JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
263

Tiina Hasu

Host-Related Factors Affecting Isopod (*Asellus aquaticus*) Susceptibility to and Interaction with an Acanthocephalan (*Acanthocephalus lucii*) Parasite

JYVÄSKYLÄN YLIOPISTO

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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ambiotica-rakennuksen salissa YAA303
elokuun 16. päivänä 2013 kello 12.

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

JYVÄSKYLÄ 2013

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JYVÄSKYLÄ 2013

Editors
Timo Marjomäki
Department of Biological and Environmental Science, University of Jyväskylä
Pekka Olsbo, Ville Korkiakangas
Publishing Unit, University Library of Jyväskylä

Jyväskylä Studies in Biological and Environmental Science

Cover picture by Tiina Hasu

URN:ISBN:978-951-39-5307-2
ISBN 978-951-39-5307-2 (PDF)

ISBN 978-951-39-5306-5 (nid.)
ISSN 1456-9701

Jyväskylä University Printing House, Jyväskylä 2013

To Tellervo

ABSTRACT

Hasu, Tiina
Host-related factors affecting isopod (*Asellus aquaticus*) susceptibility to and interaction with an acanthocephalan (*Acanthocephalus lucii*) parasite
Jyväskylä: University of Jyväskylä, 2013, 46 p.
(Jyväskylä Studies in Biological and Environmental Science
ISSN 1456-9701; 263)
ISBN 978-951-39-5306-5 (nid.)
ISBN 978-951-39-5307-2 (PDF)
Yhteenveto: Vesisiira (*Asellus aquaticus*) -isäntään liittyvien tekijöiden vaikutuksista väkäkärsämatoloisintaan (*Acanthocephalus lucii*)
Diss.

Trophically-transmitted *Acanthocephalus lucii* parasite castrates female isopod (*Asellus aquaticus*) hosts and manipulates the phenotypic traits of hosts making infected isopods more prone to predation. How certain host-related factors affect isopod susceptibility to the parasite and how *A. lucii* exposure and infection affect isopod survival, growth and female reproductive output were examined in this thesis. Under laboratory culture conditions growth factors did not affect the reproductive output of the isopods, but unfiltered tap water decreased the survival of the new-born offspring. Juvenile isopods were less susceptible to *A. lucii* and survived better than adults under exposure to the parasite. Furthermore, cystacanth-infected isopods were larger than uninfected isopods. Gravid isopods were susceptible to infection, but infected mothers survived well, although infection slightly decreased their offspring length, whereas resistant mothers paid survival costs and had significantly smaller offspring. These results suggest a functional or physiological mechanism that buffers juvenile isopods against *A. lucii* establishment and possible related harmful effects. The results further indicate the parasite's ability to interfere with host energy allocation in a way that does not increase host mortality. Naïve isopods suffered from survival costs of *A. lucii* resistance and were more susceptible to *A. lucii* than isopods from allopatric populations having likely a long history with the parasite species. The isopod population sympatric to the *A. lucii* parasite used in the exposure was as susceptible to *A. lucii* as naïve populations but did not show survival costs of resistance, suggesting that co-existence with the parasite shapes the defences of the isopods. Behavioural manipulation was most intense in spring, the optimal time for *A. lucii* transmission to the definitive fish host and the time when isopod populations are dominated by large old adults.

Keywords: *Acanthocephalus lucii*; *Asellus aquaticus*; costs of resistance; host–parasite interaction; resistance; survival; susceptibility.

Tiina Hasu, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, 40014 University of Jyväskylä, Finland

Author's address Tiina Hasu
Department of Biological and Environmental Science
P.O. Box 35
40014 University of Jyväskylä
Finland
tiina.m.hasu@jyu.fi

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

Professor Jouni Taskinen
Department of Biological and Environmental Science
P.O. Box 35
40014 University of Jyväskylä
Finland

Reviewers Dr. James C. Chubb
Department of Evolution, Ecology and Behaviour
Institute of Integrative Biology
University of Liverpool
Liverpool L69 7ZB
United Kingdom

Professor Raine Kortet
Department of Biology
University of Eastern Finland
P.O. Box 111
80101 Joensuu
Finland

Opponent Docent Riitta Rahkonen
Finnish Game and Fisheries Research Institute
P.O. Box 2
00791 Helsinki
Finland

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The thesis is based on the following original articles, which will be referred to in the text by their Roman numerals I–V.

I was responsible for the majority of the planning, execution and data analyses of studies I-IV. The main responsibility for writing articles I–IV was mine. All co-authors contributed to planning and writing paper V and I was responsible for execution of experiment 3 in paper V.

I Hasu T., Jokela J. & Valtonen E.T. 2008. Effects of growth factors and water source on laboratory cultures of a northern *Asellus aquaticus* (Isopoda) population. *Aquatic Ecology* 42: 141–150.

II Hasu T., Holmes J.C. & Valtonen E.T. 2007. Isopod (*Asellus aquaticus*) size and acanthocephalan (*Acanthocephalus lucii*) infections. *Journal of Parasitology* 93: 450–457.

III Hasu T., Valtonen E.T. & Jokela J. 2006. Costs of parasite resistance for female survival and parental care in a freshwater isopod. *Oikos* 114: 322–328.

IV Hasu T., Benesh D.P. & Valtonen E.T. 2009. Differences in parasite susceptibility and costs of resistance between naturally exposed and unexposed host populations. *Journal of Evolutionary Biology* 22: 699–707.

V Benesh D.P., Hasu T., Seppälä O. & Valtonen E.T. 2009. Seasonal changes in host phenotype manipulation by an acanthocephalan: time to be transmitted? *Parasitology* 136: 219–230.

1 INTRODUCTION

1.1 Susceptibility to parasites

Parasites are an unavoidable fact of life for all free-living organisms (e.g. Poulin 2007, Schmid-Hempel 2011) but susceptibility to parasites (i.e. the likelihood of becoming attacked and infected by the parasite) may vary largely among host individuals and populations (Hudson et al. 2002). Typically, many parasite species are found aggregated among their hosts, so that most host individuals harbour only a few parasites or none at all, while a few hosts harbour many parasites (e.g. Shaw et al. 1998, Poulin 2007). The factors and processes contributing to heterogeneities in parasite infections are the basic information needed to understand the dynamics, and to evaluate the consequences or the evolutionary causality in any host-parasite system. For instance, divergent parasitic exposure between populations and individuals in the wild can generate susceptibility differences, for example by way of local adaptation (e.g. Bryan-Walker et al. 2007). Therefore, it is important to distinguish differential susceptibility from differential exposure, which requires controlled experimental work.

Natural populations vary in their susceptibility to parasites for various abiotic and biotic reasons (e.g. Corby-Harris & Promislow 2008, Hartson et al. 2011). When parasite exposure varies spatially it is likely to influence also the parasite-mediated selection pressure on host defences among the host populations (e.g. Carius et al. 2001, Buckling & Rainey 2002). In theory, if the parasite has a strong negative effect on the fitness of the host, selection is expected to favour less susceptible or more resistant individuals in parasitized populations. Therefore, hosts evolving with and without parasites presumably invest differently in resistance. Over time this may be seen in trade-offs between immunity and other fitness-related traits, like reproductive output (e.g. Sheldon & Verhulst 1996, Rigby & Moret 2000, Schmid-Hempel 2003). Evidence for parasite-mediated selection for resistance has started to accumulate from

different host–parasite systems (e.g. Kalbe & Kurtz 2006, Bryan-Walker et al. 2007, Duffy & Sivars-Becker 2007, Duncan & Little 2007).

Several host-related factors can affect susceptibility to parasites and many of these are also interconnected depending on the host–parasite system. Body size of the host often constrains the trophic strategy used by the parasite (Lafferty & Kuris 2002) and may also affect susceptibility (e.g. Wegeberg et al. 1999). Larger host individuals may provide more resources or space to grow for a parasite. Therefore, selection is expected to favour parasites targeted for infecting larger hosts, particularly if available resources for growth are a central fitness factor for the parasite (Parker et al. 2003). On the other hand, host ability to resist parasites often increases with age, size and growth due to advanced immunological function (e.g. with the development of acquired resistance) (Schmid-Hempel 2011). In many animal species sexual selection maintains size dimorphism between males and females and may also mould other ecological (e.g. behaviour), physiological (e.g. hormones) or structural (e.g. ornamentation) characteristics of males and females that can predispose that one sex to be more susceptible to parasites than the other (Poulin 1996, Zuk & McKean 1996). The mechanistic cause for sex bias in susceptibility is often found in the link between immunological and hormonal functions. For example, androgens typically suppress immune function and render males more susceptible to infections compared with females (e.g. Zuk & McKean 1996, Klein 2004), whereas female oestrogens are thought to boost humoral immunity (Grossman 1985). Furthermore, invertebrates have several multifunctional hormones, some of which are known to affect immune system function (e.g. Adamo 2012, Webster et al. 2012). For example, juvenile hormone of both sexes of the mealworm beetle, *Tenebrio molitor*, has been shown to down-regulate immune function (Rolff & Siva-Jothy 2002). In general, male vertebrates are more parasitized than females (e.g. references in Poulin & Forbes 2012). However, Sheridan et al. (2000) did not find any general sex bias in parasite infections among arthropod hosts, although male *Macrocyclops albidus* copepods have been shown to be significantly more susceptible to *Schistocephalus solidus* cestode parasites than females of the species (Wedekind & Jakobsen 1998).

Host condition is an important determinant of susceptibility. Available resources affect host condition and this may impact immune function (e.g. Seppälä & Jokela 2010) or the effects of infection (e.g. Krist et al. 2004). Mitchell et al. (2005) showed that temperature change alone could affect the susceptibility and the nature of host–parasite interaction and, more importantly that host genotypes responded differently to temperature variation. This genotype–environment interaction effect is likely to maintain the heterogeneity of host resistance among populations in varying habitats. Susceptibility to parasites may vary also according to the host life history stage. For instance, the brooding state of female *Potamopyrgus antipodarum* snails has been shown to increase susceptibility to *Microphallus* sp. trematodes, possibly due to the trade-off between investments in reproduction and in immune defence (Dybdahl & Krist 2004). Most populations, at least at northern latitudes, are adapted to seasonal variation, which shapes their life cycles and interactions with other species.

Abundances of parasites and their hosts may vary with the season creating variation in exposure, but host susceptibility can also depend on the season (e.g. Kortet & Vainikka 2008). For example, the immunocompetence of wild rabbits (*Oryctolagus cuniculus*) against the *Trichostrongylus retortaeformis* nematode was shown to depend on the current season and the host's month of birth (Cornell et al. 2008).

1.2 Host–parasite interaction

In theory, the antagonistic interaction manifested in the fight between attacking parasite and defending host affects life histories of both, and potentially shapes the traits of all living beings (e.g. Zuk & Stoehr 2002). Many hypotheses have been presented and models built to predict and describe how coevolutionary processes like local adaptation, parasite virulence or host resistance are affected by the reciprocal interaction (e.g. Thompson 1999, Poulin et al. 2000, Gandon et al. 2002). However, obtaining evidence for these ongoing processes in the wild may be challenging, because there is diversity of possible interactive factors affecting the outcome of these processes in the wild populations and controlling or measuring even some of them is laborious or practically impossible.

