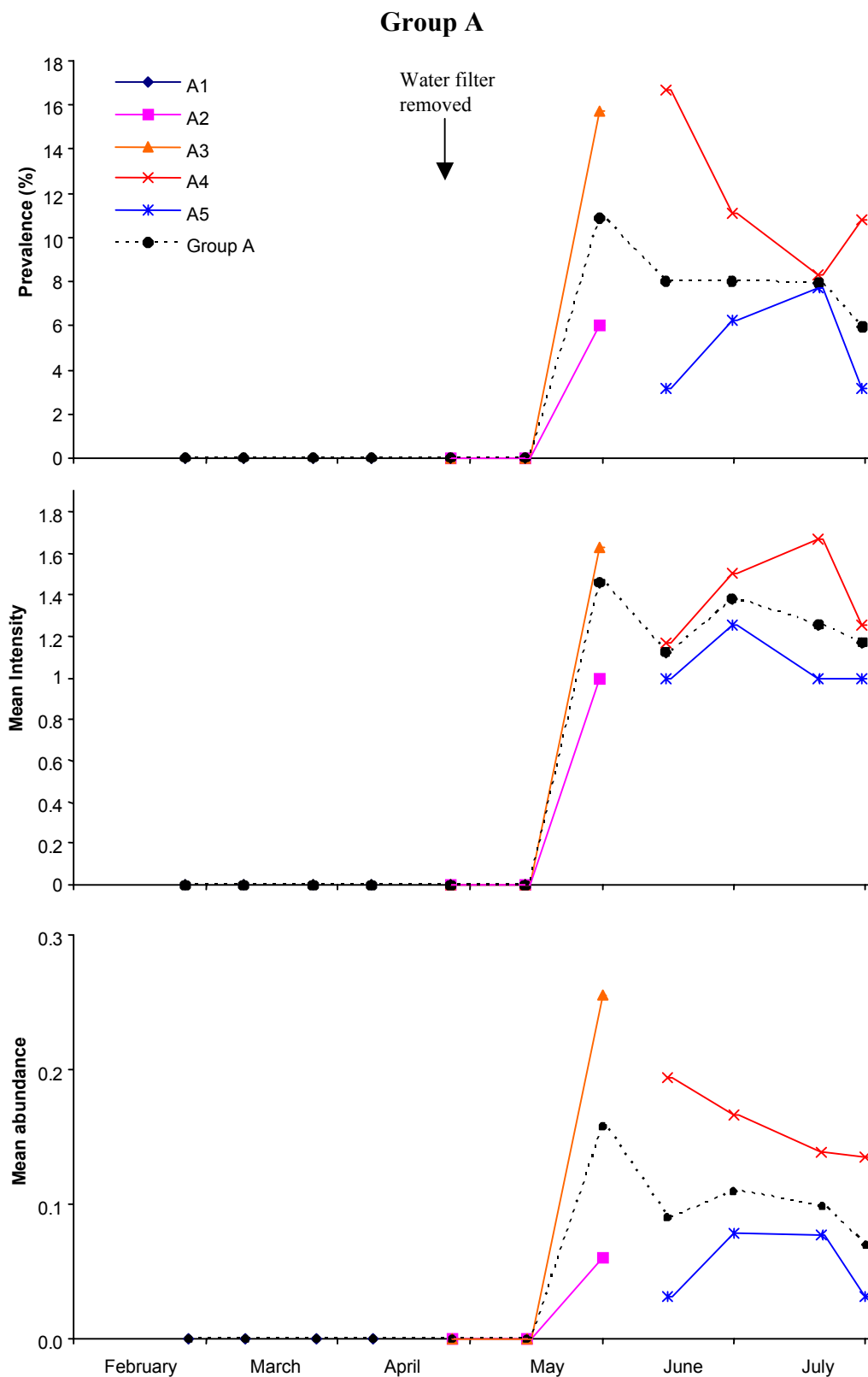**Group A**

Figure 6 Infection pattern of *Eubothrium crassum* in hatchery reared salmon (*Salmo salar*) during the study period (February – July 2000) of group A. Sample prevalence, mean intensity and mean abundance of *E. crassum* for each tank and for group A total. On June 14th the fish in A2 and A3 were mixed and then sorted into new tanks with large (A5) and small (A4) sized fish.

Host size and infection

There were significant negative correlations between host size (length and weight) and abundance of *E. crassum* following the initial infection. A pattern with strong correlations was observed during the first two months of infection in group B. These correlations disappeared by mid June (Figure 7). The same pattern was found in group A prior to the sorting of the fish (Figure 8). The relationship between host size and *E. crassum* infection is also reflected in sample prevalence, mean abundance and mean intensity following sorting of group A (Figure 6).

Figure 7 Spearman rank-order correlation coefficients (r_s) between abundance of *Eubothrium crassum* and salmon (*Salmo salar*) size (length and weight) in group B. Parallel tanks had identical water temperatures and are pooled. *p<0.05, **p<0.01 and ***p<0.001
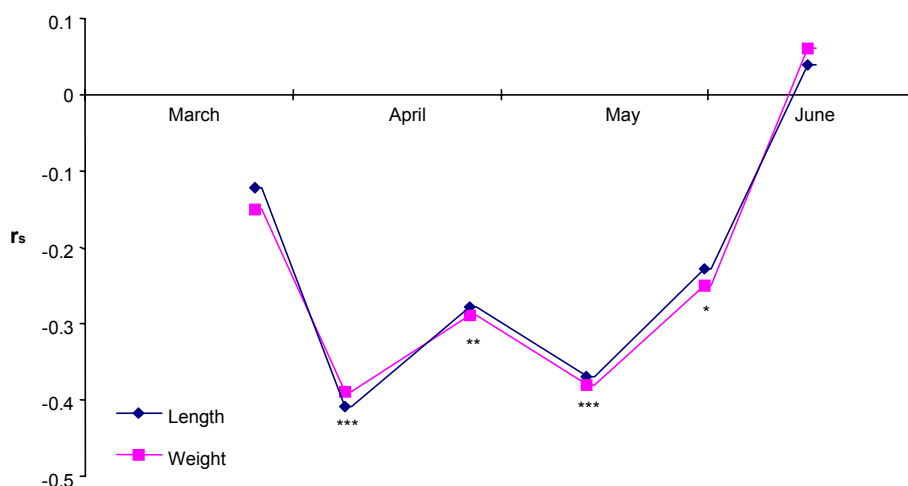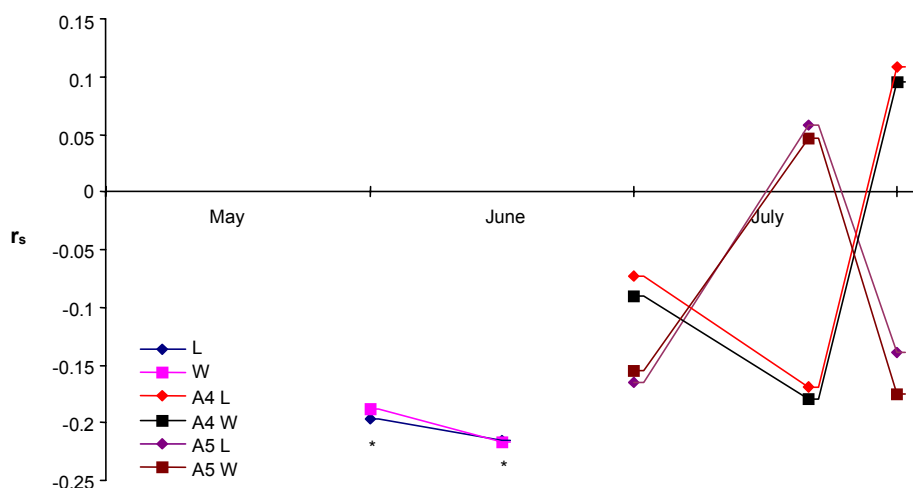

Figure 8 Spearman rank-order correlation coefficients (r_s) between abundance of *Eubothrium crassum* and salmon (*Salmo salar*) size (length (L) and weight (W)) in group A. Parallel tanks with identical temperatures, i.e. A2 and A3, are pooled. A4= small fish and A5= large fish after the sorting. *p<0.05

Maturation and growth

Gravid *E. crassum* were first observed in mid May (group B), ca. two months after infection, and the cestodes in that sample were a maximum of 980 day-degrees old (i.e. earliest possible date of infection March 9th) (Figure 9). The first observation of gravid *E. crassum* in group A was in mid June (Figure 10). The cestode was then a maximum of 490 or 740 day-degrees old depending on the earliest possible date of infection, filter removal (April 27th) or last infection free sample (May 13th), respectively (Figure 10). Considering peak incidence of infection (Group B: late March - early April and A: mid May – late May) and respective peak incidence of gravid worms (Group B: mid May – late May and A: late June – mid July), the development of eggs took ca. 51 days or 770 DD in group B, and ca. 50 days or 720 DD in group A. Plerocerciform stages were observed from late March till mid June in group B, and in mid July in group A (Figure 9 and 10). There was an overlap in length and weight between cestode stages, but almost every cestode over 100mm was gravid (Table 4). The smallest cestode measured 0.3mm (plerocerciform) and the largest 198mm (gravid), and the most heavily infected fish harboured 20 *E. crassum*. The length distribution and maturation shows a shift towards larger and more mature cestodes with time (Figure 9 and 10). The mean weight of *E. crassum* increased with time in both groups (Table 5).

