JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 139

Anssi Karvonen

Transmission of Diplostomum spathaceum between Intermediate Hosts

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ambiotica-rakennuksen salissa (YAA303)
toukokuun 8. päivänä 2004 kello 12.

Academic dissertation to be publicly discussed, by permission of
the the Faculty of Mathematics and Science of the University of Jyväskylä,
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2004

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Editors
Jukka Särkkä
Department of Biological and Environmental Science, University of Jyväskylä
Pekka Olsbo, Marja-Leena Tynkkynen
Publishing Unit, University Library of Jyväskylä

Cover photo: Intensive parasite-induced cataract in the eye of rainbow trout. Photo taken by Andy Dobson.

URN:ISBN 9513917746
ISBN 951-39-1774-6 (PDF)

ISBN 951-39-1749-5 (nid.)
ISSN 1456-9701

Jyväskylä University Printing House, Jyväskylä 2004

ABSTRACT

Karvonen, Anssi
Transmission of *Diplostomum spathaceum* between intermediate hosts
Jyväskylä: University of Jyväskylä, 2004, 40 p.
(Jyväskylä Studies in Biological and Environmental Science,
ISSN 1456-9701; 139)
ISBN 951-39-1774-6
Yhteenveto: *Diplostomum spathaceum* -loisen siirtyminen kotilo- ja kalaisännän välillä
Diss.

Transmission rate between hosts is a major determinant of fitness in parasites with complex life cycles and this has favoured the development of sophisticated adaptations in parasites to increase the probability of transmission. On the other hand, increasing pressure by parasites has forced the target hosts to develop means to control or prevent the infections. This study examined the transmission of the trematode parasite *Diplostomum spathaceum* between the two intermediate hosts, the freshwater snail (*Lymnaea stagnalis*) and rainbow trout (*Oncorhynchus mykiss*), focusing on the parasite strategies in cercarial production from the snail host in laboratory conditions and responses of the fish host to cercarial infection. Infected snails released cercariae for an extended period of time in accordance with the bet hedging hypothesis, which suggests spreading the risk of transmission failure in an environment with an unpredictable host contact probability. Bet hedging was not found in association with cercarial quality, but cercariae were released in higher numbers during day compared to night. High prevalence and intensity of infection in wild fish populations suggests that the parasite strategies are likely to maintain effective transmission to fish. As a response against the cataract-causing parasite, fish hosts acquired physiological resistance, which decreased the number of establishing parasites although the resistance proved to be insufficient to prevent cataract formation. However, this study demonstrated for the first time that the fish also decreased exposure to the parasite by avoiding the cercariae. Thus, a combination of resistance and avoidance behaviour may provide more efficient defence against the parasite. Individual differences between fish in exposure to the parasite and ability to avoid cercariae probably cause the parasites to aggregate in a few host individuals, which has important implications for the parasite population dynamics and transmission. As a practical application, the results of this study allow the estimation of key parameters in the parasite transmission dynamics, which can be used in designing preventative methods against *D. spathaceum* outbreaks in fish farming industry.

Key words: Acquired resistance; bet hedging; exposure; infectivity; *Lymnaea stagnalis*; *Oncorhynchus mykiss*; parasite aggregation; parasite avoidance; parasite-induced cataract; survival; susceptibility; terminal investment.

Anssi Karvonen, Department of Biological and Environmental Science, P.O. Box 35, FI-40014, University of Jyväskylä, Finland

Author's address Anssi Karvonen
Department of Biological and Environmental Science
University of Jyväskylä
P.O. Box 35
FI-40014 University of Jyväskylä
Finland
e-mail: anskarv@bytl.jyu.fi

Supervisors Professor Tellervo Valtonen
Department of Biological and Environmental Science
University of Jyväskylä
P.O. Box 35
FI-40014 University of Jyväskylä
Finland

Professor Peter Hudson
Biology Department
Penn State University
208 Mueller Lab
University Park, PA 16802
USA

Reviewers Professor Robert Poulin
Department of Zoology
University of Otago
P.O. Box 56
Dunedin
New Zealand

Docent Jukka Jokela
Department of Biology
University of Oulu
P.O. Box 3000
FI-90014 University of Oulu
Finland

Opponent Dr. David Marcogliese
Environment Canada
St. Lawrence Centre
105 McGill Street
Montreal, Quebec
Canada H2Y 2E7

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on five original papers, which will be referred to in the text by their Roman numerals (I-V). I am responsible writing the papers, except for II, which was written jointly with Peter Hudson. I also carried out a large part of the planning, data collection and analysis in each paper, except for I where the data were collected by Satu Kirsi.

I Karvonen, A., Kirsi, S., Hudson, P. J. & Valtonen, E. T. 2004. Patterns of cercarial production from *Diplostomum spathaceum*: terminal investment or bet hedging? Parasitology, in press.

II Karvonen, A., Paukku, S., Valtonen, E. T. & Hudson, P. J. 2003. Transmission, infectivity and survival of *Diplostomum spathaceum* cercariae. Parasitology 127: 217-224.

III Karvonen, A., Seppälä, O. & Valtonen, E. T. Eye fluke-induced cataract formation in fish: quantitative analysis using an ophthalmological microscope. Submitted.

IV Karvonen, A., Hudson, P. J., Seppälä, O. & Valtonen, E. T. 2004. Transmission dynamics of a trematode parasite: exposure, acquired resistance and parasite aggregation. Parasitology Research 92: 183-188.

V Karvonen, A., Seppälä, O. & Valtonen, E. T. 2004. Parasite resistance and avoidance behaviour in preventing eye fluke infections in fish. Parasitology, in press.

1 INTRODUCTION

1.1 General introduction

Parasites are organisms, which utilize the resources of free-living organisms, the hosts, causing harm to them (Price 1980). The parasitic way of life is probably one of the most common life forms on earth. Indeed, it has been suggested that more than half of the species known to science are parasitic (May 1988, Windsor 1998), being either obligatory parasites (i.e. totally dependent on their hosts) or having a parasitic stage in their life cycle. Because of this astonishing diversity of parasite species, host organisms often provide a resource for entire parasite communities (Holmes & Price 1986, Esch et al. 1990, Esch & Fernandez 1993, Karvonen & Valtonen 2004). Parasites were almost completely neglected in ecological studies until the 1970s, but studies such as those of Anderson & May (1978) and Hamilton & Zuk (1982) focused the attention of ecologists and evolutionary biologists towards the role of parasites in host population dynamics and sexual selection. Subsequently, parasites have received increasing attention in scientific literature concerning the ecology and evolution of their hosts (see Poulin 1995). There is now mounting evidence suggesting that parasites, for instance, affect life history traits of their hosts such as reproduction and behaviour (Price 1980, Esch & Fernandez 1993, Combes 2001, Moore 2002), shape the structure of host populations (Dobson & Hudson 1992, Hudson et al. 1998) and maintain sexual reproduction in host populations (Hamilton 1980, Lively 1987, Hamilton et al. 1990).

