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ABSTRACT

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Diss.

The interaction between *Diphyllobothrium dendriticum* (Nitzsch 1824) (Cestoda) and trout was studied using the following materials: 1) brown trout *Salmo trutta* m. *lacustris* (L.) and sea trout *Salmo trutta* m. *trutta* (L.) from the Muonio Fish Farm; 2) brown trout from Lake Inari from 1994 and 1995; and 3) data from four laboratory experiments with *D. dendriticum* and brown trout. *D. dendriticum* was found to invade the heart atrium of fish in varying prevalences at the Muonio Fish Farm, Lake Inari and in experiments. In experimental studies the size and migration activity of *D. dendriticum* in brown trout increased along with water temperature and *D. dendriticum* infection had an increasing impact on the blood lymphocyte and neutrophil counts. The same dose of intubated infective procercoids caused overdispersed distributions of plerocercoids in brown trout, which was obviously due to individual differences in the susceptibility of fish to *D. dendriticum* infection. Negative effects on feed intake and growth rate were not observed when fed *ad libitum*. Mortality induced by a few *D. dendriticum* was observed in brown trout and sea trout at the Muonio Fish Farm in the early 1990s when plerocercoids penetrated the heart of the fish. Direct or indirect evidence of *D. dendriticum* induced mortality of the stocked brown trout could not be found in Lake Inari. Moreover, *D. dendriticum* did not cause a provable mortality in brown trout aged 0+ - 1+ in experimental studies. It is concluded that brown trout normally respond to the harmful effects of *D. dendriticum* successfully. The observed mortality at Muonio Fish Farm shows, however, that the balance between *D. dendriticum* and fish may collapse under certain circumstances. These studies indicate that a small proportion (maybe 5-10%) of the trout population will be lost annually to *D. dendriticum* heart infection, at least in lakes with strong *D. dendriticum* infection.

Key words: blood leucocytes; *Diphyllobothrium dendriticum*; feed intake; growth; heart infection; mortality; pathogenicity; *Salmo trutta*; temperature.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by Roman numerals I-VI:

I Rahkonen, R., Aalto, J., Koski, P., Särkkä, J. & Juntunen, K. 1996. Cestode larvae, *Diphyllobothrium dendriticum* as a cause of heart disease leading to mortality in hatchery reared sea trout and brown trout. Diseases of Aquatic Organisms 25: 15-22.

II Rahkonen, R. & Koski, P. 1997. Occurrence of cestode larvae in brown trout after stocking in a large regulated lake in northern Finland. Diseases of Aquatic Organisms 31: 55-63.

III Rahkonen, R. & Valtonen, E. T. 1997. Infection of brown trout with *Diphyllobothrium dendriticum* procercoids. International Journal for Parasitology 27: 1315-1318.

IV Rahkonen, R. & Valtonen, E. T. Role of water temperature on the size, migration activity and lethality of *Diphyllobothrium dendriticum* (Cestoda) plerocercoids in brown trout *Salmo trutta* m. *lacustris* (L.). Manuscript (submitted)

V Rahkonen, R. & Pasternack, M. Effect of experimental *Diphyllobothrium dendriticum* infection on the blood leucocyte pattern of brown trout at two temperature levels. Manuscript (submitted)

VI Rahkonen, R., Koskela, J. & Jobling, M. The effect of *Diphyllobothrium dendriticum* (Cestoda) infection on feeding and growth of brown trout (*Salmo trutta* L.). Manuscript (submitted)

1 INTRODUCTION

1.1 Taxonomy and life-cycle of *Diphyllobothrium* species

Tapeworms of the genus *Diphyllobothrium* Cobbold 1858 (Cestoda; Pseudophyllidea) are common in European and North American freshwater fishes and three species have been distinguished (Halvorsen 1970, Bylund 1975, Andersen *et al.* 1987, Andersen & Gibson 1989): *D. latum* (L. 1758), *D. dendriticum* (Nitzsch 1824) (syn. *D. norwegicum* Vik 1957), and *D. ditremum* (Creplin 1825). The validity of the fourth species, *D. vogeli* Kuhlow 1953, is still under review (Andersen & Gibson 1989, Bylund & Andersen 1994). Procercoid larvae develop in planktonic copepods (first intermediate host) and plerocercoid larvae in fish (second intermediate host) for all these species. *D. latum* is a human tapeworm and the fish intermediate hosts include pike (*Esox lucius* L.), perch (*Perca flavescens* (L.)), ruffe (*Gymnocephalus cernuus* (L.)) and burbot (*Lota lota* (L.)) (e.g. Vik 1957, Wikgren 1963, Andersen & Valtonen 1992). For *D. dendriticum* and *D. ditremum*, salmonids, coregonids and three-spined stickleback (*Gasterosteus aculeatus* L.) mainly serve as the second intermediate hosts (Vik 1957, Bylund 1966, Andersen & Valtonen 1992). The ability of plerocercoids to pass from prey fish to predatory fish is well developed in the case of *D. latum* and *D. dendriticum* while poor for *D. ditremum* (Vik 1957, Halvorsen 1970, Halvorsen & Wissler 1973). The final hosts are piscivorous birds, mainly *Larus* species for *D. dendriticum* and *Mergus* and *Gavia* species for *D. ditremum* (Fig. 1). However, the egg production of *D. dendriticum* has been demonstrated to succeed in various mammals as well (Vik 1957, Bylund 1969, Halvorsen 1970).

When *Diphyllobothrium* larvae are ingested by a fish host, they penetrate through the oesophagus or stomach and are usually encapsulated on the anterior part of the digestive tract and adjacent tissues or on other visceral organs (liver, gonads, swim-bladder, peritoneum) or even in musculature (Vik 1957, Halvorsen 1966, Henricson 1978, Andersen *et al.* 1987, Andersen & Valtonen 1992). Fish host are shown to react to *D. dendriticum* and *D. ditremum*

infection with an inflammatory response which encapsulates the worms with varying efficiency (Bylund 1972, Sharp *et al.* 1989, 1992). Salmonids possess a less developed encapsulation process against *D. dendriticum* than whitefish (*Coregonus lavaretus* (L.)) (Bylund 1972). *D. latum* are mostly found unencapsulated in the body cavity and musculature of fish (Vik 1957).

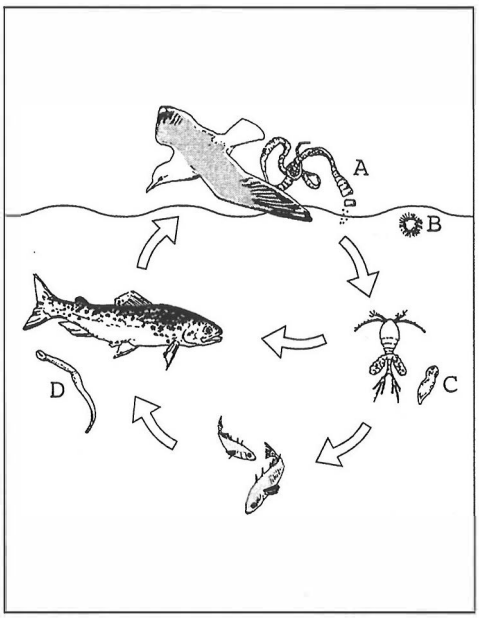


FIGURE 1 The life-cycle of *Diphyllobothrium dendriticum*. A=mature worm; B=coracidium; C=procercoid; D=plerocercoid.

1.2 Pathogenicity of *D. dendriticum*

According to Crofton (1971a) the term *parasitism* refers to an ecological relationship between the populations of two different species of organisms: parasite and host. The features of this ecological relationship include: a) physiological dependence of the parasite on the host, b) the production or tendency towards production by the infection process of an overdispersed distribution of parasites within the host population; c) death of the heavily infected host; d) a higher reproductive potential in the parasite species than in the host species.

It has been established that aggregation or overdispersion (when the majority of parasites are found in only a few hosts) is a central element in the regulation of host and parasite populations (Crofton 1971a,b, Anderson & May 1978, 1979, Anderson & Gordon 1982, Gordon & Rau 1982). According to Anderson & May (1978) increasing aggregation has a stabilizing effect on the host-parasite interaction: host individuals with the greatest numbers of parasites

will die, eliminating a lot of parasites from the ecosystem as well. A decrease in the degree of overdispersion within the older age classes of the hosts, concomitant with a decline in abundance, has been suggested to be indirect evidence of the mortality of heavily parasitized individuals in the wild (Anderson & Gordon 1982). These authors used data from Henricson (1977 1978), among others, to suggest that the observed decrease in the abundance and variance-to-mean ratios of *D. dendriticum* and *D. ditremum* in the oldest age groups in Arctic char (*Salvelinus alpinus* (L.)) was the result of host death.

In the case of *D. dendriticum* there is evidence, however, that the site of larvae in fish is also very important concerning the lethality of this species. *D. dendriticum* was shown to cause remarkable mortality at the Muonio Fish Farm in northern Finland in 1991 and 1992 (I). Among dead juvenile sea trout (*Salmo trutta* m. *trutta* (L.)) and brown trout (*Salmo trutta* m. *lacustris* (L.)), 60-90% were found to harbour usually one plerocercoid inside the heart atrium. *D. dendriticum* was found in the heart of fish caught live as well, although in clearly lower prevalences. The lethality of *D. dendriticum* seemed to be temperature dependent since the heaviest mortality occurred at the warmest time of the summer and a clearly higher mortality as well as water temperature level was observed in July 1991 compared to 1992. The observed lethality of *D. dendriticum* may refer to mortality among wild trout populations as well but losses caused by 1-2 worms in the heart cannot be seen when using the model by Anderson & Gordon (1982).

Reports on a few other cases were found where *D. dendriticum* (or species closely resembling *D. dendriticum*) were shown to kill salmonids at fish farms (Hoffman & Dunbar 1961, Berland 1987, Sharp 1991) and salmonids (Duguid & Sheppard 1944, Hickey & Harris 1947, Fraser 1960) and vendace (*Coregonus albula* L.) (Bylund 1972) in the natural environment during summer. Infection of *D. dendriticum* in the heart was only observed by Hoffman & Dunbar (1961) in brook trout (*Salvelinus fontinalis* Mitchill) and by Bylund (1972) in vendace. Except for these reports on heart infection, the pathogenicity of *D. dendriticum* has been related to heavy infections and high summer temperatures. Hickey & Harris (1947) studied the activity of free and encapsulated *D. dendriticum* plerocercoids placed in physiological saline and found an increasing trend of activity with increasing temperature.

According to Goater & Holmes (1997), any organism that uses the tissues or food reserves of another organism may have the potential to cause some negative effects. However, the degree of that depends strongly on the number of parasites present and/or the ecological context. Systematic studies on circumstances where *D. dendriticum* have negative effects on their host fish have not been done, although this kind of information could help us estimate the impact of this common parasite on fish populations.

1.3 Questions asked

The unusual case at Muonio Fish Farm in 1991 and 1992, where a few parasites were found to invade the heart of the fish and cause severe mortality among brown trout and sea trout fry at warm temperatures, raised the question of whether this kind of phenomenon is common in the natural environment and under what circumstances it may happen. The aim of this study was to systematically clarify the relationship between *D. dendriticum* and trout.

The questions asked pertained to the:

1) occurrence of *D. dendriticum* in the heart region of brown trout and sea trout;
2) effects of water temperature on the size and migration activity of *D. dendriticum* in brown trout;
3) trout response to the harmful effects of *D. dendriticum* infection; the response factors studied were the establishment of the intubated procercoids and the blood leucocyte response, feed intake, growth and mortality.

On the basis of former observations (e.g. I, Hickey & Harris 1947, Bylund 1972, Hoffman & Dunbar 1961) it was predicted that the size and migration activity of *D. dendriticum* plerocercoids will be enhanced along with water temperature. Consequently, it was expected that the occurrence of plerocercoids in the heart of fish increases in warm water, leading to increased *D. dendriticum* induced mortalities. Conversely, it is known that the fish defends itself against worms with an inflammatory response, which is known to be temperature related as well (e.g Finn & Nielsen 1971a), so it was expected that blood lymphocyte counts would be enhanced in warmer water. Two alternative hypotheses were tested regarding the effect of *D. dendriticum* on fish growth: a) brown trout are unable to compensate for any negative effects of *D. dendriticum* infection and this will result in poorer growth amongst exposed fish; b) brown trout are able to compensate for the negative effects induced by *D. dendriticum* infection, so that there are no differences in growth between exposed and control fish.

