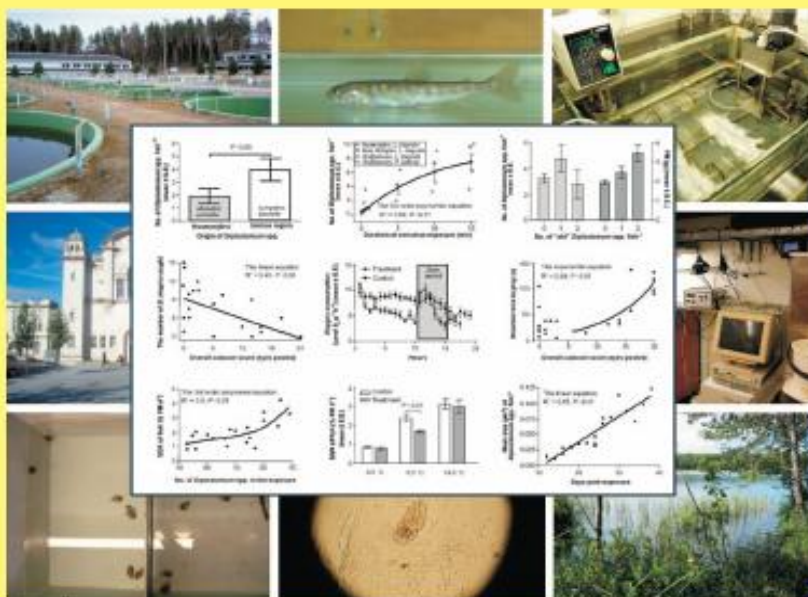University of Joensuu, PhD Dissertations in Biology

No:63

Ecophysiological approach to host-parasite interaction between Arctic charr and *Diplostomum* spp.

by
Ari Voutilainen

Joensuu
2009

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Ecophysiological approach to host-parasite interaction between Arctic charr and *Diplostomum* spp.

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Biosciences of the University of Joensuu, for public criticism in the Auditorium M100 of the University, Yliopistokatu 7, on 13th November, 2009, at 12 noon

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Parasites are ubiquitous and active members of ecosystems. The relationship between parasites and their hosts is interactive; parasites have multiple effects on their host organisms and *vice versa*. In general, parasites are predicted to be harmful and to cause energetic costs to their hosts. In the present thesis, the host-parasite interaction between young-of-the-year Lake Saimaa Arctic charr *Salvelinus alpinus* (L.) and trematode parasites *Diplostomum* spp. (Digenea: Diplostomatidae) was studied. *Diplostomum* spp. have a complex life-cycle with lymnaeid snails and fish as intermediate hosts and piscivorous birds as final hosts. The *Diplostomum* spp. that locate in the eye lens of fish and cause cataracts, have been found to have the potential to increase mortality and reduce the growth of their hosts. The Lake Saimaa Arctic charr is classified as critically endangered and the reasons for the low stocking success and yield of hatchery-reared fish have remained unresolved. Therefore, it was necessary to investigate the effects of *Diplostomum* spp. specifically on the Arctic charr. In this thesis, the *Diplostomum* spp. individuals that infected fish eye lens were termed *Diplostomum* cf. *spathaceum*.

 The main objective of the research was to answer the following questions. 1) How does the geographical origin of *D.* cf. *spathaceum* affect its infectivity in the Arctic charr? 2) Does *D.* cf. *spathaceum* infection cause energetic costs to the Arctic charr? 3) Does *D.* cf. *spathaceum* infection alter the allocation of energy in the Arctic charr? 4) Does *D.* cf. *spathaceum* induced cataract impair the foraging efficiency of the zooplanktivorous Arctic charr? The questions were addressed by exposing the Arctic charr to *D.* cf. *spathaceum* cercariae under controlled laboratory conditions and by carrying out oxygen consumption measurements and feeding experiments. In addition to the main objective, *D.* cf. *spathaceum* infection was linked to measures of the immune function (spleen size) and physiological condition (liver size) of the parasitised fish. Furthermore, the short-term effects of the fish on the release of *D.* cf. *spathaceum* cercariae from snails were investigated.

 The Arctic charr were more susceptible to *D.* cf. *spathaceum* that had sympatric origin with the fish. Duration of exposure affected the proportion of presented cercariae that were able to infect the fish, and the strength of the effect was related to the parasite's origin. The migration success of *D.* cf. *spathaceum* to the fish eye lens was associated with the previous infection status of the fish and the number of cercariae that had penetrated the fish. Interestingly, *D.* cf. *spathaceum* infection had the potential to stimulate the young-of-the-year Arctic charr to grow faster at the temperature optimal for growth of the fish (15 °C). On the other hand, when the charr were reared at 10 °C, the growth rates of the parasitised fish were lower than those of the unparasitised conspecifics. The cataract-bearing charr showed a weaker response to darkness, i.e. lower increase of oxygen consumption after switching off the lights, compared to the healthy-eyed controls. Moreover, wide and dense parasite-induced cataract impaired the feeding capability of the zooplanktivorous Arctic charr. The infection did not induce an immune response in terms of increased size of the fish spleen. No evidence of influence of the presence of fish on the release of *D.* cf. *spathaceum* cercariae from snails was found.

 The results suggested three main conclusions. First, *D.* cf. *spathaceum* was able to adapt to its local fish hosts. Second, the infection had temperature-dependent effects on the growth of Arctic charr. Third, *D.* cf. *spathaceum* affected the feeding and behaviour of the zooplanktivorous charr. Consequently, the overall effect of the parasite on the Arctic charr may be detrimental to the fish.

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To my beloved daughters Isla and Saga

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications which are referred to in the text by their Roman numerals **I-VII**. In addition, some unpublished results are presented in the summary.

I   Voutilainen, A. & Taskinen, J. (2009) Infectivity of *Diplostomum* spp. in Arctic charr: aspects of exposure duration and cercariae morphology. *Journal of Parasitology* **95**, 527–531.

II   Voutilainen, A., Huuskonen, H. & Taskinen, J. (2009) Penetration and migration success of *Diplostomum* spp. cercariae in Arctic charr. *Journal of Parasitology* (accepted manuscript).

III   Voutilainen, A., Valdez, H., Karvonen, A., Kortet, R., Kuukka, H., Peuhkuri, N., Piironen, J. & Taskinen, J. (2009) Infectivity of trematode eye flukes in farmed salmonid fish – effects of parasite and host origins. *Aquaculture* **293**, 108–112.

IV   Voutilainen, A., Taskinen, J. & Huuskonen, H. (2009) Temperature-dependent effect of the trematode eye flukes *Diplostomum* spp. on the growth of Arctic charr. Manuscript.

V   Seppänen, E., Kuukka, H., Voutilainen, A., Huuskonen, H. & Peuhkuri, N. (2009) Metabolic depression and spleen and liver enlargement in juvenile Arctic charr *Salvelinus alpinus* exposed to chronic parasite infection. *Journal of Fish Biology* **74**, 553–561.

VI   Voutilainen, A., Taskinen, J. & Huuskonen, H. (2009) Association between energetics and parasitism in a fish-trematode system. Manuscript.

VII Voutilainen, A., Figueiredo, K. & Huuskonen, H. (2008) Effects of the eye fluke *Diplostomum spathaceum* on the energetics and feeding of Arctic charr *Salvelinus alpinus*. *Journal of Fish Biology* **73**, 2228–2237.

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The author's contribution

I: the author planned the study together with Jouni Taskinen, carried out the experiments and had the main responsibility for analysing the data and writing the article.

II, **IV** and **VI**: the author planned the study with Jouni Taskinen and Hannu Huuskonen, carried out the experiments and had the main responsibility for analysing the data and writing the article.

III: the author planned the Arctic charr experiments together with Jouni Taskinen, carried them out and had the main responsibility for analysing the data and writing the article.

V: the author participated in writing the article.

VII: the author planned the study with Hannu Huuskonen, carried out the experimental infections and oxygen consumption measurements and had the main responsibility for analysing the data and writing the article.

1 INTRODUCTION

1.1 Host-parasite interactions

Parasites are ubiquitous animals whose life-cycle is dependent on one or several host organisms. However, they are not merely passive passengers but active members of ecosystems. Parasites are believed to play a significant role e.g. in the evolution of sexual selection and the immune system and in maintaining the genetic diversity of their hosts (Poulin 2007). Parasites shape the population and community dynamics of their hosts, affect the allocation of energy in food webs and maintain biodiversity (see Hudson *et al.* 2006 for a review). The relationship between parasites and their hosts is interactive, so that in the same host-parasite system, the parasite affects e.g. the morphology of the host (Zbikowska & Zbikowski 2005) and the host has effects on the reproduction of the parasite (Seppälä *et al.* 2008a). Parasitism is assumed to cause costs to the hosts, but manipulation of the hosts is also suggested to cause costs to the parasite itself (Poulin *et al.* 2005). Parasites, including their free-living stages, are not immune from effects arising from the environment and from anthropogenic activities, such as pollutants, alterations in habitat and temperature, introduced species, aquaculture and fishery (Lafferty & Kuris 1999; Pietrock & Marcogliese 2003; Hakalahti *et al.* 2006). In fact, parasite communities are potential bioindicators of ecosystem health (Valtonen *et al.* 1997, 2003; Morley & Lewis 2007).

The virulence of parasites, i.e. their ability to infect and harm hosts, varies among host-parasite systems (Schall 2002). According to the general assumption, parasites that do not reduce the lifespan of their hosts – which, in any case, are ephemeral – are, in the long term, favoured by natural selection (Schall 2002). However, the virulence of a parasite is not constant, but depends on intra- and interspecific interactions between the parasites (Kalbe *et al.* 2002; Lafferty 2002; Rauch *et al.* 2008) and the effects of the hosts on the parasite's evolution (coevolution) (Ebert 1998; Kaltz & Shykoff 1998; Kalbe & Kurtz 2006; MacColl 2009). The coevolution between parasites and their hosts is concluded to benefit the parasites so that they adapt to their local host populations (see Kaltz & Shykoff 1998 for a review).

Many animals especially those belonging to the phyla Platyhelminthes and Aschelminthes have adopted a parasitic way of life that has originated independently in several taxa (Poulin 2007). The species that belong to the classes Trematoda and Cestoda are all parasites. They have an indirect life-cycle in which the larvae infect host animals at a different trophic level than do the adults (Combes *et al.* 2002; Sukhdeo & Sukhdeo 2004). The larvae of the digenean trematodes can be found in molluscs (e.g., Väyrynen *et al.* 2000; Faltynkova 2005) and in fish (e.g., Leong & Holmes 1981; Conneely & McCarthy 1984), while the adults of the species infect birds (e.g., Pojmanska *et al.* 1984), for example. It has been proposed that this type of complex life-cycle with free-living stages and several host species enhances the chances of trematode parasites reaching the final vertebrate host (Choisy *et al.* 2003) and increasing genetic variability in the parasite population (Rauch *et al.* 2005).

Infecting hosts is not a straightforward task for parasites, as animals have several direct and indirect mechanisms for avoiding them (Wakelin 1996). Fish, for example, can change their behaviour in the presence of parasites (e.g., Karvonen *et al.* 2004a) and their active surface mucus acts as a physiochemical barrier against pathogens (Shephard 1994). The parasites that have invaded the fish's body could be eliminated by the immune system, which includes innate immunity and acquired resistance (Secombes & Chappell 1996; Jones 2001). Furthermore, fish also avoid parasites indirectly through evolutionary mechanisms, such as sexual selection, by signalling their high resistance to parasites with sexual ornaments. (e.g., Taskinen & Kortet 2002).

In general, the population dynamics of parasites is tightly bound up with that of hosts, also across trophic levels (e.g., Marcogliese *et al*. 2001a, b), and the effects of parasitism on host populations are naturally based on its effects on host individuals. Fish-parasite systems are no exceptions to the rule, and parasitism affects the growth, condition and reproduction of the fish (e.g., Pennycuick 1971a) and manipulates the behaviour and phenotypes of the hosts (see Kuris 1997; Barber *et al*. 2000; Thomas *et al*. 2005; Barber 2007 for reviews). Furthermore, the effects of parasitism on fish interact with other effects arising from the host's size and age (Pennycuick 1971b; Poulin 2000), its trophic status (Hammar 2000; Knudsen *et al*. 2008a), sex (Pennycuick 1971b; Skarstein *et al*. 2001; Måsvær *et al*. 2004), immune functions (Karvonen *et al*. 2009) and genotype (Kekäläinen *et al*. 2009).

Parasites can also affect the energy metabolism of their hosts. In most host-parasite systems, parasitism increases the host's metabolic rate (Booth *et al*. 1993; Shinagawa *et al*. 2001; Khokhlova *et al*. 2002; Devevey *et al*. 2008) and, thus, its demand for energy. The effects of parasitism on the host's growth depend on the characteristics of the parasite (Poulin 2007) and the host (e.g., Johnson & Dick 2001) as well as on the availability of nutritional resources (e.g., Schwarzenbach & Ward 2006; O'Brien & Dawson 2008). In fish, parasitism may either reduce (Moles 1983; Johnson & Dick 2001) or enhance (Ballabeni 1994; Arnott *et al*. 2000; Ondrackova *et al*. 2004) the growth of hosts. The faster growing fish are assumed to provide a better environment for parasites (Barber 2005) and, therefore, the relationship between the growth of parasites and the growth of fish is interactive.