Obligatory castrating parasites specialized to infect only one host species are ideal systems to study these reciprocal interactions, because "winning the fight" is an essential fitness factor for both hosts and parasites. The hosts are likely to be under strong selection for defences to resist the attacking parasite while the parasites should be selected for overcoming the host defences (e.g. Gandon et al. 2002, Woolhouse et al. 2002). There are many possible outcomes of this process, but most importantly the reciprocal evolution of the host's resistance/immune system and parasite's virulence/immune evasion is dynamic and affects both host and parasite. Parasites are usually thought to be ahead in these adaptations because of their shorter generation times and higher migration rates. This gives them an advantage of evolving more rapidly than their hosts, which can lead to locally adapted parasites performing better on local/sympatric host populations than on allopatric hosts (Kaltz & Shykoff 1998, Kawecki & Ebert 2004). Nonetheless, parasite maladaptation has also been documented (see Greischar & Koskella 2007) as well as host local adaptation (Roth et al. 2012).

Although efficient resistance is beneficial for the host it may be costly to maintain or use (Sheldon & Verhulst 1996). Besides defences against parasites hosts have to allocate their resources to various other fitness components like reproduction (e.g. Rolff & Siva-Jothy 2002), parental care (e.g. Deerenberg et al. 1997), predator avoidance (e.g. Rigby & Jokela 2000) and somatic growth (e.g. Brommer 2004). Because of limited resources trade-offs are commonly found between host defences and other fitness related traits. These trade-offs can be seen for example in decreased reproductive output, deteriorated quality of the progeny or in increased risk of mortality among the resistant or immunocompetent hosts. Life history theory is based on optimization models

(Stearns 1992) and thus an optimal strategy maximising the overall fitness is expected (Sheldon & Verhulst 1996, Rigby & Moret 2000). In addition to immune defences animals may have non-immunological defences, which are traits that help to prevent the infection like behavioural avoidance, symbiont-mediated immunity or fecundity compensation (e.g. Parker et al. 2011, Schmid-Hempel 2011). How intensively these are used may vary among individuals and populations, and may depend on individual condition or life history stage. Non-immunological defences can also be costly for hosts. Nevertheless, it is important to bear in mind that a trait expressed by the attacked host may well be a product of parasite manipulation, and thus the trait should be demonstrated as adaptive for the host before it can be defined as a host defence (Parker et al. 2011).

Invertebrates use humoral and cellular components of the immune system and often these components interact (e.g. Schmid-Hempel 2003). The major element of arthropod immune defence is the melanisation-encapsulation cascade (Schmid-Hempel 2011). Mechanical injuries or presence of foreign objects activate the prophenoloxidase (PPO) enzyme cascade. This PPO system is triggered by various recognition proteins followed by the release of serine proteases, which activate the prophenoloxidase cascade resulting in production of active PO enzyme. Active PO enzyme is responsible for producing cytotoxic compounds like phenols, quinones and reactive oxygen species capable of killing pathogens. Eventually the PPO cascade is needed to melanise the foreign object encapsulated by the circulating haemocytes. Activation of the PPO enzyme cascade is also involved in other immune reactions like phagocytosis. Clotting and nodule formation are also important cellular responses among invertebrates. Many humoral defence molecules such as agglutinins, lysins and antimicrobial factors may be synthesized and secreted by haemocytes, but at least for crustaceans hepatopancrea is also regarded as an important tissue involved in immune defences. (Schmid-Hempel 2003, Cerenius et al. 2010, Schmid-Hempel 2011). Although invertebrates lack the acquired specific antibody-based immunological memory found in vertebrates, invertebrates are found to have immunological priming (enhanced protection resulting from past experience with a pathogen) and some degree of specificity in their immune response against parasites (e.g. Kurtz & Franz 2003, Little & Kraaijeveld 2004, Kurtz 2005, 2007, Schmid-Hempel 2005, 2011).

Immunopathology (damage to the host caused by an inappropriate response of the immune system) is an important factor shaping the host defences against parasites and also the evolution of parasite virulence (Graham et al. 2005, Day et al. 2007, Long & Boots 2011). Immunopathology is less studied among invertebrates, but the potential risk of self-reactivity is reality among invertebrates relying on PO-cascade-based processes producing cytotoxic intermediates capable of damaging a variety of cells and tissues. Immunopathology can occur by misdirection or overproduction of immune factors (Schmid-Hempel 2011). For example, Sadd & Siva-Jothy (2006) found that activation of the phenoloxidase cascade reduced significantly the Malphigian tubule function, which is vital for the *Tenebrio molitor* beetle in maintaining the water balance in drying environments. Immunopathology can also be caused by

various immune evasion mechanisms employed by the parasite. On the other hand, immune evasion can lead to downregulation of the host immune response and thus lower the risk of self-reactivity and damage. This kind of downregulation may go together with the interests of parasites needing a long time for development in the host (Schmid-Hempel 2009).

1.3 Acanthocephalans and their arthropod hosts: is the host just an extension of the parasite phenotype?

While adult acanthocephalans do not normally alter the behaviour of their vertebrate hosts, the larval parasite stages are more likely to harm the intermediate hosts merely due to their trophic transmission mode (Kennedy 2006). The intermediate host has to be eaten by the definitive vertebrate predator host before the parasite can reach adulthood (e.g. Moore 2002). This already gives an advantage to the parasite for all adaptations which favour increased probability of the isopod host to become more susceptible to predation by the suitable definitive host at the right time. Acanthocephalans are one of the groups best known or suggested to manipulate the phenotypic traits (colour, morphology and behaviour) of their intermediate hosts (Moore 2002). One classic example of a fine-tuned manipulation is *Polymorphus paradoxus* (acanthocephalan) in its *Gammarus lacustris* (amphipod) host. Bethel & Holmes (1973, 1974, 1977) showed that evasive behaviour of cystacanth infected amphipods increased the host vulnerability to the predation by suitable definitive hosts thus clearly benefiting the parasite. Although, host manipulation is common among acanthocephalans (Thomas et al. 2012), infection may induce other effects on the host, that cannot always straightforwardly be determined as beneficial to the parasite (e.g. in Moore 2002). Furthermore, unequivocal interpretation of the changed host trait as an adaptive manipulation by the parasite seems not to be that simple, because there may be variation in intensity and frequency of the trait alteration among infected hosts and between populations. This has led to recognition that manipulation can actually be a more complicated trait with a multidimensional nature, where the host is not just a passive target of manipulation but an actively responsive organism (e.g. Poulin 2007, Poulin 2010, Thomas et al. 2012).

Nevertheless, all known acanthocephalans are trophically-transmitted parasites and successful infection by an acanthocephalan is inevitably fatal for their intermediate host (Kennedy 2006). However, there may be a long-term relationship between the host and the parasite before the detrimental end is at hand. This is because acanthocephalan larval development from acanthor to infective cystacanth stage in the intermediate host may take weeks or more depending on the species and prevailing temperature (Nickol 1985, Schmidt 1985, Kennedy 2006). Kennedy (2006) describes the acanthocephalan cystacanth stage as a resting stage capable of surviving unfavourable conditions; for

example, the seasonal absence of the suitable host. Acanthocephalan parasite *Polymorphus marilis* in amphipod *Gammarus lacustris* undergoes a true diapause, with a halted development of the pre-cystacanth stage at cold winter temperatures until warmer temperatures resume the development (Tokeson & Holmes 1982). Therefore, from the point when the acanthocephalan larva has established itself in the haemocoel of the intermediate host to the point when the parasite larva is capable of transmission to the definitive host, the host and the parasite both share the same interest in surviving. Though it is expected that selection should favour efficient defences against the developing parasite as well as efficient evasion of those defences by the parasite, responses of either one should not increase the risk of host mortality. Therefore, some degree of tolerance is also expected from the host, which may be more beneficial than costly defences (Schmid-Hempel 2011). After all, host defences are never expected to completely eliminate the risk of infection (Jokela et al. 2000). Furthermore, the lifespans of the intermediate host species for acanthocephalans are seldom longer than a year (Kennedy 2006) and depending on the life history stage of the host at time of infection the fitness value of the rest of the lifespan of the host may vary greatly. Thus the expected resource allocations among different traits of the host (e.g. reproduction versus defences) may also vary accordingly.

Moreover, trophically-transmitted parasites are not only constrained by the possible costs associated with the host manipulation, but their exploitation strategies are constrained by their mode of transmission, requiring predation by the right definitive host (e.g. Poulin 2010). For a trophically-transmitted acanthocephalan host death is not the goal; the goal is to get to the definitive host to reproduce and this will not be achieved if the host dies before the parasite has developed to the cystacanth stage. Every genetically-determined strategy or trait that increases the likelihood of getting to the target should be favoured, regardless of whether it is induced by the host or the parasite (Poulin 2010). For isopods, which would admittedly do well without the parasite, living in a wormy world means adaptations. Adaptations in the evolutionary sense usually require time scales of at least few generations. The relationship can turn out to be a fitness catastrophe for either one or end up in a situation where both parties are accepting some risks or disadvantages and compromise. Most importantly in a coevolutionary relationship: if you are not challenged, you don't get a chance to respond. The impressive finale of a successful parasite in a manipulated host is captured in the expression of a host being an extension of the parasite phenotype (Dawkins 1982). However, to see the manipulated host just as an extension of the parasite phenotype, is an extremely one-sided and partial perspective of a multidimensional entirety, where the interactive play of both protagonists is continuously (in an evolutionary sense) challenged in varying space and time.

2 AIMS OF STUDY

The main goal of this thesis was to investigate how host-related factors of an isopod intermediate host (*Asellus aquaticus*) affect its susceptibility to an acanthocephalan parasite (*Acanthocephalus lucii*) and the nature of the interaction between the host and the parasite. The host-related factors emphasized were isopod size, age, gender, reproductive stage of females and parasite history of the isopod population. The nature of the host-parasite relationship was studied by assessing survival of exposed isopods and seasonal variation in host manipulation by the parasite. The susceptibility of isopods to parasites, virulence of parasite infection, costs of parasite resistance and the strength of parasitic manipulation imposed on hosts measured in terms of altering host phenotypic traits were examined.

All the studies were done experimentally in the laboratory requiring long-term maintenance of wild-collected isopods under laboratory conditions. Therefore, the effects of three different water sources on the maintenance of isopod cultures in the laboratory were compared (I). Also an experiment was carried out to explore whether isopods kept in laboratory cultures can gain growth, reproductive or survival benefits from certain growth factors, vitamins or bright light treatments. II focuses on the relationship between isopod size and *A. lucii* infections. It also addresses survival effects of *A. lucii* exposure on different size and age groups of isopods.