2 MATERIALS AND METHODS

Dead sea trout and brown trout aged 0+ - 2+ were collected from the Muonio Fish Farm in north-western Finland, from 1991-1993 (I). Live fish from the farm were used as control fish. In most cases only the heart of the fish was studied for the presence of *D. dendriticum*. Mortality of fish and water temperature was monitored throughout the year. Plankton samples were taken from inlet water from May to August 1993 to study the source of the infection. During the peak of mortality in 1991 and 1992, the normal autopsy and examination of the presence of ectoparasites on the gills and skin, and bacteriological and virological examinations were carried out according to Midtlyng *et al.* (1992). For histopathology samples of gill, skin, heart, liver, anterior and posterior kidney, spleen and pyloric caeca from moribund fish were fixed in neutral buffered 10% formalin, embedded in paraffin and stained with haematoxylin and eosin.

Stocked brown trout were collected for larval cestode analysis in Lake Inari, a large regulated lake in Finnish Lapland, in 1994 and 1995 (II). All organs were studied and the level of infection as prevalence and abundance of infection was monitored in relation to the age of the fish. A normal pathoanatomical necropsy procedure was performed on six heavily infected brown trout. Larval cestodes were also examined from the visceral organs of potential prey fishes of brown trout: small whitefish, nine-spined stickleback (*Pungitius pungitius* (L.)) and vendace.

For experimental studies (experiment nos. 1-4, III, IV, V, VI) *D. dendriticum* eggs were produced in golden hamsters (*Mesocricetus auratus*) intubated about 11 days earlier with plerocercoids obtained from brown trout in Lake Inari, northern Finland. Eggs were incubated in dark, aerated bottles at room temperature for 10 days and after that stored in the dark at 4°C. Hatching of mature eggs took place immediately when eggs were exposed to light. Hatched coracidia were poured into an aquarium containing a laboratory culture of *Cyclops strenuus* in copepodite stages III to V.

An exposed copepod culture was kept at a temperature of 14-15°C. About three weeks post infection (p.i.) a few dozen to 100 copepods were studied and the level of infection was estimated. Anaesthetized brown trout (MS-222) were intubated with a known copepod dose into their stomach so that every exposed fish within an experiment received approximately the same amount of infective procercoids (III, IV, V, VI). CO_2 was used to anaesthetize the copepods for counting. In the first experiment (III) the intubated copepods were diluted in a drop of water but later on (IV, V, VI) a drop of 0.3% (weight/volume) pepsin in physiological saline (0.9%), pH 2, was used. Control fish received a drop of 0.3% pepsin solution. The brown trout aged 0+ - 1+ originated from a fish farm in northern Finland (Lake Inari stock) (III, IV, V) and from a farm in central Finland (Rautalampi stock) (VI). All fish were bathed with 1:4000 formalin for 20 min before the exposure (IV, V, VI) or during the experiment (III).

D. dendriticum was studied from fresh fish during and at the end of the experiment when the surviving fish were killed. In the first experiment (III) the inner organs, heart and muscle tissues were compressed between glass plates (8x20 cm) and were examined at 10-20 x magnification using transmitted light. In experiments 2, 3 and 4 (IV, V, VI) 0.5% (w/v) pepsin solution in physiological saline (0.9%), pH 2 was also used to remove the larvae from fish tissues. The solution was sieved and studied at 10-20 x magnification using transmitted light. The worms found were relaxed in tap water in a refrigerator overnight and measured.

The factors studied in each experiment were the prevalence, and mean intensity (or abundance) of *D. dendriticum* infection. The term *prevalence* refers to the proportion of fish individuals infected with *D. dendriticum*, while the term *mean intensity* (intensity in some tables) indicates the mean number of *D. dendriticum* individuals per infected fish in a sample, and the term *abundance* refers to the mean number of individuals of *D. dendriticum* per fish examined (Margolis *et al.* 1982). The size and location of the larvae as well as fish mortality were also studied. In addition, blood samples were taken in experiment no. 2 from the caudal vessels of fish to study the effect of temperature and *D. dendriticum* infection on the circulatory leucocytes (lymphocytes, neutrophils, thrombocytes) (V). For total leucocyte counts, blood was diluted 1:50 (volume/volume) in Shaw´s solution (Shaw 1930) and counted using a Neubauer haemocytometer. Differential counts (150 leucocytes/smear) were made from air-dried, methanol-fixed and stained (May-Grünwald-Giemsa) blood smears. Absolute lymphocyte, neutrophil and thrombocyte concentrations were calculated from the total and differential blood cell counts.

To study the impact of *D. dendriticum* on the feed intake and growth of brown trout (VI) the fish were individually tagged by injecting a PIT tag (Trovan) into the body cavity. Feed intake was measured using an X-radiographic technique (Talbot & Higgins 1983, Jobling *et al.* 1993). Diets used for feed intake measurements were prepared from the normal feed by grinding, homogenization and incorporation of known quantities of X-ray dense ballotini (size 8.5; Jencons Ltd Leighton Buzzard, UK.). Samples of diets were X-rayed

and standard curves for the relationships between the number of ballotini (X) and diet weights (g) calculated: diet g $=0.059+0.014\times X$, $R^2=0.87$, $P<0.001$, $N=8$.

Feed intake measurements were made by providing the ballotini marked feed during a four-hour feeding period (08.00-12.00), followed immediately by anaesthetizing the fish (MS-222), X-raying (Kostix 30 X-ray machine; Kodak X-OMAT MA film), weighing to the nearest 0.1g and identification of individuals by reading the PIT tag. X-ray plates were then developed and the mean amount of feed consumed by the fish in the tank was estimated.

Growth rates (SGR) in terms of weight were calculated according to the formula: $SGR = [(\ln X_2 - \ln X_1)/t] \times 100$, where X_1 is the weight of the fish at the start, X_2 is the weight at the end of period and t is the duration of the period in days.

The feed:gain ratio was calculated dividing the amount of food consumed (feed intake) by wet weight gain.

Statistical analyses were performed using SYSTAT statistical software (SYSTAT 1992, 1996). The χ^2 and G^2 tests were used when comparing the frequency data and the nonparametric Kruskal-Wallis test for abundance and mean intensity data. For parametric data, possible differences among treatments were tested using either a nested ANOVA model in cases in which individual responses had been measured or ANOVA when group responses were examined (Sokal & Rohlf 1981). Homogeneity of variances was examined using Cochran's test (Day & Quinn 1989), and the Lilliefors' method was used to test for normality. The Tukey-Kramer and Fischer's LSD tests were used to make *post-hoc* comparisons between sample means, and Spearman's test was used when correlations were tested. $P < 0.05$ was taken as the level of significance. The power of the F test was calculated according to Lindman (1992).

3 RESULTS AND DISCUSSION

3.1 Occurrence of *D. dendriticum* in the heart region of brown trout and sea trout

At the Muonio Fish Farm in northern Finland, the prevalence of the intracardial *D. dendriticum* infection in sea trout and brown trout aged 1+ - 3+ found dead varied from 73 to 86 % and 63 to 86% in July 1991 and June-September 1992, respectively. The average number of larvae per infected heart varied between 1.1 and 1.5, with a maximum of 6 worms in one heart (I). In random samples taken from fish caught live in the same tanks in 1991 and 1992, the prevalence of heart infection varied from 10 to 39% and 0 to 13%, respectively, with one to two worms per infected heart. In addition, among the fish studied before stocking at the same farm in March, 1993, one-year-old fingerlings were not infected, while 19% of the two-year-old sea trout and 4% of the brown trout harboured one larva in the heart of each infected fish (I). Trout aged 0+ were never found to be infected. It was proposed that the warmer water temperature in 1991 promoted the migration of *D. dendriticum* into the heart compared to 1992 (I) (see also section 3.3.4.).

Brown trout studied in Lake Inari in 1994 and 1995 harboured 1-2 *D. dendriticum* larvae, mostly unencapsulated in the atrium of the heart in about 13% of the fish aged 5+ in both years and in 5% of the 6+ and older trout in 1995 (II). The fish had been stocked as three-year-olds in the spring. Some encapsulated larvae were found in the pericardium in 3-20% of the 4+ and older trout but *D. dendriticum* was not found in the heart region after the first summer in the lake (age 3+) (II).

D. dendriticum was found to penetrate the heart of experimentally infected brown trou aged 0+-1+ as well (III, IV) (Table 1). Up to 20% of the exposed brown trout harboured a plerocercoid in the heart region (including pericardium) in experiment no. 3 where water temperature was raised gradually to close to the lethal level of brown trout (27-28°C) (IV). The maximum

prevalence of *D. dendriticum* in the atrium of the heart (7.5%) was also obtained in experiment no. 3 (IV). Plerocercoids were not found inside the ventricle or bulbus arteriosus. The proportion of brown trout with *D. dendriticum* in the heart region increased slightly along with the temperature (Table 1), which is obviously at least partly connected to the increased migration activity of plerocercoids in warmer water (see 3.2.1, Hickey & Harris 1947) (Table 1). Another contributing factor may be the clearly bigger procercoid dose in experiment no. 3 (IV) compared to other experiments.

TABLE 1 Proportion of the heart-infected fish with *D. dendriticum* plerocercoids and the mean intensity of worms per site in three experiments (III, IV).

					Atrium		Pericardium		Atrium+peric.	
	No. of		Procerc.	Weeks	Prevalence		Prevalence		Prevalence	
	fish	°C	dose	p.i.	%	Intensity	%	Intensity	%	Intensity
Exp. 1(III)	89	11-12	3-15	8.5	0.0	0.0	3.4	1.0	3.4	1.0
Exp. 2(IV)	68	11->7.5	8	12	2.9	1.0	4.4	1.0	7.4	1.0
	68	14-15	8	12	2.9	1.0	8.8	1.2	8.8	1.5
Exp. 3(IV)	55	11->28	18-20	8	7.5	1.0	14.5	1.0	20.0	1.1

The present studies indicate that it is not uncommon for *D. dendriticum* to penetrate the heart region of fish. *D. dendriticum* occurred in the heart atrium in around 8% of the fish in a single exposure in an experiment with warm water (IV) and in 5 to 13% of brown trout harbouring a strong natural infection in Lake Inari (II). However, warm water temperature alone did not generate such high *D. dendriticum* prevalences in the heart as at Muonio, especially in 1991 (I). Although it was possible to obtain experimentally some indications concerning the phenomenon that caused mortality at a fish farm, it became obvious that the interaction of various factors at farms and in the natural environment are difficult to mimic and generate in experiments. Further studies with *D. dendriticum* originating from the Muonio farm could not be carried out since the infection decreased dramatically after the rebuilding of the farm in 1993 when all the tanks were situated indoors. In autumn 1995, a total of 136 sea trout aged 1+ were again studied from the farm and a single small-sized *D. dendriticum* was found in only three of them, with two fish harbouring a plerocercoid in the heart. These plerocercoids did not, however, mature in golden hamsters. Apparently they were not infective at the time of collection. Moreover, 150 sea trout aged 2+ were studied in the following autumn but no worms were found.

Heart infections with *Diphyllobothrium* were only previously reported in reared brook trout from Canada(Hoffman & Dunbar 1961), in vendace from a lake in Finland (Bylund 1972) and recently from a rainbow trout (*Oncorhynchus mykiss* (Walbaum)) net-cage farm at a lake in Canada (D. Groman, pers.comm.). These cases were noted because *D. dendriticum* in the heart caused heavy mortalities. The reason for such few reports might be that heart has not been studied separately for infection. This detail has not been mentioned in most of the previous papers on *D. dendriticum*.