An essential feature in parasite life histories is the evolution of life cycles. The diversity of life cycles ranges from simple ones including one host species to increasingly complex cycles including interactions between the parasite and multiple host species (Esch & Fernandez 1993, Combes 2001, Moore 2002). The fundamental evolutionary reasons for the development of complex life cycles are unclear (Poulin 1998, Choisy et al. 2003, Parker et al. 2003), but current hypotheses argue for adjustments to historical events or accidents, parasite

fitness benefits and increased cross-fertilisation rate in trophically transmitted parasites (Poulin 1998, Brown et al. 2001, Choisy et al. 2003 for review). Complex life cycles have developed independently in several taxonomic parasite lineages including for instance nematodes, cestodes and trematodes (Mackiewicz 1988, Shoop 1988, Rohde 1994, Poulin 1998). The taxonomic group of trematodes is one of the largest parasite taxa, containing more than 15000 described species (Poulin 1998). Trematode life cycles generally include three hosts: molluscan first intermediate host, poikilothermic second intermediate host and poikilo- or homoiothermic vertebrate definitive host, but shorter life cycles, for instance with two hosts, are also known (Poulin & Cribb 2002).

Whether or not complex life cycles have evolved as a result of natural selection, the inclusion of additional phases in the cycle has also imposed costs for the parasites. A major challenge is the transmission from one host to another, which has led to the development of specific infective stages with intricate properties to complete this task (Esch & Fernandez 1993, Combes et al. 1994, Poulin 1998, Combes 2001). In the course of the life cycle, parasites also encounter different physiological host environments and are exposed to attacks from a range of host immune responses. Understanding of the transmission biology and dynamics of any parasite-host system therefore requires estimates of the production of the infective stages and their survival and transmission, but also how target hosts respond to infection. This information has an essential role in studies concerning the evolution of parasite-host relationships, but also in practical applications such as prevention of parasitic threats in food production.

Transmission is also a major determinant of parasite fitness (Anderson & May 1991, Hudson et al. 2002), but it generally is a highly unpredictable event since it requires both spatial and temporal overlap between the hosts and the infective stages and the evasion of host defence mechanisms. This is particularly obvious in parasites with complex life cycles, such as trematodes, where the parasites pass through a series of hosts and the parasite fitness is determined by a cascade of successful transmission events. As a consequence of this uncertainty, a majority of the infective stages used in the transmission may be lost because of mortality and parasite-induced mortality of the hosts (Dobson et al. 1992). However, such conditions have created evolutionary pressure for the development of various reproductive and transmission strategies in each step of the life cycle to increase the probability of successful transmission.

1.2 Reproductive strategies and transmission probability

One obvious strategy to enhance transmission is to produce high numbers of infective stages to increase contacts with the target hosts. Parasites are not limited by the amount of available resources and therefore they generally have

a reproductive potential several orders of magnitude greater than free-living counterparts (Dobson et al. 1992, Poulin 1998). However, the exploitation of host resources (parasite virulence) cannot be increased indefinitely. Theoretical models predict that virulence should evolve towards an optimal level, which is specific for each system and determined first by maximal parasite reproductive output through resource depletion from the hosts, and on the other hand, by risk of host death (Anderson & May 1982, Ebert & Herre 1996). Negative effects of infection on host condition, however, may alter the optimal reproductive strategy of the parasite and lead to changes in the host exploitation rate as the infection proceeds.

A distinctive feature in life cycles of trematode parasites is the transmission using free-living cercaria larvae released from the molluscan first intermediate host to infect the second intermediate host (Combes et al. 1994, Fried 1997). According to general life history theory, trematode cercariae are equal to reproductive units and the reproductive value of these parasites is maximal just prior to the release of the first cercaria, decreasing thereafter as more cercariae are released (Williams 1966, Stearns 1992, Jokela et al. 1999). If it was advantageous to maximise the production of cercariae to increase contact probability with the next host, trematodes should elevate the rate of host exploitation as the remaining reproductive value decreases, although this would result in earlier death of the molluscan host (Williams 1966, Jokela et al. 1999). In the life history theory, this is known as the 'terminal investment' or 'terminal effort' hypothesis (Clutton-Brock 1984, Candolin 1999, 2000, Ericsson et al. 2001, Yoccoz et al. 2001, Weladji et al. 2002).

Once trematode cercariae have been released from a molluscan host, they usually face an unpredictable environment in terms of probability to contact the next host. As life history theory predicts an increase in cercarial production with age of infection, an alternative strategy to increase the probability of transmission could be to maximise the period of cercarial release by reducing the rate of host exploitation and saving the host resources as the infection proceeds. This is a form of "bet hedging", which seeks to spread the risk of reproductive failure in an unpredictable environment, where the probability to complete a stage in the life cycle is low. Bet hedging has been considered for several systems of free-living organisms (Philippi & Seger 1989, Bradford & Roff 1993, Simovich & Hathaway 1997, Hopper 1999, Menu et al. 2000, Menu & Desouhant 2002), but only recently for parasites (Fenton & Hudson 2002).

In addition to patterns of cercarial release from molluscan hosts, bet hedging in trematodes may operate on the scale of resource allocation to cercariae. Since the contact rate between the infective stages and desired host is variable in both space and time, parasites should not necessarily allocate resources equally between the infective stages but should generate variability between individuals to ensure that at least some infection is taking place. Trematode cercariae are equipped with a limited set of non-renewable energy resources, usually in the form of glycogen (Anderson & Whitfield 1975, Smyth & Halton 1983, Tielens 1997), and once these are depleted, the cercariae die. In

this respect, there is a trade-off between the two components of cercarial quality, survival and infectivity; highly infectious cercariae will not survive for long and may fail to contact a host whereas long-lived individuals may have insufficient resources for infection once the contact with the host finally occurs. Parasites could use one of several alternative strategies in cercarial energy allocation to maximise transmission probability and these strategies can be interpreted indirectly by examining the characteristics of cercarial survival and infectivity in relation to age of cercariae.

1.3 Responses by target host

As parasites, such as trematodes, evolve strategies to enhance their transmission to the second intermediate hosts, hosts are likely to experience increasing pressure from the potentially harmful or even life-threatening parasites (Holmes & Bethel 1972, Price 1980, Combes 1991, 2001, Esch & Fernandez 1993, Poulin 1994, Lafferty & Morris 1996, Moore 2002). This pressure generated by parasites has led to the evolution of an array of counter-adaptations in hosts to prevent infections or minimise their effects. A major physiological barrier against parasites is the immune system, which in vertebrates generally includes non-specific immunity replenished by specific responses and immunological memory after the first encounter with a pathogen (Manning 1994, Turner 1994, Wakelin 1996). However, maintaining physiological immunity is energetically costly; resources needed for immunity are diverted from other life history traits such as growth and reproduction. In this respect there is trade-off between immunity and other life history traits (Sheldon & Verhulst 1996, Zuk & Stoehr 2002). Therefore, organisms should not invest maximally in immunity, but the optimal level of investment depends first on the degree of parasite exposure and parasite virulence, and on the other hand, on factors such as risk of autoimmunity (Zuk & Stoehr 2002). In general, it could be expected that highly virulent parasites with a marked impact on host condition should elicit stronger counter-responses in hosts (Ewald 1995). Moreover, because of costs associated with immunity, natural selection may favour development of alternative defence mechanisms, provided that these are energetically less expensive that the costs of infection (Moore 2002, Rigby et al. 2002). This would include avoidance of circumstances where the infection is most likely to take place, which would decrease the exposure to parasites. Such avoidance behaviour has been described in several parasite-host systems (Poulin & FitzGerald 1989a, Folstad et al. 1991, Hart 1994, 1997, Hutchings et al. 1998, Moore 2002, Wilson et al. 2002).

