ABSTRACT

Among Unionidae clams, the parasite *Paraergasilus rylovi* was specific to *Anodonta piscinalis*. The parasite occurred in southern Finland. The mean intensity of infection of *P. rylovi* was higher in lake populations compared to rivers. Within a lake, *P. rylovi* was more abundant at moderate depth compared to shallow or deep habitats. *Paraergasilus rylovi* produced eggs between June and August and new infections were established in clams in August. The abundance of *P. rylovi* increased with proportionate burrowing depth of *A. piscinalis*. There was an inverse relationship between *P. rylovi* intensity and age/size of the host, but no dependence between the intensity of infection and host sex. Female *A. piscinalis*, which brooded glochidia larvae were more abundantly infected by *P. rylovi* than non-reproducing females. The parasites were possible to expel from the gills of *A. piscinalis* by using high water temperature or low oxygen. *Paraergasilus rylovi* were expelled before an experiment conducted to study local adaptation of *P. rylovi*. In the reciprocal transplant experiment between two locations, both infectivity and reproductive performance of *P. rylovi* were higher in home-clams in both transplant sites, indicating that *P. rylovi* may be genetically specialized to its local host populations. In other a field experiment, anoxia-stressed clams were more susceptible to infection by *P. rylovi* than unstressed individuals when exposed to parasites about 11 months after the stress treatment. The stressed clams also showed significantly lower growth, lower reproduction and lower survival than the unstressed clams. The result indicates that stress may have long-lasting effect on host susceptibility to parasitism in natural populations.

Key words: *Anodonta piscinalis*; behaviour; coevolution; freshwater; local adaptation; *Paraergasilus rylovi*; reproduction; stress; survival; susceptibility.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-VI. I am the main writer in the papers I-II and IV-VI and I have planned and performed large part of the work in each paper. I collected the materials together with Jouni Taskinen. In paper I, some of the material was from earlier, unpublished collections made by Jouni Taskinen. I planned the experimental studies in papers IV-VI with Jouni Taskinen, and I was responsible for the performance of those studies. Paper III was written from my M. Sc Thesis so that the main writer was Jouni Taskinen. I had the main responsibility of writing in papers I, II, IV, V and VI.

I   Saarinen, M. & Taskinen, J. 2004. Aspects of the ecology and natural history of *Paraergasilus rylovi* (Copepoda, Ergasilidae) parasitic in unionids of Finland. Journal of Parasitology (accepted for publication).

II   Saarinen, M. & Taskinen, J. 2004. Burrowing behaviour, parasitism and growth in the freshwater clams *Anodonta piscinalis* and *Pseudanodonta complanata*. Submitted.

III   Taskinen, J. & Saarinen, M. 1999. Increased parasite abundance associated with reproductive maturity of the clam *Anodonta piscinalis*. Journal of Parasitology 85(3): 588-591.

IV   Saarinen, M. & Taskinen, J. 2003. Reduction in the level of infection of the bivalve *Anodonta piscinalis* by the copepod *Paraergasilus rylovi* using high temperature and low oxygen. Journal of Parasitology 89(6): 1167-1171.

V   Saarinen, M. & Taskinen, J. 2004. Long-lasting effect of stress on susceptibility of a freshwater clam to parasitism. Submitted.

VI   Saarinen, M. & Taskinen, J. 2004. Local adaptation in a crustacean parasite-molluscan host interaction: a field experiment. Submitted.

1 INTRODUCTION

1.1 General introduction

Biological diversity of parasites is overwhelming. It has been estimated that among animals, parasitism as a way of life is more common than all other feeding strategies combined (Price 1980). Therefore, our knowledge on the taxonomy and ecology of parasitic species is limited. Among those described, many of the species remain unknown by their ecology and natural history, even though they may have an important ecological impact on their host individuals and populations.

Parasitism is affected by ecological/environmental factors such as age, sex and behaviour of host and habitat structure, but also by genetic factors, manifested as host specificity and local adaptation. There is increasing evidence that genetic factors are important in determining susceptibility of hosts to parasitic infections, and that coevolutionary processes influence the genetics of both host susceptibility and parasite infectivity (e.g. Frank 1993, Webster & Davies 2001, Agrawal & Lively 2003). However, in natural populations, ecological and environmental variables, such as habitat structure or stress (see reviews by Sousa & Grosholz 1991, Lafferty & Kuris 1999), may have a large effect on the outcome of a host-parasite relationship. For example, poor quality of the environment can affect the physiological condition of the host and thereby influence the amount of resources that can be allocated to defence against parasites, thus making the host more susceptible to parasitism than would otherwise be expected by their genotypes (Jokela et al. 2000).

Host specificity is defined as the extent to which a parasite taxon is restricted in the number of host species used at a given stage in the life cycle (Poulin 1998). Highly host-specific parasites are restricted to one host species and specificity declines with the number of suitable hosts used by the parasite. The number of hosts used by parasite is determined by two factors. First, only a subset of all potential host species is actually encountered by the parasite. Second, in only part of those potential host species encountered, the parasite is

enough adapted to the morphological, physiological and immunological features of host, that it survives and continues its development (Wakelin 1984, Combes 1991, Poulin 1998). Close adaptation to one host species can usually be achieved only at the expense of adaptations to other host species (Fry 1990, Poulin 1998).

Parasite accumulation over time increases the intensity of parasite in older age classes of host, but if the virulence of parasite is density dependent and correlates positively with host mortality, maximum parasite loads occur in hosts of intermediate age (Anderson & Gordon 1982). Decreasing parasite loads with age may also be a consequence of decreased exposure or susceptibility of host to parasites due to altered behaviour or diet (Anderson & Gordon 1982, Lozano 1991, Hart 1994), changed utilization/preference of habitat or particularly due to acquired immunity (Mitchell et al. 1977, Bayne 1991, Folstad & Karter 1992). Few studies have found negative relationship between the age of animal host and the numbers of parasites they harbour (Humphrey et al. 1978, Zuk 1988, Borgia & Collis 1989).

Reproduction has generally been suggested to be a factor subjecting individuals to a higher risk of parasitism. Studies on parasites of vertebrates have demonstrated that increased reproductive effort may result in higher prevalence and intensity of parasitism (e.g. Festa-Bianchet 1989, Gustafsson et al. 1994, Oppliger et al. 1996, Allander 1997). Since both reproduction (Reznick 1985, Nur 1988) and immune responses (Klasing et al. 1991, Ilmonen et al. 2000) are costly, reproduction may increase susceptibility to parasites by reducing energy available for immunological defence. This is supported by finding that reproductive effort decreased bird antibody production (Deerenberg et al. 1997). Reduced immunocompetence and increased parasitism may also be related to hormonal changes that accompany competition for mates (Christian & Davies 1964, Folstad et al. 1989), pregnancy (Alexander & Stimson 1988, Roberts et al. 1996), or lactation (Agyemang et al. 1992). In addition, higher parasite load associated with reproduction may result from increased exposure to parasites due to reproductive activities (e.g. Tinsley & Jackson 1988, Zuk 1990, Richner et al. 1995).

The sex-related differences in the occurrence of infections within a population are mainly caused by differences in exposure to parasites or susceptibility of the host. Parasitism has been reported to be more intense in females (Drobney et al. 1983, Taskinen & Valtonen 1995), in males (Tinsley & Jackson 1988, Folstad et al. 1989, Richner et al. 1995, Poulin 1996, Wedekind & Jakobsen 1998), or to be unrelated to host sex (Bull & Burzacott 1993, Allander & Bennett 1994). In addition, the relationship between host sex and parasite load may be seasonally variable (Drobney et al. 1983, Van Damme & Ollevier 1994). For example the consumption of invertebrates, which serve as intermediate host for many avian helminths (McDonald 1969), is considerably higher in female wood ducks (*Aix sponsa*) in breeding time in spring compared to males (Drobney et al. 1983). On the other hand, sex-bias in resistance due to testosterone may make males more susceptible to infections in mammals (Alexander & Stimson 1988, Folstad et al. 1989).

During their lives, organisms are subject to stress, which is a condition in which the internal or external stressors threaten or disturb the internal homeostasis, which every organism is maintaining for survival (Chrousos & Gold 1992). Stress can vary in its duration and quality (physical, chemical, biotic or social), and it may weaken the condition of the individual and, consequently, reduce the fitness of the individual, decrease the size of a given population or limit the distribution of a species (Alpert et al. 2000, Hoffmann & Hercus 2000, Whittier et al. 2002). Parasites and diseases are an important component of any ecosystem (Price 1980), and therefore stress and parasitism frequently interact in natural populations. The interplay between stress and parasitism can be complex (Lafferty & Kuris 1999). Parasitism itself can impose a stress on the host individual, and result in fitness costs such as reduced survival, growth or reproduction of host (e.g. Lehman 1993). On the other hand, stress can lead to increased parasitism by impairing the host immune system (reviewed e.g. by Murray & Young 1992, Pruett et al. 1993). The negative effect of parasites and pathogens on a host can be magnified by stress, such as starvation, both at individual and population level, due to the condition-dependent nature of host resistance and parasite virulence (Brown et al. 2000, Krist et al. 2004).

Various stressors have been shown to increase susceptibility of vertebrates to a wide range of parasites and pathogens (e.g. Peters et al. 1988, Folstad et al. 1989, Carballo et al. 1995, Roberts et al. 1996, Davis et al. 2002) Contrary to vertebrates, among invertebrates studies demonstrating the relationship between stress and susceptibility to parasites and diseases are scarce. An increased susceptibility due to stress was reported by Van Banning (1991), Abrous et al. (2001) and Lacoste et al. (2001). In addition, Hine et al. (2002) studied the effects of different stressors (e.g. stirring, air exposure, hot water, cold water, hypersaline water, starvation) on the dynamics of protistan *Bonamia exitiosus* infections in flat oyster *Ostrea chilensis*, and demonstrated an increase in susceptibility of stressed oysters to infections. However, even a severe stress does not necessarily result in increased susceptibility to parasitism, as observed by Krist et al. (2004) in a freshwater snail-trematode parasite system.