3.2 The effect of water temperature on the migration and size of *D. dendriticum* in brown trout

3.2.1 Migration activity

The activity of plerocercoids in different temperatures was studied only by Hickey & Harris (1947) with free and encapsulated plerocercoids placed in physiological saline. They showed that free plerocercoids were non-motile at 10°C, sluggish at 12°C and active at 14°C. In the present experimental studies the activity of *D. dendriticum* was measured as a proportion of the plerocercoids which migrate outside the body cavity to the heart, pericardium and muscle (Table 2).

TABLE 2 The proportion and mean intensity of plerocercoids outside the body cavity (in the heart atrium, pericardium and muscle) at the end of the experiment. Fish that died during the experiment are not included (III, IV).

	No. of			Outside the body cavity	
	worms	°C	Weeks p.i.	%	mean intensity
Exp. 1 (III)	58	11-12	8.5	10.3	1
Exp. 2 (IV)	70	11->7.5	12	14.3	1.3
	58	14-15	12	31.0	1.5

In experiment no. 2 (IV) the proportion of plerocercoids that migrated outside the body cavity was statistically significantly greater in heated (31%) compared to non-heated (14.3%) water, indicating that the migration activity truly increased along with the temperatures used.

3.2.2 Size

The growth rate of *D. dendriticum* procercoids in copepods has been demonstrated to increase along with the increase in temperature (Vik 1957, Halvorsen 1966), but whether this is also true for plerocercoids in fish has not been studied. In this study it was expected that the development of plerocercoids is temperature dependent as well, and experiment no. 2 (IV) (see Table 3) clearly showed that *D. dendriticum* grow faster in warmer water. In addition, the mean length of the plerocercoids was about the same 8 weeks p.i. in experiment no. 3 (IV) as in non-heated aquaria (about 10°C) 12 weeks p.i. (no. 2, IV) (Table 3).

TABLE 3 Mean length of *D. dendriticum* plerocercoids in brown trout in experiments of different duration and water temperature (III, IV, VI).

	Duration		No. of	Length mm	
	weeks	°C	worms	mean	min-max
Exp. 1 (III)	8.5	11-12	52	9.1	1.5-16
Exp. 3 (IV)	8	11->28	104	10.7	2.0-22.0
Exp. 4 (VI)	10.5	10-12	142	10.1	4.0-19.0
Exp. 2 (IV)	12	11->7.5	66	10.2	4.0-21.0
Exp. 2 (IV)	12	14-15	52	22.1	11.5-44.0

3.3 Trout responses to the harmful effects of *D. dendriticum* infection

3.3.1 Establishment of procercoids

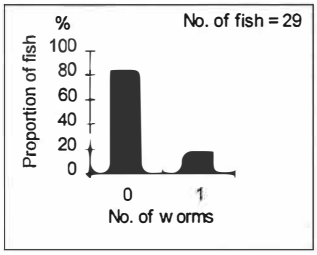
In natural habitats the generative mechanisms of overdispersion are many and varied (Crofton 1971a). Two of the most important are heterogeneity in host susceptibility to infection, and variability in exposure to infection. Anderson *et al.* (1978) found in their experiments that overdispersion of *Transversotrema patialense* (Trematoda) in a fish host (*Brachydanio rerio* (H.-B.)) increased with exposure density and time. A stochastic simulation model was used to demonstrate that small differences between the hosts in susceptibility to infection is the probable cause of such patterns (Anderson *et al.* 1978).

In all of the present experiments (III, IV, VI) approximately the same amount of infective procercoids (with cercomer) were intubated into the fish stomachs, thus minimizing the variability in exposure to infection per each experiment (see Crofton 1971a). As in the results of Anderson *et al.* (1978) the frequency distributions of *D. dendriticum* in fish went from underdispersed (var<mean) to overdispersed (var>mean) with the increasing procercoid dose (Fig. 2). The present results confirm the ideas of Anderson *et al.* (1978): differences in host susceptibility to *D. dendriticum* infection are the most important causes of aggregation. As a consequence, it is suggested that it is not possible to generate experimentally high and even *D. dendriticum* infection in fish, although it would be an advantage in many experiments.

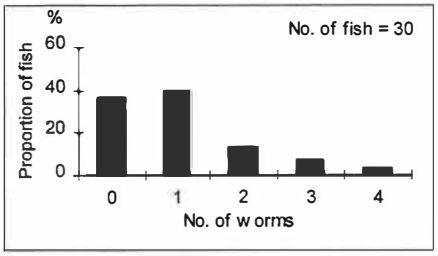
Parasites may induce selection pressure among the host population by reducing the fitness of the host. Freeland (1986) has concluded that new host defences develop against those parasites which are common and have an intermediate to high level of virulence. These requirements are met in the case of *D. dendriticum,* so it is obvious that resistance among brown trout has evolved, leading to individual variation in susceptibility. Resistant host genotypes obtain selective advantage by lowering the establishment rate of the parasites in the host or by inhibiting the effects which impair the fitness of the

host (Freeland 1986). Indications of the former mechanism were obtained in relation to establishment of the plerocercoids, while the latter mechanism was observed concerning the effects of *D. dendriticum* on the feed intake, growth (see 3.3.3) and mortality (see 3.3.4) of trout.

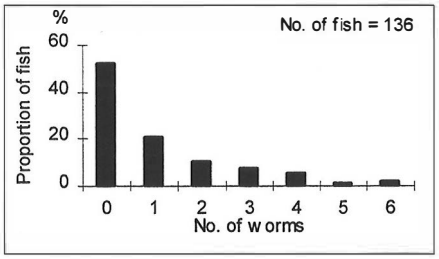
Parasite numbers should not be used as a surrogate measure of host resistance (Goater & Holmes 1997) because there are many other mechanisms as well (see Crofton 1971a) which affect the number of parasites able to become established in a host. However, as stated by Goater & Holmes (1997) too little research effort has been focused on features such as the resistance or tolerance of the host, or its ability to compensate for the damage done by parasites, when studying parasite-mediated selection in host populations. As indicated by the present results, such traits are likely to exist in trout - *D. dendriticum* associations.



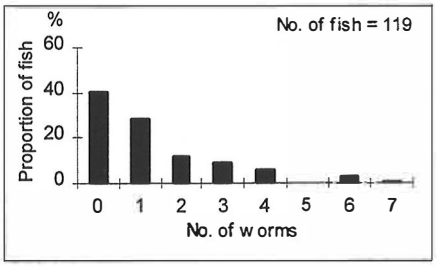
Experiment 1 (III)
Dose: 3 procercoids (water)
Prevalence: 17.2%
Abundance: 0.17
Var-to-mean: 0.88



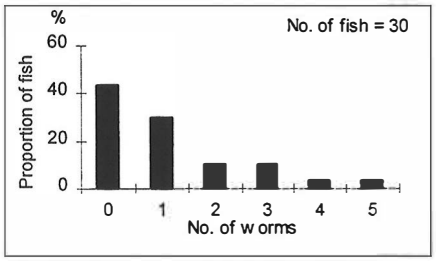
Experiment 1 (III)
Dose: 7 procercoids (water)
Prevalence: 63.3%
Abundance: 1.0
Var-to-mean: 1.1



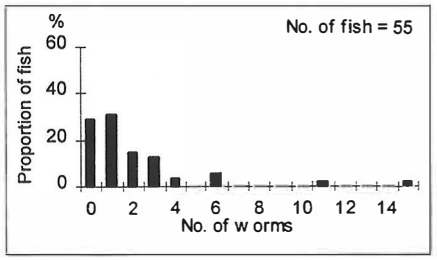
Experiment 2 (IV)
Dose: 8 procercoids (pepsin)
Prevalence: 47.8%
Abundance: 1.1
Var-to-mean: 2.0



Experiment 4 (VI)
Dose: 10 procercoids (pepsin)
Prevalence: 59.7%
Abundance: 1.3
Var-to-mean: 1.9



Experiment 1 (III)
Dose: 15 procercoids (water)
Prevalence: 56.7%
Abundance: 1.1
Var-to-mean: 1.6



Experiment 3 (IV)
Dose: 18-20 procercoids (pepsin)
Prevalence: 70.9%
Abundance: 1.9
Var-to-mean: 3.8

FIGURE 2 Distribution of *D. dendriticum* plerocercoids in brown trout intubated with different numbers of procercoids, diluted in water or pepsin-HCl solution.

3.3.2 Blood leucocyte response

Fish hosts have been shown to react to *D. dendriticum* infection with humoral and cellular responses resulting in the encapsulation of the plerocercoids within about two months (e.g. Sharp *et al.* 1989, 1992). A strong infection may lead to severe chronic granulomatous peritonitis, which was found to occur among the heavily infected brown trout in Lake Inari (II), for example. A cellular response by the host fish with leucocyte infiltration has been demonstrated against other helminths as well: e.g. *Triaenophorus crassus* Forel (Rosen & Dick 1984a,b), *Ligula intestinalis* (L.) (Hoole & Arme 1986), *Diphyllobothrium ditremum* (Rodger 1991) and *Raphidascaris acus* (L.) (Valtonen *et al.* 1994).

Information about the defence reactions of brown trout against *D. dendriticum* in different temperatures was considered important when studying the circumstances under which this parasite might be fatal to fish. In the present experiment (2.), circulatory leucocytes were studied to determine the effect of infection and water temperature on defence reactions against *D. dendriticum* (V). It has been shown that blood leucocytes reflect both the tissue damage (Finn & Nielsen 1971b) and antibody synthesis of fish (Rahkonen *et al.* 1996). It is also known that inflammation responses of fish (Finn & Nielsen 1971a) increase along with temperature.

In the present study, a clear increase was observed in lymphocyte and neutrophil counts in the peripheral blood of the brown trout infected with *D. dendriticum* plerocercoids at both temperature levels (heated 15°C and non-heated about 10°C) 12 weeks p.i. (V). On the other hand, the number of thrombocytes was lower in infected fish, particularly in non-heated aquaria. As a consequence there was no significant difference in the total leucocyte counts between infected and control fish (V). High blood lymphocyte counts in the infected fish were most probably an indication of antibody synthesis in brown trout.

Against expectations, the present two temperature levels did not create differences in the number of lymphocytes and neutrophils (V). Both temperature levels fall in the "optimum temperature range" for brown trout (4-19°C) (Elliot 1981). In addition, immune responses (antibodies and lymphocyte counts in blood) against *Aeromonas salmonicida* -bacteria, for example, have been shown to develop in brown trout at low temperatures (≤7°C) as well (e.g. Rahkonen *et al.* 1996). Thus, it is likely that the present temperatures were too similar to create real differences in lymphocyte and neutrophil numbers. Further immunological studies, including blood leucocytes, are needed using larger temperature variation and repetitive measurements.

In the same experiment (2.), however, the mean size of the plerocercoids doubled in heated compared to non-heated water, and they migrated more actively out of the body cavity (into the heart region and muscles) in the heated water (IV). Thus, there might exist certain occasions where the prevailing temperature increases the growth and activity of plerocercoids at a rate greater than the defence reactions of the host can respond to. No increased mortality was detected in the present experiments to indicate this threshold temperature,

but the results concerning the size and activity of *D. dendriticum* in contrast to the blood leucocyte response in fish in experiment 2 (IV, V) indicate that the probable threshold temperature might be around 15°C. This is in accordance with the experiences at the Muonio Fish Farm in the early 1990s (I).

3.3.3 Effect of *D. dendriticum* on the growth and feed intake of brown trout and sea trout

As presented in the Introduction, any organism that uses the tissues or food reserves of another organism has the potential to cause some negative effects (Goater & Holmes 1997). A parasitized host may undergo a nutrient deficit by one or more of the following four mechanisms (Holmes & Zohar 1990) caused by parasites: a) direct or indirect nutritional drains (competition with the host for energy or nutrients; damage to host tissues, thereby stimulating costly repair responses; or otherwise stimulation of energy and nutrient-requiring host defensive responses); b) altering gastrointestinal functions such as absorption or gut motility, thereby affecting the assimilation efficiency of the affected host; c) eating less (exhibit anorexia); d) an impaired acquisition and delivery of oxygen to the tissues caused by parasites found in pulmonary tissues or in the circulatory system, or that feed extensively on blood cells. The first mechanism can be thought to be the most important one concerning *D. dendriticum* infection where larvae encapsulate inside the body cavity, but the fourth mechanism may take place when a larva/larvae penetrates the heart.