1.4 Aims of the study

In this study, I examined the transmission of the trematode parasite *Diplostomum spathaceum* between the two intermediate hosts, the freshwater snail (*Lymnaea stagnalis*) and the rainbow trout (*Oncorhynchus mykiss*), focusing on the parasite reproductive strategies within the snail host (I, II), effects of the parasite on its fish host (III) and responses of the fish against the potentially harmful infection (IV, V). The first paper examined the patterns of cercarial release from snail hosts and tested two hypotheses of host exploitation: terminal investment and bet hedging. In the second paper, I explored the characteristics of cercarial survival and infectivity in relation to cercarial age, and examined if there was variation in these features in accordance with bet hedging hypothesis. Furthermore, in this paper I studied epidemiological aspects related to the process of cercarial transmission to the fish host (II). In the third paper, I focused on the effects of the parasite on the fish host after successful establishment by quantifying the relationship between the intensity of parasite-induced cataracts and parasite numbers in relation to different patterns of exposure. In papers IV and V, I examined if fish developed physiological resistance against this potentially harmful parasite in natural conditions for infection and how effectively this resistance could protect the fish against the parasite. Moreover, defence against parasites may also include other aspects such as parasite avoidance by the host and therefore in the last paper I explored if such behaviour occurred in the present study system, in the infection of fish hosts by the parasite cercariae (V). This study is a part of a larger project, which aims to capture the essential parameters in all steps of the parasite life cycle to find ecologically friendly and cost-effective ways to control parasite outbreaks in fish culture.

2 MATERIALS AND METHODS

2.1 Study species (I-V)

The complex taxonomy of the genus *Diplostomum* has been under considerable debate, and is still not completely resolved (Valtonen & Gibson 1997 and references therein). According to morphological measurements of metacercariae, fish in northern Finland carry two forms of diplostomids in the lens (Valtonen & Gibson 1997), the majority of which resemble *D. indistinctum*, referred to as *D. spathaceum* by Niewiadomska (1986). In this study, I considered parasites found in the lens as *D. spathaceum*, but recognise that other species may have also been present. Diplostomids are also found in the vitreous body of the eye (e.g. Kennedy & Burrough 1977, Kennedy 1981, 1987), but these were not covered in this study.

Diplostomum spathaceum s.l. is one of the most common parasites in fish and has received considerable attention in various contexts in the literature (e.g. Burrough 1978, Brassard et al. 1982, Whyte et al. 1991, Haas et al. 2002, Morley et al. 2003). The parasite is found abundantly both in fresh water and brackish water habitats (e.g. Valtonen & Gibson 1997, Valtonen et al. 1997) and occasional outbreaks have also been reported in fish farms (Stables & Chappell 1986a, Field & Irwin 1994). The life cycle of the parasite includes three hosts, fish eating bird, snail and fish (Fig. 1). Adult parasites mature in the intestine of the bird definitive host, where they produce eggs as a result of sexual reproduction (e.g. Chappell et al. 1994). Several species of birds are likely to be suitable definitive hosts for the parasite, although fish eating birds belonging to the genus Laridae and Sternidae are the most commonly proposed. Eggs are released to aquatic systems along with bird faeces and hatch into miracidia, which seek out the snail first intermediate hosts. Successful infection in a snail gives rise to sporocysts in which cercaria larvae are formed through asexual reproduction. Therefore, in the case of a single miracidial infection, cercariae originating from one particular snail are clones and carry identical genetic

information. The development of the patent infection may take 4-10 weeks depending on the water temperature (Chappell et al. 1994). Cercarial release from snails is also temperature dependent (Lyholt & Buchmann 1996) and the transmission to fish takes place in temperatures exceeding 10 °C (Stables & Chappell 1986a, b, McKeown & Irwin 1997). Cercariae penetrate the fish mainly through the gills, but also through other parts of the body (Whyte et al. 1991, Höglund 1995). Once cercariae come in contact with fish epithelium, they shed their tail and migrate through host tissues settling in the lens of the eye. Establishment into the lens must take place within 24 hours from infection (Erasmus 1959, Ratanarat-Brockelman 1974, Whyte et al. 1991) and parasites not succeeding in this are subsequently killed by host defences. In the lens, parasites develop into long-lived metacercarial stages and are protected from host defences since the lens lacks blood vessels and circulating antibodies. Metacercariae excrete metabolites to the lens, which may cause opacity or even blindness in heavy infections (Shariff et al. 1980), often referred to as a parasite-induced cataract. The life cycle is completed when an infected fish is eaten by the avian definitive host.

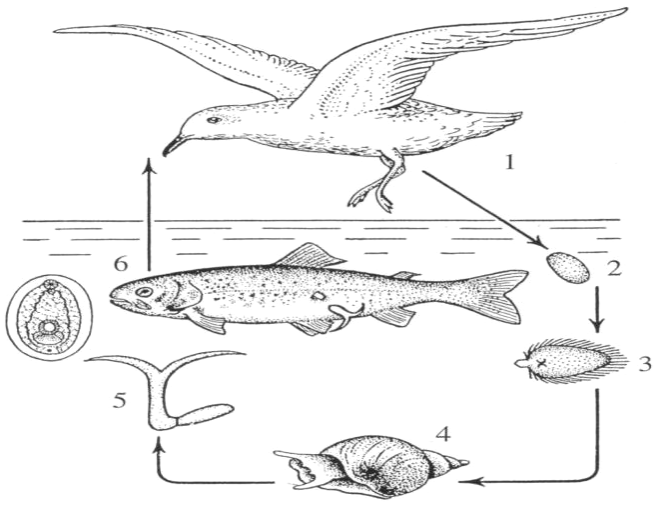

FIGURE 1 The life cycle of *Diplostomum spathaceum*; 1 = sexual reproduction in the avian definitive host, 2 = egg, 3 = miracidium, 4 = asexual reproduction in the snail first intermediate host, 5 = cercaria, 6 = metacercaria in the lens of the fish eye (Modified from Dogiel et al. 1961).

2.2 Fish material (II-V)

I used rainbow trout (*Oncorhynchus mykiss*) of 0+ or 1+ year of age as a model fish species in all studies (II-V). Fish were obtained from two commercial fish farms, one in Central Finland and the other in Southern Finland. Rainbow trout was chosen because of its easy availability and relatively high susceptibility to *D. spathaceum* infection (e.g. Betterton 1974). It could also be expected that

farmed fish raised in the same shoal would have a similar background, which would minimise individual differences in host age, size, physiological condition and previous exposure to the parasite.

2.3 Procedures for exposing fish to the parasite

The experiments of this study used either controlled laboratory exposures with known parasite doses or natural exposure with temporally variable exposure level. In the laboratory exposures (II, III, V), parasite cercariae were obtained from naturally infected *Lymnaea stagnalis* snails collected from a commercial fish farm in Central Finland. Infected snails were separated from uninfected by following the cercarial production in a small amount of water. Prior to the experiments, snails were maintained in 30 l aquaria in cold temperature (5 ^{o}C) and provided lettuce in excess. Afterwards, cercarial production was induced by bringing the snails to room temperature. In each experiment, cercariae were produced from 10 or more infected snails and the combined cercarial suspension from the snails was used in the infection trials. Cercarial density in the suspension was estimated from a minimum of ten 1 ml sub-samples from which the number of cercariae was counted under a dissection microscope. Cercarial infectivity decreases with age (II) and therefore no cercariae older than six hours were used in the experiments, except for paper II in which I examined age-related changes in cercarial infectivity. Fish were exposed individually (II, V) or in small groups (III). I used cages anchored to the littoral zone of Lake Konnevesi for the infection experiments in natural conditions. Cages were 120 x 80 x 100 cm (IV, V) or 150 x 100 x 100 cm (III), made of wood strips and covered with soft net (mesh size 10 mm), which allowed the parasite cercariae to pass through. Cages were placed in shallow lake water (depth ca. 1.5 m) amongst the densely vegetated littoral zone where the intermediate snail hosts for the parasite were known to reside.