Table 4 Weight range (mean±S.D.) and length range (mean±S.D.) of different stages of all *Eubothrium crassum* from group A and B during the study period (February – July 2000). N=number of observations, S.D. =standard deviation

	Group A			Group B		
Stages	N	Weight (mg)	Length (mm)	N	Weight (mg)	Length (mm)
1: Plerocerciform	1	0.1	0.9	32	>0.0-0.5 (0.2±0.1)	0.3-1.8 (0.9±0.4)
2: 1-20 proglottids	9	0.3-1.3 (0.6±0.3)	1.9-5 (3.2±1.0)	63	0.1-1.6 (0.4±0.3)	0.5-4.8 (2.5±1.1)
3: Immature	29	0.6-122.9 (11.2±25.1)	4.3-134 (27.9±31.6)	221	0.3-43.3 (6.4±7.4)	3.8-84 (23.1±15.5)
4: Mature	2	29.9-47.4 (38.7±12.4)	66-83 (74.5±12.0)	7	10.9-32.7 (23.9±8.0)	49-90 (68.6±16.7)
5: Gravid	9	45.4-239.1 (120.0±74.5)	81-190 (130.6±42.4)	37	16.2-205.4 (53.8±44.1)	37-198 (97.0±34.4)

Group B

Figure 9 Maturation and length distribution of *Eubothrium crassum* from hatchery-reared salmon (*Salmo salar*) during the study period of group B (February - June 2000). Only infected samples are shown, and parallel tanks that had identical water temperatures, i.e. B1 and B2, are pooled. Late June sample: survivors after hyper-oxygenation (95% mortality). DD=day-degrees, using March 9th as earliest possible date of infection. Cestode stages: 1) plerocerciform, 2) 1-20 proglottids, 3) immature, 4) mature and 5) gravid**.**
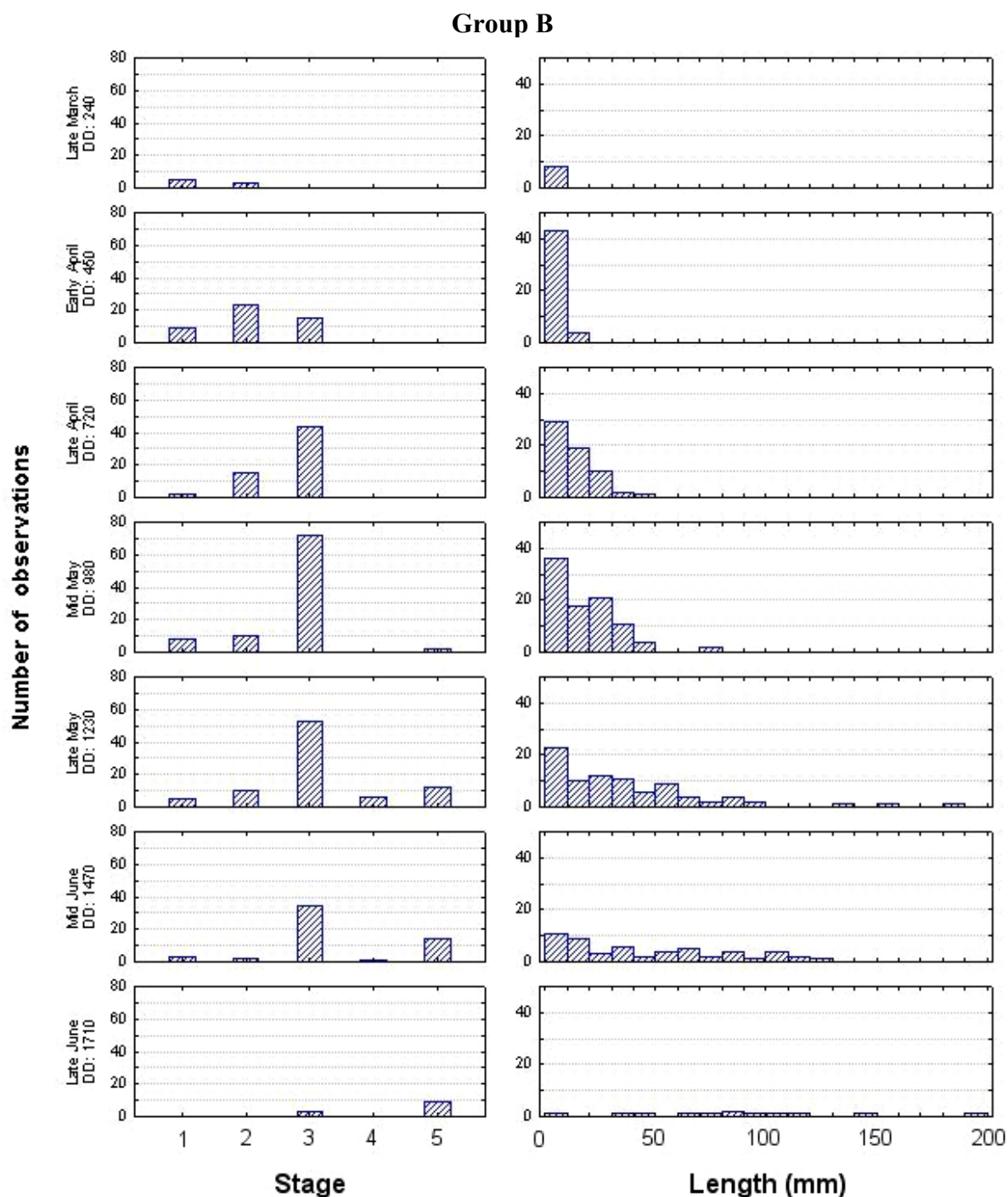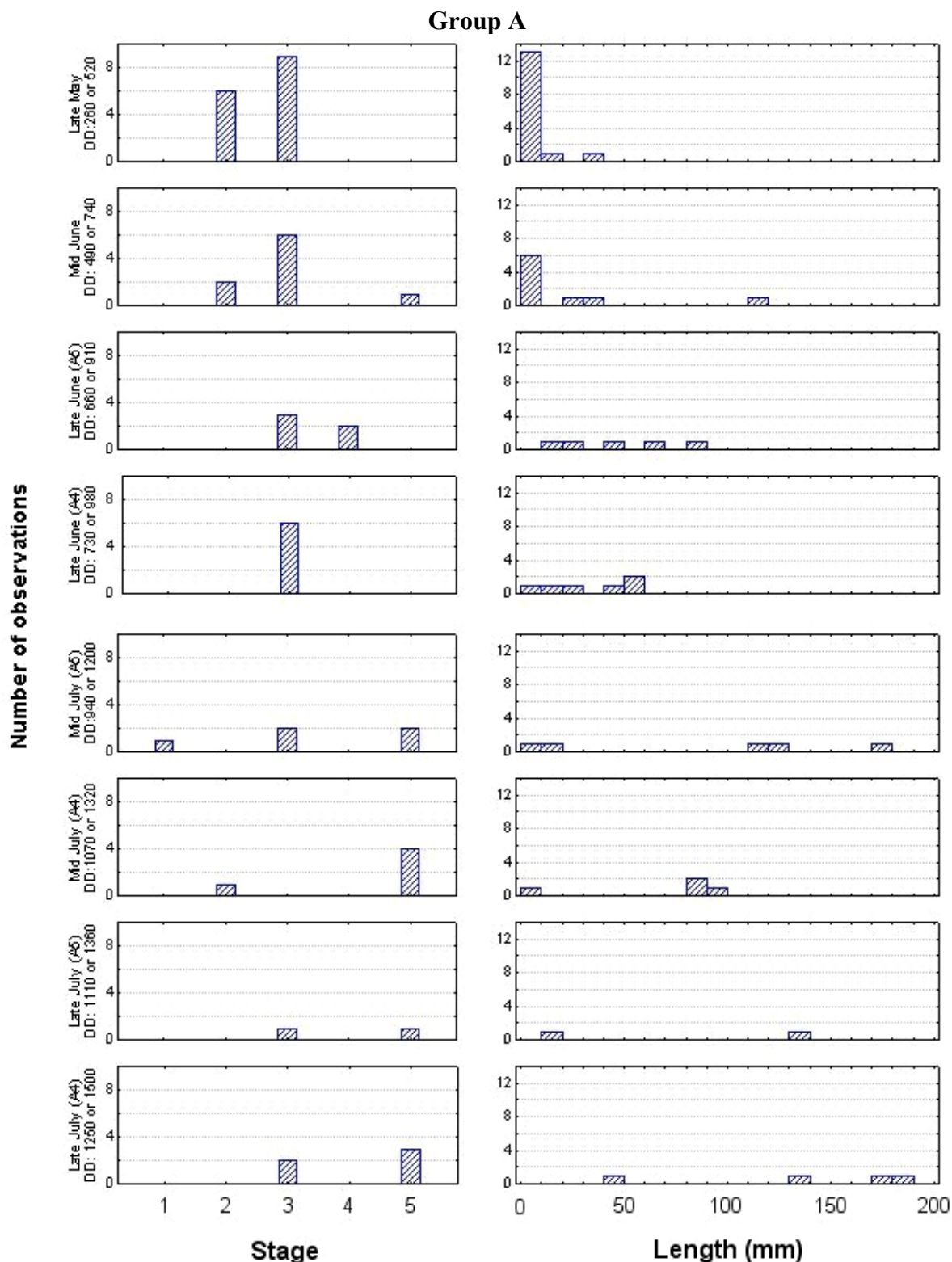
Group A

Figure 10 Maturation and length distribution of *Eubothrium crassum* from hatchery-reared salmon (*Salmo salar*) during the study period of group A (February - July 2000). Only infected samples are shown, and parallel tanks that had identical water temperatures, i.e. A2 and A3, are pooled. After sorting the fish into small (A4) and large (A5) sized fish, the parallel tanks had different water temperatures, hence were not pooled. DD=day-degrees, using April 27th (filter removal) or May 13th (last infection free sample) as earliest possible date of infection, respectively. Cestode stages: 1) plerocerciform, 2) 1-20 proglottids, 3) immature, 4) mature and 5) gravid.