One of the most frequently reported symptoms of parasitic infection is a depletion of energy stores (e.g. Walkey and Meakins 1970, Lemly & Esch 1984). Host animals may attempt to offset such depletion by increasing feeding activity at the expense of predator avoidance (Milinski 1985, Giles 1987). A negative effect of parasites on the size or condition of fish has been demonstrated in cases where the parasites had damaged the skin (Lemly & Esch 1984, Singhal *et al.* 1990, Urawa & Yamao 1992, Urawa 1995) or liver (Szalai & Dick 1991) of their host. In addition, reduced growth of salmonids has been attributed to *Eubothrium* sp. (gut tapeworm) infections (Bristow & Berland 1991) However, the growth effects of *D. dendriticum* on its host fish have not been studied.

Indications of the effect of *D. dendriticum* on the growth and condition of trout were obtained from the Muonio Fish Farm (VI), Lake Inari (II) and experimentally (VI). At Muonio, among 161 two-year-old sea trout examined for *D. dendriticum* before stocking in June 1993, the heart-infected fish were statistically significantly shorter than the healthy-hearted fish (Rahkonen, unpubl., see VI). On the other hand, in Lake Inari, the condition factor of brown trout seemed to increase along with the number of *D. dendriticum* plerocercoids, indicating that the more the fish eats, the more larvae it accumulates (II).

In an experiment on individually marked brown trout (VI), *D. dendriticum* infection did not have any negative effects on feed intake and growth. On the contrary, the mean feed intake, calculated for the 4 post-exposure measurement days, was 23% higher in exposed (3.8 g kg^{-1}, SD 0.4) than in control groups (2.9 g kg^{-1}, SD 0.8). The mean daily growth rate (SGR) in exposed groups (0.57%, SD

0.33) was also slightly better than amongst control groups (0.48%, SD 0.36). Moreover, there was a trend towards a somewhat higher feed:gain ratio amongst the brown trout that had been exposed to *D. dendriticum* (0.64, SD 0.07) compared to controls (0.58, SD 0.14), indicating feed conversion efficiency to be poorer amongst the exposed fish. The clearest difference between these three parameters was found in the exposed-uninfected fish and the controls. None of the obtained difference was statistically significant, however. The problem that emerged was the low power of the tests to reveal any significant differences. Consequently, in further experiments the number of fish should be increased and the initial weight of the fish should be as uniform as possible in order to minimize the background variation in their growth and feed intake.

The trend towards differences in feed:gain ratios between treatment groups suggests that there might be differences in metabolic energy demands between the exposed and control fish. It is possible that the growth of plerocercoids, and especially the defence processes activated by *D. dendriticum* infection, increase the energy demands of the fish host leading to increased feeding as a compensatory mechanism. The cost of mounting an immune response has not been measured, but Holmes & Zohar (1990) assume it to be high. Walkey & Meakins (1970) suggested that when fed *ad libitum,* sticklebacks bearing *Schistocephalus solidus* (Müller) plerocercoids ingest considerably more food than do uninfected fish but, on the other hand, during starvation the mortality of infected sticklebacks was clearly higher compared to uninfected fish. Moreover, sticklebacks infected with *S. solidus* are shown to take greater risks than non-infected individuals when foraging (Milinski 1985, Giles 1987, Godin & Sproul 1988). According to Giles (1987), seventy-two hours without food is sufficient to suppress the fright response in parasitized fish, and causes them to forage at the same rate as when undisturbed. Non-infected controls failed to forage successfully after a frightening stimulus, even having been without food for 96h.

Thus, it seems that the feed intake and growth of brown trout are mostly well adapted to low-level *D. dendriticum* infection, at least when the food supply is not restricted. This result is in accordance with the suggestion presented by Holmes & Zohar (1990), that when adequate nutrient supplies are available, minor damage or reductions in the share of the nutrients that go to the host can probably be compensated for by increased feeding. When increased feeding can occur without increased exposure to predators, the resulting loss in fitness may be negligible.

Assuming that there is a greater energy demand imposed upon *D. dendriticum*-infected fish, negative effects could be expected with either a poor food supply or a heavier infection, but this would need to be verified by further studies. These criteria both occurred in Lake Inari (II), though the negative effects of *D. dendriticum* on the brown trout condition could not be demonstrated. On the other hand, the larvae and their capsules increase the weight of the fish but unfortunately, the gutted weight was not measured to study this. The only negative growth effect was obtained concerning heart-infected sea trout at the Muonio Fish Farm, but the cause of this phenomenon

remains unclear. However, the cost of heart damage in fish may well be more severe than inflammations and encapsulation in the body cavity. The acquisition and delivery of oxygen to the tissues may be impaired, which is one of the mechanisms causing nutrient deficiency (Holmes & Zohar 1990).

On the whole, the growth effects of parasites upon hosts are likely to be a complex function of the amount of damage done and the ability of the host to compensate for the damage. However, the methods applied in experiment no. 4 (VI) would seem to have the potential for the examination of the energetics of fish-parasite relationships.

3.3.4 *D. dendriticum* induced mortality

3.3.4.1 Direct *D. dendriticum*-caused mortality

A clear mortality peak occurred at the Muonio Fish Farm when around 13% and 18% of brown trout and sea trout aged 2+ died in July 1991, respectively (I). Sudden deaths were observed in particular when fish were disturbed. Fish began to show violent swimming movements and died within a few minutes. More than 80% of the dead fish harboured *D. dendriticum* plerocercoids inside their heart atrium. In 1992, a mortality peak was not observed but increased mortality, ≤ 6% per month, took place in the sea trout aged 1+ and 2+ during June-September and 83% and 63% of them were heart-infected by *D. dendriticum*, respectively (I).

The high prevalence of intracardial infection of dead fish by *D. dendriticum* shows that mortality and infection are strongly associated with each other. The symptoms seen in dying fish and the pathoanatomical lesions in the infected heart suggest that *D. dendriticum* was the direct cause of death in a large proportion of the dead fish. On the basis of these facts and the absence of other pathogens it is concluded that *D. dendriticum* can cause considerable mortality in sea trout and brown trout in their second and especially their third summer, at least under farming conditions (I). The surprising phenomenon in the Muonio case was that in 90% of the fish where *D. dendriticum* had penetrated the heart, larvae were not found elsewhere. Only about 10% of the heart-infected fish had 1-2 *Diphyllobothrium* larvae encapsulated on the digestive tract so the general level of infection was low and most of the worms seemed to migrate directly into the heart (I).

In contrast to experiments by Kuhlow (1953) and Bylund (1972) mortality among infected fish in the present experiments (III, IV, VI) was generally low (Table 4). Contrary to expectations, the increased activity and larger size of the plerocercoids in warm water did not cause *D. dendriticum*-induced mortality under the present experimental conditions (IV). These results indicate that juvenile brown trout are able to tolerate for at least a few months a moderate number of *D. dendriticum* plerocercoids (<15) under various stress factors: protozoa infection (III), gas bubble disease (IV), heat stress (IV) and handling (VI). The death of individual brown trout due to *D. dendriticum* larvae in these

experiments, or some contribution of larvae to the mortality of fish suffering from other stress factors, cannot be totally excluded, however.

TABLE 4 Observed mortality of brown trout in the experiments (III, IV, VI).

	No. of fish	°C	Mortality %	No. of inf. dead fish	No. of worms	Main cause of mortality
Exp. 1(III)	89	11-12	6.7	4/6	2, 3, 3, 5	*Ichthyobodo necator*
Exp. 2(IV)	68	11->7.5	2.9	1/2	3	gas bubble disease
	68	14-15	11.7	6/8	1,1,1,2,4,6	gas bubble disease
	34*	11->7.5	0.00	0/0		gas bubble disease
	34*	14-15	20.6	0/7		gas bubble disease
Exp. 4(VI)	120	10-12	0.8	-		?
	120*	10-12	0.8	-		?
Exp. 3(IV)	55	11->28	14.5	6/8	1,2,2,3,4,6	heat
	58**	11->28	75.9	4/44	1,1,2,6	heat

*uninfected control fish

**four control fish harboured natural infection

The models of Anderson & May (1979) are based on the assumption that the parasite increases the rate of host mortalities and that mortality increases with the number of parasites per host. Our results are in accordance with Henricson's (1978) conclusions that in the case of *D. dendriticum* mortality not only correlates with the number of parasites but also with their location in the fish: a fish is able to survive with hundreds of larvae on their body cavity organs (II) but a single larva inside the heart atrium may be fatal (I). Thus it is complicated to determine the "lethal level" of *D. dendriticum* (see Crofton 1971a). However, Henricson (1978) concluded using Crofton's (1971a) model and Lopukina's *et al.* (1973) formula that Arctic char infected with only 4 to 10 *D. dendriticum* run an increasingly greater risk of death.

On the whole, we were not able to create such conditions where *D. dendriticum* migrates generally into the heart of brown trout and causes provable mortality, as was the case at the Muonio Fish Farm (I). The fish host seemed to be well buffered against the harmful effects of *D. dendriticum*. The widespread occurrence of *D. dendriticum* and also its ability to develop occasional strong infections (e.g. Vik 1957, Halvorsen 1970, Henricson 1977, 1978, Kennedy 1978, Wootten & Smith 1979, Curtis 1983, Ching 1988, Valtonen *et al.* 1988, Andersen & Valtonen 1992, Hartvigsen & Halvorsen 1993, Hartvigsen & Kennedy 1993) suggest that the *D. dendriticum*-brown trout association has reached a low degree of pathogenicity during their long co-evolution. This conclusion is in accordance with Freeland's (1986) suggestion that the host genotypes which are able to develop resistance to certain common parasites of intermediate to high virulence are likely to gain selective advantage. Freeland (1986) suggests that a host may reduce the fitness depressing effects of an infestation by limiting, a) the quantity and quality of its resources used by parasites; b) the cost of tissue

damage; c) the cost of any defence, and d) the cost of damage caused by a defence mechanism. There are indications in the present studies that all these features might be valid in the *D. dendriticum*-trout relationship, but as already suggested in 3.3.1, more research effort should be invested in the evolutionary ecology of fish-parasite relations to be able to understand the mechanisms more precisely.

The case at the Muonio Fish Farm (I) and a few other observations (Duguid & Sheppard 1944, Hickey & Harris 1947, Fraser 1960, Hoffman & Dunbar 1961, Bylund 1972) indicate, however, that the balance between *D. dendriticum* and fish may collapse in certain circumstances though this equilibrium did not break down in the present experimental conditions.

Although the heart-infected fish were not particularly poor survivors in the present experiments (III, IV), it is suggested, on the basis of our findings at Muonio (I), that fish with *D. dendriticum* plerocercoids inside the heart atrium will probably be eliminated sooner or later in the natural environment. The growth of plerocercoids over the years in fish (I) and the pathological reactions in the infected heart (I) support this opinion. In the present experiments a single dose of 10-20 procercoids resulted in up to 8% of fish having *D. dendriticum* inside the heart (Table 1). Fish are exposed to new infections in lakes every summer and, for instance, in Lake Inari 13% of brown trout aged 5+ harboured this cestode in the atrium. Our studies indicate that a small portion (maybe 5-10%) of the trout population will be lost due to *D. dendriticum* heart infection annually, at least in lakes with strong *D. dendriticum* infection.

3.3.4.2 Indirect evidence of *D. dendriticum* induced mortality

Indirect evidence of the mortality of heavily parasitized hosts in the wild is considered to be a decrease in the degree of overdispersion within the older age classes of the hosts, concomitant with a decline in abundance (Anderson & Gordon 1982). According to Goater & Holmes (1997) a crucial part of any study on parasite-mediated natural selection must be to show that variation in parasite numbers leads to variation in host fitness.