1.2 Outline of the thesis

The aim of the present research was to investigate the short- and long-term effects of infection by digeneans *Diplostomum* spp. (Trematoda:

Diplostomatidae) on young-of-the-year Arctic charr *Salvelinus alpinus* (L.) from an ecophysiological viewpoint. In this thesis, the term ecophysiological refers to the interaction between the parasite and the fish. It includes the infection dynamics of *Diplostomum* spp. cercariae in the fish and the effects of *Diplostomum* spp. infection on the fish's bioenergetics. The Arctic charr used were from Lake Kuolimo stock that is maintained in hatcheries. The fish represented the critically endangered piscivorous and fast-growing landlocked Lake Saimaa population (Seppovaara 1969). The reasons for the low stocking success and yield of the hatchery-reared Lake Saimaa Arctic charr (Piironen *et al*. 2006) have remained unresolved. It was therefore necessary to investigate the effects of *Diplostomum* spp. specifically on the Arctic charr.

The main questions were: 1) how does the geographical origin and species of the second intermediate molluscan hosts of *Diplostomum* spp. cercariae affect their infectivity in the Arctic charr? (The question has been addressed in publications I–III.) 2) Does *Diplostomum* spp. infection cause energetic costs to the Arctic charr? (IV–VII) 3) Does *Diplostomum* spp. infection alter the allocation of energy in the Arctic charr? (IV–VII) 4) Does *Diplostomum* spp. induced cataract impair the foraging efficiency of the zooplanktivorous Arctic charr? (VII) The questions were addressed by exposing the Arctic charr to the cercariae of *Diplostomum* spp. under controlled laboratory conditions (I–V and VII) and by carrying out oxygen consumption measurements (V–VII) and feeding experiments (VII). The bioenergetic investigations (IV–VII) made it possible to associate the infection of *Diplostomum* spp. with the activity of Arctic charr during the non-feeding period and with the growth rate of individual hosts at different temperatures. *Diplostomum* spp. infection was also linked to measures of the immune defence (spleen size) and physiological condition (liver size) of the parasitised fish (V). The

standard metabolic rates (SMR) of the Arctic charr were measured and related to the measures of *Diplostomum* spp. infection before (VI) and after (V) the infection had caused cataracts in the fish eye lenses. SMR refers to the minimal energetic costs required for maintaining basic body functions (Jobling 1994) and is associated with growth so that fast-growing individuals have higher SMR (Arnott *et al.* 2006; Seppänen *et al.* 2009a, b). In addition to the main objectives, the influence of the Arctic charr on the shedding of *D.* cf. *spathaceum* cercariae from infected snails was studied by carrying out two experiments. The results of these experiments were not reported in a separate publication but in the thesis summary only. In the summary, I aimed at pointing out the main findings of the original publications and at synthesising the results of independent experiments, but also at discussing the results from different viewpoints than those taken in the original publications.

Diplostomum spp. are ubiquitous trematode parasites that use three hosts during their life-cycle (see Ashton *et al.* 1969; Chappell *et al.* 1994; Chappell 1995; Niewiadomska 1996 for reviews). In the second intermediate host of the parasites, i.e. fish, *Diplostomum* spp. are found e.g. the in eyes and the brain. Due to the complex taxonomy of the parasite, the individuals of *Diplostomum* spp. that lodge in the fish eye lens are termed *Diplostomum* cf. *spathaceum* (Knopf *et al.* 2007) in the text of this summary. Evidently, these parasites include more than one species, including *Diplostomum spathaceum* and *Diplostomum pseudospathaceum* (Niewiadomska 1996; Galazzo *et al.* 2002; Niewiadomska & Laskowski 2002).

In publication III, additional species, Atlantic salmon *Salmo salar* L. and *Tylodelphus clavata* (Digenea: Diplostomatidae), were also included, but they are not dealt with in the summary.

1.3 *Diplostomum* spp. and Arctic charr in scientific literature

In international scientific journals, 293 peer-reviewed articles concerning diplostomid eye flukes *Diplostomum* spp. with fish as second intermediate hosts were published between the years 1971 and 2008 (Fig. 1) (Science Citation Index 2009).
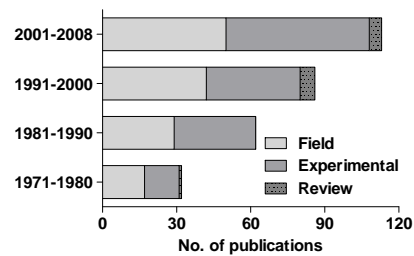
Figure 1. The number of peer-review international scientific articles (*n* = 293) that have dealt with *Diplostomum* spp. eye flukes, in relation to the decade of publication. The type of data (field, experimental, review) used in the articles is indicated by different colours. The data sampled from fish farms have been included in the field data.
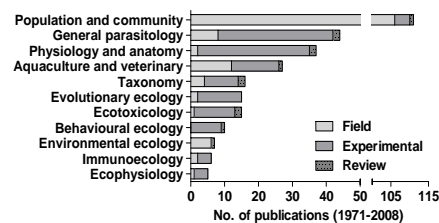
Figure 2. Topics of the peer-review scientific *Diplostomum* spp. articles (*n* = 293) published in international journals between the years 1971 and 2008. The type of data (field, experimental, review) used in the articles is indicated by different colours. The data sampled from fish farms have been included in the field data.

In 111 out of the 293 *Diplostomum* spp. articles (38%), the topic of the study was the population and/or community ecology of the parasites and/or their hosts (Fig. 2). These articles include investigations of parasite fauna in molluscan, piscine and avian hosts of *Diplostomum* spp. The next most frequently investigated topics in the articles were ecological and experimental

parasitology (15% of the publications) and the physiology and anatomy of the parasites and/or hosts (13%) (Fig. 2). Ecophysiology, the topic of the present thesis, was dealt with in only five out of the 293 articles (5%) (Fig. 2). In this case, ecophysiology refers to the effects of parasitism on the host's physiology (and *vice versa*) at the organismal level under different environmental conditions, such as varying temperature. The articles concerning ecotoxicology and evolutionary ecology, including genetics, were published in the 21st century. The *Diplostomum* spp. articles were grouped into different categories according to their abstract and/or the category stated by the journal. If more than one topic was dealt with in the article, it was placed in the category emphasised in the abstract.

The authors who participated in writing the *Diplostomum* spp. articles totalled 409, and the four most productive authors had been involved in publishing 33% of the articles, i.e. 96 out of 293. Most of the articles were published by journals specialising in parasitology, such as Parasitology Research (formerly Zeitschrift für Parasitenkunde) and Parasitology (Fig. 3). Also Journal of Fish Biology and Canadian Journal of Zoology, which do not specialise in parasitology, have published several *Diplostomum* spp. articles (Fig. 3).

Figure 3. The international scientific journals that published most of the peer-reviewed *Diplostomum* spp. articles (*n* = 293) between the years 1971 and 2008. The type of data (field, experimental, review) used in the articles is indicated by different colours. The data sampled from fish farms have been included in the field data.
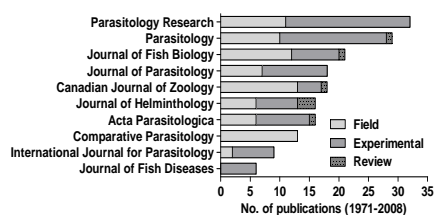
In the same period (1976–2008), Arctic charr was the study species in some 750 international scientific peer-reviewed articles (Science Citation Index 2009). Dozens of these articles dealt with Lake Saimaa Arctic charr, the study animal of the present thesis. The response to predation (Laurila *et al.* 1998; Hirvonen *et al.*, 2000; Vilhunen & Hirvonen 2003; Vilhunen *et al.* 2005; Laakkonen 2006; Vilhunen 2006; Laakkonen & Hirvonen 2007; Lautala & Hirvonen 2008) and the effect of group dynamics on individual fitness (Seppä *et al.* 1999, 2001; Lahti & Lower 2000; Björklund *et al.* 2003) were the most actively studied topics in Lake Saimaa charr. Other investigated topics concerning Lake Saimaa charr were morphology (Pakkasmaa *et al.* 1998; Janhunen *et al.* 2009), genetics (Primmer *et al.* 1999, 2003) and the function of gametes (Piironen 1993; Pakkasmaa *et al.* 2001, 2006; Babiak *et al.* 2002) and red blood cells (Lecklin & Nikinmaa 1998, 1999a, b). Pylkkö *et al.* (2000, 2002, 2005) and Madetoja *et al.* (2003) studied the relationship between *Aeromonas salmonicida* bacteria and Lake Saimaa Arctic charr.

Parasitology has been one of the main topics in Arctic charr research since the 1970s. The research resulted in 57 scientific peer-review articles published between the years 1976 and 2008 (Science Citation Index 2009). The relation of Arctic charr with several directly and trophically transmitted parasites has been well-investigated and -reported. For example, Arctic charr has been found to have a close interaction with the monogenean *Gyrodactylus salaris* (Malmberg) (Bakke *et al.* 1996; Knudsen *et al.* 2007; Robertsen *et al.* 2007, 2008; Winger *et al.* 2008a, b, 2009) and with the cestodes *Diphyllobothrium* spp. (Henricson 1977, 1978; Berube & Curtis 1984, 1986; Halvorsen & Andersen 1984; Knudsen & Klemetsen 1994; Knudsen *et al.* 1996, 2004, 2008b; Hammar 2000; Wright & Curtis 2001; Blanar *et al.* 2005). Arctic charr has also been found to be a suitable host for digeneans, such as *Cryptocotyle lingua* (Creplin) causing the so-called Black Spot (Kristoffersen 1988, 1991; Kristoffersen *et al.* 1994), as well as for *Diplostomum* spp. (e.g., Bouillon & Curtis 1987; Dorucu *et al.* 1995; Due &

Table 1. The species, the class, the prevalence (**P**, mean, %) and the intensity (**I**, mean, parasites infected fish^{-1}) of the parasites found in the wild Arctic charr, as well as the population (**A**nadromous or **R**esident) and the number of examined fish (*n*). In addition to the below listed parasites, Arctic charr have been reported to be a suitable hosts for *Caligus elongatus* (Crustacea) (Mustafa & MacKinnon 1999), *Crepidostomum metoecus* (Digenea) (Knudsen et al. 1997), *Phyllodistomum conostomum* (Digenea) (Kennedy 1978), *Spironucleus barkhanus* (Protozoa) (Sterud *et al.* 1998, 2003; Jørgensen & Sterud 2004) and for amoebae causing nodular gill disease (Speare 1999).

Parasite species (class)	P	I	Population	n	Reference(s)
Echinorhynchus gadi (Acanthocephala)	25	9.3	A	130	Due & Curtis 1995
Metechinorhynchus lateralis	19	18	A and R	172	Bouillon & Dempson 1989
Bothrimonus sturionis (Cestoda)	40	140	A	164	Bouillon & Dempson 1989; Due & Curtis 1995
Cyathocephalus truncatus	44	25	R	843	Liljedal et al. 1999; Liljedal & Folstad 2003; Skarstein et al. 2005; Knudsen et al. 2008b
Diphyllobothrium ditremum	73	81	A and R	1384	Kennedy 1978; Bouillon & Dempson 1989; Due & Curtis 1995; Hammar 2000; Knudsen et al. 2008b
D. dendriticum	44	5.5	A and R	1081	Bouillon & Dempson 1989; Due & Curtis 1995; Knudsen et al. 2008b
Eubothrium salvelini	51	18	A and R	1017	Kennedy 1978; Bouillon & Dempson 1989; Frandsen et al. 1989; Due & Curtis 1995; Liljedal et al. 1999; Hammar 2000; Liljedal & Folstad 2003; Skarstein et al. 2005
E. crassum	14	4.8	A and R	172	Bouillon & Dempson 1989
Proteocephalus exiguus	42	260	R	226	Kennedy 1978; Hammar 2000
P. longicollis	25	59	R	517	Bouillon & Dempson 1989; Frandsen et al. 1989; Due & Curtis 1995
P. tumidocollus	3.5	16	A and R	172	Bouillon & Dempson 1989
Lepeophtheirus salmonis (Crustacea)	57	15	A	290	Bjørn & Finstad 2002
Salmincola edwarsii	11	5.9	A and R	547	Kennedy 1978; Bouillon & Dempson 1989; Due & Curtis 1995
S. carpionis	12	1.9	A and R	307	Bouillon & Dempson 1989; Due & Curtis 1995
Brachyphallus crenatus (Digenea)	69	77	A	92	Bouillon & Dempson 1989
Bunodera luciopercae	8.2	15	A and R	172	Bouillon & Dempson 1989
Crepidostomum farionis	37	30	A and R	598	Bouillon & Dempson 1989; Frandsen et al. 1989; Due & Curtis 1995
Cryptocotyle lingua	7.8	-	A	1008	Kristoffersen et al. 1994
Derogenes varicus	14	2.5	A	222	Bouillon & Dempson 1989; Due & Curtis 1995
Diplostomum spp.*	84	350	R	539	Bouillon & Dempson 1989; Frandsen et al. 1989; Due & Curtis 1995; Liljedal et al. 1999; Liljedal & Folstad 2003; Skarstein et al. 2005
Lecithaster gibbosus	30	52	A	222	Bouillon & Dempson 1989; Due & Curtis 1995
Neascus sp.	34	3.3	A and R	165	Due & Curtis 1995
Phyllidistomum umblae	96	150	R	84	Liljedal et al. 1999; Liljedal & Folstad 2003
Phyllodistomum limnosa	2.5	18	R	80	Bouillon & Dempson 1989
Gyrodactylus salaris (Monogenea)	34	19	A and R	839	Robertsen et al. 2008; Winger et al. 2008a
Tetraonchus alaskensis	1.4	1.0	A and R	72	Due & Curtis 1995
Anisakis simplex (Nematoda)	6.0	1.3	A	149	Due & Curtis 1995
Capillaria salvelini	2.9	8.6	A and R	172	Bouillon & Dempson 1989
Contracaecum sp.	7.6	1.4	R	66	Due & Curtis 1995
C. osculatum/phocae	5.9	1.1	A	130	Due & Curtis 1995
Cystidicola farionis	91	82	R	728	Liljedal et al. 1999; Liljedal & Folstad 2003; Skarstein et al. 2005; Knudsen et al. 2008b
C. cristivomeri	46	230	A and R	212	Eddy & Lankester 1978
Cystidicoloides tenuissima	9.9	4.0	A and R	172	Bouillon & Dempson 1989
Hysterothylacium aduncum	3.8	1.5	A	150	Bouillon & Dempson 1989; Due & Curtis 1995
Philonema onchorynchi	50	3.9	R	247	Kennedy 1978; Knudsen et al. 2008b
P. agubernaculum	26	3.9	A and R	467	Bouillon & Dempson 1989; Due & Curtis 1995
Pseudocapillaria salvelini	26	14	A and R	137	Due & Curtis 1995
Pseudoterranova decipiens	5.3	1.0	A	19	Due & Curtis 1995

*Diplostomum spp. include the species of the genus that locate in the fish eye.