In III the costs of *A. lucii* resistance for survival of gravid female isopods and their parental care was explored. Whether survival costs of parasite resistance are equally applicable to non-gravid and male isopods was assessed in IV. With regard to the effect of host parasite history, the differences in susceptibility to *A. lucii* and costs of resistance between naïve and coexisting isopod populations were investigated. V focuses on the host manipulation by *A. lucii* and assesses whether the manipulation is more connected to seasonal changes in host/parasite age than the physical environmental conditions. The information gained from these studies was intended to form a foundation for further specialized studies to reveal the actual mechanisms behind the reciprocal action between the isopod host and its acanthocephalan parasite.

3 MATERIALS AND METHODS

3.1 Study system

The freshwater isopod *Asellus aquaticus*, serves as an obligate intermediate host for the trophically-transmitted acanthocephalan parasite, *Acanthocephalus lucii* (Schmidt 1985, Kennedy 2006). Life history studies of northern *A. aquaticus* populations (Andersson 1969, Økland 1978, Iversen & Thorup 1988) and observations on isopod occurrence and abundance made alongside other studies in Central Finland (e.g. Rask & Hiisivuori 1985) suggest that the lifespan of isopods at these latitudes is under a year. The reproductive period of isopods is from spring to autumn lasting 4 – 5 months and the overwintered isopods die soon after having reproduced by the early summer (own unpubl. data). Some isopods of the new generation may reproduce even twice by the end of autumn (own unpubl. observations) but it is not known how many of them is able to survive over the winter. At least isopods born in late summer – early autumn overwinter.

While grazing on biofilms and associated decaying organic matter on the substratum, isopods are exposed to the shelled acanthor larvae ("eggs") of the parasite. Ingested acanthors hatch in the isopod intestine and penetrate through the intestinal wall into the haemocoel of the isopod. Penetration seems to be a rather slow process, taking about 16 d at 19 ± 2 °C (Brattey 1986). In the haemocoel, larvae grow and develop to the cystacanth stage, which is infective to the fish definitive host. Development to the cystacanth stage is temperature-dependent, taking 70 – 90 d at 15 – 16 °C (Andryuk 1979, Brattey 1986) and from 32 d to 50 d at 22 °C (Andryuk 1979, Brattey 1986, Pilecka-Rapacz 1986). The variation in development time may suggest parasite adaptations to local climatic environments, or to life cycle rhythms of the local host populations. However, according to Brattey (1986) *A. lucii* prevalence in isopods is rather low throughout the year (generally < 10 %) and especially in summer when overwintered isopods die and the new generation of isopods starts to take over.

Fish, the definitive hosts, become infected with *A. lucii* when preying upon isopods that carry fully developed cystacanths (Andryuk 1979, Brattey 1988, Kennedy 2006). In the intestine of the definitive fish host, usually perch (*Perca fluviatilis*) (Crompton 1985, Brattey 1988, Karvonen et al. 2005a), cystacanths attach to the intestinal wall using their hooked proboscis. The dioecious adult worms mate, the fertilized eggs develop inside the female worm, and fully developed shelled acanthor larvae (the eggs) are shed into the water with the host's faeces (Schmidt 1985) (Fig. 1). According to Andryuk (1979) excretion of the *A. lucii* eggs starts 57 – 60 d post infection. Brattey (1988) found the highest prevalence (70 – 90 %) and abundance (14 – 16 worms/fish) in perch during late spring and summer. *A. lucii* prevalence and abundance seemed to decline from autumn reaching a minimum during winter (50 – 60 % and 2 – 3 worms/perch) (Brattey 1988). The seasonal occurrence of *A. lucii* parasite seems to follow the typical "spring recruitment cycle" (Chubb 1982, Nickol 1985, Brattey 1986, 1988, Kennedy 2006). Significantly higher *A. lucii* abundance in summer compared to winter has also been found for perch in Central Finland (Karvonen et al. 2005a).

Like many trophically-transmitted parasites *A. lucii* infection alters the phenotype of the isopod intermediate host. Isopods carrying cystacanth stages of *A. lucii* have strikingly darkened respiratory opercula (Brattey 1983, Benesh et al. 2008), which is likely to predispose infected isopods to increased risk of fish predation (Brattey 1983). Furthermore, cystacanth-infected isopods hide less than uninfected isopods (Benesh et al. 2008) making infected isopods more susceptible to predation especially by perch (Seppälä et al. 2008).

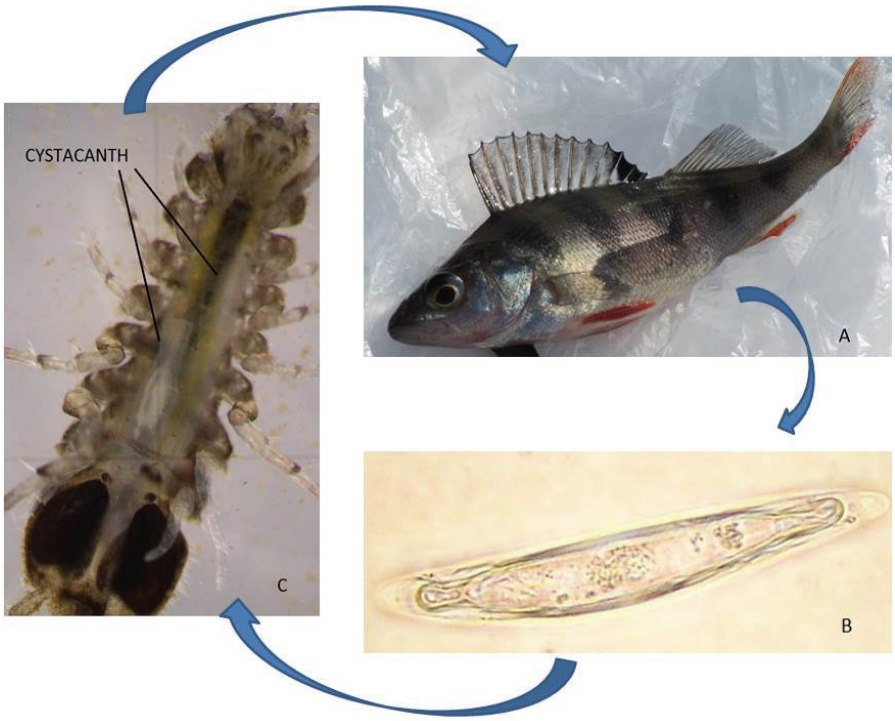

FIGURE 1 The life cycle of *Acanthocephalus lucii*. (A) European perch (*Perca fluviatilis*) is typically the definitive fish hosts. The adult worms reproduce in the intestine of the perch and (B) shelled acanthor larvae called "eggs" are shed into the water with the fish faeces. Isopods (*Asellus aquaticus*) serve as intermediate hosts and while foraging they ingest the eggs. In the intestine of the isopod the acanthor larva hatches and penetrates to the haemocoel, where it starts to grow and (C) develop to the cystacanth stage, which is infective to the fish host. When a cystacanth-infected isopod is predated by a fish the cycle is completed. In the picture above (C) two larvae can be seen in the haemocoel from the abdominal side of the isopod host. (Photos taken by the author.)

3.2 Effects of growth factors and water source on *A. aquaticus* laboratory culture (I)

Isopods were collected from a lake, Niemisjärvi (62°16'N 26°21'E) in November after the animals had been naturally exposed to winter conditions (Light:Dark = 8:16, temp = 4 °C). After 2 weeks acclimatization to laboratory conditions (L:D = 16:8; temp = 21 °C) isopods were grouped into 2 size classes based on their length: size class I (4 – 9 mm) and size class II (2 – 3.5 mm). To test the effects of 3 water sources on the reproductive performance of isopods, groups of isopods consisting of 12 isopods from size class I were assigned to either tap water, borehole water or spring water treatments (6 replicates of each). To test the effects of growth factors on the isopod reproductive performance, survival and

size of offspring 5 different treatments were assigned in another experiment to groups of isopods (12 ind./group). Each treatment consisted of 6 replicates from size class I and 3 replicates from size class II isopods. The growth factor treatments were: control, L-carnitine, vitamin mix, L-carnitine and vitamin mix, and bright light treatment. In both the water and growth factor experiments, whenever a gravid female was noticed among isopods it was transferred to a separate individual container. Gravid females were maintained and treated according to the assigned treatment. At the end of the experiments (after 11 weeks) length of all isopods and their reproductive output was measured.

3.3 *Asellus aquaticus* size and *Acanthocephalus lucii* infections (II)

Isopods originated from 3 central Finland populations; one from a eutrophic pond without *A. lucii* parasitism and 2 populations from eutrophic lakes with known *A. lucii* parasitism. Isopods were grouped as juveniles, maturing adults, younger adult generation or older adult generation according to their length. From every age/size class at least 8 replicates of groups of 5 isopods were exposed to *A. lucii* parasites while the same number of replicates served as unexposed controls. To collect *A. lucii* eggs, 10 perch from a population harbouring 67 % *A. lucii* infection were kept in a laboratory tank for 12 d. During that time all the perch faeces were collected. The sludge water was diluted, mixed well and divided equally among the groups of exposed isopods. The experiment lasted 6 – 7 weeks, after which isopods were sexed, their lengths were measured and their haemocoels were examined for *A. lucii* parasites. This experiment tested the effects of isopod size, maturity and gender on the *A. lucii* susceptibility by comparing *A. lucii* prevalence and abundance, as well as the development of *A. lucii* parasites between the exposed groups. The effects of exposure and infection on different age/size classes of isopods were evaluated by comparing the survival and length of isopods at the end of the experiment.

3.4 Costs of *A. lucii* resistance for gravid isopods (III)

This experiment aimed to determine whether resistance to *A. lucii* incurs costs, which can be measured directly as deterioration in survival or in reproductive performance of the gravid female isopods. Females with developing eggs or embryos in their brood pouch originating from two natural populations differing with respect to their history of *A. lucii* parasitism were individually and continuously exposed to *A. lucii* eggs in the laboratory. The reproductive performance and survival of susceptible (infected) and resistant (uninfected) isopods were compared along with control (unexposed) isopods for a maximum of 8 weeks. The time of release of the young, brood size, mean length of the young at birth and at 10 d post release were recorded.

3.5 Effects of parasite history on isopod susceptibility to *A. lucii* infection and costs of resistance (IV)

To study whether the previous parasite history of the isopod population affects the susceptibility and costs of *A. lucii* resistance in isopods an experimental exposure was conducted. Adult isopods from 3 lake populations with *A. lucii* as a common fish parasite (parasitized populations), isopods from 3 pond populations without *A. lucii* parasitism (naïve populations), and isopods from a population which was 10 years ago isolated from *A. lucii* parasitism (recently isolated population) were exposed for 3 d to *A. lucii* eggs under controlled laboratory conditions. Isopods were grouped as susceptible (exposed and infected), resistant (exposed but not infected) and control (unexposed to *A. lucii* during the experiment). The proportion of susceptible isopods, *A. lucii* intensity, *A. lucii* development, and the survival of isopods in each of the populations were recorded for 90 d post exposure.