The abundance of *D. dendriticum* infection in brown trout increased significantly with age in Lake Inari in 1994 and 1995 and the distributions were overdispersed (variance>mean) (Table 5) (II). The variance-to-mean ratio dropped in the oldest age group in 1994 while it increased in the same age group in 1995 indicating that the most heavily parasitized individuals are not eliminated efficiently from the brown trout population.

TABLE 5 The occurrence of *D. dendriticum* plerocercoids in brown trout in Lake Inari in 1994 and 1995 (II).

	1994					1995				
	no. of	prevalence	abundance			no. of	prevalence	abundance		
age	fish	%	mean	SD	var/mean	fish	%	mean	SD	var/mean
3+	12	75	5.3	5.0	4.7	13	46.1	0.9	1.4	2.3
4+	45	84	17.2	21.7	27.4	29	79.3	7.4	14.5	28.4
5+	29	100	86.9	83.1	79.6	24	91.7	53.2	57.9	63.0
>=6+	15	100	128.7	72.8	41.1	22	100	132.3	113.6	97.5

On the other hand, Anderson & Gordon (1982) used Henricson's (1977, 1978) data to suggest that the observed decrease in the abundance and the variance-to-mean ratios of *D. dendriticum* and *D. ditremum* in the oldest age groups in Arctic char was a result of the death of the most heavily parasitized individuals. However, the decrease did not occur until the ages of 9+ and 10+, so it is possible that the same phenomenon occurs in brown trout in Lake Inari, but that too few older fish were captured to make any interpretations. For example, Halvorsen & Andersen (1984) have also concluded that *D. ditremum* induces density-dependent mortality among Arctic char, but Pacala & Dobson (1988) have re-analysed their data and indicated that the decrease in variance-to-mean ratio among age-classes 8-13 was probably caused by a small number of fish in these oldest age groups.

As in Lake Inari (II) the cause of a heavy *D. dendriticum* infection in northern lakes is obviously connected to overcrowding and the poor nutritional condition of the brown trout/char stock (see also Vik 1957, Bylund 1966, Curtis 1983). Under these circumstances it is very difficult to prove the contribution of *D. dendriticum* to the regulation of the fish population. Regulation processes are likely to be more indirect than in the case of heart infections, such as impairing the general condition of fish so that they do not survive harsh conditions like winter (see e.g. Henricson 1978) or reducing their reproduction capacity (see e.g. Vik 1957). Holmes & Zohar (1990) stated as well, that because of differences in the compensatory ability of fish, a given number of parasites may have a considerably weaker effect on a host in good condition vs. poor. Hickey & Harris (1947) made observations on the feeding habits of gulls (*Larus* spp.), which are known to be the most important definitive hosts for *D. dendriticum* (see e.g. Vik 1957, Andersen *et al.* 1987). They never observed a gull attacking a trout unless it was moribund or dead. However, changes in behaviour which cause the infected fish to become more susceptible to predators, including fish-eating birds, needs experimental verification.

3.4 General remarks on heavy natural *D. dendriticum* infections

3.4.1 Causes of heavy infection

No detailed study has been undertaken on the factors which lead to the heavy infection of brown trout and Arctic char in some northern lakes with either *D. dendriticum* or *D. ditremum* (Vik 1957, Halvorsen 1970, Bylund 1972, Henricson 1977, 1978, Curtis 1983, Halvorsen & Andersen 1984, Bérubé & Curtis 1986, Gustafsson 1996). Curtis (1983) compared the level of *Diphyllobothrium* infection in Arctic char in four lakes in Canada and concluded that the differences in prevalence and intensity of infection between the lakes are related to the food web structures, which are influenced by the number and composition of fish species in the lake, for instance. In the lakes inhabited only by Arctic char and sticklebacks, a significant proportion of the char population may become piscivorous and heavily parasitized with *Diphyllobothrium* by transmission from prey fish (Curtis 1983).

In the case of Lake Inari the causes of heavy infection are likely to be complicated: overcrowding due to extensive brown trout stocking, concominant with poor vendace and small whitefish stocks which serve as the most important food resource for brown trout (II). Moreover, as proposed in section 3.3.3, a heavy infection may even increase the energy demand of fish. In addition, the water level regulation destroys the littoral bottom fauna (II). All these factors might lead fish to feed more on copepod intermediate hosts. On the other hand, the poor food supply may decrease the defence mechanisms of the fish against *D. dendriticum.* Overall, there seems to be a "vicious circle" of many factors that maintains the heavy infection in Lake Inari. Heavy *D. dendriticum* infection was also observed in Lake Inari in the 1960s, about 20 years after the start of water level regulation (Bylund 1966, II).

The origin for both *D. dendriticum* plerocercoids and brown trout in the present experiments was Lake Inari. In the growth experiment (VI), however, the brown trout originated in central Finland. As concluded in section 3.3.1 most of the fish harboured only 1-3 plerocercoids regardless of the procercoid dose (max 20), so they had been able to prevent the establishment of most of the procercoids. It is clear that the development of a heavy infection like that of Lake Inari, (e.g. a mean number of 130 worms per fish, four years after stocking) requires repetitive exposure to *D. dendriticum* over several years. It is also possible that the defence capability of brown trout decreases after certain *D. dendriticum* load leading to a greater establishment rate. Some indication of dose dependent immunosuppression of the response of pronephric lymphocytes of carp (*Cyprinus carpio* L.) against the extracts of *Bothriocephalus acheilognathi* Yamaguti was obtained by Nie *et al.* 1996.

3.4.2 Hygienic aspects

One harmful consequence of particularly heavy *D. dendriticum* infection is the occurrence of larvae capsules in the muscles of fish, which decreases the commercial value of the infected fish. Worms were first found in the muscles (i.e. clearly outside the body wall) of brown trout in Lake Inari after their second summer in the lake following stocking (age 4+) (II). The occurrence of larvae in the muscles increased with the age of the fish, the prevalence being 73% (abundance 3.2) in 1994 and 95% (abundance 7.1) in 1995 at the age of ≥6+ after at least four years in the lake (II). *D. dendriticum* capsules were encountered in the lateral muscles only within the area below the lateral line and in front of the ventral fins.

A single dose of about 8 procercoids in experimental infections caused 1-2 plerocercoids to occur in the muscle in 12% of the fish at 14-15°C (IV) (Table 6). This is close to Henricson's (1977) results with around 15% of wild Arctic char harbouring muscle infection. Concerning brown trout with muscle infections in Lake Inari, the minimum number of worms on their visceral organs was 16 in 1994 and 12 in 1995. On the other hand, the maximum number of *D. dendriticum* on the visceral organs without muscle infection was 258 and 86 in 1994 and 1995, respectively (Rahkonen, unpubl.). However, on the basis of the experimental studies and findings in Lake Inari, *D. dendriticum* plerocercoids can migrate into the muscles of fish even in rather light infections.

TABLE 6 The prevalence and mean intensity of *D. dendriticum* plerocercoids in the muscle (outside the body wall) of brown trout at the end of the experimental infections (III, IV). Fish that died during the experiment are not included.

	No. of fish	°C	Weeks p.i.	Prevalence %	Mean intensity
Experiment 1(III)	83	11-12	8.5	4.8	1
Experiment 2(IV)	66	11->7.5	12	7.1	1
	60	14-15	12	11.7	1.3

Infectivity of *D. dendriticum* to humans has been demonstrated experimentally by Vik (1957), Bylund (1969) and Halvorsen (1970). *D. dendriticum* has also been reported in natural human infections in Canada (Rausch & Hilliard 1970, Ching 1984). Andersen *et al.* (1987) have concluded that at least some of the reported human infections of *D. latum* might have been *D. dendriticum*, especially if diagnosed only from eggs. Curtis & Bylund (1991) assumed that *D. dendriticum* may be of particular importance as a human parasite in the Arctic and Subarctic regions, but verifications in Finland, for example, are lacking. The growing popularity of lightly salted or inadequately cooked ethnic fish dishes, especially salmon, along with improved transportation systems, caused an increase in human diphyllobothriasis in Canada and the US in the 1980s (Ching 1984, Ruttenberg *et al.* 1984). According to Ching (1984) the possible causative species are *D. dendriticum* and *D. ursi* Rausch 1954 which occur in salmonids and are

known to infect man. However, thorough epidemiological studies on *D. dendriticum* in humans are still needed in light of the results in Lake Inari (II).

3.4.3 What can be done in heavy natural infections?

At the Muonio Fish Farm *D. dendriticum* practically vanished within a couple of years after the rebuilding of the farm when all tanks were located indoors, resulting in a considerable reduction in the number of gulls in the farm region (K. Juntunen, pers.comm.). In a large lake system, however, the situation is much more complicated.

Heavy *Triaenophorus crassus* (Forel) infections in the muscle of stunted whitefish (*Coregonus lavaretus* L. s.l.) were reduced considerably through the intensive fishing of whitefish and pike, the second intermediate host and the definitive host for *T. crassus,* respectively, over six years in a lake in northern Norway (Amundsen & Kristoffersen 1990). In contrast to a marked decline in *T. crassus* occurrence in the whitefish population, the prevalence of *D. ditremum* infection in the same population never fell below 85%. Amundsen & Kristoffersen (1990) concluded that the reduction of pike, i.e. the final host, was seemingly the cause of the significant decrease in the prevalence of *T. crassus*. In the case of *D. dendriticum* the manipulation of its first intermediate host (copepods) and final host (gulls) populations is not possible so management of the fish population remains the only alternative.

The present results from Lake Inari (II) support the ideas suggested by local fish biologists to considerably reduce the number of stocked brown trout in years when the food supply, i.e. vendace and whitefish stocks, is poor. The dynamics of *D. dendriticum* infection have been monitored annually since 1994 as part of other studies in Lake Inari to verify the alterations in the infection level relative to other changes such as variations in vendace and whitefish populations. Some indication has already been obtained that the infection is decreasing in the youngest age classes of stocked brown trout along with the increasing vendace stock (II, Mutenia *et al.* 1997).

An interesting observation in the present data (II) is that the infection level in wild brown trout is clearly lower than in stocked fish of the same age. One reason for this might be that wild fish are not exposed to *D. dendriticum* infection while spending 4-5 years as parrs in local rivers before they enter the lake. Another explanation could be the differences in the diet of wild and farmed fish. In the light of this result, the increase in the brown trout stocking of rivers instead of Lake Inari might inhibit, at least to some extent, *D. dendriticum* infection, but this needs to be studied further.

4 CONCLUSIONS

It is concluded that, for the most part, a balance exists between the harmful effects of *D. dendriticum* and the responses of brown trout and sea trout which is obviously a result of their long co-evolution. The observed mortality cases by *D. dendriticum* heart infections at the Muonio Fish Farm indicate, however, that this balance may collapse in certain circumstances. The size and activity of *D. dendriticum* in brown trout increased with water temperature. Consequently, the prevailing temperature may sometimes increase the pathogenicity of plerocercoids more than the defence reactions of trout are able to cope with. The critical temperature level using the mortality of brown trout as a criterion was not shown experimentally. However, the results on the size and activity of *D. dendriticum* in contrast to the blood leucocyte response in fish indicate that the probable critical temperature might be around 15°C. This is in accordance with the experience at the Muonio Fish Farm.

In the present experiments approximately the same amount of infective procercoids were intubated into the stomach of each brown trout so variability in exposure to infection was minimized. However, the distributions of established *D. dendriticum* plerocercoids in fish went from underdispersed (var<mean) to overdispersed (var>mean) along with the increasing procercoid dose. Most of the brown trout were able to eliminate a great proportion of the intubated procercoids while some of the fish harboured a bigger plerocercoid load. It was shown in the present experiments that differences in host susceptibility to *D. dendriticum* infection may be the most important causes of aggregation.

It was established experimentally that the feed intake and growth of brown trout are well adapted to a low-level *D. dendriticum* infection, at least when the food supply is not restricted. Any evidence of a negative impact of *D. dendriticum* on the feed intake and growth of brown trout could not be found either in an experiment or in a heavy natural infection in Lake Inari.