Curtis 1995; Liljedal *et al.* 1999; Skarstein *et al.* 2005). More than 40 freshwater and marine parasite species have been reported to infect resident and/or anadromous Arctic charr. These parasites include acanthocephalans, arthropods, cestodes, digeneans, monogeneans, nematodes and protists (Table 1). Some parasites, such as the crustacean *Caligus elongatus*, have been found to infect Arctic charr in aquaculture conditions (Mustafa & MacKinnon 1999), but rarely in the wild (but see Bjørn & Finstad 2002). To my knowledge, investigations concerning the interactions between parasites and Lake Saimaa Arctic charr have not been reported prior to this thesis.

2 MATERIALS AND METHODS

2.1 *Diplostomum* cf. *spathaceum* – the parasite

2.1.1 Life-cycle

Eggs of *D.* cf. *spathaceum* are sexually reproduced in the intestine of fish-eating birds, such as gulls. The miracidia larvae, hatched from the eggs into the water, infect lymnaeid snails, including e.g. *Lymnaea stagnalis* (L.) and *Radix balthica* (L.). In the snails, the larvae develop from miracidia into cercariae. The cercariae are free-swimming and photo- and surface-orientated after they have emerged from the snails (Haas *et al.* 2008). The contact between cercariae and their fish hosts is passive, so that the cercariae do not actively swim towards the fish (Höglund 1991, 1995; Karvonen *et al.* 2003). After contact the cercariae penetrate the fish skin and migrate into their final site in the fish, the eye lens, where they may remain active for years (Hendrickson 1978). The complex life cycle with three host species and both asexual and sexual stages adopted by *D.* cf. *spathaceum* and many other trematode parasites is suggested to be advantageous for maintaining high genetic variability within the parasite population (Reusch *et al.* 2004; Rauch *et al.* 2005) and for

success in reaching the final vertebrate host of the parasites (Choisy *et al.* 2003).

In the fish, the larvae of *D.* cf. *spathaceum* develop from cercariae into metacercariae, which cause the formation of a cataract in the eye lens by their movements and metabolic excretions (Shariff *et al.* 1980; Karvonen *et al.* 2004b). Moreover, the size of the infected eye lens may be reduced, at least in some fish species (Karvonen & Seppälä 2008). Due to impaired vision as a result of the infection and cataract, both the anti-predator behaviour (Seppälä *et al.* 2004, 2005a, b, 2008b) and the foraging success of the fish (Crowden & Broom 1980; Owen *et al.* 1993; VII) are affected. The life-cycle of *D.* cf. *spathaceum* has been established in the laboratory and described in detail by Ferguson & Hayford (1941), Niewiadomska (1984, 1986), Field & Irwin (1995) and McKeown & Irwin (1995).

2.1.2 Infection dynamics in fish

The infection dynamics of *D.* cf. *spathaceum* has been investigated for decades. The association between *D.* cf. *spathaceum* infection and damage to fish eyes was described in detail first by Palmer (1939) and Ferguson & Hayford (1941). In Europe, the common eye fluke *D. spathaceum* was well-known as much as a hundred years ago (Plehn 1924).

Since the very first studies concerning the biology of *D.* cf. *spathaceum*, the rainbow trout *Oncorhynchus mykiss* (Walbaum) has been the most frequently studied fish species in the experiments. The naïve rainbow trout are highly susceptible to the parasite, and as a result a high proportion of the presented cercariae become established in the eye lenses of the exposed fish (e.g., Betterton 1974; Karvonen *et al.* 2005a; Larsen *et al.* 2005; Seppälä *et al.* 2007). On the other hand, after exposure to the orally administered cercariae of *D.* cf. *spathaceum*, rainbow trout show a longer survival time compared to some other species of salmonids (Speed & Pauley

1984). In addition to rainbow trout, the cyprinid minnow *Phoxinus phoxinus* (L.) has been found to be an especially susceptible species to *D.* cf. *spathaceum* (Sweeting 1974; Ratanarat-Brockelman 1974). In conditions similar to nature, fish react to the *D.* cf. *spathaceum* cercariae by escaping (Karvonen *et al*. 2004a), and it is highly probable that the behavioural response of fish to the presence of *D.* cf. *spathaceum* differs between fish species. Moreover, the penetrating *D.* cf. *spathaceum* cercariae induce the release of alarm substances in the fish skin and conspecifics of the challenged fish react to the signals by changing their behaviour, which in turn reduces their risk of becoming infected (Poulin *et al*. 1999). This causes difficulty in ecological generalisation of the experimental results concerning the infectivity of *D.* cf. *spathaceum* cercariae.

The infection dynamics of *D.* cf. *spathaceum* is also influenced by several abiotic factors, of which water temperature is the definitive one. Temperature has effects on the ability of miracidia to infect the first intermediate snail hosts (Waadu & Chappell 1991), on the shedding of cercariae from the snails (Lyholt & Buchmann 1996), on the migration of larvae into the second intermediate fish hosts (Stables & Chappell 1986a; Lyholt Buchmann 1996) and on the growth of metacercariae in the fish eye lens (Sweeting 1974; IV).

In general, the cercariae of *D.* cf. *spathaceum* are highly infectious to naïve fish, which have no previous contact with the parasite (e.g., Whyte *et al*. 1991). However, the infectivity of the cercariae, as well as the mortality they elicit in fish, varies between the species of parasite (Larsen *et al*. 2005) and fish (Betterton 1974; Sweeting 1974; Speed & Pauley 1984; Karvonen *et al*. 2005b). The infectivity of the cercariae also varies within a snail host population, between the snail individuals from which the larvae have been released (Seppälä *et al*. 2007, 2009). Furthermore, as the miracidia of *D.* cf. *spathaceum* develop into cercariae in snail tissue, the quality

and, presumably, the infectivity of the cercariae are associated with the condition of the host snail (Seppälä *et al*. 2008a).

The cercariae of *D.* cf. *spathaceum* recognise the appropriate fish hosts on the basis of unique chemical cues, which stimulate the larvae to remain attached to the host and to penetrate the skin (Haas *et al*. 2002). The gill region of the fish is the major site for cercarial penetration (Höglund 1991). It is suggested that the cercariae migrate from the site of penetration into the fish eye mostly through the subcutaneous tissue and muscles (Ratanarat-Brockelman 1974), although migrating *D.* cf. *spathaceum* have also been found in the bloodstream (Betterton 1974; Höglund 1991; Haas *et al*. 2007). The circulatory system cannot, however, be the final route for the larvae, because their target in the fish, the eye lens, is avascularised. The rate of migration depends on the secretions of the penetration glands and adequate glycogen reserves of the larvae (Ratanarat-Brockelman 1974). The migration rate of the parasite inside the fish slows down after 20 h at 15 °C, which corresponds to the maximum life-span of *D.* cf. *spathaceum* cercariae at 20 °C (Karvonen *et al*. 2003). The larvae that have not settled in the fish eye lens during that time are phagocytised (Ratanarat-Brockelman 1974).

2.1.3 Fish resistance

After the first contact with the cercariae of *D.* cf. *spathaceum*, the fish become partially resistant to further infections (Höglund & Thuvander 1990; Whyte *et al*. 1990; Karvonen *et al*. 2004c, 2005a). Measures of fish resistance to trematode parasites, in general, have been associated with activity of the phagocytic cells (e.g., Hakoyama *et al*. 2001; Vainikka *et al*. 2009). Furthermore, infection by *D.* cf. *spathaceum* increases the proportion of these cells in the blood of the infected fish, which indicates that the immune system of the fish has been activated (Höglund & Thuvander 1990; Kurtz *et al*. 2006). The question of whether the

increased proportion of phagocytic cells in the presence of *D.* cf. *spathaceum* relates particularly to the killing of the parasites or mainly to the ingestion of those that are already dead by phagocytosis has not been resolved (see Whyte *et al*. 1989). The infective stages of *D.* cf. *spathaceum* are capable of activating fish macrophages (Whyte *et al*. 1990), which in turn have the ability to kill the parasites (Whyte *et al*. 1989). However, the fish macrophages are able to kill the larvae of *D.* cf. *spathaceum* even without activation (Whyte *et al*. 1989). The adaptive, i.e. acquired or specific, immune system is based on a large cluster of genes known as the Major Histocompatibility Complex (MHC) (Frank 2002) and the work of antibodies. Its role in fish resistance to *D.* cf. *spathaceum* is equivocal. The presence of antibodies to *D.* cf. *spathaceum* in the bloodstream of the infected fish has been demonstrated (Bortz *et al*. 1984; Whyte *et al*. 1987), but these demonstrations can be questioned (see Stables & Chappell 1986b). Parasites, in general, show a wide variety of antigens, of which only certain ones are relevant to host immunity (Wakelin 1996). Moreover, trematodes are able to mask their antigenic determinants (epitopes) by absorbing the host-derived macromolecules onto their surfaces and to mimic the host's epitopes by producing host-like determinants (Adema & Loker 1997; Graczyk 1997). Consequently, the presence of antibodies to *D.* cf. *spathaceum* cannot be perceived as unambiguous evidence of the expression of a specific immune response.

In fish immunity to *D.* cf. *spathaceum*, the key point is that the parasite lodges in the fish eye lens – which is out of reach of the immune system – within 24 h (Ratanarat-Brockelman 1974; Höglund 1991; Whyte *et al*. 1991), which may be a too short time to mount the adaptive immune system. Resistance to *D.* cf. *spathaceum* is therefore probably related both to innate immunity and to the slowly mounted adaptive immune mechanisms (Rauch *et al*. 2006) (see Magnadottir 2006 for a review of the innate immunity

of fish). Indeed, it is strongly argued that the innate immunity has immunological memory too (Kurtz 2005). The innate and adaptive immune systems of fish have been found to be co-operative, so that the adaptive system is active especially in the genetic lines of fish that are susceptible to *D.* cf. *spathaceum* due to their ineffective innate immune response (Wegner *et al*. 2007).

2.1.4 Host manipulation

Diplostomum cf. *spathaceum* alters the behaviour of its fish host as a result of impaired vision. The fish that have a heavy infection and/or a cataract are more vulnerable to avian predation (Seppälä *et al*. 2006a) due to the parasite-induced alterations in their escape (Seppälä *et al*. 2004, 2005a), shoaling (Seppälä *et al*. 2008b) and feeding behaviour (Crowden & Broom 1980) and in crypsis (Seppälä *et al*. 2005b). As the piscine predators cannot act as the final hosts for *D.* cf. *spathaceum*, from the parasite's point of view it is not expedient to increase the likelihood that its second intermediate fish hosts will be eaten by another fish. Indeed, chronic *D.* cf. *spathaceum* infection has not been found to increase the risk of piscine predation of the parasitised fish (Seppälä *et al*. 2006b). During the first hours after exposure to *D.* cf. *spathaceum* cercariae, the fish are also more susceptible to piscine predation due to their reduced activity (Brassard *et al*. 1982a), but this is not an actual result of parasite manipulation. In general, if the natural risk of predation in an infected host is low, the parasite's success in transmission may increase if the overall predation risk of the host increases (Seppälä & Jokela 2008).