3.6 Seasonal changes in host phenotype manipulation by an acanthocephalan: time to be transmitted? (V)

A. lucii infections have been shown to alter hiding behaviour and coloration of the isopod host (Benesh et al. 2008). Therefore, 3 separate experiments were run to study how isopod manipulation by its *A. lucii* parasite varies between the seasons, and whether the manipulation is related to environmental abiotic cues or host ageing. Isopods were collected from Lake Jyväsjärvi. In the first experiment the hiding behaviour of individual isopods was observed in the laboratory (15 – 17 °C; 16L:8D) for 15 d during three different seasons: spring, late summer and end of the fall. At the end isopod size, sex, infection status and abdomen coloration were measured. For the second experiment isopods were collected at the end of August. Isopods were sorted into 9 containers (30 in each; 15 infected and 15 uninfected) in the laboratory. To test whether the host manipulation changes with environmental conditions, containers were divided to 3 different light and temperature regimes: 1) warmer/lighter (15 – 17 °C; 18L:6D), 2) colder/darker (10 – 12 °C; 12L:12D) and 3) over-winter condition (4 – 6 °C; no light). After 4 weeks maintenance under these conditions isopods were isolated into individual containers and their hiding behaviour was observed as in the first experiment. The third experiment tested the effect of increasing probability of host mortality on parasite-induced behavioural changes over the winter. Infected and uninfected isopods kept at higher temperature (15 – 17 °C) were expected to develop faster and have higher mortality than isopods kept at low temperature (4 – 6 °C) for 7 months. Once a month hiding behaviours of individual isopods were observed to assess how temperature and infection affected hiding behaviour over time. Survival of isopods was also recorded.

4 RESULTS AND DISCUSSION

4.1 *Asellus aquaticus* laboratory cultures (I)

Isopods kept in tap water cultures suffered from significantly increased offspring mortality compared with isopods in borehole or spring water cultures. These results indicate that aerated but non-filtered tap water can be lethal to developing or newly emerged isopod offspring (mancae), possibly due to a relatively high Cu concentration (De Nicola Giudici et al. 1988). Despite that, dechlorinated tap water has been used widely in laboratory cultures of isopods and other freshwater invertebrates (McCahon & Pascoe 1988). Therefore, if tap water is to be used for experiments or for isopod cultures in laboratory, it should be pre-filtered and checked for Cu, because even concentrations as low as 5 $\mu g\ l^{-1}$ affect developing embryos and juveniles in the long run (De Nicola Giudici et al. 1988).

Although generation-specific survival effects of nutrient and light treatments were found between the old and young parent generations, none of the treatments had any significant effects on adult survival when compared to controls in either parent size class. The tested nutrient and light treatments did not affect any of the reproductive success variables of isopods in the laboratory. Overall, the average reproductive success of isopods (measured as number of brooding females, or as number of live offspring per reproductive female) was rather weak in this study compared to previous laboratory experiments done during spring or early summer (own unpubl. data). Previous studies on isopods have reported significant seasonal variation in the brood size (e.g. Johnson et al. 2001). Typically, for *A. aquaticus* it has been found that the females are on average larger in spring (e.g. Økland 1978) and produce more eggs compared to the females in the autumn generation (e.g. Andersson 1969, Adcock 1979, Ridley & Thompson 1979, Flössner 1987). However, some of the aforementioned lack of response to the tested treatments could be explained by the interrupted (isopods collected in November) or atypical length of reproductive diapause experienced by the test population (only about 2 months). The natural reproductive diapause

of the study population is from September to April and lasts about 8 months, which is comparable to studies on *A. aquaticus* populations in Sweden and Norway (Andersson 1969, Økland 1978). It has been suggested that *A. aquaticus* populations from Scotland and Holland are genetically determined to reproductive diapause (Vitagliano et al. 1991). However, the exact duration of the diapause seems not to be genetically pre-programmed. Instead, there seems to be some temperature regime and photoperiodicity dependent plasticity in the timing, onset and duration of reproductive processes of *A. aquaticus* populations. According to their experimental studies using an *A. aquaticus* population from Holland, Vitagliano-Tadini et al. (1982) found that the reproductive diapause is terminated within 2 months, and a strong increase in temperature induces sexual activity. However, considering adaptations of Finnish populations to the much longer winter season than elsewhere in Europe, 2 months of reproductive diapause may be too short for the local populations to fully recover their reproductive capacity and total vitality.

Another interesting consideration resulting from the lack of any significant responses to the tested growth factor treatments is the role of the gut and endosymbiotic microbes in *A. aquaticus* nutrition. The endosymbiotic hepatopancreatic bacteria are known to contribute to *A. aquaticus* digestion by producing digestive enzymes (cellulases and phenol oxidases), which facilitate the use of leaf litter as a food source (Zimmer & Bartholmé 2003). Moreover, the importance of fungi in *A. aquaticus* diet is well documented (e.g. Graça et al. 1993a,b). In addition, *A. aquaticus* populations show local adaptations in life history traits (e.g. Maltby 1991; reproductive effort) and phenotypic traits (e.g. Hargeby et al. 2005; cryptic pigmentation) as a response to abiotic and biotic environment. Therefore, it is likely that isopods could adapt to utilize the local composition and sources of nourishment. Indeed, Wang et al. (2007) found that the hepatopancreatic community in *A. aquaticus* was complex and showed individual as well as population specific heterogeneity. How do the local dietary preferences and variations in endosymbiotic bacterial composition and activity affect isopod susceptibility to *A. lucii* infections? Because the infection route of *A. lucii* is via ingestion of the parasite egg, isopod foraging and digestion have a central role when the parasite is about establish a successful transmission.

To set up optimal and sufficiently long-lasting stable environmental conditions for a system with two interactive and possibly continuously counteracting living species, basic information on the biology and life cycle of both parties is essential. Although *A. aquaticus* isopod has long been used as a test organism in toxicity tests and methods for continuous laboratory culture have been developed (McCahon & Pascoe 1988, Bloor 2010), there is still a lack of knowledge of basic issues. It is not known e.g. how the different and seemingly locally adapted (e.g. Hargeby et al. 2005, Eroukhmanoff et al. 2011) populations manage under standard laboratory conditions, or how strictly are populations from different latitudes adapted to their annual rhythms. Experimental work is based on controlled environmental conditions, which are especially important when trying to disentangle factors affecting two closely interacting species, for

one of which the other is the only lifeline; like *A. aquaticus* host is for larval *A. lucii* parasite.

4.2 Isopod susceptibility to *A. lucii*, effects of exposure and infection on isopod growth (II, IV)

Contrary to the findings of previous studies on different isopod–acanthocephalan systems (Nickol & Dappen 1982, Oetinger & Nickol 1982), the infection experiment showed clearly that juvenile and small *A. aquaticus* isopods were less susceptible to *A. lucii* infection than adult isopods (II). Although, grazing methods and habits of different sized and aged isopods differ slightly (Marcus et al. 1978, Smock & Harlowe 1983), Moore (1975) found that the mean size of ingested particles by isopods remained the same regardless of size of the animal. Furthermore, examination of the gut contents of isopods have shown that small isopods are capable of ingesting even larger particles than *A. lucii* eggs (Marcus et al. 1978, Smock & Harlowe 1983, Petridis 1990). Hence, if the size of the *A. lucii* egg is not the obstacle, it is possible that juveniles may avoid ingesting *A. lucii* eggs for some other reason. Another plausible explanation for the lower prevalence in juveniles is that ingested food may travel more quickly through the juvenile gut compared to adult isopods giving less time for the acanthor larva to hatch. According to Moore (1975) adult isopods empty their guts in 22 – 30 h at 15 °C, and the rate of evacuation is accelerated with the temperature rise. Hatching of acanthocephalan eggs apparently requires contributions from both the host and the parasite larva and it takes hours, even days, before the larva is freed from embryonic membranes and able to start penetrating to the haemocoel of the host (Nickol 1985). Brattey (1986) observed free acanthors in the lumen of isopod guts 10 h post exposure to *A. lucii* eggs at 19 ± 2 °C. Although adult isopods are capable of launching at least cellular immune defences against parasites, juvenile isopods are not known to be able to induce immune responses (Nickol & Dappen 1982, Nickol 1985). Thus, based on current knowledge, the most convincing explanations for the lower susceptibility of juveniles compared to adult isopods are their faster evacuation of gut contents or that the alimentary tract of juveniles lacks the effective stimulus for the acanthor larva to start the hatching. These possible explanations need to be tested experimentally.

Another clear outcome from II was that infected isopods (juveniles and adults harbouring fully developed *A. lucii* larvae) grew on average larger than uninfected or unexposed isopods. Previous studies on other acanthocephalan species have found infections only to increase the body size of their female isopod host possibly as a result from ability of the parasite to interfere with reproductive development (Oetinger & Nickol 1981, Kakizaki et al. 2003). In the experiment exposure was done using diluted sludge of perch faeces containing *A. lucii* eggs, so that all containers that were exposed to *A. lucii* also got an extra dose of nutrients and microbes compared to unexposed i.e. control containers.

Therefore, it is possible that the extra nutrient or microbe load amplified the conditioning of the food, so the alder leaves were then more palatable or edible to isopods explaining the better growth of exposed isopods compared to controls. Nevertheless, that cannot explain the growth difference between infected and uninfected isopods. One fascinating possibility to explain the better growth of infected isopods is that the presence of a parasite in the host gut or within haemocoel increases enzymatic activity of isopod digestion and with the boosted digestion the host gains extra growth. The regulation of enzymatic activity could be a host response to the parasite or a product of parasite manipulation. For example, terrestrial *Porcellio scaber* isopods are able to respond to changed pH levels of ingested food by buffering and maintaining pH homeostasis in their gut (Zimmer & Topp 1997). More detailed studies of the physiological properties and processes of digestion in the gut of *A. aquaticus* or other freshwater isopods would certainly illuminate these issues.

The positive correlation between mean cystacanth intensity and isopod length (II) could simply reflect a positive link between isopod foraging intensity and susceptibility to parasitism. An alternative explanation is that the parasites are interfering with the host energy economy. Although Benesh & Valtonen (2007a) did not find a correlation between *A. lucii* intensity and isopod length, they noticed that infection accelerated isopod moulting rate. They suggested that, instead of indicating host growth, moulting would be favourable for parasite growth and therefore may represent adaptive manipulation by the parasite. The isopods used in II were on average smaller/younger than those used by Benesh & Valtonen (2007a), which could explain the observed differences in isopod growth. Nonetheless, whether the reasons behind the observed results in II originate from different foraging habits or different behavioural or physiological resistance among isopods of different size/age or developmental stage, or whether they are truly parasite-driven (manipulation of host resource use, energy reserves, foraging behaviour or hormonal control) remains to be resolved in more specific studies on physiology and behaviour in this host-parasite system.