In the present experiments a single dose of 10-20 procercoids resulted in up to 8% of fish acquiring *D. dendriticum* inside the heart. Moreover, in Lake Inari 13% of brown trout aged 5+ harboured this cestode larva in the heart. It is suggested, on the basis of our findings at Muonio, that fish with growing *D. dendriticum* plerocercoids inside their heart atrium probably will be eliminated eventually in the natural environment. The present studies indicate a small proportion (maybe 5-10%) of the trout population will be lost due to *D. dendriticum* heart infection annually, at least in lakes with a heavy *D. dendriticum* infection.

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YHTEENVETO

Lokkilapamadon, *Diphyllobothrium dendriticum* (Cestoda), ja taimenen vuorovaikutus

Väitöskirjassani tutkin, miten taimen kykenee kompensoimaan lokkilapamadon, *Diphyllobothrium dendriticum*, haitallisia vaikutuksia. Lokkilapamato kuuluu heisimatoihin ja se on mm. lohikalojen tavallinen loinen Pohjois-Euroopassa ja Pohjois-Amerikassa, ja sillä on monivaiheinen kiertokulku luonnossa. Pääasiassa *Larus* -suvun lokit ovat pääisäntiä, joiden suolessa loinen kasvaa pitkäksi, jaokkeiseksi, munia tuottavaksi madoksi. Madon on todettu asettuvan ja tuottavan munia myös monien nisäkkäiden suolessa, ihminen mukaanluettuna. Loisen munat kulkeutuvat lokin ulosteen mukana veteen, ja kesälämpötiloissa munista kuoriutuu vajaassa kuukaudessa pieniä ripsellisiä korakidiatoukkia. Planktiset hankajalkaisäyriäiset syövät näitä toukkia, ja ne kasvavat prokerkoidiasteelle noin kahdessa viikossa. Kun mm. siiat, taimenet ja kolmipiikit syövät puolestaan loisittuja hankajalkaisäyriäisiä, pienet prokerkoiditoukat vapautuvat kalan mahalaukussa, tarttuvat mahalaukun seinämään ja tunkeutuvat sen läpi ruumiinonteloon. Kalan puolustusreaktiot heräävät ja toukan ympärille alkaa kertyä valkosoluja. Useimmiten siiat onnistuvat kapseloimaan loisen heti mahalaukun pinnalle, mutta taimenilla nämä plerokerkoiditoukiksi kutsutut loiset voivat vaeltaa kalan eri elimiin, myös sydämeen ja lihakseen.

Kiinnostukseni tähän loiseen heräsi, kun Riista- ja kalatalouden tutkimuslaitoksen hoitamassa Muonion kalanviljelylaitoksessa todettiin loisen aiheuttavan merkittävää kuolleisuutta kesällä 1991 ja 1992 tunkeutumalla meri- ja järvitaimenen poikasten sydämen eteiseen. Isokokoinen loinen aiheutti eteisen repeytymisen ja tukki eteisestä kammioon johtavan aukon. Koska kuolleisuus ajoittui keskikesälle ja oli suurempaa lämpimänä kesänä 1991 kuin vuonna 1992, jolloin veden lämpötilat olivat alhaisempia, oletettiin lokkilapamadon haitallisuuden kasvavan lämpötilan myötä. Muonion tulokset

nostivat esiin kysymyksen, tapahtuuko vastaavaa myös luonnonvesissä. Kaikenkaikkiaan loisten merkityksestä kalakannoille on hyvin vähän tietoa, joten tähän aiheeseen päätettiin perehtyä lokkilapamadon avulla.

Muonion kalanviljelylaitoksella tutkittiin noin 1600 kuolleen kalan sydän (pääasiassa 1-2 -vuotiaita meri- ja järvitaimenia) heinäkuussa 1991, kesä-syyskuussa 1992 ja maalis-kesäkuussa 1993. Vertailukaloina tutkittiin eri vuodenaikoina samalta laitokselta myös elävinä pyydystettyjä kaloja. Istutettuja järvitaimenia kerättiin näytteeksi Inarijärvestä vuosina 1994 ja 1995 kaikkiaan 209, ja heisimatojen toukkavaiheet tutkittiin kaikista kalan elimistä. Myös taimenen potentiaalisten saaliskalojen, muikun ja siianpoikasten sekä kymmenpiikin, heisimadot tutkittiin. Lisäksi toteutettiin neljä laboratoriokoetta, joissa 0+ - 1+ -ikäisille nukutetuille järvitaimenille annettiin tietty loisittujen planktonien annos suoraan mahalaukkuun. Tälloin voitiin arvioida kalojen saama prokerkoiditoukkien lukumäärä. Kokeissa tutkittiin lämpötilan vaikutusta lokkilapamadon kasvuun ja vaellukseen sydämen alueelle ja lihakseen järvitaimenessa. Taimenen vasteista loista kohtaan tutkittiin kalan kykyä torjua infektio, infektion vaikutusta veren valkosoluihin kahdella eri lämpötilatasolla, noin 10°C ja 15°C, sekä tutkittiin loisen vaikutusta taimenen kasvuun ja ravinnonottoon sekä kuolleisuuteen.

Tulosten perusteella sekä lokkilapamatojen koko että vaellusaktiivisuus ruumiinontelosta sydämen alueelle ja lihaan kasvavat lämpötilan myötä. Lämpimässä vedessä tehdyssä kokeessa (>15°C) noin 8%:lla järvitaimenista oli loisia sydämessä. Lokkilapamatotartunta nosti veren lymfosyyttien ja neutrofiilien lukumääriä, mutta kahden lämpötilatason (kts. edellinen kappale) välillä eroja löytyi vain trombosyyttien määrissä, joita oli selvästi enemmän viileässä. Vaikka kussakin kokeessa kalojen mahaan siirrettiin kutakuinkin sama prokerkoidiannos, niin kaloista löydettyjen plerokerkoiditoukkien frekvenssijakaumat osoittivat suurimman osan loisista kerääntyneen muutamille yksilöille. Varianssi-keskiarvosuhde oli sitä suurempi mitä isompi oli loisannos. Tämä on mitä todennäköisimmin seurausta kalayksilöiden välisistä eroista kyvyssä torjua infektio. Lokkilapamatoaltistus ja -tartunta eivät vähentäneet kalojen kasvua eikä ravinnon ottoa kontrolleihin verrattuna. Altistetut kalat näyttivät syövän jopa enemmän kuin kontrollit, ja on mahdollista, että loisten aikaansaamat puolustusreaktiot sekä loisten kasvu lisäävät kalan energian tarvetta, jonka kala kompensoi lisääntyneellä ruokailulla. Lokkilapamatoinfektio saattaa siten heikentää kalan kasvua huonon ravintotilanteen aikana.

Lokkilapamadon aiheuttamaa kuolleisuutta osoitettiin Muonion kalanviljelylaitoksella, jossa loisia (tavallisesti 1-2 per sydän) tunkeutui kalanpoikasten sydämeen. Kuitenkaan Inarijärven taimenpopulaatiossa ei voitu osoittaa suoraa tai epäsuoraa lokkilapamadon aiheuttamaa kuolleisuutta, vaikka loisia kertyi keskimäärin 130 yksilöä kalaa kohden noin neljän vuoden aikana istutuksen jälkeen. Lokkilapamadon aiheuttamaa kuolleisuutta ei voitu osoittaa myöskään kokeellisissa töissä. Tämän tutkimuksen perusteella on ilmeistä, että taimen pystyy kompensoimaan lokkilapamadon haitalliset vaikutukset yleensä hyvin. Lokkilapamadon yleisyys vesistöissämme viittaa myös siihen, että loinen

ei voi olla kovin haitallinen kaloille. Pitkän yhteisen evoluution kuluessa tämän loisen ja taimenen välille on siten kehittynyt tasapainotila. Muonion kalanviljelylaitoksessa esiin tullut kuolleisuus kuitenkin osoittaa, että tasapaino voi joskus murtua - esimerkiksi jos loisen koko ja vaellusaktiivisuus kasvavat nopeammin kuin mihin taimenen puolustusmekanismit ehtivät vastata. Tulokset kokeellisista töistä ja Muonion laitokselta viittaavat siihen, että riskilämpötila on noin 15°C vaiheilla. Voidaan kuitenkin olettaa, että lokkilapamadon sydämeensä saaneet kalat eivät ole luonnossa kovin pitkäikäisiä. Tutkimusteni perusteella ainakin voimakkaasti infektoituneissa järvissä pieni osuus taimenpopulaatiosta (ehkä 5-10%) voi menehtyä lokkilapamadon sydäntartuntoihin vuosittain.

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ORIGINAL PAPER

VI

Rahkonen, R., Koskela, J. & Jobling, M.
The effect of Diphyllobothrium dendriticum (Cestoda) infection
on feeding and growth of brown trout
(Salmo trutta L.)

Manuscript (submitted)

The effect of *Diphyllobothrium dendriticum* (Cestoda) infection on feeding and growth of brown trout (*Salmo trutta* L.)

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ABSTRACT

The effect of *Diphyllobothrium dendriticum* (Cestoda) infection on the feeding and growth of 0+ brown trout (*Salmo trutta* L.) was studied experimentally. A total of 120 individually marked fish was infected with a dose of 10 *D. dendriticum* procercoids per fish by stomach intubation, and fish were then distributed amongst 4 tanks. Corresponding control groups were established. Feed intake was measured 5 times during the 74-days experiment using an X-radiographic technique. At autopsy 60% of the intubated fish were found to be infected, with a mean of 2.2 plerocercoids (1-7) per infected fish. Feed intake tended to be higher in fish groups exposed to procercoids than in controls, particularly amongst those fish that had been able to eliminate the procercoids. There also was a slight, but not significant, tendency for growth rate (SGR % d^{-1}) to be higher in exposed (0.57%) than in control (0.48%) fish. There was also a tendency for feed conversion to be somewhat poorer amongst exposed, than control, fish but the difference was not significant. In conclusion, *D. dendriticum* exposure did not have any major negative impact on the feed intake and growth of 0+ brown trout. On the contrary, there were indications that low level plerocercoid infections may have increased the energy demand of the host, and this was compensated for by increased feeding.

Keywords: brown trout, *Salmo trutta*, Cestoda, *Diphyllobothrium dendriticum*, parasitism, growth, feed intake

INTRODUCTION

The effects that infection with parasites have on fish growth have been little studied even though reduced growth may have a direct impact on the economy of fish farming, and on the survival of fish in natural environments. A negative effect of parasites on the size or condition of fish has been demonstrated in cases where the parasites had damaged the skin (Lemly & Esch, 1984; Singhal *et al.*, 1990; Urawa & Yamao, 1992; Urawa 1995) or liver (Szalai & Dick, 1991) of their host. In addition, reduced growth of salmonids has been attributed to *Eubothrium* sp. infections (gut tapeworm) (Bristow & Berland, 1991). On the other hand, when infected with large *Schistocephalus solidus* (Müller) (Cestoda) larvae, sticklebacks *Gasterosteus aculeatus* L. seem to have an increased energy demand, and compensate with increased feeding (Walkey & Meakins, 1970; Giles, 1987). The parasite-induced changes in energy demand may lead to increased mortality of the fish during periods of food deprivation (Walkey & Meakins, 1970). Larvae of another tapeworm, *Ligula intestinalis* (L.), are also known to have adverse effects on the growth of cyprinids (Hoole, 1994).

Diphyllobothrium dendriticum (Nitzsch, 1824) (syn. *D. norwegicum* Vik) (Cestoda) is a tapeworm that is commonly found in natural stocks of salmonids

and coregonids (Halvorsen, 1970; Henricson, 1977, 1978; Kennedy, 1978; Curtis, 1983; Ching, 1988; Valtonen *et al.*, 1988; Andersen & Valtonen, 1992; Rahkonen & Koski, 1997), and it also occurs in farmed fish (Hoffman & Dunbar, 1961; Berland, 1987; Sharp, 1991, Rahkonen *et al.*, 1996). Procercoid larvae are released from ingested copepods, penetrate the stomach wall of the host fish, and are encapsulated as plerocercoids on the visceral organs through the fish inflammatory response (Sharp *et al*, 1989, 1992; Rahkonen & Valtonen 1997). The fish hosts may accumulate varying numbers of plerocercoids, and pathogenicity seems to be related both to the level of infection and to the site of the migrating worms (Duguid & Sheppard, 1944; Hickey & Harris, 1947; Fraser, 1960; Bylund, 1972; Sharp *et al.*, 1989, Rahkonen *et al.*, 1996).