2.1.5 Role in ecosystem and aquaculture

Diplostomum cf. *spathaceum* is widespread; in Finland the distribution area basically covers the entire country. Infections of the parasite are found in lymnaeid snails (Väyrynen *et al*. 2000; Voutilainen *et al*. 2009a) and fish

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(Ruotsalainen & Ylönen 1987; Valtonen & Gibson 1997; Valtonen *et al.* 1997) both in large and small lakes, and even in fish sampled from the Bothnian Bay in brackish water (Valtonen & Gibson 1997). On average, the infection prevalence appears to be higher in piscine than in molluscan hosts. In the latter, the infection varies seasonally, and the prevalence is observed to be highest during the period of warm water (Brassard *et al.* 1982b), i.e. in Finland in August (Karvonen *et al.* 2006). *Diplostomum* cf. *spathaceum* is able to over-winter, both in snails (Karvonen *et al.* 2006) and in fish (Valtonen & Gibson 1997; Knopf *et al.* 2007).

Table 2. The mean abundance (metacercariae fish^{-1}) and prevalence [%, proportion of the sampled fish (*n*) that were infected] of *D.* cf. *spathaceum* infection in 19 fish species. The fish were sampled in lentic fresh/brackish waters in Europe, including the British Isles between the years 1950 and 2002. The fish represent the species that are found in Finland, including rainbow trout, which is not an original species but dependent on stockings. The young-of-the-year fish had been omitted from the data.

Fish species	*n*	Abundance (Prevalence)	Ref.
Rainbow trout	138	56 (97)	8, 16
Roach	1992	22 (73)	2, 6, 7, 9, 13, 14, 15, 16
Bream	465	20 (78)	4, 6, 9
Silver bream	59	12 (36)	6, 9
Eel	39	8 (92)	3
Ruffe	459	7 (39)	13, 16
Ide	110	6 (74)	12
Gudgeon	10	3 (70)	6
Brown trout	98	3 (26)	5, 8, 16
Burbot	18	3 (17)	13
Perch	1380	2 (29)	5, 7, 10, 13, 14, 15, 16
Tench	39	1 (7)	9
Northern pike	186	<1 (20)	6, 13
Rudd	454	<1 (16)	1, 2, 6, 11
Whitefish	232	<1 (15)	13
Vendace	155	<1 (14)	13
Crucian carp	133	<1 (13)	13
Minnow	68	<1 (11)	13
Nine-spined stickleback	85	<1 (5)	13, 16

References (Ref.): [1]Aydogdu *et al.* 2008, [2]Burrough 1978, [3]Conneely & McCarthy 1986, [4]Dzika *et al.* 2007, [5]Kennedy & Burrough 1978, [6]Kozicka 1958, [7]McGuigan & Sommerville 1985, [8]Moody & Gaten 1982, [9]Rolbiecki *et al.* 1999, [10]Ruotsalainen & Ylönen 1987, [11]Shukerova & Kirin 2008, [12]Sobecka *et al.* 2004, [13]Valtonen & Gibson 1997, [14]Valtonen *et al.* 1997, [15]Valtonen *et al.* 2003 and [16]Wootten 1974.

The mean abundance of *D.* cf. *spathaceum*, metacercariae fish^{-1}, has been observed to be low (<10) in most fish sampled in fresh and brackish water lakes and lagoons in Europe, including the British Isles (Table 2). The highest abundances have been detected mainly in rainbow trout, but also in cyprinids, especially in the roach *Rutilus rutilus* (L.) and the bream *Abramis brama* (L.) (Table 2). Within a fish community, in general, the prevalence and abundance of *D.* cf. *spathaceum* appear to be highest in the abundant cyprinid species and lower in salmonids and percids, excluding the ruffe *Gymnocephalus cernuus* (L.) (e.g., Kozicka 1958; Valtonen & Gibson 1997).

The problem of *D.* cf. *spathaceum* is prevalent in hatcheries and fish farms worldwide. What is worse, in most cases the observed infection has been prevalent and abundant, especially in rainbow trout (Stables & Chappell 1986c; Buchmann & Bresciani 1997; Buchmann *et al.* 1997) but also in walleye *Stizostedion vitreum* (Mitchill) (Muzzall *et al.* 1990), for example. In the case of salmonid fish, many lake populations are maintained by active stocking, which affects the distribution and abundance of the fish. Fish stocking may also affect the abundance and prevalence of *D.* cf. *spathaceum*, because the fish might have been exposed to the parasite during their early life in hatcheries before stocking. The rearing conditions of fish in hatcheries are evidently favourable to the infection by *D.* cf. *spathaceum* (Palmer 1939; Stables & Chappell 1986c; Muzzall *et al.* 1990; Field & Irwin 1994; Buchmann & Bresciani 1997; Buchmann *et al.* 1997; Sangster *et al.* 2004; Larsen *et al.* 2005; III).

As cataracts in farmed fish cause financial loss for aquaculture (e.g., Menzies *et al.* 2002), measures to prevent the presence of *D. spathaceum* in hatcheries have been actively investigated. For example, mechanical filtration of the incoming water and treatment of the water with sodium percarbonate are suggested as sustainable methods for reducing the number of

infective stages of *D.* cf. *spathaceum* in hatcheries (Larsen *et al.* 2005). *Diplostomum* cf. *spathaceum* is also sensitive to anthelmintic drugs (e.g., Björklund & Bylund 1987; Voutilainen *et al.* 2009b), and it is possible to eliminate the parasite in fish e.g. by the use of praziquantel (Bylund & Sumari 1981; Szekely & Molnar 1991).

2.1.6 Research material

To obtain the cercariae of *D.* cf. *spathaceum* for the experiments reported in this thesis, lymnaeid snails *L. stagnalis* and *R. balthica* were sampled from three lakes in Finland, Ylä-Enonvesi (62°03'N, 29°00'E), Pieni Hietajärvi (62°28'N, 30°13'E) and Huumonjärvi (65°06'N, 26°08'E) (Voutilainen *et al.* 2009a) between July 2006 and August 2008. Two snail species were sampled, as one of the aims of the research was to investigate whether the species of snail host affects the infectivity of *D.* cf. *spathaceum* cercariae within a lake. *Lymnaea stagnalis* were found in all the lakes, but *R. balthica* only in Lake Ylä-Enonvesi. Altogether 963 snails were collected and 170 (17.7%) of them were infected with *D.* cf. *spathaceum*. I acknowledge that the species of *D.* cf. *spathaceum* released from *L. stagnalis*, especially in the case of Lake Huumonjärvi, was, most likely, *D. pseudospathaceum*, not *D. spathaceum* (I) (A. Karvonen, pers. comm.). However, because the ultimate objective of this thesis was ecological, the parasites were not identified to the species level, but all of them that infected the fish eye lens were termed *D.* cf. *spathaceum* – as stated in the introductory section. The parasite fauna of the sampled snail populations were investigated at the laboratory of the Ecological Research Institute (University of Joensuu, Finland). The snails that were parasitised by *D.* cf. *spathaceum* were gradually acclimated to non-chlorinated tap water and fed with lettuce *ad libitum*, when it was necessary to keep the snails longer in the laboratory.

2.2 Arctic charr – the host

Arctic charr is the northernmost freshwater fish in the world (Maitland 1995). It is assumed to be the first fish species that colonized the area of Finland after the last glacial period (Seppovaara 1969). This Holarctic species has several morphs and both landlocked and anadromous populations that are ecologically (Klemetsen *et al.* 2003a; Larsson *et al.* 2005) and energetically (Larsson & Berglund 2005) adapted to low water temperatures. Arctic charr are able to grow at 0–22 °C and the growth rate of the species reaches its maximum at 15–17 °C (Wandsvik & Jobling 1982; Brännäs & Wiklund 1992; Larsson & Berglund 1998; Thyrel *et al.* 1999; Larsson & Berglund 2005; Larsson *et al.* 2005). Low temperature is advantageous for the embryos and larvae of the Arctic charr in terms of survival and effective utilisation of yolk reserves (Huuskonen *et al.* 2003). Different morphs of Arctic charr are adapted to foraging either zooplankton, zoobenthos or fish (e.g., Frandsen *et al.* 1989). During the first two years of life, however, all Arctic charr feed almost entirely on zooplankton (e.g., Forseth *et al.* 1994). Arctic charr spawn in autumn and the eggs hatch in spring (see Klemetsen *et al.* 2003b for a review).

Although prevalent and abundant eye fluke infections have been found in lake resident Arctic charr stocks (Bouillon & Curtis 1987; Bouillon & Dempson 1989; Frandsen *et al.* 1989; Dorucu *et al.* 1995; Due & Curtis 1995; Liljedal *et al.* 1999; Liljedal & Folstad 2003; Skarstein *et al.* 2005), there are no reports of the species having been used in experimental exposures to *D.* cf. *spathaceum*.

2.2.1 Research material

The studied Lake Saimaa Arctic charr were hatched at Saimaa Fisheries Research and Aquaculture (Finnish Game and Fisheries Research Institute) in Enonkoski, Finland (62°5'N, 28°54'E) in May 2006, 2007 and 2008. This Lake Saimaa population is critically endangered, and viability of the

population is dependent on stocking and hatcheries that rear the parent fish. Unfortunately, the stocking success of the charr has been low (Piironen *et al*. 2006), and prevalence and intensity of cataract in farmed stocks of the population have been found to be high (Kuukka *et al*. 2006). For the experiments, the Arctic charr were transported from Enonkoski to the laboratory of the Ecological Research Institute (University of Joensuu) mainly in June before the main shedding season of *D*. cf. *spathaceum* cercariae. There the fish were acclimated to tap water and thereafter maintained in flow-through aquaria and fed on commercial dry feed (BioMar®, Aqualife, Denmark) or nauplii of *Artemia franciscana* (Argentemia®, Argent Chemical Laboratories, Redmond, WA, U.S.A.) using a semi-automated feeding system (Fig. 4). The light-dark cycle during the maintenance period was set to 16L:8D or 18L:6D to simulate the natural conditions with a long illuminated period at northern latitudes in summer. The illumination, water temperature and oxygen content in the aquaria were computer-controlled. When necessary, the fish were marked with Visible Implant Elastomer tags (Northwest Marine Technology, Inc., U.S.A.) for individual or group identification. Only young-of-the-year Arctic charr were used in the experiments.
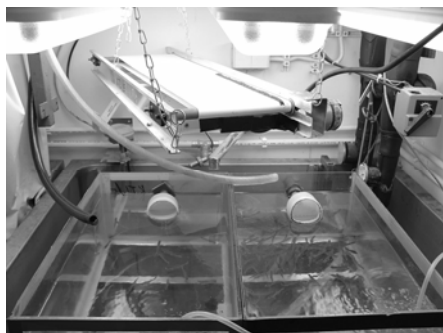

Figure 4. Young-of-the-year Arctic charr in aerated 45 litre flow-through tap water aquaria. The fish were fed by an automated feeding system during the illuminated period.

As an exception to the above stated practice, the experiments reported in the publication V were carried out at the hatchery in Enonkoski (see Seppänen 2008). At the hatchery, the young-of-the-year Arctic charr were "naturally" exposed to *D*. cf. *spathaceum* cercariae via the incoming water from Lake Ylä-Enonvesi.

2.3 Exposure of Arctic charr to *Diplostomum* cf. *spathaceum*

Release of the cercariae of *D*. cf. *spathaceum* from the randomly sampled infected snails *L. stagnalis* or *R. balthica* was stimulated by raising the temperature from 15 to 20 °C within hours (see Lyholt & Buchmann 1996). The cercariae that had emerged from several snails were mixed into the exposure suspension in a bucket (see Seppälä *et al*. 2007). When the aim was to compare the infectivity of the cercariae among the individual snail hosts (II), the fish were exposed to the cercariae that had emerged from a single snail. In most of the challenge exposures, single Arctic charr were placed in transparent plastic beakers in 500 ml of non-chlorinated tap water at 15 °C. After the fish had settled down, *D*. cf. *spathaceum* cercariae were pipetted into the beakers (Fig. 5). The number of cercariae was counted microscopically prior to being added to the exposure suspension.

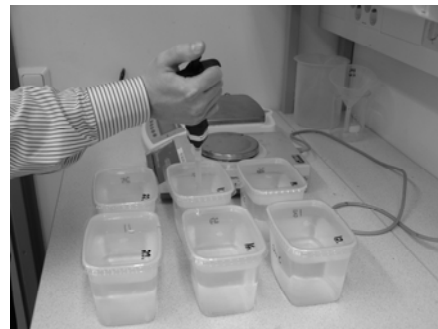*Figure 5.* For exposing the Arctic charr to *D*. cf. *spathaceum* cercariae, single fish were placed in transparent plastic beakers in 500 ml of non-chlorinated tap water. After that, the parasites were pipetted into the beakers.

Depending on the aim of each experiment, the duration of exposure ranged from 5 s to 40 min and the number of parasites from 26 to 2000 cercariae fish^{-1} (Table 3).

Table 3. Summary of the experiments in which young-of-the-year Lake Saimaa Arctic charr ($n = 268$) were exposed to the cercariae of *D.* cf. *spathaceum* at 15 °C and then maintained at the same temperature for 24–48 h. The origin of the parasites (lake and species of their snail hosts) is indicated as follows: A = Lake Pieni Hietajärvi, *Lymnaea stagnalis*, B = Lake Ylä-Enonvesi, *L. stagnalis*, C = Lake Ylä-Enonvesi, *Radix balthica* and D = Lake Huumonjärvi, *L. stagnalis*.