2.4 Determination of cataract and infection intensity

Parasite-induced cataract intensity was assessed from intact eyes using the Kowa SL-14 portable slit-lamp microscope (Wall & Bjerkås 1999). In the examination, a narrow slit of light is directed into the lens which gives a three-dimensional view into the lens, and provides a comprehensive and actual picture of the position and intensity of the cataract. Cataract intensity was assessed using a categorical scale from 0 to 4: 0 = no cataract, 1 = cataract covering horizontally < 50 % of the lens area, 2 = cataract covering < 100 %, 3 = cataract covering 100 % and 4 = cataract covering 100% and lens appearing totally opaque and white. In paper III, cataract intensity was determined from

dead fish immediately after euthanasia whereas in paper V, the intensity was assessed repeatedly from the same fish individuals under anaesthesia (MS-222 as anaesthetic). The cataract intensity was recorded from both eyes of the fish. The number of *D. spathaceum* metacercariae in fish was determined by dissecting both lenses and compressing them individually between two glass plates under a microscope. Newly established parasites could be distinguished from older, fully developed ones according to their size and morphology (Sweeting 1974). In paper IV, the slit-lamp microscopy was used in determining the number of parasites in living, anaesthetised fish, followed by dissection of the eye lenses from euthanised fish at the end of the experiment. Parasite numbers in fish given in papers II-V indicate the sum for both lenses.

3 RESULTS AND DISCUSSION

3.1 Transmission strategies

In the complex life cycles of trematode parasites, the transmission between the first and the second intermediate hosts is principally carried out using cercaria larvae. Because the probability of reaching the next host is low, several trematodes have developed intrinsic behavioural and physiological adaptations to enhance the transmission of cercariae (e.g. Kennedy 1979, Théron 1984, Lewis et al. 1989, Combes et al. 1994, Haas 1994). In this study, I focused on the reproductive strategies of *Diplostomum spathaceum* within its snail host by studying cercarial production patterns from the snail host and variability associated with survival and infectivity of cercariae.

3.1.1 Patterns of cercarial release (I)

General life history theory suggests that we can consider cercariae as reproductive units and, therefore, if it were advantageous to maximise the cercarial numbers in order to increase transmission, the rate of host exploitation should increase with period of infection (Williams 1966, Jokela et al. 1999). In support of this hypothesis, reduced survival of trematode-infected molluscs has been reported, especially in harsh environmental conditions (Meuleman 1972, Becker 1980, Lauckner 1986, Jensen et al. 1996, Jokela et al. 1999, Krist et al. 2004). Alternatively, if the parasite was maximising the period of cercarial release instead of numbers produced, then, according to the bet hedging theory (Fenton & Hudson 2002), the parasite should reduce cercarial production with time to save the host resources (McCarthy et al. 2002). When naturally infected snails collected from a fish farm were kept under constant laboratory conditions, the production of cercariae decreased as the death of the host was approached, in support of the second strategy (I). It should be noted, however, that the parasite could increase the relative host exploitation as the infection

proceeds without consequent increase in cercarial production if the amount of resources available to the parasite had declined (I).

The snail host *Lymnaea stagnalis* usually has two overlapping annual generations in Finland; over-wintered snails reproduce during spring and early summer giving rise to the second generation (Väyrynen et al. 2000). Since the development of the parasite sporocysts within the snail takes several weeks and is dependent on the ambient water temperature (Waadu & Chappell 1991, Chappell et al. 1994), the second generation snails infected in late summer and autumn probably do not produce cercariae until next spring. The snails used in this study were collected from the fish farm in July when they were already shedding cercariae (I). Therefore, the actual age of the infection and the length of the total production period could not be determined, but the maximal production time of cercariae in natural conditions is probably substantially longer than the period of 67 days observed in the laboratory conditions (I). The transmission of the parasite to fish occurs during summer months when water temperature exceeds 10 ^{o}C (Stables & Chappell 1986a, McKeown & Irwin 1997), which in Finnish conditions corresponds to a period of ca. 4 months. Thus, it is possible that the optimal strategy for the parasite to increase the transmission probability to the fish host would be to extend the cercarial shedding to encompass the whole available transmission period (Fenton & Hudson 2002, McCarthy et al. 2002). However, the mortality of the infected snails was higher compared to uninfected individuals indicating that ultimately infection by the parasite leads to earlier death of the host (I). This seems reasonable since after an extended period of cercarial production, the parasite should not necessarily save the resources of the weakened host for winter when conditions are harsh and probability of host death is high (e.g. Meuleman 1972, Lauckner 1986, Jensen et al. 1996, Jokela et al. 1999). These aspects need further experiments on the parasite life history strategies operating in natural conditions as well as studies on the infection dynamics in natural snail populations.

When the snails were held under constant laboratory conditions with a natural light-dark cycle, they shed significantly more cercariae during day compared to night (I). Several trematode species show temporal changes in diurnal shedding of cercariae (Rees 1948, Asch 1972, Lewis et al. 1989), and in schistosomes infecting humans and other mammalian hosts, shedding patterns clearly correspond to the active time of their desired definitive host (Theron 1984, Combes at al. 1994). *Diplostomum spathaceum* uses several species of fish as the second intermediate hosts and most of these are active during daytime (Helfman 1986, Wootton 1990, Wieser 1991). Since the parasite transmission to fish is passive (see below), the synchronisation of the cercarial shedding with the activity of the host may increase the probability of successful transmission. If the number of cercariae was relative to the amount of resources extracted from the host, reduced shedding during night could also save host resources and extend the total period of cercarial production in accordance with the bet hedging hypothesis (Fenton & Hudson 2002, McCarthy et al. 2002). However,

the mechanisms underlying the diurnal cercarial release need more detailed studies (see Shostak & Esch 1990).

3.1.2 Variability in the properties of cercariae (II)

The bet hedging theory suggests that a single genotype could code for several different phenotypes to spread the risk of reproductive failure in an unpredictable environment (Philippi & Seger 1989, Fenton & Hudson 2002). Since trematode cercariae originating from an individual snail are likely to carry identical genetic material (see Dybdahl & Lively 1996, Sire et al. 1999) and have variable probability of host contact, it could be expected that natural selection would have favoured bet hedging operating on the level of cercarial energy allocation. The results in paper II do not support this as both mortality and infectivity of *D. spathaceum* cercariae showed an age-dependent pattern indicating that the cercariae carried roughly equal resources and once these were depleted, the cercariae died. This is surprising since *D. spathaceum* cercariae usually have low probability to contact a suitable fish host after leaving the snail.