Table 5 Mean weight (W) of all *Eubothrium crassum* during the study period of group A and B (February - July 2000). Parallel tanks are pooled within group A and B. N= number of observations, S.D. = standard deviation, *=non-random sample in group B (survivors from hyper oxygenation (95% mortality)).

Samples	Group B			Group A		
	N	Mean W (mg)	S.D.	N	Mean W (mg)	S.D.
Late March	8	0.08	0.06			
Early April	47	0.66	0.68			
Late April	61	3.43	4.64			
Mid May	92	5.54	7.78			
Late May	86	12.53	23.83	15	1.44	1.61
Mid June	54	15.68	15.45	9	9.34	22.38
Late June*	12	78.06	53.19	11	14.29	14.52
Mid July				9	61.16	70.92
Late July				6	112.15	89.23

Effects on the host

The abundance of *E. crassum* was low in group A, and small and large fish in that group experienced different water temperatures following sorting and could not be pooled. Consequently, effects of *Eubothrium* on the salmon were mostly examined in group B.

The correlation between cestode abundance and host condition was examined in the samples with high *E. crassum* abundance following cestode growth, i.e. group B mid May - mid June (see Figure 5 and 9). There were negative correlations between condition factor (CF) and *E. crassum* abundance in the May samples, but not in June. Simultaneously, there was a negative correlation between fish length and *E. crassum* abundance (Figure 7 and Table 6), and a positive correlation between fish length and CF. Since the negative correlation between CF and *E. crassum* abundance therefore could be a length effect, partial rank order correlation coefficients were calculated. After correcting for host length, there were significant negative correlations between CF and *E. crassum* abundance in the May samples (Table 6).

Table 6 Kendall partial rank-order correlation coefficients of host (*Salmo salar*) condition factor (CF) against *Eubothrium crassum* abundance (AbE) correcting for associations of the variables with host length (L). Samples from mid May – mid June (group B) with high *E. crassum* abundance and relatively large worms are examined. N=valid sample size, **$p<0.01$, ***$p<0.001$.

Samples	Kendall *T*				Partial *T*
	N	CF:L	CF:AbE	L:AbE	CF:AbE
Mid May	100	0.34***	-0.25***	-0.30***	-0.17**
Late May	100	0.53***	-0.26***	-0.18**	-0.20**
Mid June	60	0.42***	-0.04	0.04	-0.07

Weight of *E. crassum* (WE) also correlated negatively with host condition and length in some samples mid May - mid June. When accounting for host length by partial rank order

correlation, there were significant negative correlations between CF and WE in the May samples (Table 7).

Table 7 Kendall partial rank-order correlation coefficients of host (*Salmo salar*) condition factor (CF) against total weight of *Eubothrium crassum* (WE) correcting for associations of the variables with host length (L). Samples from mid May – mid June (group B) with high *E. crassum* abundance and relatively large worms are examined. N=valid sample size, *p<0.05, **p<0.01, ***p<0.001.

		Kendall *T*			Partial *T*
Samples	N	CF:L	CF:WE	L:WE	CF:WE
Mid May	100	0.34***	-0.17*	-0.20**	-0.12*
Late May	100	0.53***	-0.18**	-0.09	-0.16**
Mid June	60	0.42***	-0.04	0.06	-0.07

The biomass of tapeworm in infected fish increases over time (Table 8), representing a potential stress to the host organism. We examined if this "load" as a fraction of host body mass (weight cestode/weight host) correlated with fish CF, using the May - June samples. "Load" did not increase in these samples, cestode growth being corresponding to host growth. There were negative correlations, becoming stronger from mid May (not significant) to mid June, when "load" explained ca. 45% of the variation in CF (Table 8).

Table 8 Pearson product-moment correlation between "load" (weight *Eubothrium crassum*/weight *Salmo salar*) and host condition factor (CF). Samples from mid May – mid June (group B) with high *E. crassum* abundance and relatively large worms are examined. N=sample size, SE=standard error of the mean.

		W *Eubothrium* (mg)		"Load" (x1000)		Correlation Load:CF	
Samples	N	Mean	SE	Mean	SE	*r*	*P*
Mid May	42	12.1	4.7	3.4	0.6	-0.07	0.68
Late May	42	25.7	4.7	3.1	0.6	-0.44	0.003
Mid June	20	42.3	6.8	3.5	0.9	-0.67	0.001

There was no pattern of correlations between *E. crassum* abundance, total weight of *E. crassum* or "load" and fish hematocrit (Hct) or leucocrit (Lct) in either group. Considering only infected fish (i.e. *E. crassum* intensity, weight and "load") and host Hct or Lct gave the same results.

Proteocephalus longicollis

Site

Proteocephalus longicollis was assigned to the sites pyloric region or post-pyloric intestine by scolex position. Of all *P. longicollis* recovered, 73.1% (N=410) were observed in the intestine, while 26.1% were located in the pyloric region (Table 9). All stages of *P. longicollis*

were located at both sites, but most of the plerocercoids were found in the intestine. A few large, gravid and apparently moribund *P. longicollis* were observed, and these were very fragile and difficult to preserve and measure.

Table 9 Occurrence of *Proteocephalus longicollis* developmental stages in the two principal sections of the intestinal tract. Frequency refers to the allocation of the stages within these sections. Pyloric region =caeca+adjoining intestine, intestine=remaining intestine, N=number of observations

Stages	Pyloric region			Intestine		
	N	Frequency (%)	Length range (mm)	N	Frequency (%)	Length range (mm)
1: Plerocercoid	69	64.5	0.5-5.0	238	78.5	0.3-6
2: Immature	19	17.8	2.8-17	33	10.9	2.6-19
3: Mature	12	11.2	12.5-71	21	6.9	12-43
4: Gravid	7	6.5	38-114	11	3.6	25-105
Total at site	107	26.1		303	73.9	

Infection pattern

Proteocephalus longicollis was first found in late March (group B), two weeks after the group was started with fish from group A. The infected fish then measured 4.3-4.7 (4.5±0.2) cm (N=3). Prevalence of *P. longicollis* in group B increased significantly from late March to early April (χ^2_1=96.33, p<0.001), reaching 20% (Figure 11), and further change in prevalence was not observed (χ^2_4=8.05, p=0.09). The abundance of *P. longicollis* in group B also increased significantly from late March to early April (Mann-Whitney U Test, p<0.001). Change in abundance was not observed thereafter (Kruskall-Wallis, H (4, N=460) =7.33, p=0.12). Intensity of *P. longicollis* in group B increased gradually to a peak in late May (Figure 11).

Fish in group A were first found infected with *P. longicollis* in late May, one month after filter removal. Prevalence was then ca. 28% (Figure 12), and further increased significantly in the period from late May – late June (χ^2_1=4.32, p=0.04), reaching 37%. Further change in prevalence was not observed (χ^2_1=0.56, p=0.46) until a significant decrease from mid July to late July (χ^2_1=14.66, p<0.001). Abundance of *P. longicollis* in group A did not change significantly in the period late May to mid July (Kruskal-Wallis, H (3,N=402) =3.65, p=0.30). A significant decrease in abundance was found from mid July to late July (Mann-Whitney U Test, p=0.002). Intensity was stable in the period with infected fish (Figure 12). There was no significant difference in *P. longicollis* abundance between small and large fish after the sorting of group A (Mann-Whitney U Test, p=0.99) (see also Figure 12).