	Arctic charr			Exposure			D. cf. spathaceum		
Publication	n	FM (g), mean ± S.E.	Date (month and year)	Cercariae fish^{-1}, range	Duration, range	Origin	Abundance, mean ± S.E.	Prevalence (%)	
I	72	1.5 ± 0.1	August 2007	41-49	5 sec - 15 min	A, B, C and D	3 ± 0	67	
VI	29	1.9 ± 0.1	August 2008	53-89	5 min	D	2 ± 0	72	
III	45	1.4 ± 0.1	August 2007	41-49	15 min	A, B and D	3 ± 0	76	
II	35	3.5 ± 0.2	September 2007	26-91	5 min	D	3 ± 0	86	
unpublished	9	2.5 ± 0.2	September 2007	42-84	5 min	D	3 ± 1	90	
IV	15	2.0 ± 0.2	August 2008	91-111	5 min	D	6 ± 1	100	
unpublished	12	1.9 ± 0.2	August 2007	41-48	2½-20 min	A and C	7 ± 2	100	
unpublished	6	0.9 ± 0.1	October 2006	200-1000	10-40 min	A	5 ± 1	100	
VII	21	0.9 ± 0.1	September 2006	300-500	25-35 min	A and D	12 ± 2	100	
VII	24	2.1 ± 0.1*	July 2007	500-2000	1-10 min	D	53 ± 8	100	

*Weighed in September 2007

To ensure high infectivity of the cercariae, they were presented to the fish within 6 h after they had been shed from the snails (see Karvonen *et al*. 2003). The challenge exposures were carried out with permission from the committee of the University of Joensuu for animal experiments (meeting 11.4.2006) (publications I, II, III and VII), the committee of the University of Kuopio for animal experiments (licence no. 05-55) (III) and the Finnish National Board of Animal Experiments (licence ESLH-2008-03722/Ym-23) (IV and VI).

After the fish had been removed from the exposure beakers, they were maintained in well-aerated tap water aquaria for 24–48 h. Then the fish were anaesthetised with sodium bicarbonate buffered tricaine methane sulfonate i.e. "MS-222" (Sigma®, Sigma Chemical Co., St. Louis, Missouri, U.S.A.) and decapitated. The eyes of the fish were dissected and compressed between glass plates. The number of *D*. cf. *spathaceum* in the eye lenses was counted using a stereomicroscope (Leica® MZ95, Leica Microsystems, Heerbrugg, Switzerland) (Fig. 6). Some of the Arctic charr had "old" *D*. cf. *spathaceum* metacercariae in their eyes as a result of "natural" exposure to the parasite at the hatchery prior to the challenge experiments. In those cases, the "old" *D*. cf. *spathaceum* were easily distinguished from the newly recruited *D*. cf. *spathaceum* on the basis of parasite size and morphology (Sweeting 1974; IV).
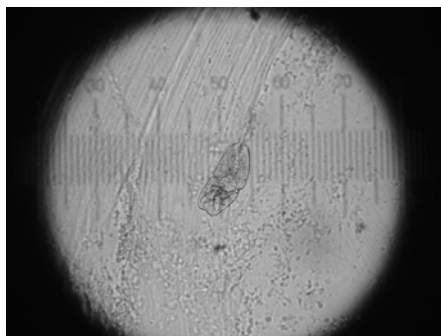
In this thesis, the proportion (%) of presented *D*. cf. *spathaceum* cercariae that were able to settle in the fish eye lens within 24 h after exposure was termed the relative infectivity of *D*. cf. *spathaceum* cercariae in distinction to their *per capita* infectivity (Karvonen *et al*. 2003).

2.4 Measurement of oxygen consumption

The oxygen consumption of the Arctic charr was measured by an automated three-chamber intermittent-flow respirometer (Cyclobios®, Innsbruck, Austria) equipped with a polarographic oxygen sensor (YSI 5750, Yellow Springs Instrument Co., Inc., Yellow Springs, OH, U.S.A.) (Forstner 1983) (Fig. 7). For the measurement, single fish were placed in transparent acrylic chambers (159-242 ml) for 24 or 48 h (Fig. 8).

Figure 7. The computer-controlled three-chamber intermittent-flow respirometer (Cyclobios®, Innsbruck) that was used to measure the oxygen consumption of the Arctic charr.

Figure 6. A light microscopic view of a larva of *D*. cf. *spathaceum* in the Arctic charr eye lens 24 h after the fish had been exposed to cercariae of the parasite.
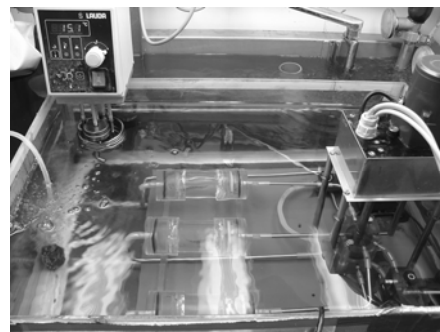

Figure 8. The acrylic chambers of the respirometer. For the measurement of oxygen consumption, single Arctic charr were placed in the chambers.

The oxygen consumption of each chamber was recorded for 15 min in every hour, and the average rate for this time was extrapolated to an hourly value. During each 15 min recording, water flow in the chamber was closed. The flow was not stopped, but the system was closed so that water circulated continuously through the fish chamber bypassing the oxygen sensor.

After the recording, the chamber was flushed for 45 min with water saturated with oxygen. Each 24 and 48 h measurement included 2 h for the determination of bacterial oxygen consumption in the empty chambers at the beginning and end of the measurement period. Most of the measurements were carried out at 15 °C (but see V) with the same photoperiod as during the maintenance (16L:8D or 18L:6D). Prior to the measurement, the fish were made to fast for 24 h.

In this thesis, the SMR of the fish (measured in μmol O_2 g^{-1} h^{-1}) was defined as the average of the two lowest hourly oxygen consumption values recorded during the 24 or 48 h period (Lahti et $al.$ 2002) (V). To control the effect of body size on SMR, the relative SMR (rSMR) for the fish was defined as the difference between the observed and expected oxygen consumption (Metcalfe et $al.$ 1995; Cutts et $al.$ 2002) (VI). To calculate rSMR, the absolute oxygen consumption of each fish (μmol O_2 g^{-1} h^{-1}) was plotted against the fresh mass (FM, g) on the double logarithmic axes. After that, the regression (oxygen consumption vs. FM) was used to calculate the expected SMR dependent on FM. Furthermore, the expected SMR value was subtracted from the observed value, and this difference was termed rSMR.

3 RESULTS AND DISCUSSION

3.1 Infectivity of *Diplostomum* cf. *spathaceum* in the Arctic charr

Three main findings concerning the infectivity of *D.* cf. *spathaceum* in Lake Saimaa Arctic charr were obtained. First, the duration of the cercariae exposure affected the relative infectivity of *D.* cf.

spathaceum, i.e. the proportion of the presented cercariae that were able to establish themselves in the fish eye lens in a given time (I). Moreover, there appeared to be an interaction between the duration of exposure and the parasite's origin in terms of infectivity (I). Second, the success of *D.* cf. *spathaceum* larvae in migrating from the site of penetration to the fish eye lens was associated with the previous infection status of the fish and with the number of penetrated cercariae (II). Third, *D.* cf. *spathaceum* were more infectious to the fish that originated from the same area as the parasite (III). In the case of the above mentioned investigations, contacts between *D.* cf. *spathaceum* and Arctic charr were not prevented or controlled prior to the experiments. Thus, none of the fish were assumed to be naïve in relation to the challenge with *D.* cf. *spathaceum*.

3.1.1 Associations between cercariae exposure, snail host and fish's infection status

In the long exposure duration, i.e. $\geq$5 min, the infectivity of *D.* cf. *spathaceum* cercariae did not vary between the snail host species (*L. stagnalis* and *R. balthica*) of the same lake (Lake Ylä-Enonvesi) or across the *L. stagnalis* populations of three different lakes (Lake Ylä-Enonvesi, Lake Pieni Hietajärvi and Lake Huumonjärvi) (I). In the short exposure duration, i.e. $\leq$60 s, however, the cercariae shed from *L. stagnalis* had higher infectivity than did the cercariae from *R. balthica* of the same lake (I). This may indicate difference in the ability to penetrate the fish host between the *D.* cf. *spathaceum* cercariae developed in the different snail host species.

Among the Arctic charr ($n = 173$) that were exposed to 47 ± 1 *D.* cf. *spathaceum* cercariae fish^{-1} (mean $\pm$ S.E.), the parasite's relative infectivity increased with the increase in exposure duration until the time exceeded 10 min (600 s, Fig. 9A) (Table 3). At this time, the infectivity reached a plateau. Furthermore, the relative infectivity of *D.*

cf. *spathaceum* also related to the number of presented cercariae (Fig. 9B, Table 3). When the fish were exposed to a higher dose of *D.* cf. *spathaceum* (≥400 parasites fish[-1]), the proportion of cercariae that reached the fish eye lens decreased.

The number of *D.* cf. *spathaceum* that settled in the fish eye lens within 24 h after the exposure related positively to the number of *D.* cf. *spathaceum* cercariae fish[-1] in the exposure (Fig. 10, Table 3). The positive relationship found between the number of presented cercariae and that of parasites that were successful in migrating to the fish eye was highly expectable on the basis of the earlier studies by Brassard *et al.* (1982c), Stables & Chappell (1986a), Whyte *et al.* (1991) and Karvonen *et al.* (2003).
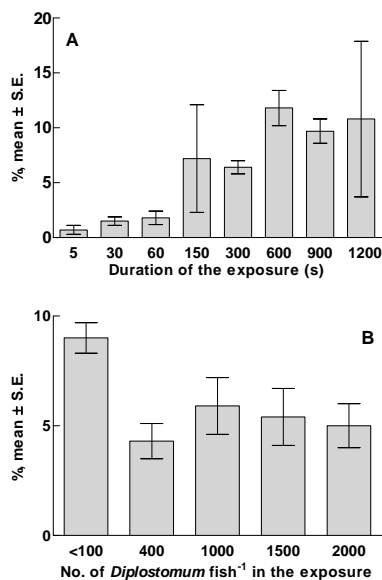

Figure 9. Proportion (%, mean ± S.E.) of the presented cercariae of *D.* cf. *spathaceum* that settled in the Arctic charr eye lens within 24 h in relation to duration of the exposure (Fig. 9A) and the number of cercariae fish[-1] in exposure (Fig. 9B). For the data see table 3.

The proportion of presented *D.* cf. *spathaceum* cercariae that were able to migrate to the fish eye lens decreased with an increase in the number of parasites that penetrated the fish skin (II). The migration success of cercariae was lower in the previously parasitised than in the previously unparasitised fish (II).

Moreover, at the snail host individual level, there appeared to be a trade-off between the penetration and migration ability of the cercariae (II). The Arctic charr (*n* = 172) included in the data presented in figures 9B and 10 were exposed to the cercariae of *D.* cf. *spathaceum* for 751 ± 37 s (mean ± S.E.) (Table 3).
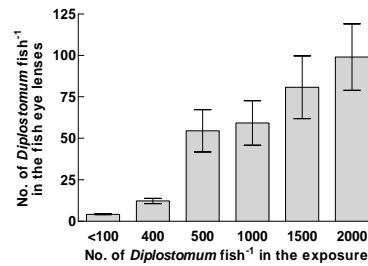

Figure 10. The number of *D.* cf. *spathaceum* fish[-1] (mean ± S.E.) that settled in the Arctic charr eye lens within 24 h in relation to the number of *D.* cf. *spathaceum* fish[-1] in exposure. For the data see table 3.

3.1.2 Geographical origin of cercariae

The cercariae of *D.* cf. *spathaceum* were found to be more infectious to Arctic charr originating from the same area as the parasite (III). The result suggested local adaptation (see Kaltz & Shykoff 1998 for a review) in the relationship between *D.* cf. *spathaceum* and the Lake Saimaa Arctic charr, i.e. the parasite has been able to adapt to its local host population. In the host-parasite coevolution both the hosts and the parasites adapt to the locally common parasite or host populations, but the parasites should have the evolutionary advantage in this arms races over their hosts, due to their shorter generation times, higher mutation rates and larger population sizes, which give them greater evolutionary potential (Kaltz & Shykoff 1998; Lively & Dybdahl 2000; Gandon & Michalakis 2002; Dybdahl & Storfer 2003). This topic was discussed in detail in publication III. I will not therefore repeat the discussion here, while, I do wish to remark on the possibility that the fish resistance is not cross-reactive to *D.* cf. *spathaceum* of different origins.

Although this remark lacks evidence, it is worth consideration and needs to be investigated in the future by carrying out cross-infections.