The relationship between parasitism and behaviour of animals has gained increasing attention among ecologists during recent years. A number of studies have concentrated on such parasite-induced behavioural alterations of host, which enhance parasite transmission (see review by Moore 2002). For example, Thomas & Poulin (1998) noticed a negative relationship between the load of trematode *Curtuteria australis* and the relative length of the foot of its intermediate host, the marine bivalve *Austrovenus stutchburyi*. Infected clams with a stunted foot were unable to bury under the sediment surface. Predation experiment conducted in natural conditions revealed that infected individuals with a lowered ability to burrow were more susceptible to predation by the definitive hosts of the parasite, shorebirds (Thomas & Poulin 1998). On the other hand, the behaviour of host may also affect the probability of infection. For instance, males of the toad *Scanhiopus couchii* spend long periods in water where the infective stages of the monogenean parasite *Pseudodiplorchis americanus* are transmitted, whereas the females enter the pond only once for a

short period to reproduce, and consequently, the males are more infected (Tinsley & Jackson 1988).

Spatial variation in parasitism is especially observed in intermediate hosts of trematodes such as clams and snails (Robson & Williams 1970, Williams & Esch 1991, Jokela & Lively 1995a, Taskinen & Valtonen 1995, Smith 2001). This variation may result e.g. from differences in exposure between habitats used by intermediate host. For example, definitive host of digenean trematode, *Rhipidocotyle fennica*, pike, *Esox lucius*, are the source of infective miracidia to first intermediate host, freshwater clam *Anodonta piscinalis*. Pikes are more abundant in the littoral zones of lakes, and consequently, clams in littoral zone are more prevalently infected by *R. fennica* than clams in sublittoral zone (Taskinen & Valtonen 1995). Habitat-specific variation in parasitism may also result from differences in host life history traits and population parameters between habitats such as age at maturity (Jokela and Lively 1995b), sex ratio (Taskinen & Valtonen 1995) and proportion of sexually reproducing individuals (Lively 1987, Jokela & Lively 1995b, Lively & Jokela 1996). In addition, the amount and quality of food may differ between habitats and result in difference in condition and consequently differences in host susceptibility to parasitism (Wahli et al. 1998) between habitats. Genetic variation in susceptibility may also result in spatial variation in parasitism, even within a host population/lake (Jokela & Lively 1995a, Lively & Jokela 1996, Krist et al. 2000). In addition, some habitats, which are suitable for host, are not preferred by the parasite, for example abundance and prevalence of ergasilid copepod parasite, *Ergasilus celestis*, of eel, *Anguilla rostrata*, were lower in stream habitats with higher water velocity (Barker & Cone 2000).

On the other hand, seasonality in production of infective stages may result in temporal variation in parasitism. For example, the prevalence of digenetic trematode *Notocotylus attenuatus* in first intermediate host, lymnaeid snail *Lymnea peregra*, increased during spring and summer when large numbers of infective miracidia are present in water (Väyrynen et al. 2000).

Because of the higher evolutionary potential of parasites, due to their larger population sizes, shorter generation times and higher mutation rates, parasites are expected to be specialized i.e. locally adapted to the most common host genotypes (e.g. Hamilton et al. 1990, Ebert 1994, Lively 1996, 1999, Krist et al. 2000, Dybdahl & Storfer 2003). Locally adapted parasites should have, on average, higher infectivity and/or greater reproductive success in the local (sympatric) host population compared to a non-local (allopatric) host population (Ebert 1994, Ebert & Hamilton 1996, Gandon & Van Zandt 1998, Kaltz et al. 1999). Local adaptation of parasites to their host have been observed in many study systems, such as plants and their pathogens (Parker 1991), plants and herbivore (Mopper et al. 1995), snails and digenean trematodes (Manning et al. 1995, Lively & Jokela 1996), fish and trematodes (Ballabeni & Ward 1993), and *Daphnia* water fleas and their microsporidian parasites (Ebert 1994). However, other studies did not observe local adaptation (Parker 1989, Ennos & McConnel 1995, Davelos et al. 1996, Dufva 1996, Strauss 1997, Imhoof &

Schmid-Hempel 1998, Mutikainen et al. 2000), or found a higher infectivity of the parasite in allopatric hosts (Roy 1998, Kaltz et al. 1999, Oppliger et al. 1999).

Most of the local adaptation studies have applied laboratory infections in common garden set up using laboratory-reared hosts and parasites. This approach has several advantages, such as the controlled source of both host and parasite, and a controlled environment for the establishment of infection. However, besides the laboratory studies, experiments of local adaptation under natural conditions would be valuable to allow better extrapolation from laboratory results to field conditions. A few studies on local adaptation have utilized natural infections in the field (e.g. Davelos et al. 1996, Dufva 1996, Roy 1998). However, naturally infected hosts collected from the field, from which parasites are expelled, have not been used in local adaptation studies so far.

1.2   Aims of the study

The aim of this thesis was to investigate the ecological/environmental and genetic factors contributing to the abundance of ergasilid copepod parasite *Paraergasilus rylovi*. In the first three papers, I studied the natural history of the parasite; the geographic distribution and host specificity of the parasite, the occurrence of the parasite in relation to host age, size, sex and habitat and for the reproductive period (occurrence of egg sacs) of the parasite (I). I examined the relationship between *P. rylovi* abundance and the behaviour (II) and the reproductive status (brooding and non-brooding females) of the host clam (III). The fourth paper describes a method to expel the parasite without harming the host clam (IV). In the fifth paper, I studied the long-lasting effect of stress on host susceptibility to parasitism. In addition, the effects of stress on clam survival, growth and reproduction, and on parasite reproduction, were examined (V). In the sixth paper, a reciprocal transplant experiment between two locations was conducted to study if the parasite is adapted to its local host population (VI).

2   METHODS

2.1   Study species (I-VI)

Anodonta piscinalis Nilsson (Mollusca, Bivalvia) is a widespread and abundant clam inhabiting slowly running waters and littoral zones of temperate lakes in northern Europe (Bauer et al. 1991). It matures at 2-4 years of age and reproduces annually (Haukioja & Hakala 1978a, Bauer 1994), reaching a maximum life span of more than 15 years (Økland 1963, Negus 1966, Haukioja & Hakala 1978a). Spawning takes place in early summer and fertilized eggs are stored in the outer gill blades of females, where they develop into glochidia larvae (Jirka & Neves 1992). The development of glochidia larvae takes place in August in Finland, and they are fully developed in autumn, when water temperature begins to fall (Jokela et al. 1991). Glochidia are stored in the gills over winter, and released next spring (Økland 1963, Negus 1966). After release, they attach to fish for a few weeks, during which they metamorphose (Ellis 1978, Jokela et al. 1991). The glochidia of *A. piscinalis* can infect several host species (Jokela et al. 1991). After successful metamorphosis, young clams drop to bottom, and their benthic live begins. (I-VI)

The natural histories of *Pseudanodonta complatata* Rössmässler, *Unio pictorum* Linnaeus and *U. tumidus* Philipsson are basically the same as of *A. piscinalis*, although the spawning of *A. piscinalis* takes place in June (Jokela et al. 1991, Taskinen et al. 1997) and that of *P. complanata* from May to July (Aldridge 1999). The glochidia of *Anodonta piscinalis* are fully developed in autumn (Jokela et al. 1991, Jokela 1996), whereas the glochidia of *P. complanata* may sometimes mature during winter (Pekkarinen & Englund 1995). The development of glochidia of *U. pictorum* lasts about one month (Tudorancea 1969, Pekkarinen 1992, 1993), and many broods per year are possible in *Unio* species in temperate zones (Dudgeon & Morton 1983, Pekkarinen 1992). (I-II)

The ergasilid copepod *Paraergasilus rylovi* Markewitsch 1937 was redescribed by Chernysheva & Purasjoki (1991) on the basis of planktonic specimens and mature specimens parasitic on the clam *Anodonta piscinalis* in

Lake Vrero, St Petersburg district. Markewitsch (1937) and Markewitsch & Kusmorska (1937) described the species from planktonic material from brackish water from the northern Caspian Sea, but subsequent findings have been mainly from freshwater; mature specimens being found from nostrils or gills of fishes (e.g. Kostarev 1977). However, Titar & Chernogorenko (1982) found their species from the freshwater clam *Anodonta piscinalis*. Since the type-material used by Markewitch (1937) has been destroyed, and due to uncertainties of original description, it is possible that instead of *P. rylovi*, Chernysheva & Purasjoki (1991) described *P. markewitsch* Titar & Chernogorenko 1982. Before this taxonomic problem is solved, the name *P. rylovi* is used.

Adult females of *P. rylovi* are parasites, inhabiting the gills of the freshwater clam, *Anodonta piscinalis* (Chernysheva & Purasjoki 1991). Other aspects of the biology of *P. rylovi* are largely unknown. Ergasilid copepods are ectoparasites of many freshwater fish, and they feed on gill tissues and blood, and may cause deformation or necrosis of the gill filaments (Bauer et al. 1973). To my knowledge, *P. rylovi* is the only ergasilid species specialized on freshwater molluscs. (I-VI)

Unionid bivalves are frequently parasitized by water mites (Baker 1977, Fisher et al. 2000). The larvae of hydrachnellid water mites leave a host clam and undergo a parasitic phase within insects, primarily chironomids (Jones 1978), after which they reinvade a host clam for further development (Böttger 1977, Gledhill 1985, Smith & Cook 1991). Parasitic adult and nymphal stages attach to gills, mantle, palps and the visceral epithelium (McMahon 1991) feeding on mucus, gill tissue or hemolymph of the unionacean host (Baker 1977, Fisher et al. 2000). (II)

The trematode parasite *Rhipidocotyle fennica* Gibson, Taskinen and Valtonen (Digenea, Bucephalidae) uses the bivalve *A. piscinalis* as first intermediate host (Taskinen et al. 1991), affecting negatively the glycogen storage (Jokela et al. 1993), reproduction (Taskinen & Valtonen 1995) and growth (Taskinen 1998) of the host. (I-III, V)

2.2 Collecting clams (I-VI)

Number of individuals of four species of unionids studied during 1987-1989, 1994, 1996 and 2002-2003 are summarized in Table 1.