Group B

Figure 11 Infection pattern of *Proteocephalus longicollis* in hatchery reared salmon (*Salmo salar*) during the study period (February – June 2000) of group B. Sample prevalence, mean intensity and mean abundance of *P. longicollis* for each tank and for group B total. Fish in tank B1 and B2 was moved to tank B3 on May 11th. *=Survivors after hyper-oxygenation (95% mortality).
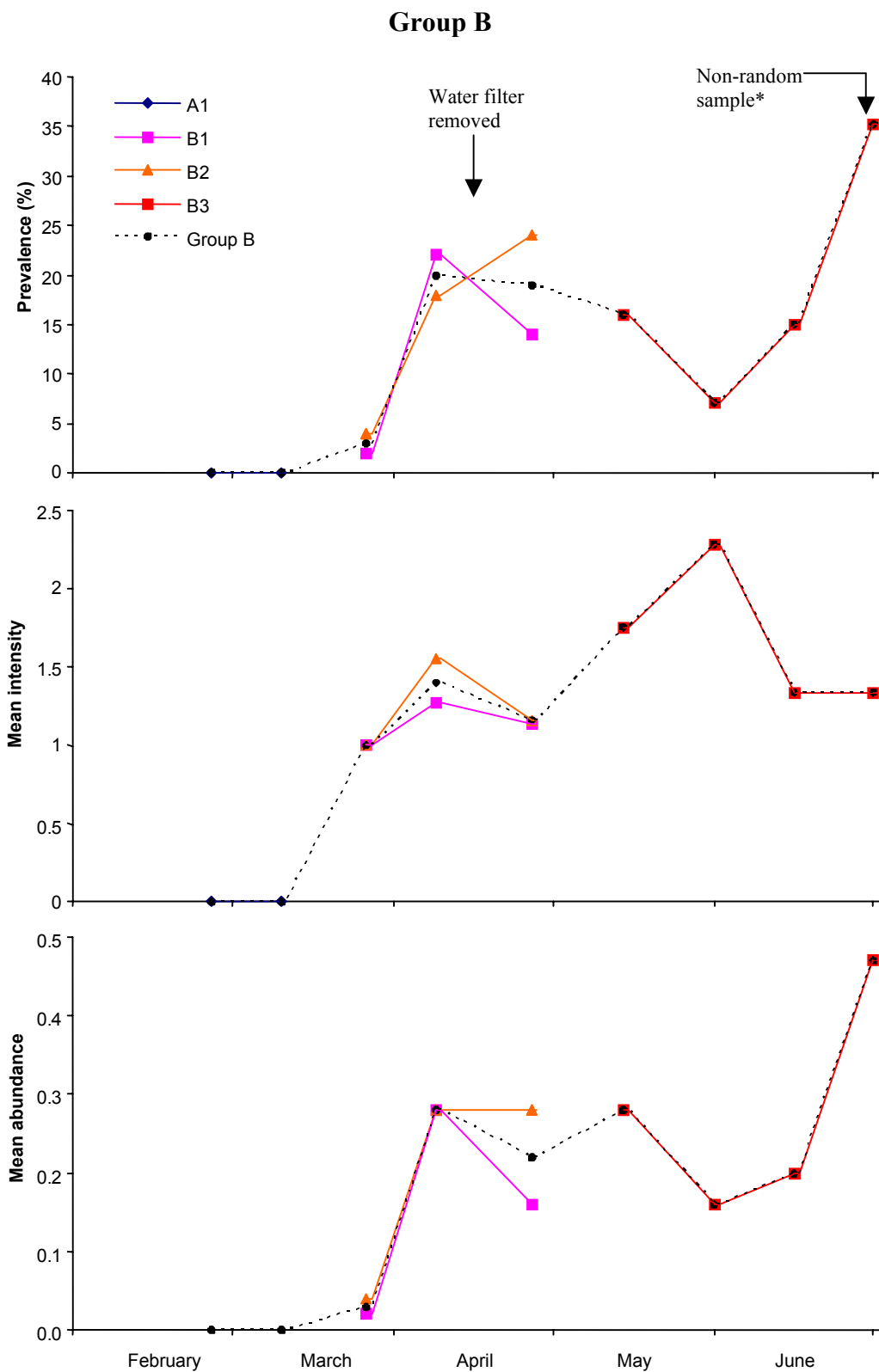
Group A
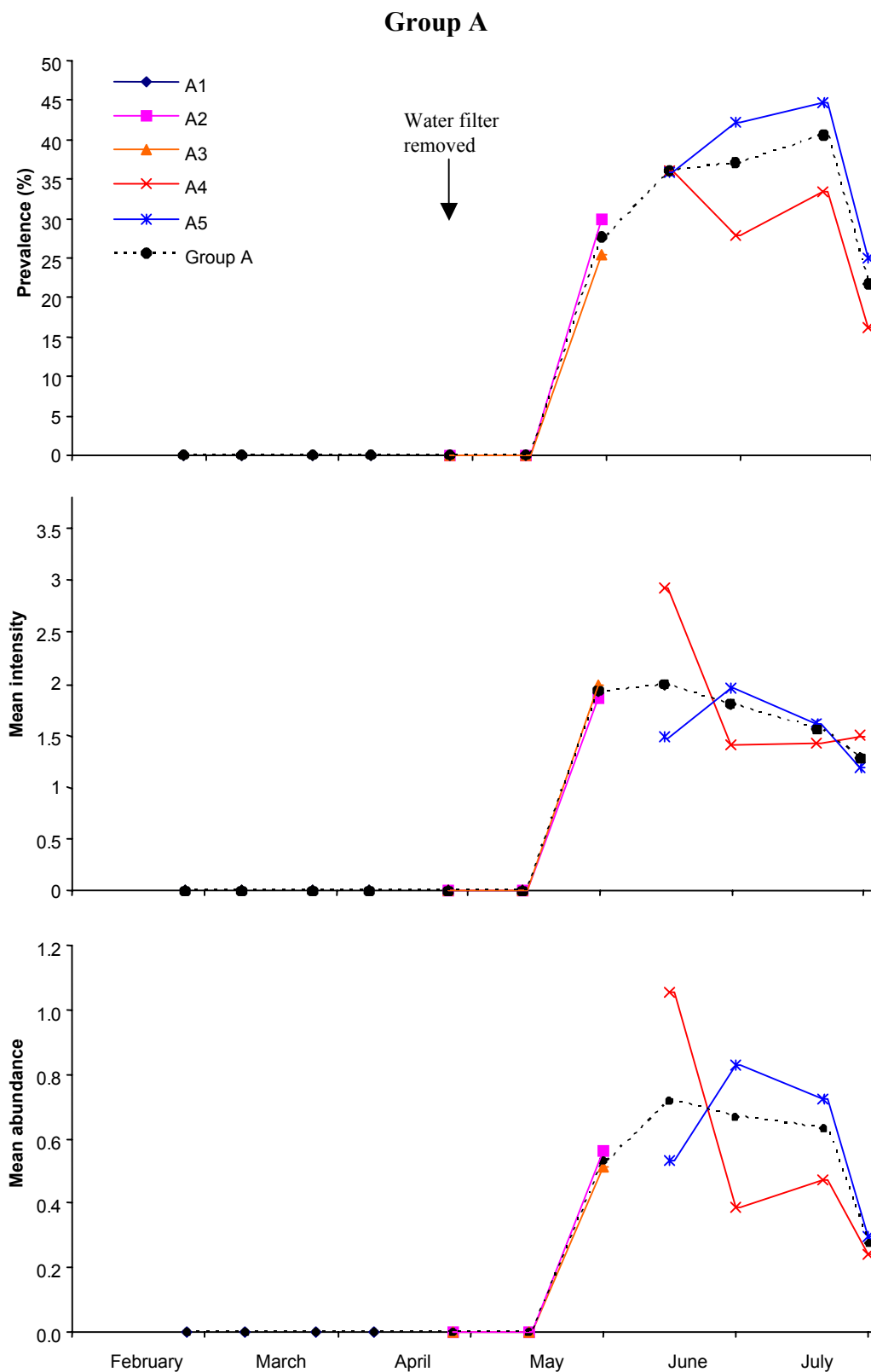

Figure 12 Infection pattern of *Proteocephalus longicollis* in hatchery reared salmon (*Salmo salar*) during the study period (February – July 2000) of group A. Sample prevalence, mean intensity and mean abundance of *P. longicollis* for each tank and for group A total. On June 14th the fish in A2 and A3 were mixed and then sorted into new tanks with large (A5) and small (A4) sized fish.

Host size and infection

There were significant negative correlations between host size (length and weight) and abundance of *P. longicollis*. A pattern with negative correlations was observed from April to May in group B (Figure 13). Negative correlations between fish size and cestode abundance were also found in the largest sorting of fish in late June and mid July (group A).