No difference was found in *A. lucii* prevalence between male and female isopods (II). The only difference between the genders was that when exposed to *A. lucii* as juveniles, male isopods harboured on average significantly more early stage parasites compared to females at the end of the experiment (i.e. males had higher mean *A. lucii* abundance). Considering that in this experiment parasite exposure was continuous, the reason for this may well stem from male juveniles exhibiting more intense foraging behaviour than female juveniles, thereby exposing themselves more intensively to parasite eggs. Male juveniles also grew faster than females (sexual size dimorphism is typical for the species) suggesting they had energetic demands to consume more food than females. On the other hand, could female isopods exhibit behavioural defences against *A. lucii*, e.g. to avoid eating parasite eggs? That kind of avoidance behaviour against starling faeces containing *Plagiorhynchus cylindraceus* eggs has been suggested for female *Armadillium vulgare* isopods (Moore 2002). *P. cylindraceus* causes sterilization of the female isopods as well as altered behaviour, which makes especially female

hosts more susceptible to predation by the definitive bird hosts, starlings (Moore 1983). Sexual divergence in trade-offs between feeding and sheltering behaviour has been proposed for marine isopods of the species *Idotea balthica* (Merilaita & Jormalainen 2000, Vesakoski et al. 2008). Therefore, the idea of female isopods evolving behavioural defences against castrating parasite appears plausible.

Significant differences in *A. lucii* susceptibility were found among isopod populations having different *A. lucii* parasite history (IV). Isopod populations lacking a history of *A. lucii* parasitism (naïve populations) were more susceptible to *A. lucii* in experimental infections than populations known to have a long history with the parasite, but with one exception: the isopod population sympatric to the parasite population used in these infections was as susceptible as the naïve populations. These results are in accordance with the idea that in the absence of the parasite, selection should not favour defences of no use if they bear extra costs. Instead, in the absence of the parasite the available resources for such defences should be traded off with other important fitness components. Indeed, in III the females from the parasite-free population from Niemijärvi (naïve population F in IV) produced bigger broods than females from Kuuhankavesi (allopatric population B in IV) suggesting that naïve females had more resources to invest in reproduction compared to females from populations with *A. lucii* parasitism. Whether this truly represents an evolutionary life history adaptation of the host population as a response to parasitism, needs more evidence. Coevolutionary history or recent epidemic history of the host population with the parasite seems to reduce host susceptibility also in other host-parasite systems, including: *Oncorhynchus mykiss* rainbow trout – *Diplostomum spathaceum* trematode (Karvonen et al. 2005b), *Gasterosteus aculeatus* three-spined stickleback – *Diplostomum pseudospathaceum* trematode (Kalbe & Kurtz 2006), *Daphnia dentifera* – *Metschnikowia bicuspidate* (Duffy & Sivars-Becker 2007), *Daphnia galeata* – microparasite (Schoebel et al. 2010), *Paracalliope novizealande amphibod* – *Maritrema novaezealandensis* trematode (Bryan-Walker et al. 2007).

The isopod population occurring sympatrically with the parasite had higher infection prevalence than the 2 allopatric isopod populations suggesting possible local parasite adaptation (IV). Although, local adaptation has not been studied in any acanthocephalan-isopod system, Franceschi et al. (2010) found indications of local parasite adaptation in two populations of an acanthocephalan-amphipod system when measuring parasite infectivity. In theory local adaptation is expected for the party whose migration rate is higher, although relative generation time might also have an effect (Greischar & Koskella 2007). Nevertheless, to demonstrate local adaptation several sympatric host–parasite population combinations should be cross-infected and both the host and the parasite main effects analyzed for host susceptibility and parasite infectivity (Greischar & Koskella 2007).

4.3 Effects of *A. lucii* exposure and infection on isopod survival (II, IV)

Isopod size or age seemed to influence the effects of *A. lucii* exposure and infection on the survival of isopods. Isopods exposed to *A. lucii* as juveniles clearly survived better than unexposed isopods (II). In addition, exposed isopods newly reaching adulthood seemed to survive marginally better than control isopods of the same size or age (II). Larger and older adult isopods suffered from increased mortality when exposed to *A. lucii* (II). Because survival was defined from the numbers of live isopods at the end of the experiment, it is not known whether those isopods that had died during the 6 – 7 weeks had been infected or not (II). Therefore, the increased adult mortality may result from the parasite infection, the costs of resisting the parasites or both. Experimental laboratory infections of arthropods easily produce unnaturally high infection intensities when the exposure is continuous and the dose is not controlled for. Massive infections or large amounts of acanthors intruding into the haemocoel of the host may cause pathology and lead to death of the host (Nickol 1985, Kennedy 2006). It is not sure if this was the case in II with the adult and larger isopods, although Brattey (1986) also found reduced survival for large *A. aquaticus* experimentally exposed to *A. lucii*.

Studies by Benesh & Valtonen (2007a,b), however, shed light on the issue from the parasite perspective. They showed that intensive *A. lucii* parasitism increased isopod host mortality only during early infection (up to approx. 40 d post infection), but no longer after that, and proposed that the rapid growth of larval parasites is worse for the host viability than the slower growth of larger parasites later in the infection. Wild-caught infected isopods have much lower acanthocephalan intensities and it is assumed that exposure rates in the wild are much lower than those in laboratory experiments (Nickol 1985). Although, there may be local, occasional and seasonal high occurrences of acanthocephalan infections, it is not known if parasites induce any direct mortality among intermediate hosts in the wild (Kennedy 2006). Moreover, growing evidence seems to suggest that trophically-transmitted parasites may not cause direct host mortality i.e. virulence by host exploitation (III, IV, V, Uznanski & Nickol 1980, Wedekind 1997, Benesh & Valtonen 2007a, Benesh 2010).

Thus, if the parasite infection was not directly behind the observed decline in survival of adult isopods (II), it may have been the consequences resulting from host responses to the parasite invasion. These costs of resistance may arise from physiological or behavioural activities. For example, activating immune defences (e.g. production of cellular or humoral components) may increase host energy consumption or divert energy reserves from other vital functions. If the host intensifies energy gains by increasing foraging it may face the risk of more parasite attacks, and therefore result in a stressful loop. Active avoidance of grazing acanthors or selective foraging behaviour may also take up extra energy and end up unprofitable. Also, immunopathology may have been the cause for

increased mortality among the exposed adult isopods. To present knowledge no melanized acanthocephalan larvae have been found within naturally infected *A. aquaticus* haemocoel (Brattey 1986, Dezfuli et al. 1994, Dezfuli 2000, own personal observations). Although, the acanthocephalan larvae have been seen partly enclosed in layers of host cells, the envelope found around the larvae seems to protect the parasite against the host response in the haemocoel (Brattey 1986, Dezfuli 2000, own personal observations). However, observations of host responses to hatched acanthors and acanthors piercing the host intestinal wall are scarce. Nickol & Dappen (1982) were able to find a haemocyte response against *P. cylindraceus* acanthors in the intestinal lumen of the *A. vulgare* host. The gut epithelium is thought to be the essential site for immune responses; for example, the stable fly (*Stomoxys calcitrans*) is able to kill trypanosomes in its midgut within a few days with the help of an antimicrobial peptide secreted exclusively in the anterior midgut of the fly (Boulanger et al. 2002). Considering the quality and the nature of the environments that isopods typically inhabit and are found foraging in, the risk of catching various pathogens via ingestion is rather high. Therefore, it would be plausible that freshwater isopods had also adapted to fight against pathogens and parasites in the immediate site where the attack takes place, in the gut. Immunopathology would also explain why juvenile isopods supposedly incapable of launching an immune response against acanthocephalans did not suffer from increased mortality either when exposed to acanthocephalans (II). Nonetheless, without further evidence II shows that juvenile isopods in this host–parasite system are more resistant to acanthocephalan infections than adults. How they achieved their resistance and whether their resistance bears any survival or other costs have to be proved in more targeted experiments.

Isopods originating from populations where no *A. lucii* parasitism exists (naïve populations) had low survival when they remained uninfected after *A. lucii* exposure in the laboratory indicating they paid survival costs of parasite resistance (IV). Isopods from populations with a long history with *A. lucii* parasitism did not show survival costs of resistance. This suggests that mechanisms of defences or the efficiency of using defences may also differ between the populations. In the absence of *A. lucii* parasitism there may not be a strong selection pressure for the traits responsible for the parasite resistance, unless these traits are somehow linked to other fitness-related traits. However, more detailed knowledge of the infection process is needed to assess what mechanisms and physiological pathways might be involved in the interaction of the present host-parasite system. Interestingly, the adult isopods suffering from significantly lower survival in II were also from the naïve isopod population (population F in IV) indicating the costs of resistance as a possible reason for adult mortality in II. *A. lucii* infection did not seem to cause extra mortality for any isopod population (IV) supporting the suggestion of non-virulent direct host exploitation for trophically-transmitted parasites.

4.4 Ovigerous females and effects of *A. lucii* exposure and infection (III)

Because successful *A. lucii* infection will eventually sterilize the female host thereby causing total loss of fitness, there should be selection for some means of parasite resistance, at least for female isopods. In spite of the continuous exposure in our infection experiment with ovigerous isopods, only 61 % of the exposed females got infected (III), which is clearly less than the 100 % prevalence obtained for adult males and non-ovigerous females (II). Although, infection experiments in II and III are not completely comparable due to different exposure methods, in both experiments isopods were exposed continuously ensuring access to parasite eggs throughout the experiments and justifying a rough comparison. One explanation for lower prevalence among the ovigerous females is that brooding females may forage on average less than non-ovigerous females and males. Johnson et al. (2001) suggested that growing embryos in the marsupium compress the female gut hindering food intake. Furthermore, the large and full brood pouch of ovigerous females may effectively slow down their locomotion and make foraging energetically costly, and also the female more prone to predation. Indeed, in addition to altered feeding patterns many brooding isopods are thought to seek protected habitats to minimize predation risk during the incubation period (Johnson et al 2001). Nevertheless, it should be noted, that this kind of behavioural change is not directed against the parasite. However, if female isopods could recognize the parasite eggs and actively avoid them, it would make the behavioural avoidance trait an adaptation against the parasite. This hypothesis should be confirmed with evidence from choice experiments.

Not necessarily excluding the former reason, the alternative explanation for the observed lower level of susceptibility among ovigerous females would be that their hormonal state boosts or affects the quality or the efficiency of the immune response against *A. lucii*. The crustacean, and also the isopod, reproductive and moulting cycle is controlled by several interacting and multifunctional hormones and molecules (Hopkins 2012, Webster et al. 2012). How this hormonal interplay can interfere with the immunological processes of female isopods is not yet known. Although many studies have found trade-offs between reproductive activity and immunity (see e.g. Zuk & Stoehr 2002), Adamo et al. (2001) found that female crickets (*Gryllus texensis*) increased their phenol oxidase (PO) activity after the onset of sexual behaviour, whereas males decreased their PO activity level at that time. Enhanced parasite resistance in female crickets for several days after mating was also shown by Shoemaker et al. (2006). One possible explanation they suggested was that chemicals in male sperm enhanced the ability to mount an immune response in female crickets. These studies (Adamo et al. 2001, Shoemaker et al. 2006) emphasize the importance of measuring sexual dimorphism of important fitness traits like immune function at different stages of the species life cycle.