During 1987, 1988 and 1989, clams were collected from the littoral (depth 1-2 m) and sublittoral (depth 3-4 m) zones of Lake Saravesi, 30 km northeast from the city of Jyväskylä, Finland, using a bottom dredge. For description of the collection site, see Taskinen & Valtonen (1995). In 1987, 344 *A. piscinalis*, 19 *P. complanata* and 11 *U. pictorum* were collected. In 1988 the corresponding figures were 151, 12 and 7, and in 1989 85, 14 and 3. Clams were collected at different times of year. In 1987 the collection months were as follows: May, June, July, August, and October. In 1988 the collections were made in July,

August, and September, and in 1989 in March and November. This material is the same used by Taskinen et al. (1994) to study the seasonality of trematode infection in *A. piscinalis*, except November and December 1987 samples, December 1988 sample, and January 1989 sample used by Taskinen et al. (1994), which were excluded, since those clams were kept for several days in a higher temperature than the lake water, which may have reduced the number of *P. rylovi* (IV). Monthly collections (numbers of *A. piscinalis* collected in parentheses) were also made in Lake Saravesi in 1996 using scuba diving: May (42), June (18) and August (25). (I)

TABLE 1   Number of studied individuals of four species of unionids studied during 1987-1989, 1994, 1996 and 2002-2003.

| | Year | | | | | | |
| Species | 1987 | 1988 | 1989 | 1994 | 1996 | 2002 | 2003 |
				N studied			
Anodonta piscinalis	344	151	85	29	902	1305	387
Pseudanodonta complanata	19	12	14	19	87		
Unio pictorum	11	7	3		64		
Unio tumidus					17		

In 1994, a sample of mature clams, *A. piscinalis* (n= 29) and *P. complanata* (n= 19), was collected by a scuba diver from the littoral zone of Lake Alvajärvi close to the city of Jyväskylä, Finland. This material was used in (II).

Between May and October 1996, *A. piscinalis* (n= 902) were collected using scuba diving from 17 locations in Finland, 11 of the locations were in southern Finland, within 100 km from the city of Jyväskylä (see Table 1 in I). The southern populations contained also *P. complanata* (n= 87), *U. pictorum* (n= 64), and *U. tumidus* (n= 17). Six of the locations were in northern Finland, between the cities of Oulu and Rovaniemi. In September (n=73) and October (n=22) 2002, *A. piscinalis* were collected from Lake Saravesi using scuba diving. (I)

The material from 1996 from Lakes Alvajärvi, Jyväsjärvi and Saravesi were partially used in (III), and from Lakes Alvajärvi, Saravesi and Tuomiojärvi in (II) The data collected from Lake Saravesi in October 2002 was used as a field reference sample in (IV).

For the 'recovery experiment' (IV), 312 *A. piscinalis* were collected using scuba from the littoral zone of Lake Saravesi in September 2002. For three other smaller experiments, 45 clams were collected from Lake Saravesi and 35 clams from adjacent Lake Ahveninen in October 2002. (IV)

To study long-lasting effect of stress on susceptibility to parasitism (V), 463 *A. piscinalis* were collected from Lake Jyväsjärvi in August and 355 *A. piscinalis* from Lake Ahveninen in September 2002.

To study local adaptation of *P. rylovi* (VI), 213 *A. piscinalis* were collected using scuba from the littoral zone of Lake Saravesi and 134 clams from River Kuusaankoski, which flows to Lake Saravesi in May 2003. The populations are 4

km apart. In August 2003, at the end of the experiment, 20 clams from each population were collected as reference samples.

2.3   Laboratory examinations (I-VI)

In the laboratory, clams were aged by counting the annual rings of the shell and their length was measured (see Haukioja & Hakala 1978b) (I-VI). Occurrence of glochidia larvae in the outer gill blades was checked visually (III, V). Active, adult stages of *Unionicola* water mites were collected and counted (II). The gill tissues were pressed between two large glass plates and examined for *P. rylovi* (I-VI) and larval stages of *Unionicola* water mites (II) using a dissection microscope and transmitted light. For each *P. rylovi* individual, the occurrence of egg sacs was examined (I, V-VI). The gonads of the clams were examined for larval trematodes and for gender using the same method (Taskinen et al. 1991) (I-III, V). Clams were considered to be females if they had egg follicles in their gonad, and sexually mature, if they brooded glochidia larvae (I, III, V).

2.4   A field survey: *Paraergasilus rylovi* in Unionidae clams (I)

The aim of this study was to investigate the natural history and ecology of *Paraergasilus rylovi*. The geographic distribution of the parasite was studied in 17 clam populations in southern and northern Finland (Table 1 in I). Host specificity of the parasite was studied in 11 southern populations, including four species of Unionidae clams (*A. piscinalis, P. complanata, U. pictorum* and *U. tumidus*), between May and October 1996. In one clam population, Lake Saravesi, the parasite was studied more closely in relation to host age, size, and sex, and for the reproductive period (occurrence of egg sacs) of the parasite in 1987, 1988, 1989, and 1996.

Seven populations, four of which were from lakes (numbers 3, 7, 8, 11 in Table 1 in I) and three from rivers (numbers 1, 2, and 4 in Table 1), were sampled in May 1996 (a total of 399 *A. piscinalis*) to study the effect of habitat (stagnant vs. flowing water) on the intensity of *P. rylovi* infection. To study the effect of infection of the trematode *R. fennica* on the intensity of *P. rylovi*, all *A. piscinalis* collections from 1987-1989, 1996, and 2002 in Lake Saravesi were combined.

2.5 A field survey: Burrowing behaviour, parasitism and growth in the freshwater clams (II)

Among bivalvian molluscs, behavioural changes related to parasitism have been studied in marine species, especially with respect to digenean trematode parasites. Many hypotheses have been proposed to explain interaction between parasites and (altered) behaviour of their marine bivalvian hosts. Trophically transmitted trematode metacercariae may make their host to remain closer to the sediment surface (Lim & Green 1991), or have a reduced ability to burrow (Lauckner 1984, Thomas & Poulin 1998) to enhance predation (and transmission) probability. As in the case of parasitism and burrowing, the few studies on the relationship between burrowing behaviour and clam growth are performed using marine species (e.g. De Goeij & Luttikhuizen 1998, Mouritsen 1997, Rde la Huz et al. 2002).

The relationship between parasitism and the behaviour of freshwater bivalves has not been previously studied. Therefore, the aim of this study was to investigate (1) the abundance of the arthropod parasites *P. rylovi* and *Unionicola* water mites, and (2) the occurrence of the trematode parasite *R. fennica*, and (3) growth of clam in relation to the burrowing behaviour of freshwater bivalve clams, *A. piscinalis* and *P. complanata* in three Finnish lakes. Since the current parasites are not transmitted trophically, any adaptive parasite manipulation of host behaviour to enhance transmission was not predicted. To study parasitism and growth of *A. piscinalis* and *P. complanata* with respect to burrowing behaviour, the height of the above-sediment part of the shell (i.e., sediment-siphon distance, SSD, the line of the sediment surface marked by a scuba diver on the shell) and the length of the clam were measured in the laboratory. The proportionate burrowing depth (% of the clam length under the surface of the sediment) was calculated as follows: ((length-SSD)/length)100%.

2.6 A field survey: Abundance of *Paraergasilus rylovi* in relation to reproductive maturity of the host (III)

Bivalves are suitable models to study the variation in parasite load in relation to host reproductive status, because their reproductive effort is high (Haukioja & Hakala 1978a, Bayne et al. 1983, Mackie 1984, Sprung 1991). Females of maturating age groups (from 3 to 5 yr) from three populations were studied to examine the relationship between abundance of *P. rylovi* and the reproductive status (brooding and non-brooding females) of the host clam, *A. piscinalis*, in August and October 1996. Female clams produce and brood glochidia larvae in that time (Jokela et al. 1991, Taskinen et al. 1997) and the reproductive status of female clams can easily be determined by the presence or absence of glochidia.

2.7   Experimental manipulation of the intensity of the parasite (IV)

The aim of this study was to develop a method to kill, or expel, the crustacean parasite *P. rylovi* from the gills of the host, the freshwater unionid clam *A. piscinalis*. This would contribute to the use of *P. rylovi* — *A. piscinalis* association as a model in studies of the ecology of host-parasite interactions by enabling experimental manipulation of the intensity of parasitism.

Ergasilid copepods seem to be sensitive to changes in temperature. Pojmanska (1984) reported free-living stages of the ergasilid copepod parasite of fish, *Ergasilus sieboldi*, to suffer from high temperature in artificially heated lakes. Therefore, the effectiveness of temperature in freeing *A. piscinalis* from *P. rylovi* infection was first chosen to test. In addition, the effect of low oxygen on *P. rylovi* was tested, since *Anodonta* clams are known to be capable of anaerobic metabolism under anoxic conditions (Van Den Thillart & De Vries 1985). The survival of the host clams was also monitored in the different water quality treatments, since the ideal method would result in complete recovery from infection combined with minimum impact on the survival of the host.

Four experiments were performed. In the 'recovery experiment', infected clams were exposed to different water quality treatments for 14-days to kill, or expel, the crustacean parasite *P. rylovi* from the gills of the host, *A. piscinalis*. Water quality treatments were: (1) 'high temperature' treatment (26° C, aeration, high oxygen); (2) 'low-oxygen, water-flow' treatment (18° C, no aeration); (3) 'low-oxygen, no water-change' treatment (18° C, no aeration) and; (4) control (18° C, aeration, high oxygen). In the 'host abandon experiment', clams were exposed to conditions that were found unfavourable to *P. rylovi* in the recovery experiment and individual clams were monitored to determine whether *P. rylovi* are able to abandon the host and move into the water column. In the 'host switch experiment', it was studied if *P. rylovi*, which leave one host, are able to infect another host individual. In the 'survival experiment', survival of individual *P. rylovi*, which were removed from the host, were monitored.

2.8   Long-lasting effects of stress (V)

Common to the studies demonstrating the relationship between stress and susceptibility to parasites is that they have usually been conducted in the laboratory, and the exposure to parasites or diseases took place during, or shortly after the stress event. The question of whether a stress event can have long-lasting effects on host susceptibility in natural populations has not been studied. This question was addressed by exposing clams from two populations, uninfected Lake Ahveninen and infected Lake Jyväsjärvi, to stress (anoxia) for 25 days in August and September 2002, after which the stressed clams were

transported to Lake Saravesi together with unstressed clams, which were caged in their home populations during the 25 days. The stressed clams from Lake Jyväsjärvi were freed from parasites during the stress treatment by the poor water quality, as it was shown in (IV). In Lake Saravesi clams were placed in the bottom of the littoral zone at 1 m depth in basins (12 L, diameter 34 cm) filled one third with sand, 5 clams from both treatments to one basin. Clams were allowed to become infected naturally about 11 months after the stress event. Samples were taken monthly, except for winter months, in order to monitor the infection of clams by *P. rylovi*. Experiment lasted 11 months; last containers were fetched back to the laboratory in August 2003. Furthermore, the effects of stress on clam survival, growth and reproduction, and parasite reproduction, were examined.