Figure 13 Spearman rank-order correlation coefficients (r_s) between abundance of *Proteocephalus longicollis* and salmon (*Salmo salar*) size (length and weight) in group B. Parallel tanks had identical water temperatures and are pooled. *p<0.05, **p<0.01
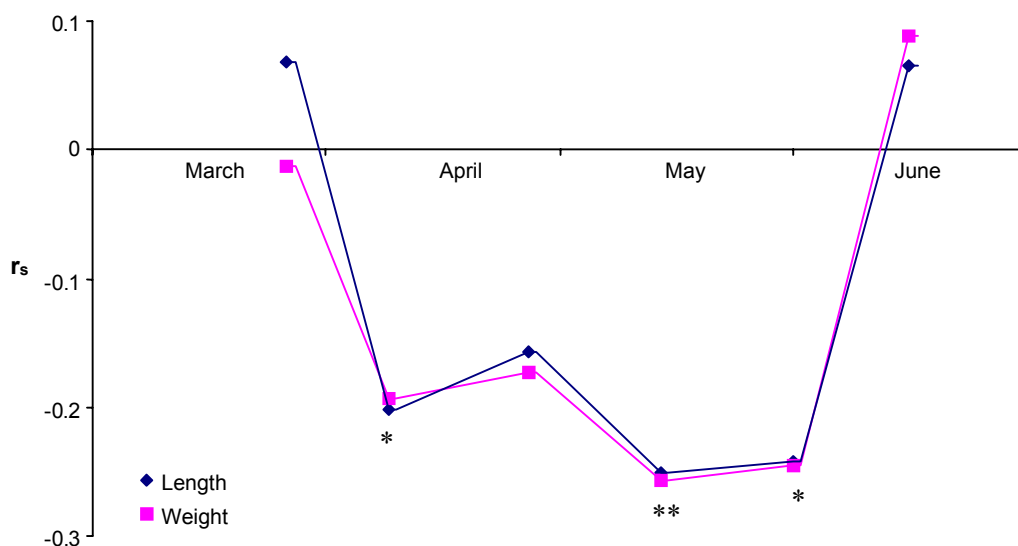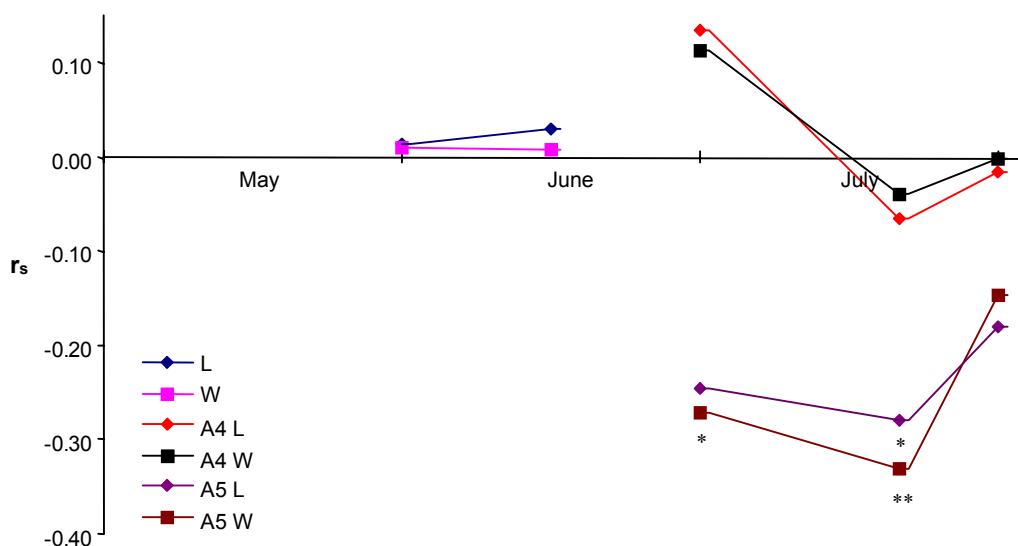

Figure 14 Spearman rank-order correlation coefficients (r_s) between abundance of *Proteocephalus longicollis* and salmon (*Salmo salar*) size (length (L) and weight (W)) in group A. Parallel tanks with identical temperatures, i.e. A2 and A3, are pooled. A4= small fish and A5= large fish after the sorting. *p<0.05, **p<0.01

31

Maturation and growth

Despite the fact that group B became infected first, gravid *P. longicollis* was not observed in that fish (Figure 15). Further, in late May were only plerocercoids found in this group. Gravid worms were first observed in group A in mid June, and were a maximum of 490 or 740 day-degrees old depending on earliest possible date of infection, filter removal (April 27th) or last infection free sample (May 13th), respectively (Figure 16). Considering peak incidence of infection (mid May – late May) and respective peak incidence of gravid worms (mid June – late June), the development of eggs took ca. 33 days or 460 DD in group A. Plerocercoids were observed in all samples from both groups, i.e. from late March till late July (Figure 15 and 16). There was an overlap in length between cestode stages, but cestodes over 80 mm were gravid (Table 10).

Table 10 Length (range (mean±S.D.)) of different stages of all *Proteocephalus longicollis* from group A and B during the study period (February – July 2000). N=number of observations, S.D. =standard deviation of the mean

	Group A		Group B	
Stages	N	Length (mm)	N	Length (mm)
1: Plerocercoid	207	0.3-6 (2.0±1.2)	100	0.3-6 (1.7±1.1)
2: Immature	32	2.8-17 (8.5±3.8)	20	2.6-19 (8.0±4.2)
3: Mature	28	12-71 (24.9±12.5)	5	17-25 (19.6±3.0)
4: Gravid	18	25-114 (54.2±26.4)	0	

The smallest cestode measured 0.3mm (plerocercoid) while the largest measured 114mm (gravid). An attempt was made to weigh the tapeworms, but the small size and their fragility in ethanol prohibited this. Length only is therefore used as a parameter for growth. Only in group A could a tendency with an increased mean length of *P. longicollis* be observed (Table 11).

Table 11 Mean length (L) of all *Proteocephalus longicollis* during the study period of group A and B (February - July 2000). Parallel tanks are grouped within group A and B. N= number of observations, S.D. = standard deviation of the mean, *= non-random sample in group B (survivors of the hyper oxygenation (95% mortality))

	Group B			Group A		
Samples	N	Mean L (mm)	S.D.	N	Mean L (mm)	S.D.
Late March	3	1.1	0.1			
Early April	28	5.5	6.1			
Late April	22	3.5	4.2			
Mid May	28	2.0	2.7			
Late May	16	1.4	0.2	54	3.5	6.3
Mid June	12	1.6	0.7	72	5.3	9.7
Late June*	16	6.0	6.0	67	11.9	16.5
Mid July				64	12.0	23.1
Late July				28	7.6	15.9

Group B

Figure 15 Maturation and length distribution of *Proteocephalus longicollis* from hatchery-reared salmon (*Salmo salar*) during the study period of group B (February - June 2000). Only infected samples are shown, and parallel tanks that had identical water temperatures, i.e. B1 and B2, are pooled. Late June sample: survivors after hyper-oxygenation (95% mortality). DD=day-degrees, using March 9th as earliest possible date of infection. Cestode stages: 1) plerocerciform, 2) immature, 3) mature and 4) gravid
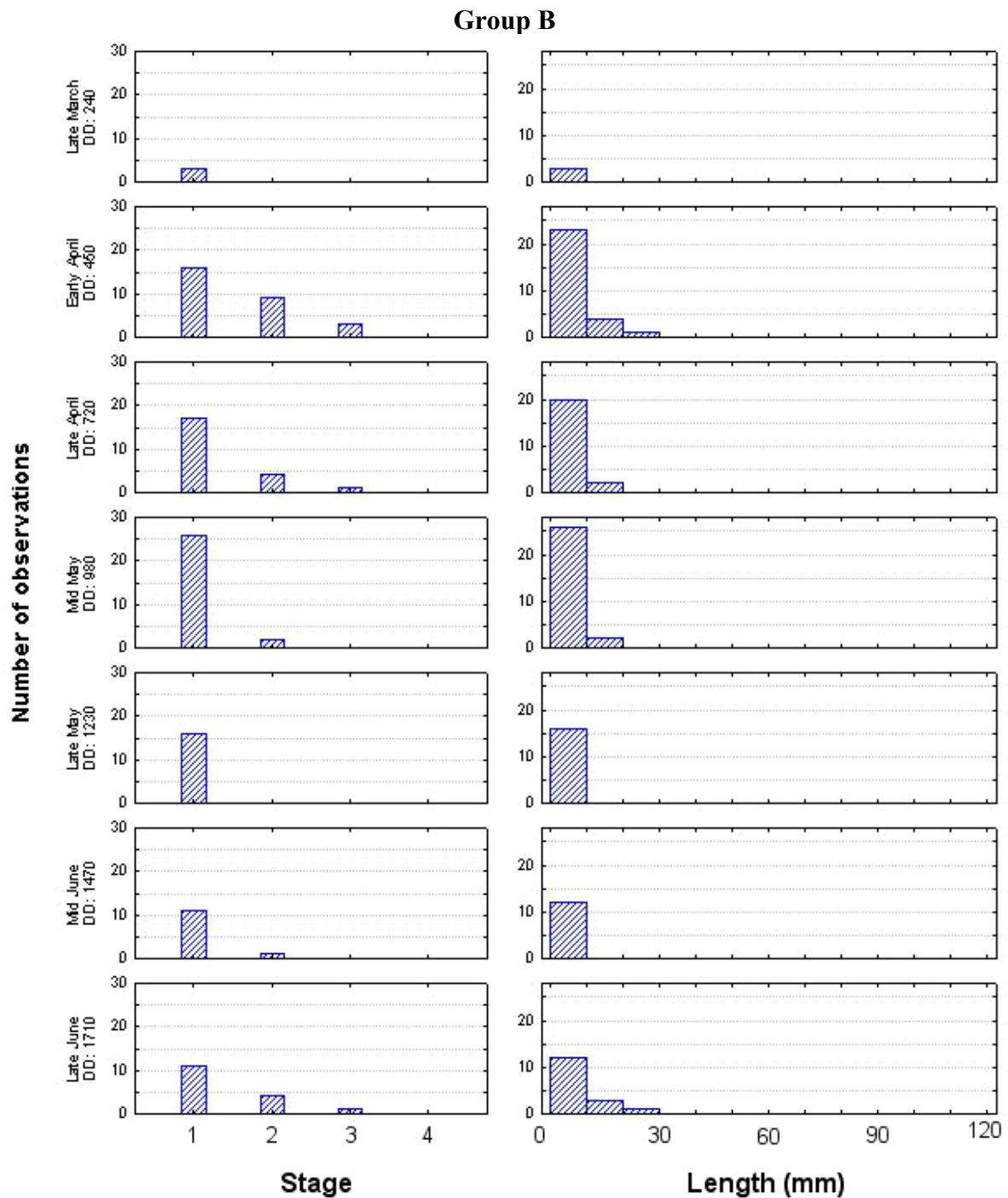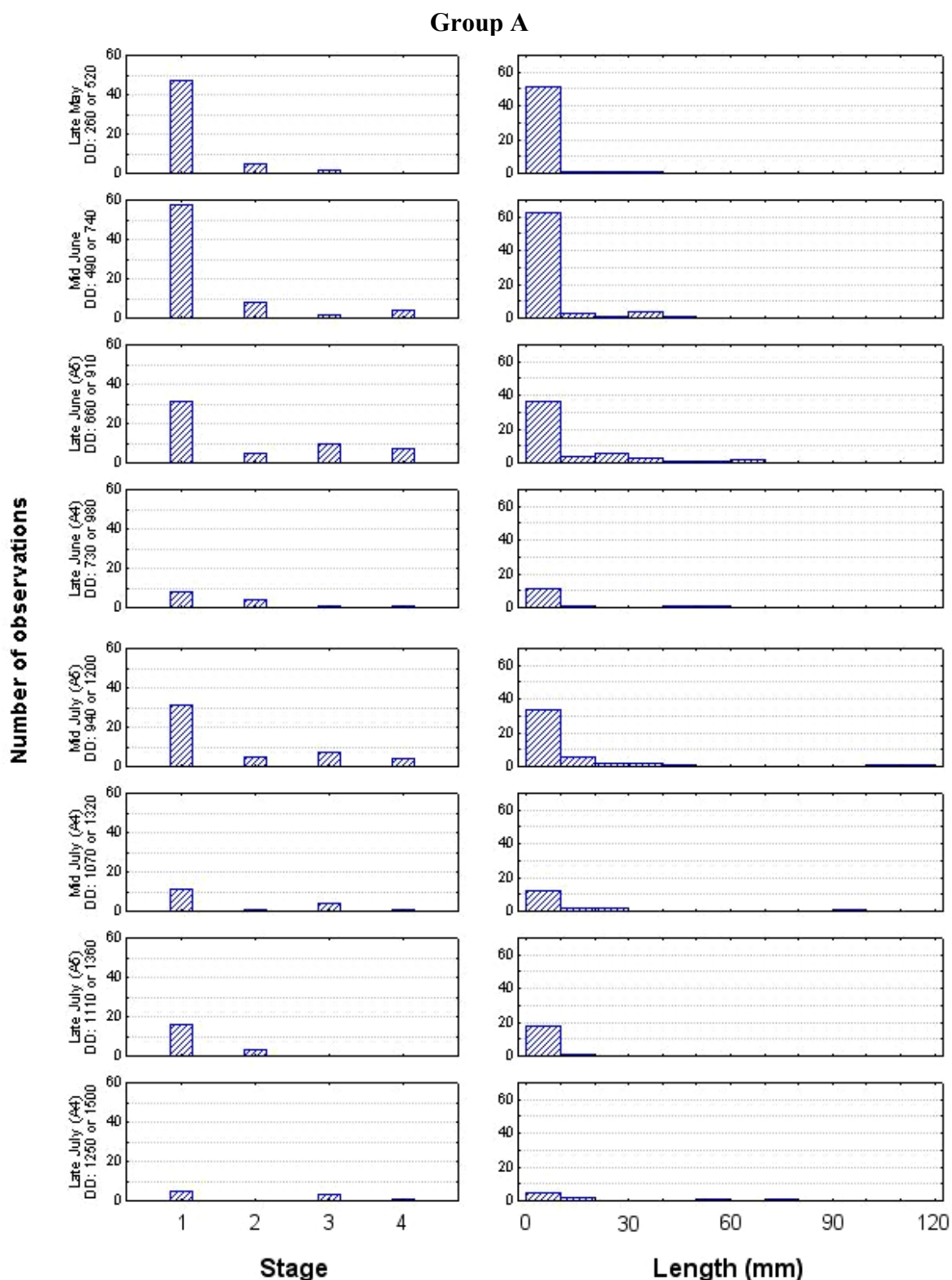
Group A