In this thesis, most of the cercariae of *D.* cf. *spathaceum* used as study parasites originated from *L. stagnalis* that had been sampled from Lake Huumonjärvi. Interestingly, in the relationship between Lake Huumonjärvi *D.* cf. *spathaceum* and Lake Saimaa Arctic charr, the naïve fish were not more susceptible to the parasite than the previously infected conspecifics (Fig. 11). This is not in accordance with earlier findings in rainbow trout (Höglund & Thuvander 1990; Whyte *et al*. 1990; Karvonen *et al*. 2004c, 2005a). I acknowledge that the naïve Arctic charr were hatched in a different year (2008) than the previously parasitised conspecifics, which had been hatched in 2007 (Table 3). Furthermore, the naïve fish were exposed to cercariae that had emerged from different snails in a different year than those to which the previously parasitised fish were exposed. Thus the infectivity of *D.* cf. *spathaceum* cercariae was not truly comparable between the naïve and the previously parasitised charr.
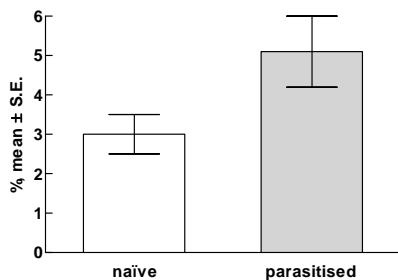

Figure 11. Proportion (%, mean ± S.E.) of the presented cercariae of *D.* cf. *spathaceum* from Lake Huumonjärvi that settled in the Arctic charr eye lens within 24 h after the challenge experiment. The naïve fish had no previous contact with *D.* cf. *spathaceum*, but the parasitised fish were exposed to *D.* cf. *spathaceum* from Lake Ylä-Enonvesi prior to the experiment.

However, the Arctic charr that had been infected in the hatchery prior to the experiments were parasitised by *D.* cf. *spathaceum* from Lake Ylä-Enonvesi –

not by *D.* cf. *spathaceum* from Lake Huumonjärvi. In this case, the key question is: Is it possible that the acquired resistance against *D.* cf. *spathaceum* from Lake Ylä-Enonvesi does not protect the fish against *D.* cf. *spathaceum* of different origin, i.e. Lake Huumonjärvi? Speculatively, if the antigens of *D.* cf. *spathaceum* are strain-specific, the antibodies of fish may recognise only antigens of the parasite strain that the fish had been exposed to. More information concerning the parasite's antigenic determinants to which the host's antibodies are able to bind is needed, before the strain-specificity of the antigens of *D.* cf. *spathaceum* can be discussed more precisely. Immunisation with *D. spathaceum* has been found to elicit no immune response against another species of the genus (*D. gasterostei*) in rainbow trout (Karvonen *et al*. 2009).

On the other hand, if the fish resistance to *D.* cf. *spathaceum* is related both to the innate immunity and to the adaptive defence mechanisms, as stated earlier, part of the immunogenic memory is possibly mediated by mechanisms that are ready immediately after the parasite challenge, not by the specific antibodies (Kurtz 2005; Rauch *et al*. 2006). Furthermore, in addition to the fish immune response, intra- and interspecific interactions between parasites also affect the infection dynamics of *D.* cf. *spathaceum*. Competition between different genotypes within a parasite population has been demonstrated to reduce the load of *D. pseudospathaceum* (Rauch *et al*. 2008) and alter the fitness rank between strains of *D. spathaceum* in the target fish (Seppälä *et al*. 2009). Both negative (Kennedy & Burrough 1977; Burrough 1978; Kennedy 1981) and positive (Karvonen *et al*. 2009) interspecific associations between the fish eye flukes *Diplostomum* spp. and *Tylodelphys* spp. in the double infections have been reported. Moreover, some fish species, e.g. crucian carp *Carassius carassius* (L.) (Karvonen *et al*. 2005b), may be physiologically unsuitable hosts for the parasite.

In conclusion to section 3.1, the infectivity of *D.* cf. *spathaceum* in the Arctic charr was affected by the duration and dose of cercariae exposure, the origin of the parasite (both geographical origin and species of snail hosts) and the previous infection status of the fish. To answer the first of the main questions presented in this thesis, the Arctic charr were more susceptible to *D.* cf. *spathaceum* that were of sympatric origin with the fish. In future, more attention should be paid to the above stated aspects when, e.g., experimental infections of fish with *D.* cf. *spathaceum* and maintenance procedures for endangered fish stocks are being planned.

3.2 Energetics of the *Diplostomum* cf. *spathaceum* infected Arctic charr

The experiments concerning the effects of *D.* cf. *spathaceum* infection and temperature on the energetics of Arctic charr resulted in four main findings. First, total oxygen consumption of the cataract-bearing Arctic charr was higher than that of the healthy-eyed controls when the fish were maintained at 15 °C (VII) (Fig. 12). On the other hand, when the fish were reared and their oxygen consumption measured at 4 °C, the cataract-bearing charr had lower consumption rates than the controls (V) (Fig. 12). Second, in the respirometer, the cataract-bearing Arctic charr showed a weaker response to darkness, i.e. a lower increase in oxygen consumption and activity after switching off the lights, than did the healthy-eyed conspecifics (Fig. 13, 14) (VII). Third, *D.* cf. *spathaceum* infection in the absence of cataract was found to have the potential to stimulate growth of the young-of-the-year Arctic charr at a temperature optimal for growth of the fish (15 °C) (VI). On the other hand, when the charr were reared at 9.5 °C, the growth rates of the parasitised charr were lower than those of the unparasitised conspecifics (IV). Fourth, wide and dense parasite-induced cataracts impaired the feeding capability of the zooplanktivorous Arctic charr (VII). The results indicated interaction between the effects of *D.* cf. *spathaceum* and temperature on the energy metabolism of the Arctic charr.

3.2.1 Associations between infection, oxygen consumption and temperature

In the pooled data of publications V and VII, the effect of temperature on total oxygen consumption of the Arctic charr was statistically significant (Two-way ANOVA: $F_{1, 59} = 50.94$, $P < 0.01$) contrary to the effect of the infection status of the fish (healthy-eyed vs. cataract-bearing) ($F_{1, 59} = 2.39$, $P = 0.13$) (see Fig. 12). However, the interaction between temperature and infection status was statistically highly significant (Two-way ANOVA: $F_{1, 59} = 15.31$, $P < 0.01$) (see Fig. 12). The mean mass-specific total oxygen consumption of the fish measured at 15 °C was higher than that of the fish measured at 4 °C (Fig. 12). But, as the charr measured at 4 °C were larger (FM 2.9 ± 0.2 g, mean ± S.E.) than the fish measured at 15 °C (FM 1.9 ± 0.1 g (mean ± S.E.), the difference in oxygen consumption was smaller than could be expected on the basis of temperature alone.

In general, the total energy costs in fish can be partitioned into three components; SMR, specific dynamic action (SDA) and activity. SMR refers to the lowest oxygen consumption of unfed quiescent fish (e.g., Metcalfe *et al*. 1995; Lahti *et al*. 2002) and SDA includes the energy costs related to the processing of food (e.g., Jobling 1994). If the demand for energy of the activity component is high, it easily masks the energy costs of the other two components (Jobling 1994). In the case of cataract-bearing charr reared at 15 °C, it seemed that the fish were active in the respirometer throughout the measuring period, and thus determining their SMR proved to be difficult (VII). Consequently, the total oxygen consumption of the fish, rather than their SMR, was used in comparing the demand for energy between the cataract-bearing and the healthy-eyed Arctic charr (VII). *Diplostomum* cf. *spathaceum* infection without causing a cataract was found not to cause alterations in the rSMR or in the total oxygen consumption of the parasitised fish (VI).
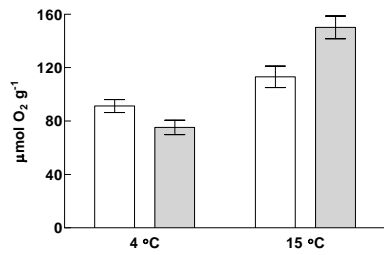
Figure 12. Mean ± S.E. total oxygen consumption (μmol O_2 g^{-1}) of the healthy-eyed controls (open bars) and the *D.* cf. *spathaceum* infected cataract-bearing Arctic charr (grey bars) during the last 20 h in the respirometer at 4 °C ($n = 42$) (V) and at 15 °C ($n = 17$) (VII).

The Arctic charr that were parasitised by *D.* cf. *spathaceum* and maintained at 4 °C ($n = 50$) had a lower SMR and heavier liver and spleen compared to the unparasitised siblings ($n = 50$) maintained at the same temperature (V). However, when three outliers – one fish that was heavier (7.1 g) than the others (2.9 ± 0.1 g, mean ± S.E.), one that had higher SMR (4.004 μmol O_2 g^{-1} h^{-1}) than the others (1.969 ± 0.044 μmol O_2 g^{-1} h^{-1}, mean ± S.E.) and one the mass of whose liver was about 5% of the fish's total mass (mean ± S.E. in the pooled data was 1.21 ± 0.03%) – were excluded from the data, no differences in the FM of the liver and the spleen between the parasitised and unparasitised fish were found. In this reanalysed data, as in the original data, the parasitised charr ($n = 48$) had a lower SMR (1.870 ± 0.065 μmol O_2 g^{-1} h^{-1}, mean ± S.E.) than the unparasitised controls ($n = 49$) (2.066 ± 0.056 μmol O_2 g^{-1} h^{-1}) (*t*-test, $t_{95} = 2.286$, $P = 0.024$). Thus the results did not indicate higher energy consumption of the parasitised fish but *vice versa*. In addition, the reanalysed data indicated that the infection with *D.* cf. *spathaceum* does not induce an immune response in terms of increased size of the spleen. Furthermore, mechanical filtering (mesh diameter 25 μm, to prevent *D.* cf. *spathaceum* infections) of the incoming water was applied for the control fish but not for the parasitised ones. This may have influenced the results, e.g. by also filtering out other cercariae/pathogens. It is also possible that no sign of the induced immune response to *D.* cf. *spathaceum* in the Arctic charr was detected in study V due to the low temperature (4 °C). Although an immune response to vaccines can be elicited in Arctic charr at <10 °C, the response is mounted slowly at such low temperatures (Pylkkö *et al.* 2002). Finally, although frequently used, the size of the spleen may not be as good a measure of immune defense as is generally believed (Vainikka *et al.* 2009).

After exposure to *D.* cf. *spathaceum* cercariae, oxygen consumption of the Arctic charr increased for several hours (VII) as a result of the reaction to the parasite impact (see also Laitinen *et al.* 1996). The observed acute increase in oxygen consumption may have masked some of the energetic costs caused by the rapid immune response. However, this is difficult to show, as a similar increase in oxygen consumption can be detected when fish are exposed to stimuli that are not related to immune defence, e.g. the presence of a predator (Huuskonen & Karjalainen 1997).

It is suggested that the energetic costs of the host's immune defence against parasites relate mainly to the MHC-mediated specific mechanisms (Kurtz *et al.* 2006). As the fish's defence against the primary challenge caused by *D.* cf. *spathaceum* is based on the innate immune system (Rauch *et al.* 2006), the inducible costs of the immune response are expected to be low. Indeed, *D.* cf. *spathaceum* infection was found to cause no direct energetic costs to the Arctic charr in terms of increased SMR (V, VI). However, it is possible that the energetic costs of an acquired immune response, which is mounted slowly (Whyte *et al.* 1987) and appears after multiple exposures to *D.* cf. *spathaceum* (Kalbe & Kurtz 2006), are higher. In the future, the energetic costs arising from the secondary challenge by *D.* cf. *spathaceum* in fish need to be investigated more precisely.

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3.2.2 Darkness induced changes in oxygen consumption

In the wild, the nocturnal feeding activity of young Arctic charr (Adams *et al.* 1988; Jørgensen & Jobling 1989) may be a strategy for reducing intraspecific competition (Alanärä & Brännäs 1997) or an adaptation in order to avoid visual predators that forage mainly in daylight. In general, nocturnal activity of fish is associated with the temperature and time of year (Adams *et al.* 1988; Jørgensen & Jobling 1989; Fraser *et al.* 1993, 1995; Gries *et al.* 1997; Greenwood & Metcalfe 1998). Many species are reported to become nocturnal at low temperatures, especially in winter (Heggenes *et al.* 1993; Contor & Griffith 1995).

In the respirometer, the unparasitised healthy-eyed Arctic charr rapidly increased their oxygen consumption after the lights were switched off (Fig. 13, 14) (V, VI, VII). When the fish's oxygen consumption had increased to the high level, it remained there for several hours, but fell back to the level preceding darkness before the lights were switched on. A similar increase in oxygen consumption was observed in the healthy-eyed fish both at low (4 °C) (V) and at moderately high (15 °C) (VI, VII) temperatures, although the relative increase in consumption was higher at the lower temperature. The highest hourly oxygen consumption rate of the charr during darkness was 2.8 and 2.3 times higher than the mean of their last hourly consumption values preceding darkness at 4 °C (V) and at 15 °C (pooled data of publications VI and VII), respectively (Fig. 13, 14). Increased activity was proposed as the reason for the increased oxygen consumption of the fish.