2.9 A reciprocal transplant experiment: local adaptation of the parasite (VI)

So far, most of the local adaptation studies have applied laboratory infections using laboratory-reared host and parasites. However, besides the laboratory studies, experiments of local adaptation under natural conditions would be valuable to allow better extrapolation from laboratory results to field conditions. To study the local adaptation of the copepod parasite *P. rylovi* in the clam host, *A. piscinalis*, a reciprocal transplant experiment between two locations, Lake Saravesi and River Kuusaankoski, was performed. Before the experiment, parasites were removed from host clams in the laboratory using the high temperature method described in (IV). During the experiment, clams were kept in their natural environment and were allowed to become infected naturally. In addition, in Lake Saravesi the clams were transplanted to three different depth-habitats (0.8, 2.0 and 4.0 m). The infectivity (parasite abundance) and reproduction (proportion of parasites carrying egg sacs) were used as measures of parasite adaptation.

3 RESULTS AND DISCUSSION

3.1 *Paraergasilus rylovi* in Unionidae clams (I)

Paraergasilus rylovi preferred the freshwater clam *A. piscinalis* as a host, while the other unionid bivalves were either only occasionally infected (*P. complanata*) or not infected (*U. pictorum*, *U. tumidus*) (Table 2 in I). In addition, no *P. rylovi* females were found with egg sacs in *P. complanata*, suggesting that the parasite may not be able to reproduce in *P. complanata*. These results indicate that *P. rylovi* is specialized to infect *A. piscinalis*, only. Host specificity of three other ergasilid copepods was studied in the present area by Tuuha et al. (1992). In an extensive investigation including four fish populations and a total of 2230 fishes, *Ergasilus sieboldi* was strictly specified to *Perca fluviatilis*, and *E. briani* and *Neoergasilus japonicus* to *Rutilus rutilus*. *Paraergasilus longidigitus* occurred both in *P. fluviatilis* and *R. rutilus*, but none of the ergasilids infected *Coregonus* sp. (Tuuha et al. 1992).

Paraergasilus rylovi occurred in southern Finland. In the material pooled over populations (southern Finland, 11 populations) and years (1987-89, 1996) the mean prevalence and intensity (± se) of infection in *A. piscinalis* was 71.2% and 16.4 ± 0.6, respectively. The parasite was absent in the northern Finland (Table 1 in I). The only other location from which *P. rylovi* has been reported in clams so far is the St. Petersburg district in Russia (Chernysheva & Purasjoki 1991). If the distribution of *P. rylovi* is limited by chance of introduction to new areas, it is not surprising that southern Finnish populations harbour the parasite, since hydrologically they share the same recipient, Gulf of Finland, Baltic Sea, with St. Petersburg region. The drainage areas of the northern populations flow in other directions, to the Gulf of Bothnia, Baltic Sea, or White Sea, Arctic Ocean. However, the possibility that the shorter summers and cooler waters in the north inhibit successful reproduction of *P. rylovi*, thus restricting the parasite to southern areas, cannot be excluded.

The mean intensity of infection of *P. rylovi* was higher in lake populations (mean ± se, 14.7 ± 1.0) compared to river populations (1.9 ± 0.3). Gee & Davey

(1986) reported lower levels of infection of a parasitic copepod, *Mytilicola intestinalis*, in its host, the blue mussel *Mytilus edulis*, in turbulent regions compared to calm regions. They hypothesised this resulting from the essentially passive nature of the transmission process, that the poor swimming, short-lived infective stages of *M. intestinalis* enter the mussel via filtration. A similar process might explain the lower prevalence and intensity of *P. rylovi* parasitism in rivers compared to lakes in the current study. In addition, increased stream velocity has also been found to decrease the abundance and prevalence of fish ergasilid copepod, *E. celestis* in wild eels, *Anguilla rostrata* (Barker & Cone 2000).

The egg sacs of *P. rylovi* in Lake Saravesi occurred during the warm water period in June-July-August. The reproductive period of the fish ergasilid, *E. briani* takes place at the same time in Lake Saravesi (Tuuha et al. 1992). There was a seasonal tendency for higher intensities of infection to be found in late autumn in Lake Saravesi. Eggs hatched in summer produce larvae that would become infective adults during the autumn, which could result in an increase in the intensity of infection at that time.

Female and male clams did not differ in *P. rylovi* infection intensity. *Paraergasilus rylovi* probably enter the clam host via filtration like the parasitic copepod *M. intestinalis* in the mussel *M. edulis*. Larger sized *M. edulis* with a larger field of filtration (Davey & Gee 1976) harbour more *M. intestinalis* (Gee & Davey 1986). A similar size (and age) dependent pattern of infection is found in the prevalence of the filtration-entering trematode *R. fennica* in *A. piscinalis* (Taskinen & Valtonen 1995). Therefore, it was surprising that there was a negative relationship between size (and age) of the host, and intensity of *P. rylovi* infection. The behaviour of the host might change with age and size so that the exposure to parasitism decreases, or there might be age-related changes in susceptibility to parasitism. Alternatively, the increasing prevalence of the trematode *R. fennica* with size (age) of *A. piscinalis* (Taskinen & Valtonen 1995) might have a negative effect on *P. rylovi*. The present results did not yield an association between *P. rylovi* and *R. fennica*, giving no support to the last hypothesis. However, experimental studies are needed to resolve alternative explanations.

3.2   Burrowing behaviour, parasitism and growth (II)

The abundance of *P. rylovi* increased with proportionate burrowing depth of *A. piscinalis*. Furthermore, the abundances of encysted water mites in *A. piscinalis* and *P. complanata* were higher in completely burrowed individuals than among partly visible ones. Among active, adult water mites, such a positive relationship between mite abundance and proportionate burrowing depth was not found. Indeed, it may be assumed that parasite-parasite interactions can be more important in harem-building adult unionicolids (Davids et al. 1988) in regulation of their population size within a host opposite to tissue-penetrating

larval water mites and blood-feeding *P. rylovi* (Bauer et al. 1973). The infection of trematode *R. fennica* had no main effect on the proportionate burrowing depth of *A. piscinalis* in Lake Saravesi.

The infective stages of parasites of bivalve molluscs, such as copepodits of copepod parasites and nymphal stages of water mites, invade or are sucked into the host clam via inhalant siphons (e.g. Gee & Davey 1986, Smith & Cook 1991, Bush et al. 2001). In the current clam species, the inhalant siphons are located at the highest point of the shell in relation to the surface of the bottom sediment. If the parasites *P. rylovi* and *Unionicola* spp. actively seek the host clam, it could be assumed that less burrowed (more visible) clams would be easier to be found, which would result in a negative relationship between parasitism and deeper location of clam. However, the present results indicate a positive association between parasitism and burrowing depth of the clam.

Several alternative hypotheses may be proposed to explain the present results. For example, increasing burrowing depth may increase exposure to parasites even if it seems intuitively improbable. Negative phototaxis, or positive geotaxis, of the infective stages of the parasites would increase the risk of infection in deeper burrowed clams. Bolster (1954) observed copepodits of *Mytilicola intestinalis* to swim downward and escape light. Indeed, *Mytilus edulis* individuals, which are growing or are cultured off the bottom, have lower infection rates than individuals, which live at bottom (Hockley 1951). Dimock and Davids (1985) studied the spectral sensitivity and photo-behaviour of *Unionicola* water mites. They showed experimentally that the positive phototaxis of *U. intermedia* became negative when the mite was exposed to water taken from the mantle cavity of its molluscan host, *Anodonta anatina* (= *A. piscinalis*). An alternative hypothesis is that the increasing burrowing depth may increase clam susceptibility to parasitism. If the feeding efficiency decreases with burrowing depth, as found by De Goeij and Luttikhuizen (1998) for *Macoma balthica*, it could be assumed that host's ability to control the infection (immune responses) would become impaired with increased burrowing depth, and consequently *P. rylovi* may survive better in burrowed host. In the present study, the relative growth of *A. piscinalis* during the growing season was lower in completely burrowed individuals than among partly visible clams. This may indicate that resources available for visible clams are more abundant than for the completely burrowed individuals.

For *P. rylovi*, *A. piscinalis* is the only host in the life cycle. For water mites, *A. piscinalis* is the final host for both adult and nymphal stages of the parasites (Baker 1987). Therefore, it is unlikely that the observed parasitism pattern with respect to host burrowing, would result from adaptive manipulation of the host behaviour by parasites, although it is possible, however, that manipulation of host burrowing depth (increasing burrowing depth, decreasing exposure) may reduce competition between parasite individuals within a host.

The results indicate that deep burrowing may be associated with increased parasitism and decreased growth. Why do clams then burrow deep? Visible clams can be assumed more vulnerable to muskrat (*Ondatra zibethicus*) predation than burrowed clams. In the present study areas, muskrats are

important predators of unionid clams (Jokela & Mutikainen 1995a). Therefore, the optimal burrowing depth of clams may vary temporally and spatially depending on the prevailing conditions with respect to risk of parasitism and predation. This study provides correlative results of field patterns in the study of relationships between the behaviour of unionid clams, growth and parasites. However, experimental work is needed to reveal the causal connections behind observed patterns, by manipulating clam burrowing, parasitism, predation and resources available.

3.3 Abundance of *Paraergasilus rylovi* and reproductive maturity of the host (III)

Female *A. piscinalis*, which carried glochidia larvae, were more abundantly infected by *P. rylovi* than non-reproducing female clams in Lakes Alvajärvi, Jyväsjärvi and Saravesi; the observed pattern was consistent between the lakes. The mean length-adjusted abundance of *P. rylovi* over populations was 15.3 and 8.4 parasites per host for reproducing and non-reproducing individuals, respectively.

Several alternative mutually non-exclusive hypotheses can be proposed to explain the present result. For example, maturation and reproduction may increase exposure of clams to infective stages of *P. rylovi*. Altered host behaviour associated with maturation may increase exposure of *A. piscinalis* to *P. rylovi* copepodits. Immature unionid clams *Elliptio complanata* are found completely buried in the substratum while mature clams are only partly burrowed (Amyot & Downing 1991). Watters et al. (2001) observed several North American freshwater clam species to surface during spawning in spring, and discovered that some of them remained exposed until autumn. Individuals of some other species re-burrowed after spring, but then surfaced again and remained there until autumn. However, my results on burrowing behaviour and *P. rylovi* parasitism suggest that the possible deeper burrowing of immature *A. piscinalis* would rather increase than decrease the abundance of *P. rylovi* infection (II). Since the biomass of glochidia larvae, that are produced and brooded by female *A. piscinalis*, constitute on average 20% of the total biomass of clam soft tissues (Jokela et al. 1993), reproducing females may have to increase their filtration rate to compensate for the increased nutritional demands of reproduction. This could lead to increased exposure to *P. rylovi*, and contribute to the observed higher parasite abundance in reproducing females. Tankersley & Dimock (1993) found no differences between the filtration rates of brooding and non-brooding females in the unionid clam *Pyganodon cataracta*, but they could not tell whether brooding females filter longer periods per day than non-brooding ones. Thus, the idea that changed filtration rate accompanied with reproduction, or changed burrowing

behaviour associated with maturation, would expose *A. piscinalis* to higher parasite abundance, cannot be rejected.