Figure 16 Maturation and length distribution of *Proteocephalus longicollis* from hatchery-reared salmon (*Salmo salar*) during the study period of group A (February - July 2000). Only infected samples are shown, and parallel tanks that had identical water temperatures, i.e. A2 and A3, are pooled. After sorting the fish into small (A4) and large (A5) sized fish, the parallel tanks had different water temperatures, hence were not pooled. DD=day-degrees, using April 27th (filter removal) or May 13th (last infection free sample) as earliest possible date of infection, respectively. Cestode stages: 1) plerocerciform, 2) 1-20 proglottids, 3) immature, 4) mature and 5) gravid.

Effect on the host

Proteocephalus longicollis abundance was at its highest in the period mid June - mid July in group A. At this time the worms were relatively large, so these samples were used to examine possible effects on host condition as measured by the condition factor (CF). The fish tanks had different water temperatures after the sorting into small (A4) and large (A5) fish, and hence could not be pooled for analysis.

There were no correlations between *P. longicollis* abundance (AbP) and host condition factor (CF) among the small fish (Table 12). There were negative correlations between cestode abundance and CF among the large fish in the June-July samples (Table 13). Simultaneously, there were positive correlations between CF and fish length (L) in June and negative correlations between *P. longicollis* abundance and fish length in late June and mid July (Table 13 and see figure 14). Accounting for host length by partial order correlation, there were negative correlations between CF and *P. longicolli*s abundance in June and July (Table 13).

Table 12 Spearman rank-order correlation coefficient of host (*Salmo salar*) condition factor (CF) against *Proteocephalus longicollis* abundance (AbP) among the small sized fish (A4). Samples from mid June – mid July with high *P. longicollis* abundance and relatively large tapeworms are examined. N=valid sample size

		CF:AbP	
Samples	N	r_s	p-level
Mid June	36	0,07	0,67
Late June	36	-0,03	0,88
Mid July	36	0,04	0,82

Table 13 Kendall partial rank-order correlation coefficients of host (*Salmo salar*) condition factor (CF) against *Proteocephalus longicollis* abundance (AbP) correcting for associations of the variables with host length (L) among the large sized fish (A5). Samples from mid June – mid July with high *P. longicollis* abundance and relatively large tapeworms are examined. N=valid sample size, *p<0.05, **p<0.01, ***p<0.001

		Kendall *T*			Partial *T*
Samples	N	CF:L	CF:AbP	L:AbP	CF:AbP
Mid June	64	0.17*	-0.22*	-0.07	-0.21**
Late June	64	0.31***	-0.18*	-0.19*	-0.16*
Mid July	65	0.10	-0.18*	-0.23**	-0.17*

Since abundance of *P. longicollis* might correlate with host CF, correlation between *P. longicollis* abundance and host CF in group B was calculated, using the mid May- mid June samples. There were negative correlations between CF and *P. longicollis* abundance in the May samples, while the June sample was positively correlated (Table 14). In addition there were positive correlations between CF and host length in May samples (Table 14), and negative correlations between host length and cestode abundance in the May samples (Table 14 and see Figure 13). Adjusting for correlations with host length by partial order

correlations, there was a negative correlation between host CF and *P. longicollis* in mid May, while the mid June sample was positively correlated.

Table 14 Kendall partial rank-order correlation coefficients of host (*Salmo salar*) condition factor (CF) against *Proteocephalus longicollis* abundance (AbP) correcting for associations of the variables with host length (L). Examined in samples May-June in group B. N=valid sample size, *p<0.05, **p<0.01, ***p<0.001

			Kendall *T*		Partial *T*
Samples	N	CF:L	CF:AbP	L:AbP	CF:AbP
Mid May	100	0.34***	-0.19**	-0.21**	-0.13*
Late May	100	0.53***	-0.16*	-0.20**	-0.06
Mid June	60	0.42***	0,20*	0,06	0,20*

There was no pattern of correlations between *P. longicollis* abundance and host Hct or Lct values in either group. Considering only infected fish (i.e. *P. longicollis* intensity) and host Hct or Lct gave the same results.

Associations between the cestode species

A total of 405 salmon were found infected by *Eubothrium crassum* and/or *Proteocephalus longicollis*, 64 (15.8%) of these were co-infected by both cestodes, and harboured in total 117 *P. longicollis* and 130 *E. crassum*. The most heavily co-infected fish had 10 *E. crassum* and 5 *P. longicollis* simultaneously.

Prevalence and abundance of *E. crassum* and *P. longicollis* were simultaneously relatively high in the early April – mid June samples (group B) (see figure 5 and 11). These samples were pooled (N=460) to examine if a potential association between the cestodes occurred. A positive association between the cestode species was found in that period (χ^2_1=17.87, p<0.001). There was also a positive correlation between the intensity of *E. crassum* and *P. longicollis* among co-infected salmon in this period (N=41, r_s=0.35, p=0.02).

Gravid cestodes from both species never occurred in a single host. However, some fish harboured up to two gravid specimens of *E. crassum* or *P. longicollis*. Fish with gravid *P. longicollis* were never infected with *E. crassum*, while gravid *E. crassum* could co-occur with small immature *P. longicollis*.