In previous studies somewhat contradictory results have been obtained regarding the impact of *D. dendriticum* on the growth and condition of fish. Infection of the heart with *D. dendriticum* was found to result in mortality of sea trout (*Salmo trutta* m. *trutta* (L.)) and brown trout (*Salmo trutta* m. *lacustris* (L.)) at a fish farm in northern Finland (Rahkonen *et al.*, 1996), and two-year-old sea trout with *D. dendriticum* inside the heart were significantly smaller than healthy fish (Rahkonen, unpubl.). On the other hand, in Lake Inari, northern Finland, where brown trout were heavily infected with *D. dendriticum* after stocking, there was a positive correlation between the Fulton's condition index of the brown trout and the number of *D. dendriticum* with which they were infected. Thus, it seemed clear that the more the fish had eaten, the greater was the accumulation of *D. dendriticum* larvae (Rahkonen & Koski, 1997).

It may not be possible to obtain an adequate picture of the impact of *D. dendriticum* infection on feeding and growth of fish by merely comparing mean lengths and weights of infected and uninfected fish. Consequently, the aim of this study was to examine the effect of *D. dendriticum* infection on brown trout, by adopting an experimental approach in which the feed intake and growth of infected and uninfected fish were monitored.

Based upon the results of previous studies concerning the effects of parasites on their fish hosts (Walkey & Meakins, 1970; Lemly & Esch, 1984; Giles, 1987; Singhal *et al.*, 1990; Szalai & Dick, 1991; Urawa & Yamao, 1992; Urawa, 1995), we tested two alternative hypotheses regarding the effect of *D. dendriticum* on fish growth:

A) brown trout are unable to compensate for any negative effects of *D. dendriticum* infection and this will result in poorer growth amongst exposed fish.

B) brown trout are able to compensate for the negative effects induced by *D. dendriticum* infection, so that there are no differences in growth between exposed and control fish.

MATERIALS AND METHODS

The 74-day experiment was carried out between 31 January and 15 April 1997 at the Laukaa Research Station of the Finnish Game and Fisheries Research Institute (62°30'N, 26°E). The fish used were age 0+ hatchery-reared brown trout (mean weight about 18g) of the Rautalampi strain, derived from broodstock held at the research station. Twelve weeks prior to the start of the experiment fish were selected from a larger population and individually tagged by injecting a PIT tag (Trovan™) into the body cavity. Acclimatization of the fish to the experimental conditions was started 3 weeks before the first measurement was undertaken. Eight groups, each comprising 30 tagged trout, were established in 0.2 m^2 (bottom area) circular black plastic tanks (volume: 55 l). This gave four tanks of fish for each of the parasite exposed (4x30 fish) and control (4x30 fish) treatments.

Fish in the parasite-exposed group were deprived of food for one day, and then on 04. February were anaesthetized and stomach intubated with 15 *Cyclops strenuus* copepods. Copepods were counted under CO_2 anaesthesia and placed in 0.3% pepsin in physiological saline, pH 2, in pipette tips. Copepods had been primed by feeding them on hatched coracidia of *D. dendriticum,* originating from Lake Inari brown trout, 25 days beforehand. Copepod infection with coracidia was checked 24 days post infection, and 70% of those examined (N=60) harboured one to three well developed procercoids. Thus, the dose of 15 copepods given to the trout was expected to contain approximately 10 procercoids. The control fish were handled in the same fashion as fish in the exposed groups, except that controls received a drop of pepsin solution rather than copepods. The method is described in more detail by Rahkonen & Valtonen (1997).

The fish were reared at 10-12°C, obtained by heating and aeration of water prior to distribution to the tanks. Water flow to the tanks was maintained at 3 l min^{-1}. Fish were exposed to a simulated natural photoperiod (07L:17D at the start and 15L:09D at the end of the experiment) provided by artificial overhead lighting. The fish were fed on commercial dry pellet feed (Tess respons 1.7 mm). Feed was distributed by belt feeders for 4 h each day between 08.00-12.00 h, at a ration of 10 g kg^{-1} d^{-1}.

Feed intake was measured using an X-radiographic technique (Talbot & Higgins, 1983; Jobling *et al.,* 1993). The first measurement was made four days (31. January 1997) before infection of fish with parasites. Thereafter, measurements were repeated at approximately 2 week intervals. Diets used for feed intake measurement were prepared from the normal feed by grinding, homogenisation and incorporation of known quantities of X-ray dense ballotini (size 8.5; Jencons Ltd Leighton Buzzard, UK) followed by compression into pellets and re-drying at 40-45°C. Samples of the diets were X-rayed and a standard curve for the relationship between number of ballotini (X) and diet weight (g) calculated:

diet g $= 0.059+0.014\times X$, $R^2 = 0.87$, $P<0.001$, $N = 8$

Feeds were stored at 5°C prior to use. Feed intake measurements were made by providing the marked feed during a four-hour feeding period (08.00-12.00), followed immediately by anaesthetizing the fish (MS-222), X-raying (Kostix 30 X-ray machine; Kodak X-OMAT MA film), weighing to the nearest 0.1g and identification of individuals by reading the PIT tag. X-ray plates were then developed and the amounts of feed consumed by the fish in a tank estimated.

Growth rates (SGR), in terms of weight, were calculated according to the formula: SGR = $[(\ln X_2 - \ln X_1)/t] \times 100$, where X_1 is the weight of the fish at the start and X_2 is the weight at the end of a period and t is the duration of the period in days.

Feed:gain ratio was calculated by dividing the amount of food consumed (feed intake) by wet weight gain. The feed consumption data used in making these calculations were those estimated from the X-radiographic measurements. The following formula was used for the calculation of the coefficient of variation:

CV = S.D./mean x 100, where S.D. is the standard deviation and mean is the population mean.

The fish were killed by a high dose of anaesthetic two weeks after the last measurement, the abdominal cavity was cut open and the viscera loosened. Fish were then placed individually in 0.5% (w/v) pepsin in physiological saline (0.9%), pH 2 and left overnight. The resulting solution was sieved and studied at 10-20 x magnification using transmitted light. Any worms found were collected and held in tap water overnight in a refrigerator. The lengths of the worms were then measured.

Statistical analyses were performed using SYSTAT statistical software (SYSTAT, 1996). The G^2 test of independence and the non parametric Kruskal-Wallis test were used to compare between tank prevalences and intensities of infection, respectively. Possible differences among treatments (exposed/control) were tested using either a nested ANOVA model in cases in which individual responses (wet weight, growth rate) had been measured, or ANOVA when group responses (intake, feed:gain ratio) were examined (Sokal and Rohlf, 1981). Correlations were tested using a Spearman's rank correlation test. Homogeneity of variances was examined using Cohran's test, and the Lilliefors' method was used to test for normality (SYSTAT, 1996). The Tukey-Kramer test was used to make *post-hoc* comparisons between sample means, and $p<0.05$ was taken as the level of significance. The power of the F test was calculated according to Lindman (1992).

RESULTS

Sixty percent of the exposed fish became infected with plerocercoids (1-7 per infected fish). Prevalence varied from 53.3% to 65.5% among the four tanks, with no significant differences (G^2 test of independence = 1.192, df 3, P>0.05) (Table 1). The mean number of worms per infected fish ranged from 1.6 to 2.6 worms (Kruskal-Wallis test statistics = 7.776, df = 3, P = 0.051) (Table 1). The mean length of the relaxed worms was 10.1 mm (SD 2.9) and the plerocercoids showed aggregated distributions (Fig. 1).

One of the 120 control fish was found to be infected with one *D. dendriticum*, and this fish was omitted from further analysis. Only one fish exposed to *D. dendriticum* and one control fish died during the experiment.

There was a tendency for feed intake (g kg^{-1} per day) to be slightly higher in exposed than in control groups on every sampling date (Fig. 2), but the differences were not statistically significant (ANOVA, Bonferroni adjusted probabilities, P > 0.05/5). When mean feed intake was calculated for the 4 post-exposure measurement days (18.02, 04.03, 19.03, 02.04), intake was 23% higher in exposed (3.8 g kg^{-1}, SD 0.4) than in control groups (2.9 g kg^{-1}, SD 0.8), but probably because of the low power of the test (30%) the difference was not significant (ANOVA, $F_{(1,6)} = 3.604$, P = 0.106).

During the course of the experiment the mean wet weight of the exposed and control groups of fish increased from 18.2g (SD 1.0) and 19.3g (SD 0.7) to 26.1g (SD 0.9) and 26.6g (SD 1.0), respectively, and final weights did not differ between the groups (nested ANOVA, $F_{(1,6)} = 1.580$, P > 0.05) (Fig. 3A). The changes in the coefficients of variation of wet weight (CV_{ww}) were also quite similar in the exposed and control groups, increasing from initial values of 28 and 29% to final values of 39 and 45% (Fig 3B).

During the first three periods of the study SGR varied between 0.3-0.6% and 0.3-0.5% for the exposed and control fish, respectively (Fig. 3C), but during the fourth period growth rate increased, being 1.0 and 0.8%, respectively. There was a tendency for growth rate to be higher in exposed fish (Fig. 3C), but there were no significant differences between groups in mean daily growth rate for the whole experiment (exposed, 0.57%, SD 0.33; control, 0.48%, SD 0.36, nested ANOVA, $F_{(1,6)} = 2.216$, P > 0.05). However, the power of the test to reveal a difference between the treatments was low (15%). The interindividual (within tank) variability in growth rate (CV_{SGR}) varied between 46-345% and 81-417% in exposed and control groups, respectively. In those periods where SGR was high, CV_{SGR} was low (Fig 3 C and D). Consequently, there were significant negative correlations between the SGR and CV_{SGR} in both groups (Spearman's rank correlation test N = 16; exposed $r_S = -0.68$, P < 0.01; control: $r_S = -0.79$, P < 0.01).

Feed:gain ratio (feed intake:wet weight gain) was somewhat higher in exposed, 0.64 (SD 0.07) than in control groups, 0.58 (SD 0.14), but the difference was not stastistically significant (ANOVA, $F_{(1,6)} = 0.547$, P > 0.05). The power of the test to detect differences was, however, weak (11%).

An attempt was made to correlate the growth rate of individual fish with the number of *D. dendriticum* plerocercoids it contained. Correlations obtained for the four tanks of exposed fish were as follows:

Tank 1: $r_s = 0.098$ (N = 30), Tank 3: $r_s = -0.519$ (N = 27), Tank 5: $r_s = -0.117$ (N = 28), Tank 7: $r_s = 0.129$ (N = 30). The only statistically significant negative ($p<0.01$) correlation was found for tank 3, where one fish harboured 6 plerocercoids and the others 1-2.

Comparisons were also made among the control and the exposed-uninfected and exposed-infected fish (Fig. 4). Feed intake, calculated for the 4 post-exposure measurement days, differed almost significantly between the groups (ANOVA, $F_{(2,9)} = 3.8$, $P = 0.063$), with a Tukey-Kramer's *post hoc* comparison revealing close to significant differences between controls and the exposed-uninfected fish ($P = 0.057$). SGRs and feed:gain ratios were slightly, but not significantly, higher among the exposed-uninfected fish than amongst those in the other two categories (SGR: nested ANOVA, $F_{(2,9)} = 1.473$, $P = 0.280$; feed:gain ratio: ANOVA, $F_{(2,9)} = 0.178$, $P = 0.840$).

DISCUSSION

As in a previous study, infection of brown trout with *D. dendriticum* procercoids was successful (Rahkonen & Valtonen, 1997), a dose of 10 procercoids resulting in 60% infection, with 1-7 plerocercoids per infected fish. The results of the present experiment conform to previous findings. The results seem to indicate that fish differ in their abilities to defend themselves against penetrating procercoids, thereby making it difficult to generate an even infection in a group of fish (Fig. 1). This supports the hypothesis that individual differences in the susceptibility of fish to parasites are an important cause of the aggregated distributions of parasites seen within hosts in the natural environment (see e.g. Crofton, 1971).