Alternatively, *P. rylovi* may survive and reproduce better in mature, reproducing clams. This may be the case if there is a trade-off in the energy allocation between reproduction and immunological defence in *A. piscinalis*, or if maturation/reproduction induces hormonal changes that suppress immunity. A prerequisite of this hypothesis, and host immune response to *P. rylovi*, is that the parasite is harmful for the host. Nagasawa & Nagata (1992) reported that the parasitic copepod *Pectenophilus ornatus* fed on host blood, and decreased condition index of the scallop host *Patinopecten yessoensis*. Ergasilid gill parasites of fish feed on gill tissues and blood, and have caused deformation or necrosis of the gill filaments (Bauer et al. 1973). Thus, *P. rylovi* may be assumed to be potentially harmful for its clam host. Therefore, and because allocation to reproduction in bivalves is high (Mackie 1984), the possibility that the higher parasite abundance in reproducing *A. piscinalis* would result from reduced immunocompetence in reproducing individuals cannot ruled out.

Reproductive maturity of the host, *A. piscinalis*, seems to increase the risk of parasitism. This may be a consequence of behavioural, physiological or immunological differences between reproducing and non-reproducing clams, but experimental work is needed to separate between the alternative explanations.

3.4 Experimental manipulation of the intensity of the parasite (IV)

All 3 different water quality treatments in the 'recovery experiment' decreased the abundance of *P. rylovi*. The most effective treatment was 'high temperature' treatment (26° C, aeration, high oxygen), followed by 'low-oxygen, no water-change' treatment (18° C, no aeration) and 'low-oxygen, water-flow' treatment (18° C, no aeration). High temperature seemed to be particularly effective, because the abundance of *P. rylovi* collapsed to near zero by day 7 (Fig. 1 in IV). The results of the 'host abandon experiment', where the clams were kept in separate small aquaria at 26° C, indicate that the increase of temperature from 18° C to 26° C (8° C/24 hr) may expel *P. rylovi* very quickly – only 1 single live parasite individual was observed in the host clam after 24 hr at 26° C. In the 'recovery experiment', dead parasites were never observed in the host clams. However, results of the 'host abandon experiment' suggest that the parasites leave the host clam in unfavourable conditions, and subsequently, die in the water, since dead parasite specimens were observed in the water during the first 3 days after the temperature increase in that experiment. The harmfulness of high temperature, or temperature increase, to *P. rylovi* was also demonstrated by the results of the 'survival experiment', in which parasites removed from the

host and kept in Petri dishes, in aged tap water at 18° C of temperature, survived up to 16 days (on average 12.7 days).

The temperature chosen for this study, 26° C, is at the limit of the natural variation in the present study area, so 26° C water temperature would occur occasionally, although not every year. Maximum surface water temperatures of 24° C are commonly reached in Lake Saravesi (Taskinen et al. 1994). The rapid increases in temperature applied in this study (8° C/24hr) are unrealistic in natural waters, so in the wild populations, high temperatures do not necessarily harm *P. rylovi.* However, in some special habitats, such as artificially heated lakes, harmful temperature regimes may occur. Indeed, Pojmanska (1984) reported that the ergasilid copepod of fish, *Ergasilus sieboldi*, suffer from high temperatures in thermally polluted lakes. Report by Valtonen et al. (1993) of higher prevalences of the crustacean fish parasite *Achtheres percarum* in cooler summers compared to warm summers is also consistent with the experimental results of this study.

The 'host switch experiment', where clams from infected Lake Saravesi and adjacent uninfected Lake Ahveninen, were placed into a single container at 18° C, showed that *P. rylovi* which abandon a host, are able to move to another host individual. However, the abundances of *P. rylovi* remained low in clams from uninfected Lake Ahveninen, so although able to infect new host individuals, not all *P. rylovi*, which abandon the Lake Saravesi host clams, were able to re-settle.

Based on the current results, it seems that *P. rylovi* tolerate low oxygen concentrations better than high temperature. On the other hand, the host *A. piscinalis* is known to be fairly tolerant to anoxic conditions (Van Den Thillart & De Vries 1985). Low oxygen concentrations are likely to be encountered, at least in the current study area, where ice covers the lakes from late November until early May, more often than high temperatures. However, the host seems to tolerate high temperatures quite well, since only 2 from a total of 72 (3%) clams died during 14 days in the high temperature treatment compared to 15% in low-oxygen, no water change treatment.

The results of the present study indicate that it is possible to expel the parasite *P. rylovi* with high water temperature without affecting the survival of the host, *A. piscinalis.*

3.5 Long-lasting effects of stress (V)

Among clams from both populations, the stressed clams were significantly more intensively parasitized by *P. rylovi*, and showed significantly lower growth, lower reproduction and lower survival than the unstressed clams, indicating that stress lowered the condition of the clams. The reproduction of the parasite was not affected by stress treatment.

The clams were exposed to natural infections about 11 months after the stress, the individuals being held in their natural environment. *Anodonta piscinalis* clams certainly face anoxic, or low oxygen conditions in their natural environment in temperate lakes, and exhibit metabolic adaptations to anoxic conditions (Lewis 1984, Van Den Thillart & De Vries 1985).

The higher intensity of infection among the stressed clams can be due to several processes, such as easier recognition of or easier penetration of stressed individuals by parasites, or better development of parasites in stressed clams. On the other hand, if above things are equal in stressed and unstressed hosts, the variation in the intensity of parasitism with respect to stress could be due to differences in the host's ability to inhibit parasite establishment, i.e. differences in host resistance to the parasite. Although the results do not allow to distinguish between these explanations, one plausible explanation could be a connection between stress, host condition and host immune defence. The immune system of bivalve molluscs is based on an innate, non-lymphoid system involving humoral responses such as agglutination and cellular components such as encapsulation and phagocytosis (Pipe 1990, Fisher & DiNuzzo 1991, Oliver & Fisher 1995, Hine 1999), and for example in oysters, noradrenaline, the principal catecholamide, released during stress, inhibits the immune functions, such as phagosytosis (Lacoste et al. 2002). Hine et al. (2002) observed significantly higher intensities of protistan parasite *Bonamia exitiosus* infections in stressed oysters than in controls. In the present study, the stressed clams showed lower growth, lower reproduction and lower survival than the unstressed clams, which indicates that they were in poorer condition than the unstressed clams. In a previous study, brooding female *A. piscinalis* were found more intensively parasitized by *P. rylovi* than non-reproducing females (III). Glochidia brooding poses a stress to the clam (Aldridge & McIvor 2003). The results indicate that the low condition of stressed *A. piscinalis* may have made them more susceptible to infection. This is in line with the results from studies on vertebrates in which a dependence between host stress, condition, immune defence and parasitism/disease has been observed (reviewed e.g. by Murray & Young 1992, Pruett et al. 1993).

The experimental design of the current study included a stress treatment in the laboratory and non-stress treatment in the field. One could argue that the observed relationship between stress and susceptibility to parasitism would be produced by some unknown difference between the laboratory and field conditions. However, the non-stressed groups were also kept in the laboratory and exposed to the laboratory conditions, such as aged tap water for 2-3 days before the non-stress treatment. The stress treatment applied in the present study, anoxia, poses such a severe physiological challenge for the clam, that this probably overrides any effects of laboratory conditions as such. The severity of the stress treatment was demonstrated by 'abortion' of the female clams during the stress treatment; they evacuated the gills by releasing the stored glochidia larvae from their outer demibranchs. This phenomenon, occurring during very stressful conditions, has been viewed as an adaptive strategy to lower stress in unionid clams (Aldridge & McIvor 2003). Indeed, results of Tankersley &

Dimock (1993) indicated that the respiratory efficiency was lower in the brooding females of the unionid clam *Pyganodon cataracta* compared to non-brooding individuals. They suggested that this was in part a consequence of reduced functional gill surface area due to brooded glochidia larvae. By evacuating the gill contents, the mussels may benefit from increased functional gill surface area and thus increased rates of oxygen uptake (Tankersley & Dimock 1993).

Handling, laboratory operations and transporting all inevitably affect the clams so that the non-stressed group was actually subjected to some stress. This was indicated by their lower growth when compared to the control clams, which were just collected, marked, measured and put back in Lake Jyväsjärvi as soon as possible. Nevertheless, survival and the relative reproduction index (the proportion of gill blade volume filled with glochidia larvae) of non-stressed clams were equal to that of the control clams kept in Lake Jyväsjärvi, suggesting that the non-stressed groups can be regarded as unstressed as measured by their reproductive performance and survival. There is also an energy-allocation explanation for the observed difference between growth and reproduction/survival of the clams with respect to stress. Jokela & Mutikainen (1995b) found a equivalent pattern in a field experiment, and concluded that allocation to maintenance and reproduction dominated over growth when resources were limited in *A. piscinalis*.

The seasonal timing of stress treatment was determined by the reproductive cycle of the parasite so that the clams were transported to the lake as soon as possible after the seasonal infective period was over, in September (I). In an organism living in seasonally fluctuating environment, such as *A. piscinalis*, the seasonal timing of stress has a large effect on the consequences of stress. It was shown in a field experiment by Jokela (1996) that a stress applied late in the season, as in the present study, had more intensive negative effect on clam performance than a stress occurring earlier in the season, when clams can better compensate and adjust their reproductive effort in accordance with resources available.

Paraergasilus rylovi have been observed to carry egg sacs in June, July and August (I). Therefore, this period should be optimal to get the experimental clams infected naturally, and for the parasites to possess egg sacs. The samples of experimental clams collected during summer 2003 indicate that only one new infection was established among the previously uninfected Lake Ahveninen clams and the stressed Lake Jyväsjärvi clams by 6 August 2003. The unstressed clams from Lake Jyväsjärvi were infected by *P. rylovi* when they were released to Lake Saravesi. However, by 6 August 2003, the mean intensity of parasitism had markedly decreased. Thus, in those clams the numbers of 'old' parasites were most probably so low that their effect on the total intensity of infection at the end of the experiment, 18 August 2003, may have been insignificant. The clams were also investigated for trematode parasites before and at the end of the experiment since trematode infection has been observed to decrease the physiological performance, especially tolerance of anoxia (Becker 1980, Lauckner 1986, Sousa & Gleason 1989). The occurrence of trematodes cannot

confound the present results, since only three out of 276 clam individuals were found to be infected by trematode parasites.