Trematodes are known to become locally adapted to their hosts (Lively 1989, Ballabeni & Ward 1993, Lively & Jokela 1996, Lively & Dybdahl 2000) and it is likely that parasites may quickly respond to changes in the host population structure. The snails used in this study were collected from a fish farm where the fish host density and consequent host contact probability are constantly high. Therefore, it could be expected that selection would favour rapid development of local parasite strains in which the individual genotypes would code for similar lifetime and infectivity traits of cercariae, such as short-lived and highly infectious, which would be beneficial in conditions of high host availability. A corresponding phenomenon has been observed in the egg hatching dynamics of the fish louse (*Argulus foliaceus*), which shows a peaked, short-term egg hatching pattern in fish farms, but an extended pattern in lakes where the fish density and host contact probability are lower (Pasternak et al. 2000). This can be seen as a bet hedging strategy aiming to maximise transmission according to host availability (Fenton & Hudson 2002).

To verify this in the case of *D. spathaceum* would require studies on cercarial qualities also in lake populations. The homogeneity of the parasite genotypes within the farm is a function of genotype mixing through the visits by different avian definitive hosts. The mobility of birds is undoubtedly higher than the fish hosts of *Argulus* and therefore it would seem unlikely that the parasite gene flow, for instance from nearby lake populations, could be inhibited. Moreover, several other trematode species belonging to different taxonomic lineages show similar age-dependent survival and infectivity of cercariae (Anderson & Whitfield 1975, Evans 1985, McCarthy 1999, Whitfield et al. 2003). This would reflect a general pattern in the survival and infectivity of trematode cercariae and suggests that there is no unequal division of resources to the infective stages, which would imply bet hedging.

In general, since trematodes are found in all types of environments infecting a wide range of host taxa, they show a variety of cercarial transmission modes and strategies with distinct species-specific differences corresponding to detailed features in the transmission process (e.g. Esch & Fernandez 1993, Combes et al. 1994). Indeed, even closely related species may have marked differences in cercarial shedding patterns and characteristics of cercariae (Taskinen et al. 1991, Gibson et al. 1992). Some trematodes have also truncated their life cycles for instance by using the same molluscan host as the first and the second intermediate host (Poulin & Cribb 2002). Therefore it may be difficult to draw general conclusions on the strategies related to production and transmission of trematode cercariae especially given that our knowledge of the majority of trematode systems is limited. However, it is probable that these elements are determined separately in each particular system as a result of specific selective forces.

3.2 Effects of *D. spathaceum* on fish host

As parasites have developed a range of adaptations to enhance their transmission (Combes 2001), and generally have negative effects on host condition by obtaining resources for their own growth and reproduction, target hosts are expected to invest into prevention of infections. Investment into defence may be a function of parasite virulence; parasites with higher virulence, such as those severely depleting the host resources or manipulating the host to enhance transmission, should generally evoke intensive counterattack by the host (Ewald 1995). In the fish host, metacercarial stages of *D. spathaceum* are found in the lens of the eye, which may predispose fish to effects of the parasite in this delicate system. Indeed, the parasite is known to induce eye cataracts (Ferguson & Hayford 1941, Marcogliese et al. 2001), which in severe cases may lead to total blindness and even destruction of the lens (Shariff et al. 1980). Infection by the parasite may impair the feeding ability of fish, reduce growth and also predispose fish to predation (Crowden & Broom 1980, Owen et al. 1993, Buchmann & Uldal 1994, Seppälä et al. 2004). Although the role of the cataract in these processes is not known, it is likely that it has a key role in impairing the vision of fish and causing the secondary effects during the chronic phase of infection.

Trematodes are known to affect their second intermediate hosts in an intensity-dependent manner, i.e. the effects of infection increase with number of parasites (e.g. Lafferty & Morris 1996). Similarly, in the case of *D. spathaceum*, cataract intensity increased with parasite burden and, on average, eyes harbouring more than 20 metacercariae had intensive cataracts (III). This is not surprising since the parasite causes cataracts by excreting metabolic waste and damaging the lens structure while moving in the lens (Shariff et al. 1980), effects of which are likely to be increased with increasing parasite numbers. However,

it is important to quantify the parasite load above which the deleterious effects in fish begin to appear as this has important implications for the development of resistance in the fish host (see below), and for the evolutionary ecology of this trophically-transmitted parasite (Seppälä et al. 2004).

The relationship between parasite burden and cataract intensity is likely to be affected by several factors such as species-specific differences, and size of the fish and corresponding volume of the lens. For instance, fish species differ in their susceptibility to infection (Betterton 1974, Hendrickson 1978, Valtonen & Gibson 1997), which reflects different physiological performance of the parasite between host fish species. The pattern of infection may also affect cataract formation; since the migrating parasites must pass through the lens surface for successful establishment, it could be expected that many concurrent infections could damage the lens and result in more intensive cataract formation. When fish were exposed to a single experimental infection or a long-term natural infection, fish in natural conditions with gradual accumulation of parasites showed more intensive cataract formation in relation to parasite burden (III). This implies that a relatively high number of parasites may enter the lens within a narrow time frame without a subsequent increase in cataract formation. This seems reasonable since, if the parasite could increase the predation susceptibility of fish by inducing cataract formation (see Seppälä et al. 2004), the cataract should not appear before the metacercariae are fully developed and infective to the definitive host, which may take several weeks from infection (Chappell et al. 1994).

3.3 Responses of fish host to infection

Since increasing parasite numbers may have serious effects on the fish host (Crowden & Broom 1980, Owen et al. 1993, Buchmann & Uldal 1994, Seppälä et al. 2004), it could be expected that fish hosts would aim to prevent infection. Indeed, workers have detected both non-specific and specific immunological responses in fish against infection in laboratory conditions (Bortz et al. 1984, Stables & Chappell 1986c, Whyte et al. 1987, 1989, 1990, Höglund & Thuvander 1990). However, studies have not considered the ecological significance of resistance or been conducted in natural conditions with a natural pattern and level of infection, which could provide more realistic insight into development of resistance and its ability to protect the fish (Hellriegel 2001). The infection trials in which rainbow trout were exposed to natural infection conditions indicated that fish developed resistance against the parasite, which decreased the number of parasites established in subsequent infections (IV). Although the data showed the development of resistance in an ecological setting, they could not be used to estimate the actual efficiency of physiological resistance. Whyte et al. (1987) showed that acquired resistance in rainbow trout decreased parasite establishment by 70-80 %, but this still allows at least 20 % of the parasites to

reach the eye. Similarly, it was observed in paper IV that parasite numbers increased in one of the cages as a consequence of increase in exposure, which indicates that large numbers of parasites may still establish in fish that have acquired some resistance. If we wish to estimate the efficiency of physiological resistance, it is essential to consider the effects caused by the parasites, which establish in fish despite resistance. Long-term experimental caging of previously exposed fish at the same location in L. Konnevesi indicated that the fish in restricted cages, without a possibility to avoid infection, developed intensive cataracts as a result of natural exposure to the parasite (V). This suggests that physiological resistance is not sufficient to protect fish against the parasite and implies that it would be necessary for the fish to reduce exposure to the parasite through other mechanisms such as avoiding the infection.