Discussion

Cestodes

The normal prevalence of cestodes in the hatchery-reared salmon used to be, according to the responsible veterinarian, 10-20% in autumn (50% in extreme cases) when treatment with anthelminthica is evaluated (pers. comm. G. Folkestad). However, apart from the presence of *Eubothrium*, species, prevalence and abundance of the cestodes was not known. An earlier parasitological study of charr in Lake Henangervatnet has revealed the presence of three cestode species, *Diphyllobothrium dendriticum*, *D. ditremum* and *Proteocephalus longicollis* (*sensu* Scholz & Hanzelova 1998) (Alexandersen 1993), and these can potentially infect the farmed fish via zooplankton. The work of Alexandersen also revealed the absence of *E. salvelini* in the watercourse, a parasite of charr, confirming that the juvenile *Eubothrium* found in the present study is *E. crassum*. Together with the study by Alexandersen (1993), the present samples from wild and farmed fish show that 4 cestodes occur in salmonids in the watercourse. Only *E. crassum* and *P. longicollis* are common in the hatchery salmon, and will be discussed accordingly.

Infection dynamics, growth and maturation

Factors affecting the observed infection dynamics

The first infections with cestodes were observed in late March (group B), group A fish being first infected in late May, i.e. one month after the filter was removed. This difference could be due to different filtering systems. In group B a disc-filter with an overflow system was used, and this allowed unfiltered water to pass if the filter got clogged-up. This implies that copepods infected with cestodes could have entered group B in pulses. The drum-filter in group A did not have an overflow system, and all water was probably effectively filtered. All fish (N=500) examined prior to filter removal in group A were uninfected.

Infections with *E. crassum* and *P. longicollis* showed differences in abundance, timing and longevity, hence, they are discussed separately.

Eubothrium crassum

Kennedy (1978a) suggested that *Eubothrium crassum* consists of three species: a European freshwater species, an Atlantic marine species and a Pacific marine species. It is still not clear whether *E. crassum* in Europe comprise a single species, or a freshwater and a marine species (Bristow & Berland 1989, Shinn *et al*. 1999, Kralova-Hromadova *et al*. 2003, Scholz *et al*. 2003). However, since worms morphologically conforming to *E. crassum* can complete the life cycle in both freshwater (cf. Kennedy 1978a) and in seawater (Saksvik *et al*. 2001b), they are considered as different species in this work.

Transmission of *E. crassum* occurs when fish ingest infected copepods, and the first site of *E. crassum* is either the stomach or intestine, where digestion of the copepod happens. Growth only commences when a plerocerciform worm moves into a caecum, and larger worms are more likely to be found in anterior caeca, the preferred site of *E. crassum* (Kennedy 1996). Salmon in the present study must be regarded as only lightly infected (cf. Kennedy 1996), so lack of suitable sites is unlikely to be a constraint on the *E. crassum* infrapopulations. Still, small and immature worms were observed in the post-pyloric intestine in the present study, and these may be about to be lost from the fish, as suggested by Kennedy (1996).

The principal increase in prevalence and abundance of *E. crassum* occurred from late March to mid May (Group B), suggesting that this was the main period of infection in the hatchery. A modest abundance of 0.16 developed in group A in late May, which supports this pattern. However, it also demonstrates an ongoing but low-grade infection that is also apparent by the presence of plerocerciform worms in late May and mid June (group B). Even so, only two newly acquired worms (i.e. stage 1 and 2) were found after mid June (group A), probably demonstrating a decreasing infection pressure by *E. crassum* infected copepods in summer. In Ånøya Drainage System in Norway Vik (1963) found that the intermediate hosts for *E. crassum* were abundant in spring, but disappeared in July and August. If a similar pattern occurs in Lake Henangervatnet, then the disappearance of intermediate hosts may explain the lack of further infections during summer. In England, recruitment of *E. crassum* in brown trout appeared to cease in July – August (Kennedy 1996), a comparable pattern.

A similar timing of infection was found by Wootten (1972) at Hanningfield Reservoir, England. Virtually uninfected brown trout stocked into the reservoir in March – April, quickly acquired infections reaching 100% prevalence by July, demonstrating that the spring infection period was important. Brown trout in Lock Leven, Scotland, harboured plerocerciform *E. crassum* mainly from July to September, but they were also present at other times of the year

(Campbell 1972/73). Further, brown trout infected with juvenile (<10mm) *E. crassum* occurred in May and June, but not in March – April in Malham Tarn, England (Kennedy 1996). In these field studies the infection appears to happen later than in the present study. However, copepods are usually scarce in early spring, and this may compel large trout to feed on more rewarding preys (e.g. small fish). Also, in both those studies gravid cestodes were observed in March – April, which may indicate that infections of copepods occurred at that time.

Little is known about the intraspecific competition of *E. crassum*, but clearly not all worms are able to establish in fish (cf. Kennedy 1996). However, it is conceivable that the establishment rates are lower in fish already harbouring large worms than in fish that are only infected by juvenile worms (Wootten 1972). Consequently, intraspecific interactions may affect the observed infection dynamics when abundance is as high as in the field studies cited.

Abundant food supply and high temperatures may have enhanced the growth of cestodes in the present study, compared to natural conditions. Plerocerciform *E. crassum* seemed to quickly start strobilization. The development of the first proglottid apparently occurred within days post-infection since cestodes from stage 2 (i.e. 1-20 proglottids) outnumbered plerocerciformes in nearly every sample. The time from infection to the development of eggs was estimated to. 50 days or 750 DD in the present study. These results are in agreement with Wootten (1972), who found mature and gravid *E. crassum* in July in trout that likely were infected in March – April. Consequently he suggested that *E. crassum* can produce eggs within 2-3 months after establishment at that time of the year. The temperatures in the present study were most likely higher than those of Wootten (not given, but natural), explaining a slightly shorter development time.

Kennedy (1996) found a seasonal infection cycle where brown trout became infected by *E. crassum* in spring and summer. These worms grew during autumn and winter, and could attain a length of 100mm by February. In March – April the largest individuals exceeded 200 mm and were gravid. From May to June all cestodes became gravid and/or lost from the fish, and were replaced by a new generation of worms. Kennedy therefore suggested that *E. crassum* survive for a maximum of 1 year in fish. The rapid development of *E. crassum* indicated by the present study supports his assumption.

Wootten (1972) did not find a fixed length at which *E. crassum* from brown- or rainbow trout became gravid. This is in contrast to the present results where almost every *E. crassum* over 100 mm was gravid. A similar tendency was observed in *E. crassum* from brown trout in northern Norway (Kennedy 1978b). This difference in growth and maturation may be due to a

shorter summer at northern latitudes, with earlier development of the worms when conditions are favourable.

Proteocephalus longicollis

Fish become infected with *P. longicollis* after ingestion of copepods harbouring procercoids (Scholz 1999a). Adult cestodes are usually located in the anterior part of the intestine or caeca, while juveniles may be found throughout the intestine (Petersson 1971, Alexandersen 1993, Scholz 1999a). Intraspecific competition between juvenile worms is likely to occur, since only a small proportion of the tapeworms are able to establish (Scholz 1999a). Hence some juveniles in the post-pyloric intestine in the present study may have been on their way to be lost from the fish.

The principal increase in prevalence and abundance with *P. longicollis* occurred from late March to early April in group B. The water to this group was then still filtered, but clearly ineffectively so. Despite the confounding effect of the filter, the infection clearly demonstrates the presence of copepods with infective procercoids in this period. A more pronounced increase in prevalence and abundance was found from mid May to late May in group A, demonstrating a major period of infection. The prevalence in this group further increased from late May to mid June, showing that further infections occur, albeit not affecting abundance significantly. The major infection period seen in group A is not detectable in group B. However, a significant decrease in both prevalence and abundance occurred in group A two months after the first major infection pulse, indicating infrapopulation mortalities (cf. Scholz 1999a). If occurring in group B, this period of worm loss coincide with the recruitment pulse seen in A, hence suggesting a masking effect. That this is the case is further suggested by the nearly complete absence of stage 2+ *Proteocephalus* in the May samples, being dominated by plerocercoids, both findings being in accordance with mortality among grown worms and recruitment of juveniles. This study therefore provides evidence for two periods of infection in the hatchery, a March-April pulse and a May-June pulse. While the relative importance of the first period cannot be fully assessed due to the filtering, the second resulted in a prevalence exceeding 40% despite that the fish at this time was larger. Between these apparent pulses there was a period with no infective stages reaching the hatchery, clearly shown by the lack of cestodes in the mid-May sample in group A two weeks after filter removal, and also supported by the unchanged prevalence and abundance of group B in this period.

Alexandersen (1993) observed an apparently different infection pattern with *Proteocephalus* sp. (=*P. longicollis*) in charr in lake Henangervatnet from June 1990 to May 1991. The highest prevalence and abundance of juveniles were found from October to April, but a low prevalence of juveniles could also be found in the remaining year. Adults mainly occurred from May to September. From September to October Alexandersen observed that the population of *P. longicollis* shifted almost entirely from adult cestodes to a population with merely juveniles. The infection pattern fitted that of *P. torulosus* in dace (*Leuciscus leuciscus* L.) (Kennedy & Hine 1969), and Alexandersen suggested that low temperatures could decrease the resistance of charr to cestode infections, hence explain why infections only occurred in the colder months of the year. Comparing the pattern observed by Alexandersen to the present observations, the spring pulse observed in the hatchery may represent the end of a winter infection period demonstrated by high abundance of plerocercoids in the charr. The May - June pulse is not reflected at all in charr, this time being a minimum in plerocercoid abundance (Alexandersen 1993). This difference is not easily explainable. Since extensive evidence show that charr feed preferentially on cladocerans during summer (cf. Hindar & Jonsson 1982, Klemetsen 1987 and references therein), it is possible that the prey reaching the hatchery differ from that used by charr in the lake, and the prey choice in the smaller salmon may still include copepods.