Moreover, costs of parasite resistance were found for gravid isopods which remained uninfected in spite of continuous parasite exposure as both their survival and the mean size of offspring was significantly lower compared with controls (III). This suggests that females which allocated resources to efficient activation of their immune system or/and used behavioural defences (avoided consuming abundant acanthors) suffered from reduced fitness. Brooding behaviour consumes female energy reserves (Fernandez et al. 2000, Baeza & Fernandez 2002, Lardies et al. 2004) and for *A. aquaticus* the incubation period takes from 2 to 6 weeks depending on the population and the prevailing temperature (Andersson 1969, Økland 1978). Therefore, it seems likely that resistant ovigerous females were not able to replenish their energy reserves used for defences or the immune reaction caused pathological effects leading to increased mortality rate and reduced parental care (III). The most common cause suggested for physiological costs of resistance is host energy depletion due to immune system activation, especially when resources are limited (e.g. Moret & Schmid-Hempel 2000, Schmid-Hempel 2011). Examples of immunopathology among invertebrates are still scarce, but it is suggested that self-reactivity does occur also in insects (Schmid-Hempel 2005, Sadd & Siva-Jothy 2006). Brooding isopods face physically and physiologically quite different circumstances than non-gravid and male isopods, and therefore it is possible that their immune activity control is also affected by their state. Like Zuk & Stoehr (2002) notice the apparent complexity and diversity of factors interacting in the immunity – life history trade-offs requires a multifaceted approach.

Acanthocephalus lucii infection seemed to increase the survival of ovigerous isopods, but also decrease slightly their mean offspring birth length (III). This indicates that the parasite is able to interfere with the host energy allocation, perhaps by diverting energy from parental care to boost host and thereby its own survival. Conversion of host reproductive resources to host somatic growth or to prolong host life is a commonly suggested exploitation strategy for various other parasites in their intermediate hosts (Minchella 1985, Taskinen et al. 1997, Hurd et al. 2001). However, in spite of the infection, female isopods were capable of producing offspring (III) suggesting that the host exploitation by the parasite was not extremely forceful. Contrary to the findings in IV no statistically significant differences were found in the costs of resistance between the naïve and parasitized population for ovigerous isopods (III). This can be explained simply by the lack of statistical power in III, although it is possible that some physiological or genetic mechanisms of gravid females prevent the elimination of fitness costs associated with resistance (e.g. Tian et al. 2003, Zhong et al. 2005).

4.5 Seasonality in the host manipulation by *A. lucii* (V)

The studied host phenotypic traits were isopod hiding behaviour and abdominal coloration. Infected isopods always had darker abdominal coloration than uninfected isopods regardless of the sampling season, although the coloration varied slightly with the season. Acclimation of the isopods to different temperature and light regimes in the laboratory did not affect the observed difference in coloration between the infected and uninfected isopods. However, isopods kept under warmer and lighter conditions (16 – 17 °C with L:D 18:6) were on average darker than isopods acclimated to the two colder and darker conditions. These rather consistent results of host coloration may indicate that the altered host abdominal coloration alone does not contribute to *A. lucii* seasonal occurrence. For two other species of acanthocephalans using *Gammarus pulex* as their host, the altered host coloration by itself was shown not to be related to enhanced parasite transmission (Kaldonski et al. 2009). On the other hand, isopod hiding behaviour changed significantly between the seasons. In general infected isopods spent more time exposed than uninfected isopods, but the difference disappeared by late autumn and was largest in the spring. Regardless of the acclimation conditions (temperature and light regime) infected isopods spent more time exposed than uninfected isopods, although the warmer and lighter environment induced an increase in hiding behaviour. Put together, these results support the earlier observations that modification of the host coloration and host behaviour may have different mechanisms (Benesh et al. 2008). Furthermore, the results suggest that the traits altered by infection, the abdominal coloration and the hiding behaviour, can vary with abiotic factors. However, the effect of infection on isopod hiding varied only with season, not with changing environmental conditions suggesting the seasonal alteration of host behaviour is dependent more on host or parasite age than on abiotic conditions.

Because the isopods collected from the lake were not a random sample, interpretations of the length differences between the infected and uninfected isopods should be treated with caution. However, the lack of length difference between infected and uninfected isopods may be an indication of selective predation. If the larger infected isopods are more heavily manipulated by the parasite as suggested by Benesh et al. (2009), and if the increased manipulation leads to enhanced predation, which was observed by Seppälä et al. (2008), one would not find the infected isopods any larger than uninfected in the wild samples of isopods, although *A. lucii* infection would increase the host length as was suggested previously (II).

Again *A. lucii* infection did not affect isopod host mortality. Furthermore, maintaining isopods at high temperature (15 – 17 °C) induced the anticipated mortality increase. However, behavioural manipulation of infected isopods was not accelerated with the decreased host survival suggesting that the parasite was not able to respond to the deterioration of host condition. Instead, refuge use by

isopods kept at low temperature tended to decrease over the 7 months; however, the divergence in refuge use between infected and uninfected isopods tended to increase significantly towards the spring. By May infected isopods were exposed 75 % but for uninfected isopods the exposure value remained at 20 %. These results suggest the following seasonal pattern in the isopod manipulation and in the host-parasite cycle for the study system at northern latitudes. Isopods born during spring and summer can be infected with *A. lucii* after reaching the size of 3 mm (II). Parasite larvae become infective to fish in late summer and autumn, but the refuge use by the infected isopods remains mostly unchanged. Some of the parasite population is likely transmitted to fish definitive hosts already during the late summer and early fall, but much of the parasite population overwinters in isopods (Brattey 1986). Gradually towards the spring manipulation of host hiding behaviour increases with increasing parasite /host age. Isopod predation by the definitive fish host, the perch, peaks in spring when large adult isopods are abundant and light conditions allow efficient hunting by the visually orientated forager (Persson 1983, Rask & Hiisivuori 1985, Brattey 1988).

Although, it has been suggested that host manipulation should be most intense when encounter rates are low (Poulin 1994), it may not be advantageous for the parasite if manipulation does not increase transmission to the right definitive host. Therefore, from the parasite perspective, intensive manipulation of isopod host behaviour during late summer and autumn would not affect parasite transmission success, if isopods are not then the major item in the diet of the preferred fish host. Delaying transmission until spring may involve increased risk of dying during the winter, but the value of the used manipulation strategy is based on the increased probability of successful transmissions. After all, it is the net fitness benefits that are counted (Poulin et al. 2005). Furthermore, because the overwintered isopods are likely to die soon after having reproduced in the spring or by early summer at the latest (Rask & Hiisivuori 1985, Brattey 1986), spring transmission and increased manipulation at that time would be favoured. However, until the net benefits of manipulation are quantified in the field, it cannot be definitely determined whether isopod behavioural alteration is an adaptive strategy for *A. lucii* (e.g. Poulin 2010). Nevertheless, these studies raise further interesting questions. Is the parasite adapted to the seasonality of the host life cycle? To what extend is the castrating parasite able to affect the seasonality of the host life cycle?

4.6 Further consideration – mostly from the host perspective

These results prompt speculation of the possible consequences and further potential bearings on the nature of isopod host – acanthocephalan parasite interaction. Here I want to reflect mainly the host perspective of the issue, because many aspects of the parasite perspective have already been dealt elsewhere (e.g. Benesh & Valtonen 2007a,b, Benesh et al. 2008, 2009, Seppälä et al.

2008). Size is an important fitness factor for male isopods, because larger males are able to outcompete the smaller males in mating (Ridley & Thompson 1979, Bertin & Cezilly 2003). Therefore the enhanced growth induced by *A. lucii* infection might have potential to benefit the isopod host. However, Benesh et al. (2007) showed that precopulatory mate-guarding behaviour of *A. aquaticus* males increased their mortality risk and that the observed mortality risk was positively related to the male length. Thus, the possible fitness benefits of large size appear to be complicated. Furthermore, infection may decrease the competitive ability of the males or willingness to mate (Zohar & Holmes 1998, Sparkes et al. 2006, Bierbower & Sparkes 2007), although this has not yet been shown experimentally for *A. aquaticus*. Instead, Brattey (1983) observed that cystacanth-infected males were at least capable of fertilizing uninfected females in the laboratory.

It is also known that fecundity of females (brood size) increases with increasing size of the female (Andersson 1969, Ridley & Thompson 1979 and references therein, Flössner 1987). Nevertheless, for a female host the potential growth benefit induced by the parasite is likely not used to boost the host reproductive output, because cystacanth-infected females are known to be sterile (Brattey 1983). Nonetheless, there is a chance for females to reproduce in spite of the infection if the timing of reproductive effort is scheduled before the parasite larva induces the sterilization. Indeed, Brattey (1986) found a few ovigerous females harbouring early *A. lucii* infections. Also, Dezfuli et al. (1994) found one of the 6 *A. anguillae* infected *A. aquaticus* females carrying 50 eggs in its brood pouch. The infection experiment with ovigerous females confirms their findings, and although the infection did seem to slightly impair the quality of the offspring (measured as mean length of the offspring) infected females were able to produce one progeny (III). These findings prove that there is a narrow window of opportunity for female isopods to restore some of their fitness in spite of the infection. How realistic it is in the wild, is a subject for another study. For example, it is not known how long it takes before the developing *A. lucii* larva induces the atrophy of isopod ovaries. However this period, although short-term, seems to be long enough to protect the production of one brood thus allowing fitness gains and compensation for the obviously inevitable later sterilization due to the infection.

Furthermore, during the reproductive period female isopods may get extra protection against *A. lucii* exposure due to the reproductive characteristics of the species. In *A. aquaticus* mating requires precopulatory pairing where the male grasps and holds the female tightly under his body (Ridley & Thompson 1979). This precopulatory pairing can last for days or even over a week and, although Manning (1975) noted that "both individuals can apparently feed normally", no experimental evidence of this exists for *A. aquaticus* yet. Anyway, Jormalainen (1998) made the point that for female isopods this mate guarding behaviour may cause qualitative and quantitative feeding costs because she cannot influence where the male will carry her.