There seemed to be differences in feed intake between exposed and control fish, the feed intake of the exposed groups being greater than that of control groups (Fig. 2), particularly among the exposed fish that had been able to eliminate the procercoids (Fig. 4). Variation, particularly among the control groups, was however, too high which decreased the power of the statistical tests to show statistically significant differences. There are few studies concerning the effects of parasites on the feed intake of host fish. Infection with *S. solidus*, a large cestode larva occurring in the body cavity of three-spined stickleback has been reported to result in increased ingestion by the host (Walkey & Meakins, 1970; Giles, 1987).

Growth rate was not reduced among the exposed fish compared to the controls (Fig. 3), but there was a trend towards a higher feed:gain ratio amongst the brown trout that had been exposed to *D. dendriticum*, indicating feed conversion efficiency to be poorer amongst the exposed fish. Reduced growth of

fish has been demonstrated when parasites damage vital organs (Lemly & Esch, 1984; Singhal *et al.*, 1990; Szalai & Dick, 1991; Urawa & Yamao, 1992; Urawa, 1995). The site of the worms was not studied in the present experiment, but most *D. dendriticum* plerocercoids seem to encapsulate in the adipose tissue around the stomach and intestine of brown trout (Rahkonen & Valtonen, 1997). The trend towards differences in feed:gain ratios between treatment groups suggests that there might be differences in metabolic energy demands between the exposed and control fish. It is possible that the growth of plerocercoids, and especially defence processes against *D. dendriticum* infection, increase the energy demands of the fish host leading to increased feeding as a compensatory mechanism. This is in accordance with the view of Holmes & Zohar (1990) who suggested that when feed supplies are adequate, minor damage or a reduction in the host's share of ingested nutrients can probably be compensated by increased feeding.

Walkey & Meakins (1970) and Giles (1987) suggested that three-spined stickleback infected with *S. solidus* eat more in order to satisfy their greater energy demand, and sticklebacks infected with *S. solidus* are also known to take greater risks than non-infected individuals when foraging (Milinski, 1985; Godin & Sproul, 1988).

The level of infection appeared to have little influence on the growth of individual fish, because a significant negative correlation between growth and number of worms was only recorded for one tank of fish. Further, there was no evidence of differences in variability in either weight (CV_{WW}) or growth rates (CV_{SGR}) within the groups of exposed and control fish (Fig. 3). An increase in heterogeneity would have been predicted amongst the exposed fish if infection with *D. dendriticum* resulted in growth suppression, especially if the level of suppression of the individual fish was related to the intensity of infection.

Thus, it seems that brown trout can tolerate low-level *D. dendriticum* infections, at least when the food supply is not restricted. Consequently, the present results support our second hypothesis (B). Assuming that there is a greater energy demand imposed upon infected fish, negative effects could be expected to occur in the case of either poor food supply or heavier infection, but this would need to be verified by further studies. Further, some refinements of experimental protocol are required to improve the power of statistical tests to reveal differences between treatments.

In conclusion, the methods applied in this study would seem to have potential for the examination of the energetics of fish-parasite relationships. Many of the exposed fish seemed to have a well developed ability to prevent the establishment of the worms, but even when small numbers of *D. dendriticum* became established they did not have negative impacts on the feed intake and growth of the brown trout. There were, however, some indications that infection with *D. dendriticum* plerocercoids resulted in increased energy demands of the fish host, which was compensated for by increased feeding.

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Table 1. Occurrence of *D. dendriticum* plerocercoids in infected brown trout.

		Prevalence	No. of worms per infected fish		
	No. of fish	%	mean	SD	min-max
Tank 1	30	53.3	1.8	1.0	1-4
Tank 3	30	56.7	1.6	1.2	1-6
Tank 5	29	65.5	2.6	2.0	1-7
Tank 7	30	63.3	2.6	1.3	1-6

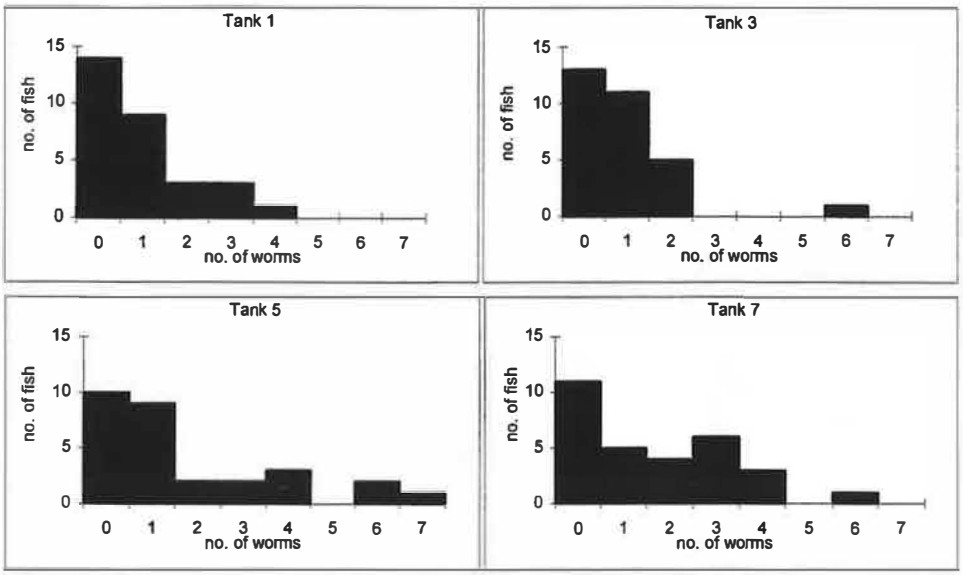

Figure 1. Frequency distributions of *D. dendriticum* plerocercoids in brown trout intubated with an estimated 10 procercoids per fish.

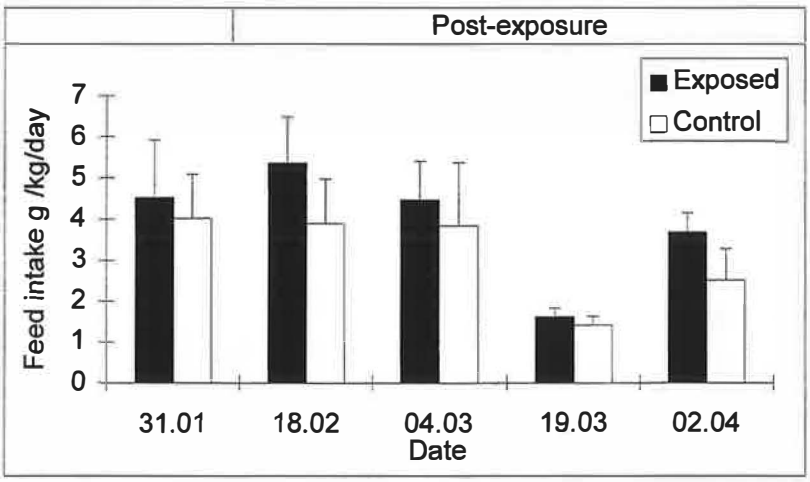

Figure 2. Temporal changes in feed intake (g kg^{-1} d^{-1}) of brown trout in exposed and control groups. The fish in exposed groups were intubated with a dose of 10 *D. dendriticum* procercoids on 04.02.1997. Data are shown as treatment means +SD (N = 4).

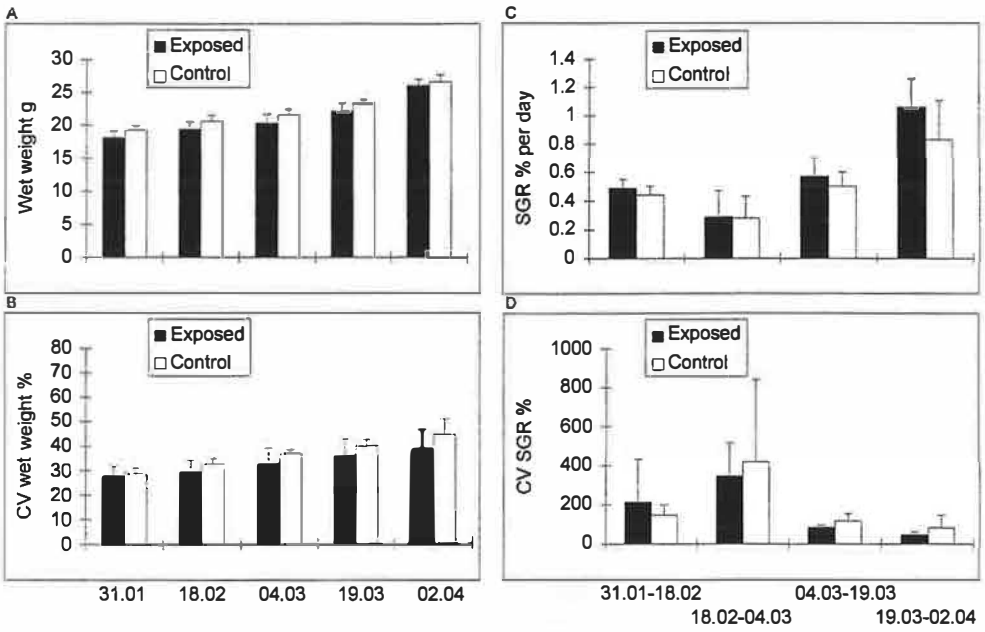

Figure 3. Wet weights (A) and growth rates (SGR) (C) and interindividual variability (CV) in wet weight (B) and growth rate (D) of brown trout in exposed and control groups. The exposed fish were intubated with a dose of 10 *D. dendriticum* procercoids on 04.02.1997. Data are shown as treatment means +SD (N = 4).

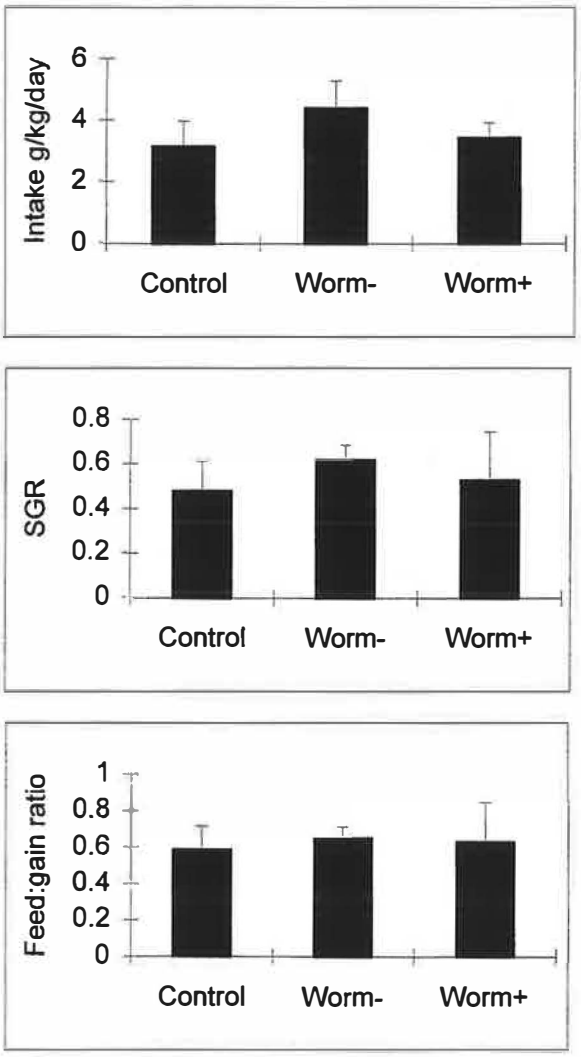

Figure 4. Feed intake (g kg^{-1} d^{-1}), growth rate (SGR) and feed:gain ratios of control, uninfected-exposed (worm-) and fish infected with *D. dendriticum* (worm+). Data are shown as treatment means +SD (N = 4). Feed intake was calculated for the 4 post-exposure measurement days and SGR and feed:gain ratios for the whole study period (60 days).