Aderounmu (pers. comm. in Chubb 1982) found *P. neglectus* (=*P. longicollis*) in brown trout at a fish hatchery in Wales in March, and the prevalence increased during spring, demonstrating a spring infection as detected in the present study. A study of the seasonal dynamics of *P. neglectus* (=*P. longicollis*) in intermediate hosts and in cage-cultured rainbow trout were carried out by Hanzelova *et al*. (1990) from May to November in Slovakia. Trout were first infected in mid June, and infection was coinciding with increasing infection levels in intermediate hosts. Two weeks later the prevalence in fish reached 100%, and the highest abundance was observed in mid July. Hence, that study also shows a different pattern of infection than in the present study, perhaps due to the use of copepod intermediate hosts with a differing seasonal incidence.

Growth and maturation of *P. longicollis* is mainly controlled by water temperature (Scholz 1999a), and the high temperatures and high food availability may have enhanced the development of cestodes in the present study. Unsegmented plerocercoids in experimentally infected rainbow trout were found 17 days post infection at 10 °C (Scholz 1991). However, due to higher temperatures in the present study, a shorter development time may be expected. *Proteocephalus longicollis* in fish from group B were small, the longest only 25mm, and no

worms became gravid. This is surprising, and not easy to explain. Size differences of *P. neglectus* (= *P. longicollis*) in different age groups (i.e. 0+ and 1+) of rainbow trout was found in the Dobsina water reservoir, Slovakia (Hanzelova & Spakulova 1992). In underyearlings the cestodes tended to be smaller than 50mm, while in yearlings they mostly exceeded 50mm. Still, gravid cestodes were found in both age groups. Hanzelova and Spakulova proposed that the observed cestode size differences were host-induced effects. This explanation may apply also to the present observations, perhaps due to dissimilar food flow in small and large fish.

The time from infection of *P. longicollis* to the development of eggs was estimated to ca. 33 days or 460 DD in the present study. Adult specimens of *P. longicollis* have been found in rainbow trout a few weeks after infection (Hanzelova *et al.* 1990), and after 1.5-2 months in experimentally infected *Coregonus peled* fry, kept at 12-21°C (Albetova 1975) (cf. Scholz 1999a). These studies show a fairly similar development time if the definition of "adult"/"mature" worms in those studies are gravid specimens. Other authors (cf. Scholz 1999a) have found a longer maturation time. Field studies suggest that specimens of *Proteocephalus* have life span of maximum one year (Scholz 1999a), and the fast development of *P. longicollis* in the present study supports that assumption.

Species of *Proteocephalus* may show different patterns of maturation and development at different latitudes (Scholz 1999a), which may explain some of the differences in growth and maturation between the present study and others. Still, the high temperatures in the present study may have enhanced the development of the worms, while other studies may experience crowding effects or change in feeding preferences of the fish, which may alter the observed development and infection dynamics of *P. longicollis*.

Host size and infection

Consistent negative correlations were found between cestode abundance and host size (length and weight) for both *E. crassum* and *P. longicollis* (group B). However, this tendency disappeared in June. The most conceivable explanation is that the smaller fish are more prone to feed on copepods, perhaps due to feed competition. However, Boyce (1974) found a similar tendency in sockeye fry (*Oncorhynchus nerka*) experimentally infected individually with approximately ten *Eubothrium salvelini* infected copepods. He suggested improved cestode attachment opportunity as an explanation, or that larger fry have a more developed immune system reducing the cestode establishment success. The disappearance of this tendency in the

June sample is difficult to explain but may be a random outcome or related to mortality in the infrapopulations due to maturation of the worms.

The present study provides evidence suggesting that the cestodes *E. crassum* and *P. longicollis* co-occur more often than expected by chance. Since infections by both cestodes are biased towards smaller fish in many samples, these results suggest the presence of a subset small-sized fish adopting copepod feeding.

Effect on host

Eubothrium crassum

The present study shows that infection by *E. crassum* may have a negative effect on host condition factor. Similar results were obtained at high abundance of marine *Eubothrium* sp. in Atlantic salmon, and intensity of *E. salvelini* in charr (Hoffmann *et al*. 1986, Saksvik *et al*. 2001a). Conversely, Gerdeaux *et al*. (1995) did not find any relationship between charr condition factor and a "parasitic index" of *E. salvelini*, but his results are not readily comparable to the present due to the unconventional way he measured worm load. The clearest effects of *E. crassum* on the hatchery reared parr were seen from worm "load", the relationship between cestode and fish weights. This result suggests that worm mass rather than abundance represents a strain on the host affecting aspects of its growth. As a consequence, only one worm might reduce growth in small fish. Substantial loads (>10%) of *E. salvelini* were found in juvenile sockeye salmon (*Oncorhynchus nerka*) (Smith 1973). Smith considered it likely that the cestode caused direct mortalities among the heavily infected sockeye, and indirect mortalities by predation from reduced growth, poor swimming performance and aberrant behaviour. The negative growth effects highlighted were considered caused either by poor host nutrition due to demands of the parasite or from reduced food gathering capabilities.

Reduced growth due to *Eubothrium* sp. infections in seawater reared Atlantic salmon have also been deduced from lower average weight of infected fish (Bristow & Berland 1991a). The problems with such field studies are, as discussed above, that the smaller fish may feed more on zooplankton. A recent experimental study examined this problem, and for the first time provided clear evidence for growth effects of *Eubothrium* sp. (Saksvik *et al*. 2001a).

While Saksvik *et al*. (2001a) found a small but significant relationship between condition factor and *Eubothrium* sp. infection in seawater reared Atlantic salmon, they found a much

clearer and more important reduction in length-growth associated with the cestode infections. If valid for the closely related (or conspecific, cf. Scholz *et al.* 2003) freshwater *E. crassum*, the rather consistent negative correlations between *E. crassum* and host size can also partly be explained by growth retardation.

Proteocephalus longicollis

Compared to *Eubothrium* spp., there is very limited evidence for pathology or growth effects of *Proteocephalus* infections in salmonids. It has been suggested that infections with *P. longicollis* reduce the growth of rainbow trout (Priemer & Goltz 1986, Engelhardt *et al.* 1988), but the evidence is scant. Heavily infected trout were a few percent (2-4%) lighter than fish with fewer worms (Engelhardt *et al.* 1988). Priemer & Goltz (1986) also reached this conclusion from the observation that the smallest fish were infected while the largest were uninfected. The present results provide no conclusive evidence for a negative effect of *P. longicollis* on host condition, since the significant correlations found were both negative and positive. Unfortunately, this aspect could not be examined using *Proteocephalus* load, but the limited effects claimed by Engelhardt *et al.* (1988) was based on relatively high loads. As length-growth effects rather than lowered condition may result from cestode infections, discussed above, such effects should be examined through experimental infections.

Conclusions and future works

Two species of adult cestodes (*Eubothrium crassum* and *Proteocephalus longicollis*) and a single encysted *Diphyllobothrium dendriticum* were found in the farmed salmon, all common in wild salmonids.

Eubothrium crassum and *P. longicollis* were first found in March due to an ineffective filter system. The principal infection by *E. crassum* took place in early spring, while infections by *P. longicollis* occurred in two pulses, the first in early spring and the second in late spring – early summer. Infections happened quickly, with cestode prevalence and abundance usually peaking within a few weeks. Prevalence of *E. crassum* and *P. longicollis* reached a maximum of 42% and 41%, respectively, but only few worms usually occurred in each infected fish. The high temperatures and food availability in hatchery fish apparently allow these cestodes to grow and mature rapidly. Evidence was found suggesting that *P. longicollis* and *E. crassum* started producing eggs after ca. one and two months, respectively. While lower temperatures occur in nature, these results suggest that these cestodes can complete their life cycle in less than a year in Norwegian lakes.

The present study demonstrates that *E. crassum* may have a negative effect on the condition of juvenile salmon, proportional to worm mass. Experimental studies on marine *Eubothrium* infections in salmon show that length-growth may be significantly reduced, hence a serious effect on growth only reflected by a modest reduction in condition. *E. crassum* infections were most common in small sized fish, in accordance with a similar impact of this cestode. However, other factors like feed competition or even wrong pellet size can potentially produce such effects by promoting copepod feeding among small fish, so evidence for length-growth reduction requires studies on experimentally infected parr.

No convincing evidence was found suggesting that *Proteocephalus longicollis* infections have similar effect on salmon as *E. crassum*, which together with findings in other studies indicate that this species is comparably benign.

For hatcheries, cestode infections in parr and smolts can have several negative effects. Reduced fish growth means increased feed expenses, and length-growth effects affect the proportion of underyearlings that can be produced. Tapeworm infected smolt may be considered unattractive for stocking, so anthelminthic treatments may be required, representing increased costs.

Hatcheries receiving water from lakes may best avoid cestode problems by utilizing effective filter systems that remove all zooplankton, at least in peak periods of infection.

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