The highest oxygen consumption rate of the cataract-bearing charr during the dark period was 2.1 and 1.4 times higher than the mean of their last hourly consumption rates preceding darkness at 4 °C (V) and at 15 °C (VII), respectively (Fig. 14). The increase in oxygen consumption as a result of the fish's response to darkness differed between the control and the cataract-bearing charr both at 4 °C (*t*-test, t_{42} = 2.224, $P = 0.032$) (V) and at 15 °C (Mann-Whitney U test, $n = 29$, $Z = -2.440$, $P = 0.013$) (VII) (Fig. 14). The tests were performed for percentual increase in the fish's oxygen consumption.
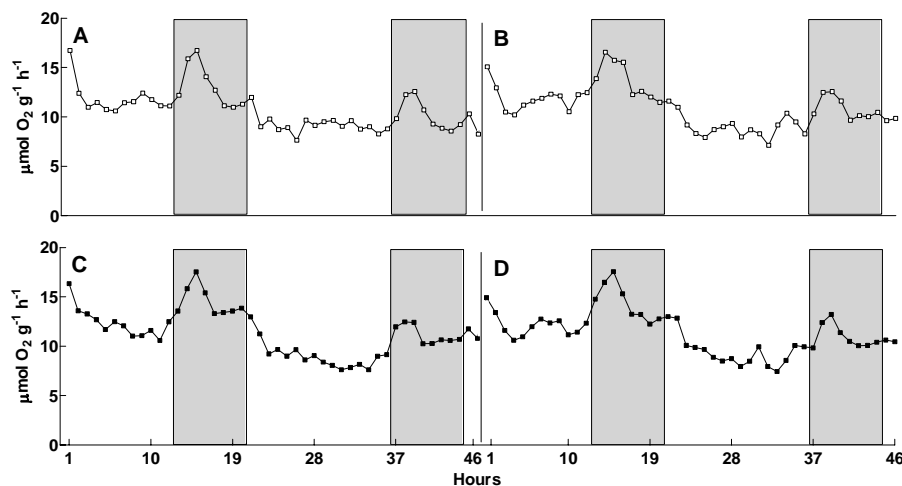

Figure 13. The oxygen consumption (μmol O_2 g^{-1} h^{-1}) of the unparasitised untreated Arctic charr (figures A and B) and that of the parasitised conspecifics that were exposed to *D.* cf. *spathaceum* (C and D), at 15 °C (VI). For the first time, the oxygen consumption was measured prior to the fish having been exposed to the parasite [(A) and C] and the second time 12–40 days (range) after the exposure [(B) and D]. The grey rectangles indicate the periods of darkness. The exposed charr had no cataracts in their eye lenses.

Moreover, the response of the cataract-bearing fish to darkness was delayed, especially at 15 °C (Fig. 14). The highest hourly oxygen consumption of the cataract-bearing fish during the period of darkness was measured 2 h 50 min ± 20 min and 3 h 20 min ± 30 min (mean ± S.E.) after the lights had been switched off, at 4 °C (V) and at 15 °C (VII), respectively (Fig. 14). The highest hourly oxygen consumption of the healthy-eyed fish during darkness was measured 2 h 20 min ± 20 min and 1 h 30 min ± 10 min (mean ± S.E.) after the lights were switched off, at 4 °C (V) and at 15 °C (VI, VII), respectively (Fig. 13, 14).
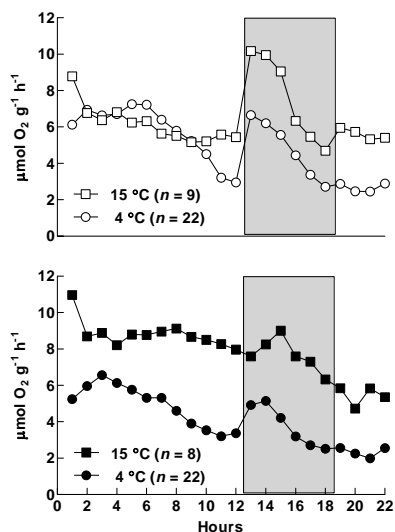

Figure 14. Oxygen consumption (μmol O$_2$ g^{-1} h^{-1}) of the unparasitised healthy-eyed (upper figure, open symbols) and the *D.* cf. *spathaceum* parasitised cataract-bearing Arctic charr (lower figure, solid symbols) measured at 15 °C (squares) (VII) and at 4 °C (circles) (V). The number of fish (*n*) has been given in brackets. The grey rectangle indicates the period of darkness.

To conclude, the altered nocturnal behaviour of cataract-bearing juvenile Arctic charr may impair their abilities to compete for food and to avoid predators. In the absence of cataracts, *D.* cf. *spathaceum* infection had no effects on the response of Arctic charr to darkness (Fig. 13).

3.2.3 Association between growth and temperature

The effect of *D.* cf. *spathaceum* infection on the growth of Arctic charr was related to temperature (IV) and the number of parasite cercariae to which the fish were exposed (VI). The positive relationship between intensity of *D.* cf. *spathaceum* infection/exposure and the growth of Arctic charr was demonstrated under experimental conditions at 15 °C (VI). On the other hand, the growth rates of the *D.* cf. *spathaceum* infected Arctic charr were lower than those of the controls when the fish were reared at 9.5 °C (IV).

The pre-exposure FM and rSMR of the fish that had been exposed to *D.* cf. *spathaceum* cercariae and had been infected by parasites from the challenge did not differ from those of the fish that had been exposed to *D.* cf. *spathaceum* cercariae but had remained unparasitised (VI). The results indicate that the fast-growing Arctic charr did not harbour more parasites, but that exposure to and the infection with *D.* cf. *spathaceum* stimulated the fish to grow faster at 15 °C (VI). Generally, fast-growing fish have high SMR (e.g., McCarthy 2000; Arnott *et al.* 2006; Seppänen *et al.* 2009b).

Among the Arctic charr that were "naturally" exposed to *D.* cf. *spathaceum* in hatchery conditions in June and/or July 2007 and reared at 15 °C from July to August 2007 (*n* = 129) (Table 3), the FM of the fish that had two *D.* cf. *spathaceum* metacercariae fish^{-1} was higher than that of the conspecifics that had only one or no metacercariae (ANOVA, *n* = 129, $F_{2, 126}$ = 22.950, *P* <0.001 and Tukey's HSD test for the post hoc comparisons) (Fig. 15). However, as the Arctic charr had not been weighed before they were "naturally" exposed to *D.* cf. *spathaceum* in the hatchery, I have to acknowledge the possibility that the fish were parasitised because they were large – not *vice versa*.

Contrary to the results reported in paper VI, no difference was found in growth rates between the parasitised and unparasitised charr reared at 15 °C in the experiment reported in publication IV.

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However, in the latter case, the parasitised charr had higher condition factors (K) than the unparasitised conspecifics (the difference in K between the infected and uninfected fish was statistically almost significant, $P = 0.088$) (IV). Thus the results reported in paper IV, do not contradict the finding that *D.* cf. *spathaceum* infection has the potential to enhance the growth of juvenile Arctic charr at 15 °C (Fig. 15) (VI).
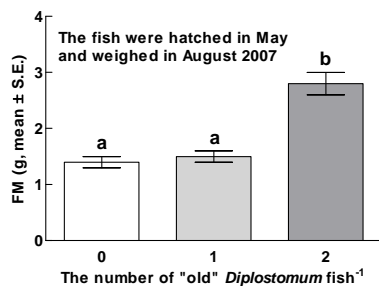

Figure 15. Mean ± S.E. fresh mass (FM, g) of the Arctic charr that had had no ($n = 105$), one ($n = 16$) or two ($n = 8$) *D.* cf. *spathaceum* fish[-1] from "natural" exposure to the parasite in the hatchery in June/July 2007. The different letters above the bars denote significant (ANOVA and Tukey's HSD, $P < 0.05$) differences between the groups. For the data see table 3.

Previously, the cestode *Schistocephalus solidus* has been shown to enhance the growth of the three-spined stickleback *Gasterosteus aculeatus* L. (Arnott *et al.* 2000), and a low dose infection with the digenean *Diplostomum phoxini* has been found to increase the growth rate of the minnow *P. phoxinus* (Ballabeni 1994). *Diplostomum phoxini* has a similar life-cycle to that of *D.* cf. *spathaceum*, but unlike *D.* cf. *spathaceum*, *D. phoxini* locates in the brain, not in the eye lens in fish. As the fish eye lens is avascularised, it is not probable that *D.* cf. *spathaceum* metacercariae could e.g. secrete growth enhancers, as some tapeworms do (Phares 1996). The ultimate mechanism underlying the increased growth rate of *D.* cf. *spathaceum* infected Arctic charr at 15 °C remained unresolved. It has been proposed that the enhanced growth of parasitised fish, in general, may be the result of their decreased spontaneous swimming activity (M. Nikinmaa, pers.

comm.). In consequence of the lower activity, the fish are able to allocate more energy to the growth – if they are reared at the temperature optimal for the growth and fed *ad libitum*.

The observed negative effect of *D.* cf. *spathaceum* on the growth of Arctic charr at 9.5 °C (IV) reveals that at lower temperatures not optimal for the growth of the fish, *D.* cf. *spathaceum* infection may lower the efficiency of its host's energy metabolism. Although the growth potential of the cold-adapted Arctic charr is high especially at low temperatures (<10 °C) (e.g., Brännäs & Wiklund 1992), the growth rate of the species reaches its maximum at 15–17 °C (Wandsvik & Jobling 1982; Brännäs & Wiklund 1992; Larsson & Berglund 1998; Thyrel *et al.* 1999; Larsson & Berglund 2005; Larsson *et al.* 2005). The same inhibiting effect of *Diplostomum* spp. on fish growth was reported by Kolman *et al.* (2001) in the Siberian sturgeon *Acipenser baerii* Brandt. The growth rates of the *Diplostomum* spp. infected sturgeons began to decrease more than those of the unparasitised controls when the temperature fell below 12 °C (Kolman *et al.* 2001).

In the future, the effects of *D.* cf. *spathaceum* infection on Arctic charr growth should also be investigated at high temperatures (<20 °C) and in the presence of food restriction (see Brännäs & Linner 2000). This would be especially interesting because high water temperatures favour *D.* cf. *spathaceum* (Sweeting 1974; Stables & Chappell 1986a; Lyholt & Buchmann 1996) and, possibly, impair the ability of Arctic charr to tolerate stress (see Lyytikäinen et al. 2002). If *D.* cf. *spathaceum* infection increases the growth rate of Arctic charr, it also increases the fish's demand for energy and thus food consumption. I hypothesise that at high temperatures close to the upper thermal limit for the growth of Arctic charr and/or in the presence of limited food resources, the unparasitised fish have a competitive advantage over the conspecifics parasitised by *D.* cf. *spathaceum*. In this case, competition refers to competition for food (zooplankton) among juvenile Arctic charr within a group.

Growth of *D.* cf. *spathaceum* in the Arctic charr. The rates of growth and development of *D.* cf. *spathaceum* larvae in the Arctic charr eye lens at 14.5 °C (IV) corresponded to the rates in the African clawed frog *Xenopus laevis* at 12 °C reported by Sweeting (1974). During the first 11 days, growth of the larvae in the Arctic charr was slow compared to the next three weeks. Five weeks after the larvae had settled in the eye lens their growth ceased. At a lower temperature (9.5 °C), the larvae did not grow at all in 48 days, although they maintained their capability to move (IV).

Development of cataracts. The *D.* cf. *spathaceum* infected Arctic charr (*n* = 50) maintained at 4 °C (*n* = 50) had 1.6 ± 0.1 metacercariae fish[-1] (mean ± S.E.), and cataracts covered 14 ± 1% (mean ± S.E.) of their eye lens area fish[-1] (see Wall & Bjerkås 1999; Karvonen *et al.* 2004b) (V). The infected charr maintained at 15 °C (*n* = 8) had 17.6 ± 2.6 *D.* cf. *spathaceum* fish[-1], and cataracts covered 20 ± 5% of their eye lens area fish[-1] (VII). Although the charr maintained at 4 °C had about ten times fewer parasites than the charr maintained at 15 °C, there was only a small difference in the cataract intensity between the fish maintained at different temperatures (V, VII). However, the fish reared at the lower temperature (4 °C) were maintained at a higher temperature (>10 °C) during the first four months after hatching, and their eyes were examined for *D.* cf. *spathaceum* and cataracts about eight months after the fish had been exposed to the parasite (V). In the case of charr maintained at 15 °C, the eyes of the fish were examined ten weeks after the fish had been exposed to *D.* cf. *spathaceum* (VII). The result demonstrates that even a low level *D.* cf. *spathaceum* infection can result in a high intensity of cataracts, if the infection period is long enough.

Foraging of the cataract-bearing Arctic charr. Infection by *D.* cf. *spathaceum*, even without cataracts, shortens the distance at which the fish react to offered invertebrate prey (Crowden & Broom 1980; Owen *et al.* 1993) and increases the number of unsuccessful attacks (Crowden & Broom 1980). Consequently, the infection reduces the overall foraging efficiency of the fish. In Arctic charr, the fish that had wide and dense *D.* cf. *spathaceum* induced cataracts had a longer reaction time to prey (*Daphnia magna*) compared to the healthy-eyed conspecifics in the same school (VII). In addition, the parasitised cataract-bearing charr caught fewer prey in a given time than did the healthy controls (VII). A low level cataract had no significant influence on the foraging efficiency of the Arctic charr. The effects of cataracts on the feeding capability of Arctic charr were easy to interpret and, therefore, they have been discussed less in the summary.