In natural conditions, *D. spathaceum* cercariae are released from infected snails in great numbers (I) after which they maintain themselves in the water column using short swimming bursts. The life span of the cercariae is also short (II). This implies that in normal lake conditions with low water currents, cercariae probably cannot disperse far within the narrow lifetime, but are mainly concentrated in proximity to the shedding snails. Furthermore, the prevalence of the parasite in natural snail populations is usually low (Väyrynen et al. 2000) with only a few percent of snails shedding cercariae. These aspects suggest that cercariae are patchily distributed in water. Moreover, epidemiological infection trials exploring the density and frequency dependent nature of the cercarial transmission process (Anderson & May 1978, McCallum 2000, McCallum et al. 2001) indicated that the transmission coefficient (β) decreased with total number of infective stages, which implies that the transmission is essentially frequency dependent (II). In other words, the relative number of parasites established in fish decreased with total number of infective stages in larger water volumes indicating that in larger tanks, fish became infected within a specific home range and that the infection was determined by host movements (II). The result also reflects the passive host finding of the parasite cercariae, supported by previous findings (Whyte et al. 1991, Höglund 1995). These aspects suggest that it would be beneficial for fish to recognise patches with high cercarial densities and initiate avoidance behaviour before heavy infection occurs.

Behavioural trials conducted in the laboratory indicated that fish recognised the presence of the cercariae and showed avoidance behaviour, which significantly decreased the number of establishing parasites (V). Parasite avoidance has been described in several parasite-host systems where hosts show a variety of means to detect infection [reviewed by Hart (1994) and Moore (2002)]. In the present system, three possible mechanisms in parasite detection can be considered. First, it is possible that fish might observe the cercariae visually, although this is unlikely since cercariae are small and virtually transparent. Cercarial density used in the experiment was also relatively low and possible grouping of cercariae (‘clouding’), which could make them visible to fish as a group, was therefore unlikely. However, this is possible in natural

conditions where cercarial densities are likely to be higher in close proximity to the snails (I). Second, cercariae could release specific odours detectable by fish, but this is also unlikely since Poulin et al. (1999) found that rainbow trout did not respond to odours of *Diplostomum* spp. cercariae. When the parasite cercariae come in contact with fish epithelium, they use enzymes stored in the specific penetration glands to enter the fish (Tielens 1997). Thus, the most probable mechanism in this system is mechanical stimuli triggered by penetrating cercariae, although this allows some cercariae to establish in fish before the avoidance takes place. This mechanism is also supported by the observations of Laitinen et al. (1996) that fish did not respond to the presence of non-invasive cercariae, but did show marked physiological reactions when exposed to *Diplostomum* spp. cercariae.

The mechanical detection of cercariae also suggests that the avoidance behaviour is not specific against *D. spathaceum*, but probably operates also against other invasive trematode species. However, although not verified in this study, it could be that changing cercarial density affects the ability of fish to detect the infection. Some trematodes shed relatively low numbers of cercariae or the cercariae are actively dispersed to the surroundings in contrast to *D. spathaceum* (e.g. Taskinen et al. 1991, Esch & Fernandez 1993, Combes at al. 1994). Thus, it is possible that these cercariae are not found in the high density patches typical of *D. spathaceum*, which might be required for detection, but further studies with different parasite species are needed to explore these aspects in detail. It is also difficult to evaluate whether the detection of cercariae and subsequent avoidance behaviour are adaptations developed by fish against trematode parasites since fish could respond to any abnormal stimuli in the ambient water. Studies on the relative effectiveness of the avoidance behaviour in fish originating from different habitats with different levels of exposure could shed light on this issue. Nevertheless, the ability of fish to respond to cercariae, adaptive or not, seem to be beneficial for the fish as it decreases the rate of exposure to harmful parasites.

The significance of avoidance behaviour for individual fish may change when fish are gathered in shoals where individuals usually are able to respond to signals from conspecifics. Therefore, avoidance responses initiated by infection in some individuals may be followed by others without an actual contact with cercariae. Fish also release alarm substances from their skin when exposed to *Diplostomum* spp. cercariae, which initiate responses in conspecifics (Poulin et al. 1999), but may also act on an individual level if fish are able to respond to alarm substances released from their own skin. In conclusion, although shoaling itself is likely to decrease the probability to encounter parasites, such as certain ectoparasites (Poulin & FitzGerald 1989b), individual responses to infection followed by responses in conspecifics may further decrease the exposure of fish living in shoals.

In general, responses of fish against trematode species have received relatively little attention compared for instance to species with medical significance to humans or livestock. However, it could be expected that fish

would respond differently to trematodes with different pattern of establishment or site of infection. Responses should also be proportional to the damage caused by infection. In the case of *D. spathaceum*, it is reasonable that fish evoke strong responses against the parasite since infection has severe effects on fish and the parasite infects an immunologically privileged site where it probably cannot be prevented once the establishment has occurred. Therefore physiological attack against the parasite must take place within 24 hours from exposure, which undoubtedly requires rapid and effective immunological responses. Indeed, the 70-80 % reduction in parasite establishment after the first exposure (Whyte et al. 1987), replenished by the avoidance of parasite cercariae (V), could be considered rather intensive response by the host. However, in the light of the current knowledge it is difficult to proportion these responses to ones acting against other fish-infecting trematodes. It may be that trematode species infecting other parts of the fish should not necessarily evoke similar immediate and intensive responses in host since these parasites can be controlled also after the establishment for instance by encysting them. These aspects need further studies especially on the interactions between the site of parasite infection, parasite harmfulness to the host and the intensity of host responses.

3.4 Parasite aggregation

Exposure to parasites and subsequent host responses have important implications for the distribution of parasites in host populations. A fundamental feature in most parasite-host interactions is the aggregated distribution of parasites where a small proportion of the host population harbours majority of the parasite population (Anderson & May 1978, Shaw & Dobson 1995, Shaw et al. 1998, Wilson et al. 2002). Parasite aggregation has an essential role in the population and evolutionary dynamics of parasite-host relationships (Poulin 1993, Wilson et al. 2002) and one of the fundamental questions in parasitology pertains to the relative importance of exposure and susceptibility as causatives of aggregated parasite distributions in host populations (Combes 2000, Hudson et al. 2002). In natural fish populations, numbers of *D. spathaceum* parasites differ markedly between host individuals and the parasite distribution is typically aggregated (e.g. Pennycuick 1971, Sweeting 1974, Burrough 1978). Although exposure and susceptibility are likely to be different between individual hosts because of clumped distribution of cercariae and varying prior experience with the parasite, the relative role of these factors in the aggregation of *D. spathaceum* in fish has received very little attention. The infection experiment in cages, where differences in exposure between fish individuals were likely to be negligible and the possibility to avoid infection was excluded, indicated that parasites were not aggregated compared to wild fish populations with presumably variable exposure (IV). This suggests

that the aggregated distribution of *D. spathaceum* might be caused by differences in exposure between individual hosts rather than differences in susceptibility.

Behaviour is undoubtedly one of the essential aspects determining how hosts are exposed to parasites (e.g. Anderson et al. 1978, Poulin et al. 1991, Moore 2002). Indeed, in the present system, movement of fish determines the encounter rate with cercarial patches around the infected snails (II). However, fish also use movement to avoid infection (V) and individual differences in their ability to do so may lead to aggregated parasite distribution in an otherwise homogeneous host population (Anderson et al. 1978, Moore 2002). In this study, fish showed considerable variation in the response time to cercariae, which led to corresponding variation in parasite numbers (V). This may have important implications in natural conditions, where parasite densities in patches close to shedding snails may be elevated (I) so that lower ability to avoid infection could lead to rapid accumulation of parasites in some fish (IV, V), shifting these individuals towards the tail of the parasite distribution. Thus, this result implies that differences in the avoidance behaviour could also generate parasite aggregation in this system, but it should be noted that the relative roles of factors contributing to aggregated parasite distribution undoubtedly vary between different host species and with different infection pressures.