The parasite point of view should also be considered when assessing factors affecting its transmission from the definitive host to the intermediate host. In the *A. lucii* life cycle, the egg (acanthor larva enclosed in layers of envelopes or

eggshells) is the only free-living stage and therefore has a crucial role for parasite fitness. Very little is known about the egg dispersal in the wild and the dynamics of transmission to isopods in their natural habitats. Nevertheless, laboratory investigations on egg viability of some acanthocephalan species suggest large variation, but the survivorship of eggs is at least time- and temperature-dependent ranging from a few weeks to over a year for some species (Kennedy 1985, 2006). Acanthocephalan eggs can be considered as resting and resistant stages. Indeed, the envelopes around the acanthor larva obviously provide protection against a wide variety of environmental conditions from fish gut via freshwater to intestine of the isopod host (e.g. Crompton 1985, Nickol 1985, Nikishin 2001). Besides offering protection the various structures and functions of the outer envelopes on the egg are suggested to be adaptations facilitating parasite dispersion and transmission to intermediate hosts (Nickol 1985, Nikishin 2001, Wongkham & Whitfield 2004). Many acanthocephalan eggs, like *A. lucii* (own observations, Amin et al. 2011), possess fibrils or filaments around them and it is suggested that they help the eggs to attach on algae and biofilms thus bringing them on offer for grazing isopods (Nickol 1985). Furthermore, the role of these fibrils in anchoring the egg on the lumen of the host gut, thus enhancing parasite establishment, may also be significant. Recent findings and observations on *A. dirus* egg dispersal (Wahl & Sparkes 2012) and attraction of the *Caecidotea intermedius* isopod host to feed on gravid female *A. dirus* worms (Kopp et al. 2011) suggest that isopods must have some means to sense the parasite in the environment. Which one is on the winning side; is the parasite luring the isopod to launch an eating response towards the egg or is the isopod able to alarm the defensive avoidance behaviour? More specific experimental approaches should elucidate these issues and the possible mechanisms behind the capability of an isopod to sense or distinguish the acanthocephalan eggs.

5 CONCLUSIONS

Unfiltered tap water was shown to decrease the survival of laboratory born *Asellus aquaticus* isopods possibly due to the concentration of Cu in it (I). The unresponsiveness of isopod reproduction to the growth factor and light treatments and the observed generation-specific survival effects of the treatments (I) indicate interesting aspects in the nutrition of isopods which have the potential to affect the isopod susceptibility to parasites transmitted via ingestion, like the trophically-transmitted *Acanthocephalus lucii*. In particular, investigations of the role and action of digestive and defensive agents in the guts of different aged isopods from different populations would be useful for initiating deeper knowledge about the interaction between the host and the parasite.

In contrast to previous studies with comparable isopod host – acanthocephalan parasite systems, juvenile *A. aquaticus* isopods were less susceptible to acanthocephalan infection than adult isopods (II). This either implies that juveniles do not prefer to eat the parasite eggs or the mechanisms operating to establish *A. lucii* infection in the juvenile gut may not be as functional as they are in the gut of adult isopods. These mechanisms may be, for example, related to different kinds of recognition agents in the interplay between the isopod digestive tract and the acanthocephalan acanthor larva. The survival effects of *A. lucii* exposure on isopods depended on the size/age of the isopod. Juvenile isopods survived better compared to controls under exposure but adults suffered from increased mortality when exposed to *A. lucii* parasites (II) suggesting an age-specific response of isopods against the attacking parasites. Experimentally infected isopods harbouring cystacanths grew larger than unexposed isopods (II) suggesting either parasite ability to affect host energy economy or indicating a positive relationship between the host growth, foraging activity, and susceptibility to *A. lucii*. Male and female isopods seemed not to differ in *A. lucii* susceptibility (II).

Gravid female isopods that were able to resist *A. lucii* infection under continuous exposure had lower survival and produced smaller offspring indicating the parasite resistance came with the costs (III). Although susceptible females had also a slight decrease in the length of offspring, their survival was

better than that of control isopods (III). This seems to be an indication of the parasite's ability to interfere with the resource allocation of the host, thus diverting energy from host parental effort into somatic maintenance to boost host and own survival. Isopod populations differed in their susceptibility to the *A. lucii* parasite depending on the parasite origin and whether or not the isopod population had a previous history with *A. lucii* parasitism (IV). Naïve isopods from populations without *A. lucii* parasitism were more susceptible to *A. lucii* infections than allopatric isopods from populations with *A. lucii* parasitism under controlled parasite exposure in the laboratory (IV). In addition the isopods sympatric to the *A. lucii* parasite population used in the exposure were as susceptible as naïve isopods (IV). However, only resistant isopods from the naïve populations, but not from the parasitized populations, exhibited increased mortality, implying that resistance entails survival costs primarily to naïve isopods (IV). This suggests that co-existence with the parasite has potential to shape the defence mechanisms of isopods.

A. lucii parasite is known to manipulate the behaviour and coloration of the isopod host so that infected isopods have darker abdominal coloration and spend more time exposed than uninfected isopods, thereby predisposing the host to increased risk of predation. Irrespective of altering the temperature and light regimes of the isopods kept in the laboratory, infected isopods were always darker and spent less time hiding than uninfected isopods (V). However, the difference between infected and uninfected isopods in hiding behaviour increased from autumn to spring (V), the season of optimal time for transmission to the definitive fish host, the perch. Thus by May the time infected isopods spent exposed was 75 % while for uninfected isopods the value remained around 20 %. These results suggest that host age and/or parasite age, rather than environmental conditions, affect the strength of parasitic manipulation of host behaviour. Furthermore, *A. lucii* infection did not affect isopod survival during the 7 months isopods were kept at cold environmental conditions in the laboratory (V). This implies that the parasite does not risk the host and its own survival by overexploiting the host.

To conclude, annual life cycle of isopods and the observed seasonality in *A. lucii* occurrence may allow some fitness compensation for the infected isopod hosts. Because juveniles are less susceptible to *A. lucii* (II), at least female isopods may have a chance to reach the reproductive age and reproduce once before the infection castrates the host (III). The developing parasite larva in the isopod intermediate host does not seem to cause direct mortality of the host (III, IV). Instead resisting *A. lucii* infection seems to incur survival costs depending at least on the life cycle stage and the parasite history of the exposed isopods (III, IV). The behavioural manipulation by the parasite seems to be most intensive especially in spring when the host and the cystacanth have grown old (V) and when the likelihood of becoming preyed upon by the preferred fish host is high. These studies yielded some unexpected results on the isopod host – acanthocephalan parasite system, but further fascinating insights into both the causality and mechanisms of the observed phenomena wait to be revealed.

Acknowledgements

First I would like to express my gratitude to Professor Emerita E. Tellervo Valtonen, my supervisor, whose persistence and never-ending confidence in succeeding kept me doing research work with this fascinating but challenging host–parasite system. Her enthusiasm inspired me during the years and left me with forever longing for scientific research, "the quest for truth". I also thank my other supervisor, Professor Jouni Taskinen for his relaxed, but firm attitude to writing this thesis. "Tiina, here's the good thing: everything has not to be completely perfect…", was the last kick I needed from him to finish the revision. Naturally, I am deeply grateful for all my co-authors for their help, advice and comments. I thank Professor Jukka Jokela for saving me from drowning in multitude of possible ways of data processing and interpretation – his expertise in seeing the profitable core in a tangled mass of data is incredible. I was honoured to share the interest in host–parasite interactions with Professor Emeritus John C. Holmes. I also acknowledge Dr. Otto Seppälä and Dr. Daniel P. Benesh: the two sharp-witted young scientists, whose talents and amazing skills of finding solutions and simply just doing it helped me to realize where I stood. Particularly, I thank Dan for finally taking care of the correspondence for the article IV during the time when the other commitments were my first priority. I must acknowledge Professor Roger Jones for kindly checking the English of this summary, the editor Dr. Timo Marjomäki for his patience and the pre-examiners Dr. James C. Chubb and Professor Raine Kortet for their comments.

During the many years at Ambiotica the staff and trainees gave valuable practical help, which I will always remember, smilingly. I warmly thank all the members of the aquatic parasitology group for their stimulating company, insightful discussions and sharing thoughts. Especially, I owe my warmest thanks to Dr. Katja Pulkkinen, Dr. Mari Saarinen-Valta, Dr. Tuula Sinisalo, Dr. Lotta-Riina Sundberg, Dr. Anssi Karvonen and Dr. Heidi Kunttu. I feel privileged to have had the opportunity to enjoy your companionship and discuss all the matters of work and life with you. I miss those coffee breaks! I also want to thank my present colleagues at the Clinical physiology and isotope laboratory for their positive attitude and understanding towards my academic commitment.

I want to express my sincere gratitude to my relatives and family. My mother Raija-Liisa, my sister Sari, my parents-in-law Aino and Väinö and my brother-in-law Juha-Pekka, whose empathy I have always felt and who have helped me with domestic backup whenever I've needed. Thank you, Jere, Joel and Anni just for being my children: your existence reminds me what is the most important in life. I owe my deepest gratitude to my husband, Hannu. His encouragement, technical support, comments on the summary and practical help, like checking the reference list at the end, speeded up the writing process and made it more bearable. Thank you, Hannu, for your endless understanding; "…and nothing else matters…"

This study was financially supported by the Finnish Cultural Foundation, Ella and Georg Ehrnrooth Foundation and the Academy of Finland.

YHTEENVETO (RÉSUMÉ IN FINNISH)

Vesisiira (*Asellus aquaticus*) –isäntään liittyvien tekijöiden vaikutuksista väkäkärsämatoloisintaan (*Acanthocephalus lucii*)

Makeiden vesien yleinen pohjaeläin vesisiira (*Asellus aquaticus*) on *Acanthocephalus lucii* -väkäkärsämatoloisen väli-isäntä. Vesisiira saa loistartunnan syötyään *A. lucii* -loisen akantor-toukan, joka tunkeutuu vesisiiran suolen läpi ruumiinonteloon kasvamaan. *A. lucii* -loisen tiedetään kastroivan naaraspuoliset vesisiiraisännät. Kun loinen on kasvanut kystakantti-toukaksi, se pystyy manipuloimaan väli-isäntänsä ilmiasua ja käyttäytymistä siten, että loisitut vesisiirat ovat takaruumiistaan tummempia ja viettävät enemmän aikaa näkyvillä kuin loisettomat vesisiirat. Tällaisen käyttäytymisen on havaittu altistavan loisittuja vesisiiroja loisettomia yksilöitä enemmän *A. lucii* -loisen kalapääisännän, ahvenen (*Perca fluviatilis*) saalistukselle.