Stress of the host can influence parasite transmission in several ways. For example, by reducing the condition of the host, stress may impair the immune system and decrease resistance of the host, increasing the number of infected hosts, and thereby increasing parasite transmission. At the same time, reproduction or replication within the host can increase due to impaired host condition (e.g. Lafferty & Kuris 1999). However, some studies of nutritional stress have shown that host stress and low host condition may decrease parasite transmission (Krist et al. 2004, Pulkkinen & Ebert 2004). In addition to infectivity, parasite reproductive performance with respect to stress was investigated. There was no difference in the proportion of parasites carrying egg sacs between stressed and unstressed clams, suggesting that parasite reproduction may not be affected by host stress in the present system, as measured about 11 months after the stress. However, it has been shown that two weeks in anoxic conditions in the laboratory kills the *P. rylovi* (IV). Therefore, although anoxia increases *A. piscinalis* susceptibility to infection, it may be catastrophic for the parasite, and it's transmission leading, perhaps, to a local extinction of *P. rylovi*. Nevertheless, the current results indicate that stress, in general, may increase host's susceptibility to parasitism, but the net effect on parasite transmission may vary depending on particular host-parasite relationship.

The results of this field experiment indicate (1) that susceptibility of *A. piscinalis* to *P. rylovi* infection may be condition-dependent, and (2) that stress may have a long-lasting and increasing effect on host susceptibility to parasitism in natural populations. It was previously known that stress can increase susceptibility, but it was not known that exposure to a stress event can increase susceptibility to infection for such a long period after the stress.

3.6 Local adaptation of the parasite (VI)

The experimental design applied transplantation of hosts, from which the parasite individuals had been expelled (IV). A reciprocal transplantation between two populations took place in the field between early June and early August 2003. This period should be optimal to get the experimental clams infected naturally, and for the parasites to possess egg sacs (I).

In the reciprocal transplant experiment, both infectivity and reproductive performance of *P. rylovi* were higher in home-clams in both transplant sites (Figs. 1-2 in VI). Thus, the present results indicate that the crustacean parasite, *P. rylovi*, may be genetically specialized to the sympatric host population i.e. locally adapted to its bivalve host, *A. piscinalis*. In addition, in Lake Saravesi, the infectivity and reproduction of the parasite were higher in home-clams

regardless of the transplant habitat (0.8, 2.0, and 4.0 m), as indicated by the non-significant interactions between host origin and transplant depth.

Anodonta piscinalis occurs densely in all the three habitats used. The most significant difference between the habitats is that the shallowest (0.8 m) is protected from wave action by macrophytes. This probably limits water currents that carry the infective stages of *P. rylovi*. The non-significant interaction between clam origin and transplant habitat in Lake Saravesi with respect to both parasite abundance and reproduction has important implications for the study of local adaptation in the current system. First, it suggests that in a small scale in Lake Saravesi, where the three transplant habitats were within 200 m, the host and parasite populations may not be genetically so fragmented that it would have resulted in an interaction between host origin and habitat. Second, the non-significant interaction between host origin and habitat, even though the parasite abundances differed between the habitats, being highest in the depth of 2.0 m and lowest in 0.8 m, suggests that local adaptation may not be affected by the exposure rate of the parasite, in the present system. Third, the non-significant interaction, in spite of the clear differences between the habitats in the egg production of the parasite, the mean proportion of female *P. rylovi* carrying egg sacs decreasing with increasing water depth, indicates that local adaptation may not be affected by the performance of the parasite in the present system.

According to simulation models, local adaptation is expected in highly virulent parasites (Gandon et al. 1996, Lively 1999) and when migration rate of the parasite is higher than that of the host (Gandon et al. 1996). The virulence of the parasite *P. rylovi* is unknown, but probably is not high, at least when compared to castrating digenetic trematodes of the host, *A. piscinalis* (see Taskinen & Valtonen 1995). In addition, the migration rate of *P. rylovi* is probably not higher than that of the host. The parasite has a planktic larva, the migration of which relies on water currents. The host has a larval phase, the glochidium, which attaches to fish, such as roach, *Rutilus rutilus* (Jokela et al. 1991). Roach is known to perform spawning migrations in spring (Mills 1991) during the period when glochidia are attached to fish (Jokela et al. 1991). In spite of this, local adaptation by the parasite was found in the present study. Conventionally, the shorter generation times and higher reproductive rates of parasites compared to those of their hosts have been thought to contribute to the higher evolutionary potential of the parasites (Hamilton et al. 1990, Hafner et al. 1994). Thus, adaptation to new host genotypes may be faster than the emergence of new resistant host genotypes. The life cycle of *P. rylovi* resembles that of other ergasilid copepods. For example, the ergasilid parasite of fish, *Ergasilus briani*, has two generations per year in the present study area (Tuuha et al. 1992). Thus, the parasite clearly has shorter generation times than the host, *A. piscinalis*, which may live up to 10 years in the study area (Taskinen & Valtonen 1995). Therefore, the current results are consistent with the conventional theory concerning local adaptation by parasites.

4   CONCLUSIONS

The results of this thesis indicate that *P. rylovi* is specialized to infect *A. piscinalis*. This in turn means that reciprocal evolution—coevolution—can be expected between *P. rylovi* and *A. piscinalis*. *Paraergasilus rylovi* occurred in southern Finland, but was absent from northern Finland. If the distribution of *P. rylovi* is limited by chance of introduction to new areas, it is not surprising that southern Finnish populations harbour the parasite, since hydrologically they share the same recipient, Gulf of Finland, Baltic Sea, with St. Petersburg region, which is the only other location from which *P. rylovi* has been reported in clams so far (Chernysheva & Purasjoki 1991). The mean intensity of infection of *P. rylovi* was higher in lake populations compared to river populations. If the infective stages of *P. rylovi* enter the clam passively, via filtration, it might be that in rivers, the larvae of *P. rylovi* are flushed away before they become established. In addition, *P. rylovi* was more abundant at moderate depth (2.0 m) compared to shallow (0.8 m) or deep (4.0 m) habitats (VI) within a lake, possibly reflecting differences between habitats in exposure rate. There was a seasonal tendency for higher intensities of infection in late autumn in Lake Saravesi. This is in line with the findings of egg production of *P. rylovi* between June and August (I) and establishment of new infections in August (V-VI). Eggs hatched in summer produce larvae that become infective adults during the autumn, which result in an increase in the intensity of infection at that time. *Paraergasilus rylovi* probably enter the clam host via filtration like the parasitic copepod *Mytilicola intestinalis* in the mussel *Mytilus edulis*. Larger sized *M. edulis* with a larger field of filtration (Davey & Gee 1976) harbour more *M. intestinalis* (Gee & Davey 1986). A similar size (and age) dependent pattern of infection is found in the prevalence of the filtration-entering trematode *R. fennica* in *A. piscinalis* (Taskinen & Valtonen 1995). Therefore, it was surprising that there was a negative relationship between size (and age) of the host, and intensity of *P. rylovi* infection (I). The behaviour of the host might change with age and size so that the exposure to parasitism decreases. Also the susceptibility of host to parasitism might decrease with age and/or size. Alternatively, the increasing prevalence of the trematode *R. fennica* with size (age) of *A. piscinalis* (Taskinen &

Valtonen 1995) might have a negative effect on *P. rylovi*. The present results yielded no association between *P. rylovi* and *R. fennica*, suggesting that the last hypothesis may not be correct (I). However, experimental studies are needed to resolve the alternative explanations.

The abundance of *P. rylovi* increased with proportionate burrowing depth of *A. piscinalis*. Furthermore, the abundances of encysted water mites in *A. piscinalis* and *P. complanata* were higher in completely burrowed individuals than among partly visible ones. The relative growth of *A. piscinalis* during the growing season was lower in completely burrowed individuals than among partly visible clams (II). This may indicate that resources available for visible clams are more abundant than for the completely burrowed individuals. If the feeding efficiency decreases with burrowing depth, it could be assumed that host's ability to control the infection (immune responses) would become impaired with increased burrowing depth, thus increasing susceptibility of clams to parasitism. The alternative hypothesis that exposure to parasites studied increases with increasing burrowing depth, seems intuitively improbable. However, observed increase in abundance of *P. rylovi* with burrowing depth of the host is in line with findings that the burrowing depth of *A. piscinalis* change with size (and age) so that bigger clams are more visible compared to the smaller individuals (Saarinen & Taskinen 2003) and with observed negative relationship between size (and age) of the host and intensity of *P. rylovi* infection (I). Experimental work is needed to reveal the causal connections behind the patterns, by manipulating clam burrowing, parasitism and resources available.

Female *A. piscinalis*, which brooded glochidia larvae were more abundantly infected by *P. rylovi* than non-reproducing female clams (III). Since the biomass of glochidia larvae that are produced and brooded by female *A. piscinalis*, constitute on average 20% of the total biomass of clam soft tissues (Jokela et al. 1993), reproducing females may have to increase their filtration rate to compensate for the increased nutritional demands of reproduction. This could lead to increased exposure to *P. rylovi*, and contribute to the observed higher parasite abundance in reproducing females. Alternatively, *P. rylovi* may survive and reproduce better in mature, reproducing clams. This may be the case if there is a trade-off in the energy allocation between reproduction and immunological defence in *A. piscinalis*, or if maturation/reproduction induces hormonal changes that suppress immunity. Nevertheless, the experimental work is needed to separate between the alternative explanations.

The parasites *P. rylovi* are possible to be expelled or killed from the gills of *A. piscinalis* by using high water temperature or low oxygen (IV). Particularly high temperature treatment turned out to be fast and effective method to expel *P. rylovi*, and in addition, seemed to be least harmful to host. This experimental manipulation of the intensity of *P rylovi* infection was subsequently used successfully in a reciprocal transplant experiment conducted to study local adaptation of *P. rylovi* (VI).