4 CONCLUSIONS

Transmission is a major determinant of parasite fitness (Anderson & May 1991, Hudson et al. 2002) and the unpredictability related to this process has favoured the development of multiple strategies in parasites to increase the probability of transmission (Combes 2001). On the other hand, infection has negative effects on the condition and fitness of the target hosts, and therefore hosts have responded to parasites with adaptations that prevent or reduce infections. The present study examined the transmission of the complex life cycle trematode *Diplostomum spathaceum* between the intermediate hosts focusing on the strategies in cercarial release from the snail host and the responses elicited by the fish host against the cercarial infection.

The examination of two reproductive hypotheses in the snail, terminal investment and bet hedging (Williams 1966, Jokela et al. 1999, Fenton & Hudson 2002), indicated that the parasite cercariae were released from the snails for an extended period of time in support of the second hypothesis (I). Furthermore, cercariae were released in higher numbers during day than night, which may increase contacts with diurnally active fish hosts (I). Although the effects of these strategies on the cercarial transmission rate were not examined directly, it could be expected that by adopting an extended pattern of cercarial production instead of maximal short-term production, the parasite could increase the probability of transmission to fish hosts with unpredictable temporal and spatial availability. The prevalence of infection in natural populations of various fish species is high (e.g. Burrough 1978, Valtonen & Gibson 1997) and diplostomids may comprise a substantial proportion of the whole parasite fauna in fish (Valtonen et al. 2003). This suggests that strategies in cercarial release could maintain rather effective transmission between snail and fish hosts, especially given that the parasite prevalence in snails is low (Väyrynen et al. 2000) and the infective stages are short-lived and passively transmitted (II).

After successful establishment in fish, the parasite has detrimental effects on the host (Crowden & Broom 1980, Shariff et al. 1980, Owen et al. 1993,

Buchmann & Uldal 1997, Seppälä et al. 2004, III), which suggests that fish should invest into defence to reduce the effects of infection. Immunological reactions in fish against the parasite are well described in the literature, but previous studies have not considered the ability of acquired resistance to protect the fish against the secondary effects of infection. In this study, fish held in natural conditions acquired physiological resistance against the infection, which decreased subsequent parasite establishment (IV), but did not prevent the secondary effects of infection i.e. cataract formation (V). However, as demonstrated for the first time in this study, fish also decreased exposure to the parasite by responding to the presence of cercariae with avoidance behaviour (V). Since the recognition of infection is probably mediated by mechanical stimuli caused by penetrating cercariae, it is possible that the acquired physiological resistance serves as an adequate protection for the fish while this recognition takes place. Thus, the combination of physiological resistance and avoidance behaviour probably provides more effective way of resistance for the fish with lower costs (see Rigby et al. 2002).

Although the high prevalence of *D. spathaceum* infection in natural populations indicates that most fish are exposed (e.g. Burrough 1978, Valtonen & Gibson 1997), the aggregated distribution of the parasite (Pennycuick 1971, Sweeting 1974, Burrough 1978) implies that only a small proportion of hosts harbour high parasite burdens. The aggregation of parasites into few host individuals is a predominant phenomenon in nature (Shaw & Dobson 1995, Shaw et al. 1998), but causes of this pattern of distribution are not well known (Wilson et al. 2002). The results in this study suggest that individual differences between fish in exposure and ability to avoid the cercariae are important (IV, V). This represents one of the few attempts to evaluate the interaction between exposure and susceptibility in causing parasite aggregation in the *Diplostomum*-fish relation, and in parasite-host systems in general (but see e.g. Tanguay & Scott 1992, Lysne & Skorping 2002). However, it should be stressed that the number of parasites acquired by an individual fish is determined separately in each particular encounter and is affected by the degree of exposure, previous infection history and the genetic compatibility between the parasite and the host.

Aggregation of *D. spathaceum* in fish may have important implications for the life cycle of the parasite, especially if cataract formation plays an essential role in enhancing the transmission of the parasite to the avian definitive host (Seppälä et al. 2004). Since the intensity of cataracts is dependent on the parasite burden (III), it may be that fish individuals in the tail of the aggregated distribution with heaviest parasite loads, are those experiencing the most severe effects of the infection and ultimately sustain the parasite life cycle (see Wilson et al. 2002). However, it is likely that both the parasite aggregation and the effects of the infection show host species-specific differences, which should be considered in studies exploring the relative roles of different fish species in maintaining the parasite populations.

Detailed knowledge of parasite transmission dynamics also has a key role when we seek ecologically friendly treatments for both well-known and emerging parasitic pathogens. *Diplostomum spathaceum* is frequently found in fish farms causing occasional epidemics and the prevention of infections by this parasite has proved difficult (Stables & Chappell 1986a, Field & Irwin 1994). One of the essential concepts in epidemiology of parasitic pathogens is the basic reproductive ratio, R_0, which is defined for macroparasites as the number of female parasites produced by each female in a completely susceptible host population where density dependent constraints are not operating (Anderson & May 1991, Hudson et al. 2002). R_0 has to be greater than 1 for the parasite to persist or spread in the host population. The estimation of R_0 in complex life cycles involves the multiplication and transmission of parasites through a series of intermediate hosts. To understand these dynamics between each life cycle stage we need estimates of the production of infective stages (I), their survival and the rate of transmission (II), and how hosts acquire resistance against further infection (IV, V). This study is part of a larger project, which examines the population and transmission dynamics of *D. spathaceum* in each step of the life cycle. One of the aims of the project is to develop a mathematical model of the parasite life cycle, which uses the information acquired from empirical field data and detailed experiments in estimation of important and tangible model parameters. The purpose of the model would be to identify the key steps in the parasite life cycle, which could be manipulated to suppress R_0 locally below 1 and cause parasite numbers to decrease in the population. In practise, by using the model dynamics to define a specific proportion of the host population for removal, parasite control could be achieved using ecologically sustainable methods and with low costs. This model could have important implications for the fish farming industry in the fight against this harmful parasite, which causes continuous economical losses to the industry.

Acknowledgements

First I would like to express my gratitude to my supervisors. Tellervo Valtonen introduced me to the world of parasites and supervised this project with her enthusiastic attitude to science and life in general. Peter Hudson brought an international touch to our team and discussions with him opened new ideas and aspects to this work.

I also thank my other co-workers in the Diplostomum project, Otto Seppälä, Satu Paukku, Satu Kirsi and Miia Savolainen who did, or are currently doing, their master's theses in the course of this project. Otto became a PhD-student exploring other aspects in the Diplostomum life cycle and our fruitful discussions on various issues benefited this work in great deal. Discussions with Andy Dobson contributed to aspects related to the life cycle modelling and parameter estimation. This work was also assisted by trainees Jani Hannula, Soile Huotari, Ville Kupari and Erkka Tawast.