Tämän väitöskirjatyön tarkoituksena oli tutkia kokeellisesti, mitkä vesisiiraan liittyvät tekijät vaikuttavat väli-isännän alttiuteen saada *A. lucii* -loistartunta ja tarkastella isäntä–lois-vuorovaikutussuhteen luonnetta. Tutkittuja isäntään liittyviä tekijöitä olivat vesisiiran koko/ikä ja sukupuoli (II) sekä vesisiirapopulaation aiempi *A. lucii* -loishistoria (III, IV). Lisäksi tutkittiin vesisiiranaaraiden alttiutta loisinnalle jälkeläisten kantovaiheen aikana sekä vertailtiin loiselle alttiiden ja vastustuskykyisten naaraiden kelpoisuutta mittaamalla niiden hengissäsäilymistä ja jälkeläistuotantoa (III). Vesisiirapopulaation aiemman *A. lucii* -loishistorian vaikutusta isäntä–lois-suhteen luonteeseen tarkasteltiin altistamalla *A. lucii* -loiselle aikuisia vesisiiroja, jotka olivat peräisin joko populaatioista, joissa ei ollut *A. lucii* -loista (*A. lucii* -loiselle naiivit vesisiirat) tai populaatioista, joissa oli ollut *A. lucii* loisintaa (IV). Kokeessa mitattiin ja vertailtiin eri populaatioista peräisin olevien loiselle alttiiden ja vastustuskykyisten vesisiirojen hengissäsäilymistä (IV). *A. lucii* -loisinnasta isännälle aiheutuvan ilmiasu- ja käyttäytymismuutoksen vuodenaikaisvaihtelua tarkasteltiin kokeissa, joissa tutkittiin, riippuuko manipulaation voimakkuus fysikaalisista muutoksista ympäristössä vai isännän/loisen ikääntymisestä (V).

Koska vesisiira–väkäkärsämato-mallisysteemin ylläpidosta laboratorio-olosuhteissa tiedetään toistaiseksi hyvin vähän ja koska kokeellisten loisaltistusten toteuttaminen vaatii kontrolloituja olosuhteita, tutkittiin ensin luonnosta kerättyjen vesisiirojen selviytymistä ja lisääntymistä laboratoriossa kolmea eri alkuperää olevissa viljelyvesissä (vesijohtovesi, porakaivovesi, lähdevesi) (I). Tämän lisäksi testattiin kolmen kasvutekijän (L-karnitiini, vitamiinivalmiste, kirkasvalo) vaikutusta vesisiirojen hengissäsäilymiseen ja lisääntymiseen (I). Tutkimuksissa selvisi, että suodattamaton vesijohtovesi lisää merkitsevästi vastasyntyneiden vesisiirojen kuolleisuutta laboratorioviljelmissä (I), mikä johtuu todennäköisesti vesijohtoveden Cu-pitoisuudesta. Laboratoriokokeissa testatut kasvutekijä- ja kirkasvalokäsittelyt eivät parantaneet vesisiirojen selviytymistä tai lisääntymismenestystä (I). Vesisiirojen ravitsemukseen ja ravinnonkäyttöön liittyvät tekijät ovat keskeisessä roolissa, kun arvioidaan vesisiirojen alttiutta

loiselle, joka siirtyy ravinnon mukana isäntäänsä, kuten väkäkärsämato väli-isäntäänsä vesisiiraan. Erityisen hyödyllistä olisi tutkia, kuinka ruuansulatus ja immuunipuolustus suolistossa eri ikäisillä ja eri populaatiosta peräisin olevilla vesisiiroilla toimii ja kartoittaa näiden mekanismien merkitystä sekä mahdollisia yhteisvaikutuksia yhtäältä isännän alttiuteen saada loistartunta ja toisaalta loisen kykyyn infektoida isäntä. Tällaisen tiedon avulla voidaan ymmärtää syvällisemmin isäntä–lois-suhteen vuorovaikutuksia.

Vastoin aiempia tutkimuksia samantyyppisillä isäntä–lois-systeemeillä tutkimukset paljastivat, että nuoret vesisiirat olivat vähemmän alttiita *A. lucii* –väkäkärsämatoloisinnalle kuin aikuiset vesisiirat (II). Tulos viittaa joko siihen, että nuoret vesisiirat eivät mielellään syö akantor-toukkia tai *A. lucii* -infektion mahdollistavat mekanismit nuorten vesisiirojen suolistossa eivät ole yhtä toimivia kuin aikuisilla vesisiiroilla. Tällaiset mekanismit voivat liittyä esimerkiksi erilaisiin vastavuoroisiin tunnistustekijöihin vesisiirojen ruuansulatuskanavan ja väkäkärsämatoloisen toukkien välillä. Väkäkärsämato-altistuksen vaikutus vesisiirojen hengissäsäilymiseen riippui vesisiirojen koosta, toisin sanoen iästä. Nuoret vesisiirat selvisivät loisaltistuksessa kontrolleja todennäköisemmin hengissä, kun taas aikuisilla vesisiiroilla loisaltistus lisäsi kuolleisuutta (II). Tämä viittaa vesisiirojen iästä riippuvaan vasteeseen hyökkääviä loisia vastaan. Laboratoriokokeessa loistartunnan saaneet vesisiirat, joissa väkäkärsämatoloinen oli kehittynyt kysta-kantti-vaiheeseen, kasvoivat suuremmiksi kuin loiselle altistamattomat kontrollivesisiirat (II). Tämä tulos vihjaa joko väkäkärsämatoloisen kykyyn vaikuttaa isäntänsä energiatalouteen tai on osoitus positiivisesta korrelaatiosta isännän kasvun, ravinnonkäytön ja *A. lucii* -väkäkärsämatoloiselle altistumisen välillä. Vesisiirojen alttiudessa *A. lucii* -loistartunnalle ei ollut eroa koiraiden ja naaraiden välillä (II).

Jatkuvasta loisaltistuksesta huolimatta *A. lucii* -loiselle vastustuskykyisinä pysyneet kantavat vesisiiranaaraat kärsivät kontrollinaaraita korkeammasta kuolleisuudesta ja tuottivat pienempiä jälkeläisiä, mikä viittaa vastustuskyvyn aiheuttamiin kustannuksiin (III). Vaikka myös loiselle alttiit naaraat tuottivat jonkin verran pienempiä jälkeläisiä altistamattomiin kontrolleihin verrattuna, nämä loistartunnan saaneet kantavat naaraat säilyivät todennäköisemmin hengissä kuin kontrollivesisiirat (III). Tämä viitannee siihen, että *A. lucii* -loinen pystyy häiritsemään isännän voimavarojen kohdentamista ohjaamalla energiaa jälkeläishoivasta isännän elimistön toiminnan ylläpitoon edistäen täten sekä omaa että isäntänsä hengissäsäilymistä.

Vesisiirapopulaatioiden välillä oli eroja *A. lucii* -loisalttiudessa riippuen vesisiirapopulaation *A. lucii* -loishistoriasta sekä siitä, oliko kokeellisessa altistuksessa käytetty *A. lucii* -loiskanta peräisin samasta järvestä kuin sille altistetut vesisiirat (IV). Kontrolloiduissa laboratorioaltistuskokeissa naiivit vesisiirat, jotka olivat peräisin populaatioista, joissa ei ollut *A. lucii* -loisintaa, olivat alttiimpia *A. lucii* -loistartunnalle kuin vesisiirat populaatiosta, joissa oli *A. lucii* -loisintaa (IV). Ainoastaan sen populaation vesisiirat, jotka olivat peräisin samasta järvestä kuin altistuksessa käytetty *A. lucii* -loiskanta, olivat yhtä alttiita loistartunnalle kuin naiivien populaatioiden vesisiirat (IV). Kuitenkin vain naiiveista populaatioista peräisin olevat loiselle vastustuskykyiset vesisiirat kärsi-

vät poikkeavan suuresta kuolleisuudesta (IV). Toisin sanoen isännän kyvystä vastustaa loistartuntaa aiheutuu kuolleisuutta enimmäkseen naiiveille vesisiiroille. Tulokset viittaavat siihen, että isäntäpopulaation aiempi yhteiselo loislajin kanssa (coevolution, yhteisevoluutio) voi muokata esimerkiksi isäntäpopulaation puolustusmekanismien energiataloutta.

Tutkimukset *A. lucii* -loisen vesisiiraisännille aiheuttamasta ilmiasun ja käyttäytymisen manipulaatiosta paljastivat, että ympäröivästä lämpötilasta tai valojaksoisuudesta riippumatta laboratoriossa pidetyt loisitut vesisiirat olivat kaikissa olosuhteissa tummempia ja viettivät aina vähemmän aikaa piilossa kuin loisettomat vesisiirat (V). Laboratoriossa marraskuusta toukokuuhun kestäneessä käyttäytymisen seurantakokeessa ero piiloutumisessa loisittujen ja loisettomien vesisiirojen välillä kuitenkin kasvoi ja oli suurinta keväällä (V), loisen kannalta optimaalisena aikana siirtyä pääisäntäänsä kalaan, tyypillisimmin ahveneen. Loisitut vesisiirat viettivät toukokuussa 75 % ajasta esillä, kun samaan aikaan loisettomilla vesisiiroilla vastaava luku jäi 20 %:iin. Tulokset viittaavat siihen, että isännän ikä ja/tai loisen ikä, eivät niinkään ympäristön fysikaaliset olosuhteet, vaikuttavat siihen, kuinka voimakkaasti loinen manipuloi isännän käyttäytymistä. Seitsemän seurantakuukauden aikana, jonka vesisiirat viettivät kylmässä laboratorio-olosuhteissa, *A. lucii* -loistartunnalla ei ollut vaikutusta vesisiirojen hengissäsäilymiseen (V). Tämän mukaan loinen ei näyttäisi riskeeraavan isännän ja samalla myös omaa selvitymistään kuormittamalla isäntäänsä liikaa.

Luonnossa oikea ajoitus ratkaisee usein kelpoisuuteen liittyvien piirteiden hyödyn. Vaikka *A. lucii* -loinen pystyy kastroimaan vesisiiranaarasisäntänsä ja manipuloimaan isäntiensä käyttäytymistä aiheuttaen täten niille huomattavaa kelpoisuuden alenemaa, peli ei vesisiiran kannalta kuitenkaan ole täysin menetetty. Vesisiirojen talvehtineet yksilöt kuolevat vanhuuttaan viimeistään alkukesän mentyä ja koska uuden sukupolven nuoret vesisiirat ovat vähemmän alttiita *A. lucii* -loistartunnalle (II), nuoret aikuistuneet naaraat voivat hyvinkin ehtiä lisääntymään kerran ennen kuin *A. lucii* -loinen on tarttunut ja pystyy kastroimaan isäntänsä (III). Kehittyvä väkäkärsämadon toukkavaihe vesisiira-väli-isännässä ei suoraan näyttäisi aiheuttavan kuolleisuutta isännälleen (III, IV, V). Kun loistoukka on saavuttanut kystakantti-vaiheen, sen aiheuttama isännän käyttäytymisen manipulaatio lisää isännän riskiä altistua predaatiolle. Tämä manipulaatio näyttäisi olevan intensiivisintä keväisin, kun vesisiiraisäntä sekä kystakantti-loinen ovat jo vanhoja sekä kasvaneet kooltaan isoiksi (V) ja kun todennäköisyys joutua sopivan kalapääisännän predaation kohteeksi on suuri. Tutkimukset vesisiira–väkäkärsämato -mallilla paljastivat joitakin odottamattomia tuloksia isäntään liittyvien tekijöiden vaikutuksesta loisalttiuteen sekä isäntä–lois-suhteen luonteeseen. Syy–seuraus-suhteet ja mekanismit havaittujen ilmiöiden takana odottavat tutkijaansa.

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