To answer the main questions of the thesis, *D.* cf. *spathaceum* infection did not cause direct energetic costs to the Arctic charr in terms of increased SMR (V) (answer to question no. 2). No evidence of the energetic costs of *D.* cf. *spathaceum* induced immune response in the Arctic charr was found (V, VI, VII). In the chronic phase of *D.* cf. *spathaceum* infection, however, the total oxygen consumption of the parasitised cataract-bearing Arctic charr was higher than that of the healthy-eyed, unparasitised conspecifics (VII). The reason for this was assumed to be the increased activity of the parasitised fish (VII). It was suggested that *D.* cf. *spathaceum* infection altered the allocation of energy in the parasitised Arctic charr so that, after exposure to the parasite, the fish allocated more energy to growth and less energy to other traits (VI) (answer to question no. 3). The suggestion was based on the finding that the increased growth rates of the parasitised fish had no effect on their SMR, which remained at the level preceding the infection (VI). Lastly, *D.* cf. *spathaceum* induced cataracts, but not the infection itself, impaired the foraging efficiency of the zooplanktivorous Arctic charr (answer to question no. 4).

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3.3 Release of *Diplostomum* cf. *spathaceum* cercariae in the presence of Arctic charr

In addition to the studies reported in the original publications, two experiments were carried out to investigate whether the presence or chemical cues of juvenile Arctic charr affects the release of *D*. cf. *spathaceum* cercariae from the infected snails *L. stagnalis*. The snails were collected from Lake Huumonjärvi in August 2008. They were fed with lettuce *ad libitum* and maintained in the laboratory under natural light conditions for days before the experiments.

In the experiment A, 100 randomly sampled snails were placed singly in transparent plastic beakers in 500 ml of non-chlorinated tap water at 14 °C. After that, 10 ml water from a 6 l bucket, in which six young-of-the-year Arctic charr were kept for 6 h, were pipetted into 50 randomly sampled snail beakers. The snails were maintained in the beakers overnight and the temperature was raised to 19 °C within 8 h. The next morning, the snails were removed from the beakers and the cercariae of trematodes shed from the snails into the water were counted and identified to the lowest taxonomic level possible (Schell 1970; Niewiadomska & Kiseliene 1994; Niewiadomska *et al.* 1997; Faltynkova *et al.* 2007; Santos *et al.* 2007) from 3×10 ml water samples using a stereomicroscope (Leica® MZ95, Leica Microsystems, Heerbrugg, Switzerland). The snails from which no trematode parasites were shed during the experiment were killed by dropping them in boiling water for a few seconds. The killed snails were then crushed and their tissues were examined microscopically for parasites.

In experiment B, first, 24 snails that were parasitised by *D*. cf. *spathaceum* were placed singly in 500 ml transparent beakers filled with tap water at 18 °C. Then, one young-of-the-year Arctic charr was placed in 12 randomly sampled beakers and maintained there for 1 min. Next, the snails were ranked according to the time needed for the release of *D*. cf. *spathaceum* to begin. The beginning of release was defined as the moment when it was possible to see actively swimming cercariae around the snail in the water. The experiment was stopped after 6 h and the snails were removed from the beakers. The released *D*. cf. *spathaceum* cercariae were counted as described in the above paragraph. Experiment B was carried out in the morning, when the natural rate of release of *D*. cf. *spathaceum* cercariae is highest (Karvonen *et al.* 2004d).

In experiment A, 29% of the snails were parasitised by *D*. cf. *spathaceum*, 10% by *Plagiorchis* sp., 3% by *Australapatemon* sp., 1% by *Notocotylus* sp. and 1% by an anonymous echinostomatid. Of the 100 snails, five were double parasitised by *D*. cf. *spathaceum* along with some other trematode. The double infected snails were excluded from the analyses, because the mixed infection might reduce the number of emerging parasite cercariae (e.g., McCarthy 1999). All other identified trematodes but *Plagiorchis* sp. shed cercariae from the snails during the experiment. Out of the 10 snails infected with *Plagiorchis* sp., seven did not shed cercariae, but the infection was detected by the crushing method. Out of the 29 snails infected solely with *D*. cf. *spathaceum*, 13 were treated with the fish water and the remaining 16 were untreated controls. The mean ± S.E. number of the released *D*. cf. *spathaceum* snail^{-1} h^{-1} was 1350 ± 149 in the control snails ($n = 16$) and 1477 ± 239 in the snails treated with fish water ($n = 13$). The rate of release did not differ between the snail groups (*t*-test, $t_{27} = -0.468$, $P = 0.644$). The FM of the control snails (5.0 ± 0.4 g, mean ± S.E.) did not differ from that of the treated ones (4.8 ± 0.4 g) (*t*-test, $t_{27} = 0.371$, $P = 0.713$).

In experiment B, the release of the cercariae of *D*. cf. *spathaceum* from the snails began within 30 min, independent of the treatment. The snails were ranked according to the time needed to induce the release of parasites. The rank was found to be random, so that the cercariae were not shed first either from the control or from the treated snails (Runs-test, r = 13, $P > 0.05$). The number of released *D*. cf.

spathaceum snail^{-1} h^{-1} did not differ between the control (3750 ± 636, mean ± S.E.) and the treated snails (2583 ± 328) (*t*-test, t_{22} = 1.632, *P* = 0.117). The FM of the control snails (6.8 ± 0.2 g, mean ± S.E.) did not differ from that of the treated snails (6.3 ± 0.2 g) (*t*-test, t_{22} = 1.784, *P* = 0.088).

In conclusion, the presence of Arctic charr did not affect the release of *D.* cf. *spathaceum* cercariae from the infected snails *L. stagnalis* in the short term. In general, the release of *D.* cf. *spathaceum* cercariae from snails is highly dependent on temperature (Lyholt & Buchmann 1996) and time of the day (Karvonen *et al.* 2004c). In August, when the experiments were carried out, the rate of release (cercariae snail^{-1} h^{-1}) reaches its maximum early in the morning (Karvonen *et al.* 2004c). Although the release of trematode parasites from the first intermediate snail hosts may be stimulated e.g. by rapid variation in light intensity i.e. "a shade response" in some host-parasite systems (e.g., Raymond & Probert 1987), *D.* cf. *spathaceum* did not respond to the chemical and/or physical presence of the second intermediate fish host. Whether the presence of fish has long-term effects on the production and release of *D.* cf. *spathaceum* cercariae, remained unresolved.

4 CONCLUDING REMARKS

Lake Saimaa Arctic charr were more susceptible to *D.* cf. *spathaceum* of the same geographical origin as the fish (answer to the first main question of this thesis) (III). *D.* cf. *spathaceum* did not cause direct energetic costs to the Arctic charr in terms of increased SMR (V), but in the presence of cataracts the infection increased the total oxygen (energy) consumption of the charr (question no. 2) (VII). However, the restlessness of the cataract-bearing fish in the respirometer at 15 °C impeded determination of their SMR. It was therefore not possible, on the basis of the results of this thesis, to distinguish the supposed energetic costs of innate immunity from those of the specific immune system. It was suggested that *D.* cf. *spathaceum* infection acted as a factor altering the allocation of energy in the Arctic charr so that after exposure to the parasite the infected fish allocated more energy to growth and less energy to other energy demanding traits (question no. 3) (VI). And, lastly, wide and dense *D.* cf. *spathaceum* induced cataracts impaired the foraging efficiency of the zooplanktivorous juvenile Arctic charr (question no. 4) (VII).

In addition to the above stated main findings, the results of this thesis revealed the following conclusions. First, there may be an interaction between duration of cercariae exposure and origin of *D. cf. spathaceum* in terms of infectivity (I). Second, the migration success of D. cf. spathaceum larvae in fish relates both to the first intermediate molluscan and to the second intermediate piscine hosts of the parasite (II). Third, the effects of *D. cf. spathaceum* infection on the growth of Arctic charr seem to be associated with temperature (IV). Fourth, *D.* cf. *spathaceum* infection did not induce an immune response in the Arctic charr at 4 °C in terms of increased size of the spleen. And fifth, the presence of charr or chemical cues from the fish had no short-term effects on the release of *D.* cf. *spathaceum* cercariae from the infected snails *L. stagnalis*.

The results suggested that the consequences of *D.* cf. *spathaceum* infection and especially those of cataracts for the zooplanktivorous young-of-the-year Lake Saimaa Arctic charr are substantial. The overall effect of *D.* cf. *spathaceum* on the Arctic charr may be detrimental to the fish.

Several factors that arise from 1) the origin of the parasite and fish, 2) intra- and interspecific associations between the parasites (the topic was not treated is this thesis), 3) the immune functions of the host and 4) abiotic factors, such as varying temperature, affect the infection dynamics of *D.* cf. *spathaceum* in fish. Moreover, the factors may interact with each other. Generally, the observed infection dynamics of *D.* cf. *spathaceum*

in Lake Saimaa Arctic charr was found to be in accordance with that found in other salmonid fish, mainly rainbow trout, used in earlier investigations. The infectivity of *D.* cf. *spathaceum* pooled over the studied groups of cercariae – which had emerged from two snail species (*L. stagnalis* and *R. balthica*) from three lakes (Lake Ylä-Enonvesi, Lake Pieni Hietajärvi and Lake Huumonjärvi) – appeared to be quite low in Lake Saimaa Arctic charr. However, it is difficult to state whether Arctic charr, in general, are resistant or susceptible to *D.* cf. *spathaceum* without carrying out experimental comparisons between fish species.

A low level of infection by *D.* cf. *spathaceum* had the potential to stimulate the juvenile Arctic charr to grow faster at a temperature optimal for the growth of the fish (15 °C). It was suggested that the increased growth rate was a result of the fish's response to the parasite impact rather than due to the fish being parasitised because they were large or fast-growing. The fish with a high rSMR were not found to harbour more parasites than the conspecifics with a lower rSMR (VI). The possibility that *D.* cf. *spathaceum* is able to manipulate the growth of its fish hosts to enhance its own transmission to the final bird hosts cannot be excluded. The increased growth of *D.* cf. *spathaceum* infected fish presumably results in increased foraging and thus increased risk of avian predation. However, this behaviour favours *D.* cf. *spathaceum* if the parasite's metacercariae located in the fish eye lens are fully-developed and ready for transmission. Speculatively, the enhanced growth of *D.* cf. *spathaceum* infected juvenile Arctic charr may, in fact, increase their survival in the short term (e.g., Rice *et al.* 1993). The increased survival of parasitised fish, in turn, improves the chances of *D.* cf. *spathaceum* metacercariae to become fully-developed. To summarise, *D.* cf. *spathaceum* appears to aim at maximising its chance of transmission, first, by increasing the survival of its fish host during the early stage of metacercarial infection, and then, when the metacercariae are fully-developed, by increasing the host's risk of avian predation (Seppälä *et al.* 2004, 2005a, b, 2008b).

An intense *D.* cf. *spathaceum* induced cataract impaired the feeding efficiency and altered the nocturnal behaviour of juvenile zooplanktivorous Arctic charr. It is highly probable that the fish that have been heavily parasitised by *D.* cf. *spathaceum* in hatcheries have a lower survival rate in the wild after stocking because of their reduced abilities to compete for food and to avoid predators. Consequently, procedures that aim at preventing a high level of infection with *D.* cf. *spathaceum* in the Arctic charr in hatcheries are of vital importance to the further existence of the fish. For example, elimination of *D.* cf. *spathaceum* cercariae from the incoming water by mechanical filtration (Larsen *et al.* 2005) and discouraging the presence of piscivorous birds (the final hosts for *D.* cf. *spathaceum*) and snails (the first intermediate hosts for *D.* cf. *spathaceum*) in vicinity to fish farms (Sangster *et al.* 2004) have been suggested as sustainable methods to prevent the infection in fish. It is also possible, but perhaps not practical, to kill the cercariae of *D.* cf. *spathaceum* before they have penetrated the fish (Larsen *et al.* 2005; Voutilainen *et al.* 2009b) or the metacercariae after they have settled in the fish eye lens by chemical treatment (Bylund & Sumari 1981; Björklund & Bylund 1987; Szekely & Molnar 1991). However, the ability of fish eye to recover from a cataract and other mechanical damage caused by *D.* cf. *spathaceum* after *in vivo* treatment with e.g. praziquantel needs to be investigated in detail prior to planning treatment protocols for fish.

Finally, I here point out some questions concerning the host-parasite interaction between Arctic charr and *D.* cf. *spathaceum* that should be addressed in the future. 1) Does *D.* cf. *spathaceum* infection affect the energy metabolism of charr reared at high temperatures (>15 °C)? This question is timely because cold-adapted charr are possibly threatened by

ongoing global warming (see Rosenzweig *et al.* 2008). 2) Does the parentage of young Arctic charr affect their susceptibility to *D.* cf. *spathaceum*, as it appears to affect their metabolic and growth rates (Pakkasmaa *et al.* 2006; Eilertsen *et al.* 2009)? 3) Does *D.* cf. *spathaceum* infection affect group and population dynamics in Arctic charr? If the infection stimulates the fish to grow faster, it is possible that the parasitised, fast-growing fish become more aggressive and dominant to satisfy their increased demands for energy and food. This in turn may alter the social hierarchy of the group to which the fish belong. 4) Do *D.* cf. *spathaceum* induced cataracts impair the foraging of benthivorous Arctic charr? In aquaculture conditions, the growth rates of *D.* cf. *spathaceum* parasitised cataract-bearing charr are not seriously affected by their impaired vision if the fish are fed dry feed that sinks to the bottom of tanks (pers. obs.). The capability of cataract-bearing Arctic charr to forage live zoobenthos must therefore be tested under controlled conditions.

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