Stress affected negatively on condition of *A. piscinalis* (V). In addition to decreased survival of stressed clams, also the growth of stressed hosts

decreased and female clams were not able to produce so much glochidia larvae compared to non-stressed females. Stressed clams were also shown to suffer increased parasitism by *P. rylovi* (V) when exposed to *P. rylovi* infection about 11 months after the stress treatment. Thus, the exposure to low oxygen conditions can increase the susceptibility of clams to parasites even for a long period.

In the reciprocal transplant experiment, both infectivity and reproductive performance of *P. rylovi* were higher in home-clams in both transplant sites compared to away-clams (VI). This indicates that the crustacean parasite, *P. rylovi*, may be genetically specialized to sympatric host populations—that is, locally adapted to its bivalve host, *A. piscinalis*. In addition, in Lake Saravesi, the infectivity and reproduction of the parasite were higher in home-clams regardless of the transplant habitat (0.8, 2.0, and 4.0 m), as indicated by the non-significant interactions between host origin and transplant depth, thus suggesting that the local adaptation may not be affected by habitat-dependent processes, such as possible differences in exposure rate or the performance of the parasite in the present system. (VI)

Taken together, the present results indicate that both ecological and genetic factors, as well as host condition, contribute to the abundance of *P. rylovi* infection in its host, *A. piscinalis*.

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Acknowledgements

YHTEENVETO

Paraergasilus rylovi -loisäyriäisen esiintymiseen ja runsauteen vaikuttavat tekijät *Anodonta piscinalis* -pikkujärvisimpukassa

Väitöskirjatyössäni tutkin *Paraergasilus rylovi* -äyriäisloisen esiintymiseen ja runsauteen vaikuttavia ekologisia ja geneettisiä tekijöitä.

Tutkin *P. rylovi* -loisäyriäisen yleisekologiaa, sen esiintymistä neljässä Unionidae-heimon suursimpukkalajissa, maantieteellistä esiintymistä, lisääntymisaikaa sekä esiintymisen runsauden riippuvuutta habitaatista, isännän iästä, koosta ja sukupuolesta (I). *Paraergasilus rylovi* esiintyi neljästä tutkitusta simpukkalajista pääosin pikkujärvisimpukassa (*Anodonta piscinalis*); muutaman kerran loinen löytyi myös litteäjärvisimpukasta (*Pseudanodonta complanata*), mutta näissä tapauksissa loisen ei havaittu lisääntyvän. *Unio*-suvun jokisimpukoista loista ei löytynyt. Tulosten perusteella voidaan sanoa, että *P. rylovi* on erikoistunut loisimaan pikkujärvisimpukassa. Loisäyriäinen esiintyi eteläisissä järvissä, mutta ei pohjoisissa. Tämä voi johtua siitä, ettei loinen ole vielä levinnyt näille alueille tai siitä, että pohjoisen lyhyempi kesä ja kylmemmät vedet estävät loisen lisääntymisen onnistumisen. Verrattaessa loisen runsautta järvissä ja virtavesissä havaittiin, että loinen oli huomattavasti runsaampi järvissä. Tämä voi olla seurausta loisen todennäköisesti passiivisesta infektiotavasta, jolloin virtaava vesi huuhtoisi loisen infektiiviset vaiheet pois ennen kuin ne kykenisivät asettautumaan simpukkaan. Myös veden syvyys vaikutti loisen runsauteen järvessä. Loinen oli runsaampi keskinkertaisessa syvyydessä (2,0 m) kuin matalassa (0,8 m) tai syvässä (4,0 m) vedessä (VI). Tämä voi indikoida eroja loisaltistuksessa syvyyshabitaattien välillä. Loisen runsaudessa ilmeni lievää vuodenaikaisuutta – lukumäärät kasvoivat syksyä kohti. Loisen havaittiin lisääntyvän lämpimän veden aikana kesä-elokuussa (I), mikä selittää suuremmat loismäärät syksyllä. Pikkujärvisimpukan sukupuoli ei vaikuttanut loisen runsauteen, mutta lukumäärä väheni simpukan koon ja iän kasvaessa. Tämä voi johtua ikä- tai kokoriippuvista muutoksista simpukan loisaltistuksen ja/tai loisiintumisalttiuden suhteen. Pikkujärvisimpukalla tutkimusalueella yleisenä esiintyvän imumadon, joka kastroi isäntänsä, esiintyminen yleistyy simpukan koon kasvaessa. Voi olla, että tämän imumadon infektio vaikuttaa jotenkin haitallisesti *P. rylovi* -loiseen. Tässä työssä loisten esiintymisien välillä ei kuitenkaan havaittu yhteyttä (I).

Olen tutkinut myös *P. rylovi* -äyriäisen sekä *Unionicola*-suvun vesipunkkien runsauden ja simpukan kasvun yhteyttä pikku- ja litteäjärvisimpukan suhteelliseen hautautumissyvyyteen (II). *Paraergasilus rylovi* -äyriäisen runsaus kasvoi pikkujärvisimpukan suhteellisen hautautumissyvyyden kasvaessa. Vesipunkkien kystittyneitä, passiivisia toukkavaiheita oli enemmän kokonaan hautautuneissa litteäjärvisimpukoissa ja pikkujärvisimpukoissa kuin osittain pinnalla olevissa simpukoissa. Kokonaan hautautuneet simpukat olivat kasvaneet suhteessa vähemmän kasvukauden aikana kuin osittain pinnalla olleet.

Tämä voisi olla seurausta siitä, että ravintoa on enemmän ja helpommin saatavilla sedimentin pinnalla. Jos ravinnonottotehokkuus todella heikkenee hautautumissyvyyden kasvaessa, voisi olettaa, että tämä vaikuttaisi myös simpukan kykyyn säädellä loismääriä energiaa vaativan puolustusjärjestelmän avulla. Vaihtoehtoinen hypoteesi, se että simpukan varsinainen altistuminen loisten infektiivisille vaiheille kasvaisi hautautumissyvyyden myötä, vaikuttaa epätodennäköiseltä. Tutkimusalueella piisamit ovat pääasiallisia simpukoiden saalistajia ja voidaan olettaa, että pinnalla, näkyvissä olevat simpukat olisivat alttiimpia piisamipredaatiolle. Optimaalinen hautautumissyvyys loisaltistuksen ja predaatioriskin suhteen voi vaihdella paikallisesti ja ajallisesti. Korrelatiivisen kenttäaineiston perusteella on kuitenkin vaikea päätellä, onko hautautumissyvyys syy loisten runsaudelle vai seurausta loisten runsaudesta tai muista seikoista. Tässä tapauksessa ei esimerkiksi tiedetä, onko simpukka ollut hautautunut vai pinnalla infektion tapahtuessa. Kuitenkin *P. rylovi* -loisen lukumäärät vähenivät simpukan koon ja iän kasvaessa (I), ja tämän lisäksi pikkujärvisimpukan koon kasvaessa hautautumissyvyys pienenee. Havaittu loisten lukumäärien pieneneminen simpukan näkyvillä olevan osan kasvaessa voi olla seurausta koko- tai ikäriippuvaisesta muutoksesta infektiodynamiikassa pikemminkin kuin seurausta itse hautautumisesta (II). Ainoastaan kokeellisella tutkimuksella on mahdollista selvittää mahdollisia syy-seuraussuhteita.

Pikkujärvisimpukan sukukypsyyden ja lisääntymisen yhteyttä *P. rylovi* -loisäyriäisen runsauteen olen tutkinut 3-5-vuotiailla simpukkanaarailla (III). Simpukoilla assimiloidusta energiasta käytetään suhteellisen suuri osa lisääntymiseen, ja glokidio-toukkien tuottaminen vaatii paljon energiaa, sillä niiden massan on arvioitu pikkujärvisimpukalla olevan 20 % pehmytosien massasta. Sukukypsillä, lisääntyvillä naarailla oli kaksi kertaa enemmän *P. rylovi* -loisia verrattuna lisääntymättömiin, immatureihin naaraisiin. Yritys kompensoida kasvanut energian tarve lisääntymisen aikaan suodatusnopeutta tai -aikaa kasvattamalla voi lisätä altistumista loisen infektiivisille vaiheille. Lisääntymiseen käytetty energia voi myös olla poissa niistä resursseista, joita voidaan käyttää loisten ja tautien vastustamiseen. Lisääntymiseen liittyvät hormonaaliset muutokset voivat myös alentaa simpukan puolustusjärjestelmän tehoa ja näin lisätä simpukan alttiutta loisiintumiselle. *Paraergasilus rylovi* voi siis säilyä paremmin hengissä simpukoissa, joiden puolustusjärjestelmän teho on laskenut.

Tässä työssä on kehitetty menetelmä, jolla *P. rylovi* -äyriäisen saa poistettua simpukasta itse simpukkaa vahingoittamatta (IV). Tämän lisäksi tutkin loisen kykyä poistua isäntäsimpukasta ja kykyä siirtyä toiseen isäntäyksilöön. Myös simpukasta poistettujen loisten eloonjäämistä tarkkailtiin laboratoriossa. Kolmesta tutkitusta menetelmästä korkea lämpötila, 26° C, osoittautui tehokkaimmaksi loisen poistomenetelmäksi; loislukumäärä laski lähelle nollaa ensimmäisen käsittelyviikon aikana. Kaksi muuta menetelmää, jotka molemmat perustuivat ensisijaisesti alhaiseen happipitoisuuteen, saavuttivat saman tuloksen kahdessa viikossa. Tutkiessani lämpötilan vaikutusta yksittäisten simpukoiden loisiin havaitsin, että lämpötilan nosto 24 tunnissa 18 ° C:sta 26° C:een häätää *P. rylovi* -äyriäisen nopeasti, sillä loisia ei havaittu enää kahden käsittelypäivän jälkeen. Havaitsin lisäksi loisten poistuvan simpukasta veteen ja lopul-

ta kuolevan epäsuotuisissa lämpötilaoloissa. Kuolleita loisia en havainnut simpukoissa minkään osakokeen aikana. Simpukoista poistetut äyriäisloiset elivät laboratoriossa 18 ° C:ssa jopa 16 päivän ajan. Osoitin myös, alun perin loisettomia isäntiä käyttäen, että *P. rylovi* -loisen on mahdollista lähteä simpukkaisännästään ja infektoida toinen simpukkayksilö. Luonnossa, ainakin Suomessa, alhainen happipitoisuus on todennäköisempi tilanne kuin korkea lämpötila, vaikkakin työssä käytetty lämpötila onkin normaalin vaihtelun rajoissa tutkimusalueella. Luonnossa korkea lämpötila ei välttämättä vaikuta negatiivisesti loisäyriäiseen, vaan voi olla, että työssä käytetty lämpötilan nostonopeus, 8° C/24h, on itse lämpötilaa tärkeämpi tekijä. Tämän tutkimuksen perusteella näyttääkin siltä, että *P. rylovi* sietää alhaista happipitoisuutta paremmin kuin korkeaa lämpötilaa ja/tai nopeaa lämpötilan nousua. Pikkujärvisimpukan, ainakin aikuisten yksilöiden, tiedetään kestävän kohtuullisen hyvin alhaista happipitoisuutta, mutta tässä kokeessa korkea lämpötila vahingoitti simpukkaa vähemmän kuin alhainen happipitoisuus. *Paraergasilus rylovi* voidaan siis poistaa simpukasta simpukkayksilöä oleellisesti vahingoittamatta käyttämällä korkeaa lämpötilaa.