I would also like to acknowledge all the members of the "parasitology group" in Jyväskylä. Special thanks to Katja Pulkkinen who has been my work colleague and friend ever since I started working with parasites. This work has benefited from discussions with Rauno Alatalo, Ellen Bjerkås, Andy Fenton, John Holmes, Harri Högmander, Jukka Jokela, Ilmari Jokinen, Markku Julkunen, Hanna Kokko, Yrjö Lankinen, Timo Marjomäki, Päivi Rintamäki-Kinnunen, Jouni Taskinen and numerous other people.

A large part of this work was carried out at Konnevesi Research Station and I thank the head of the station Jussi Viitala for providing the working facilities, and technical staff Janne Koskinen, Risto Latvanen and Jyrki Raatikainen for their invaluable help in various practical aspects of this work. Thanks also to the people at our Department in Jyväskylä. Roger Jones kindly checked the English of this summary and the manuscript included in this thesis.

Above all, I thank my wife Minna-Liisa, my parents, sister and brothers, and my relatives and friends for their love and support during these years.

This work was financed by Biological Interactions graduate school, Finnish Cultural Foundation, Savon Taimen Ltd. and the SUNARE project of the Academy of Finland.

YHTEENVETO

Diplostomum spathaceum -loisen siirtyminen kotilo- ja kalaisännän välillä

Monivaiheisten eli useita isäntälajeja käsittävien loisten elinkiertojen keskeinen haaste on transmissio eli siirtyminen elinkierron seuraavaan isäntään. Loisen kelpoisuus riippuu oleellisesti transmission onnistumisesta, ja tästä syystä useille loislajeille on evoluution seurauksena kehittynyt pitkälle erikoistuneita sopeumia, joiden avulla loiset voivat lisätä transmission onnistumisen todennäköisyyttä. Toisaalta, koska loiset ovat haitallisia, isännät pyrkivät vastaavasti pienentämään infektion todennäköisyyttä ja/tai sen vaikutuksia. Tässä väitöskirjassa tutkin *Diplostomum spathaceum* -imumatoloisen transmissiota kahden väli-isännän, kotilon (*Lymnaea stagnalis*) ja kirjolohen (*Oncorhynchus mykiss*), välillä. Tutkimukseni keskittyivät loisen kerkariatuotantostrategioihin kotiloväliisännässä ja siihen, miten elinkierron seuraava isäntä, kala, vastaa loisinfektioon.

Loisen kerkariatoukat vapautuivat kotiloista pitkän ajan kuluessa, mikä tukee 'bet hedging' hypoteesia. Tämän hypoteesin mukaan kerkariatuotannon pitkäkestoisuus, tehokkaamman ja lyhytkestoisen tuotannon sijaan, voi pienentää loisen transmission epäonnistumisen riskiä ympäristössä, jossa seuraavan isännän kohtaaminen on epävarmaa. Toisaalta 'bet hedging' hypoteesin mukaista vaihtelua kerkarioiden ominaisuuksissa ei havaittu. Kerkarioita vapautui kuitenkin enemmän päivällä, jolloin kalat ovat aktiivisia ja altistuvat todennäköisemmin loisinfektiolle. Vaikka näiden strategioiden suoria vaikutuksia transmission tehokkuuteen ei tutkittu, voidaan olettaa, että pitkäkestoinen kerkariatuotanto lisää transmissiota satunnaisesti infektiolle alttiina oleviin kaloihin. *D. spathaceum* -loinen on hyvin yleinen monissa kalalajeissa, ja loismäärät ovat paikoin korkeita, mikä viittaa siihen, että loisen kerkariatuotantostrategiat voivat ylläpitää tehokasta siirtymistä kaloihin.

Voimakas *D. spathaceum* -loisen infektio kalan silmän linssissä aiheuttaa kaihia, jolla voi olla merkittäviä vaikutuksia kalan kasvuun, kuntoon ja alttiuteen joutua saaliiksi. Tästä syystä voidaan olettaa, että kalan tulisi pyrkiä vähentämään silmään pääsevien loisten määrää minimoidakseen infektion haitalliset vaikutukset. Kun kirjolohia altistettiin *D. spathaceum* -loisille luonnossa, kaloille kehittyi fysiologinen resistenssi loista vastaan, mikä vähensi seuraavissa altistuksissa kalaan pääsevien loisten määrää. Resistenssi ei kuitenkaan tarjonnut riittävää suojaa kaihin muodostumista vastaan. Tutkimuksessa havaittiin ensimmäistä kertaa, että kalat reagoivat loisen kerkariatoukkien läsnäoloon vedessä ja pyrkivät välttämään infektiota. Kalojen puolustautuminen loista vastaan tapahtuu siis todennäköisesti sekä fysiologisen resistenssin että välttämiskäyttäytymisen avulla. Tämä voi tarjota kaloille tehokkaamman suojan loista vastaan ja vähentää fysiologisen resistenssin mahdollisia kustannuksia.

Loisen korkea infektioprosentti kaloissa osoittaa, että suuri osa kalapopulaatiosta altistuu loiselle. Toisaalta, loismäärät ovat korkeita vain pienessä osassa isäntäyksilöitä. Loisten kertyminen muutamiin yksilöihin on tyypillinen piirre useimmissa lois-isäntä -vuorovaikutussuhteissa, mutta ko. ilmiöön johtavat syyt ovat usein huonosti tunnettuja. Tutkimukseni tulokset osoittavat, että *D. spathaceum* -loisen kohdalla loisten kertyminen tiettyihin isäntiin voi johtua isäntäkalojen yksilökohtaisista eroista altistumisessa loiselle sekä kyvyssä välttää loisen kerkarioita. Lisäksi havaitsin, että loisen aiheuttaman kaihin voimakkuus on suhteessa silmän loismäärään, millä voi olla merkittäviä vaikutuksia loisen elinkierrolle, erityisesti jos loinen pystyy vaikuttamaan kalan käyttäytymiseen ja fysiologiaan lisätäkseen transmissiota lintupääisäntiin. Tällöin on mahdollista, että pieni, voimakkaimmin loisittu osa kalapopulaatiosta kokee voimakkaimmat infektion vaikutukset ja mahdollisesti myös ylläpitää loisen elinkiertoa. On kuitenkin huomioitava, että kalaisäntälajien välillä on eroja infektioalttiudessa ja altistumisessa loiselle. Onkin tärkeää tarkastella em. aspekteja myös eri kalalajien suhteen.

Loisten transmissiodynamiikan yksityiskohtainen tuntemus on avainasemassa etsittäessä kestävän kehityksen mukaisia keinoja torjua loisinfektioita esimerkiksi vesiviljelyssä. *D. spathaceum* -loinen esiintyy yleisesti kalanviljelylaitoksilla aiheuttaen taloudellisia tappioita mm. heikentyneen kalan kasvun ja yleiskunnon seurauksena. Tämä työ on osa laajempaa projektia, jossa tutkitaan *D. spathaceum* -loisen elinkiertobiologiaa ja -dynamiikkaa elinkierron kaikissa vaiheissa. Projektin yhtenä tavoitteena on kehittää elinkierron matemaattinen malli estimoimalla keskeisiä loisen transmissioparametreja empiiristen kenttäaineistojen sekä kokeellisten tutkimusten perusteella. Mallin avulla voidaan suunnata torjuntatoimet tarkasti määriteltyyn osaan loisen elinkiertoa ja kontrolloida loisinfektioita kalanviljelylaitoksilla ekologisesti kestävillä menetelmillä ja pienimmillä mahdollisilla kustannuksilla.

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