Tutkin myös stressin pitkäaikaisia vaikutuksia pikkujärvisimpukan loisiintumisalttiuteen, simpukan elossapysymiseen, kasvuun ja lisääntymiseen (V). Simpukat olivat peräisin kahdesta populaatiosta, Jyväskylän Jyväsjärvestä, jossa *P. rylovi* -loista esiintyy ja Laukaan Ahvenisesta, jossa *P. rylovi* -loista ei ole havaittu. Stressikäsittelyssä simpukoita pidettiin laboratoriossa alhaisessa happipitoisuudessa 25 päivän ajan elo-syyskuussa 2002. Edellisen työn perusteella (IV) tiesin, että jo kahden viikon alhainen happipitoisuus poistaa *P. rylovi* -äyriäisen simpukasta, joten syyskuussa 2002 stressiryhmien loisettomat simpukat siirrettiin Saraveteen yhdessä ei-stressiryhmien simpukoiden kanssa, jotka olivat tuon 25 päivän ajan omissa järvissään. Ahvenisen ei-stressiryhmässä ei ollut loisia, mutta Jyväsjärven ei-stressiryhmässä oli. Vaikka olisikin ollut mahdollista poistaa loinen tästä ryhmästä lämpötilamenetelmää (IV) apuna käyttäen, tutkimuksen tarkoituksen, stressin vaikutusten selvittämisen vuoksi tähän ei ryhdytty. Vaikka lämpötilamenetelmä ei näytä vahingoittavan simpukkaa, voi korkea lämpötila silti aiheuttaa stressireaktion. Saravedessä simpukoista otettiin osanäytteitä kuukausittain talvea lukuun ottamatta elokuuhun 2003 asti. Uusia *P. rylovi* -äyriäisiä alkoi ilmestyä simpukoihin elokuun alussa 2003. Kun koe lopetettiin 18. elokuuta 2003, havaittiin, että *P. rylovi* -äyriäisiä oli runsaammin stressikäsittelyn saaneissa simpukoissa. Stressiryhmissä kuolleisuus oli huomattavasti korkeampaa kuin ei-stressiryhmissä. Simpukoiden kasvu oli pienempää stressiryhmissä, kuten myös naarassimpukoiden tuottamien glokidio-toukkien määrä. Loisen lisääntyminen ei sen sijaan eronnut stressi- ja ei-stressiryhmien välillä. Altistuminen stressille heikensi siten simpukan kuntoa ja lisäsi loisiintumisalttiutta. Stressin on tiedetty lisäävän loisiintumisalttiutta, mutta uutta tässä työssä oli se, että osoitin stressin voivan lisätä simpukan loisiintumisalttiutta pitkänkin ajan kuluttua luonnonolosuhteissa.

Tutkin myös ristiininfektiokokeella (VI) kahden populaation välillä, kykeneekö *P. rylovi* infektoimaan paremmin oman, sympatrisen pikkujärvisimpukkapopulaationsa yksilöitä ja onko sen lisääntymismenestys parempi oman po-

pulaationsa simpukoissa verrattuna vieraan, allopatrisen populaation simpu-
koihin. Ennen varsinaisen kokeen suorittamista simpukoissa jo olleet *P. rylovi* -
loiset poistettiin laboratoriossa lämpötilamenetelmää (IV) apuna käyttäen. In-
fektiokokeissa havaitsin, että *P. rylovi* infektoi paremmin oman, sympatrisen
populaationsa simpukoita kummassakin tutkitussa populaatiossa. Loinen myös
lisääntyi paremmin oman populaationsa isännissä. Nämä tulokset tukevat hy-
poteesia, jonka mukaan loinen sopeutuu evolutiivisesti omaan, sympatriseen
isäntäpopulaatioonsa. Tulokset indikoivat myös, että ekologisten tekijöiden li-
säksi myös geneettisillä tekijöillä on selkeä merkitys *P. rylovi* -loisen runsauteen.

REFERENCES

Abrous, M., Rondelaud, D. & Dreyfuss, G. 2001. The stress of Lymnaea truncatula just before miracidial exposure with Fasciola hepatica increased the prevalence of infection. Exp. Parasitol. 99: 49-51.

Agrawal, A. F. & Lively, C. M. 2003. Modelling infection as a two-step process combining gene-for-gene and matching-allele genetics. Proc. R. Soc. Lond. B 270: 323-334.

Agyemang, K., Dwinger, R. H., Little, D. A., Leperre, P. & Grieve, A. S. 1992. Interaction between physiological status in N´Dama cows and trypanosome infections and its effect on health and productivity of cattle in Gambia. Acta Tropica 50: 91-99.

Aldridge, D. C. 1999. The morphology, growth and reproduction of Unionidae (Bivalvia) in a fenland waterway. J. Molluscan Stud. 65: 47-60.

Aldridge, D. C. & McIvor, A. L. 2003. Gill evacuation and release of glochidia by Unio pictorum and Unio tumidus (Bivalvia: Unionidae) under thermal and hypoxic stress. J. Molluscan Stud. 69: 55-59.

Alexander, J. & Stimson, W. H. 1988. Sex hormones and the course of parasitic infection. Parasitol. Today 4: 189-193.

Allander, K. 1997. Reproductive investment and parasite susceptibility in the Great Tit. Funct. Ecol. 11: 358-364.

Allander, K. & Bennett, G. F. 1994. Prevalence and intensity of haematozoan infection in a population of great tits Parus major. J. Avian Biol. 25: 69-74.

Alpert, P., Bone, E. & Holzapfel, C. 2000. Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. Perspect. Plant Ecol. Evol. Syst. 3: 52-66.

Amyot, J. P. & Downing, J. A. 1991. Endo- and epibenthic distribution of the unionid mollusc Elliptio complanata. J. N. Am. Benthol. Soc. 10: 280-285.

Anderson, R. M. & Gordon, D. M. 1982. Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. Parasitology 85: 373-398.

Baker, R. A. 1977. Nutrition of the mite Unionicola intermedia, Koenike and its relationship to the inflammatory response induced in its molluscan host Anodonta anatina, L. Parasitology 75: 301-308.

Baker, R. A. 1987. Aspects of the life history of Unionicola ypsilophora (Bonz 1783), a freshwater mite living in the swan mussel, Anodonta cygnea (L.). Naturalist 112: 53-58.

Ballabeni, P. & Ward, P. L. 1993. Local adaptation of the trematode Diplostomum phoxini to the European minnow Phoxinus phoxinus, its second intermediate host. Funct. Ecol. 7: 84-90.

Van Banning, P. 1991. Observation on bonamiasis in the stock of the European flat oyster, Ostrea edulis, in the Netherlands, with special reference to the recent developments in Lake Gravelingen. Aquaculture 93: 205-211.

Barker, D. E. & Cone, D. K. 2000. Occurrence of Ergasilus celestis (Copepoda) and Pseudodactylogyrus anguillae (Monogenea) among wild eels (Anguilla rostrata) in relation to stream flow, pH and temperature and recommendations for controlling their transmission among eels. Aquaculture 3-4: 261-274.

Bauer, G. 1994. The adaptive value of offspring size among freshwater mussels (Bivalvia, Unionidae). J. Anim. Ecol. 63: 933-944.

Bauer, G., Hochwald, S. & Silkenat, W. 1991. Spatial distribution of freshwater mussels – The role of host fish and metabolic rate. Freshwat. Biol. 26: 377-386.

Bauer, O. N., Musselius,V. A. & Strelkov, Y. A. 1973. Diseases of pond fishes. 312 p., Israel Program for Scientific Translations, Jerusalem, Israel.

Bayne, B. L., Salkeld, P. N. & Worall, C. M. 1983. Reproductive effort and value of different populations of marine mussel, Mytilus edulis L. Oecologia 59: 18-26.

Bayne, C. J. 1991. Invertebrate host immune mechanisms and parasite escapes. In: Toft, C. A., Aeschlimann, A. & Bolis, L. (eds), Parasite-host associations: coexistence or conflict?: 299-315. Oxford Univ. Press, Oxford.

Becker, W. 1980. Metabolic interrelationship of parasitic trematodes and molluscs, especially Schistosoma mansoni in Biomphalaria glabrata. Z. Parasitenkunde 63: 101-111.

Bolster, G. C. 1954. The biology and dispersal of Mytilicola intestinalis Steuer, a copepod parasite of mussels. Fishery investigations, Series II, Marine Fisheries 18: 1-30. Great Britain Ministry of Agriculture, Fisheries and Food.

Borgia, G. & Collis, K. 1989. Female choice for parasite-free male satin bowerbirds and the evolution of bright male plumage. Behav. Ecol. Sociobiol. 25: 445-454.

Böttger, K. 1977. The general life cycle of fresh water mites (Hydrachnellae, Acari). Acarologia 18: 496-502.

Bull, C. M. & Burzacott, D. 1993. The impact of tick load on fitness of their lizard hosts. Oecologia 96: 415-419.

Brown, M. J. F., Loosli, R. & Schmid-Hempel, P. 2000. Condition-dependent expression of virulence in a trypanosome infecting bumblebees. Oikos 91: 421-427.

Bush, A. O., Fernández, J. C., Esch, G. W. & Seed, J. R. 2001. Parasitism. The diversity and ecology of animal parasites. 566 p., Cambridge Univ. Press, Cambridge.

Carballo, M., Munoz, M. J., Cuellar, M. & Tarazona J. V. 1995. Effects of waterborne copper, cyanide, ammonia, and nitrite on stress parameters and changes in susceptibility to saprolegniosis in rainbow trout (Oncorhynchus mykiss). Appl. Environ. Microbiol. 61: 2108-2112.

Chernysheva, N. B. & Purasjoki, K. J. 1991. A redescription of Paraergasilus rylovi Markevich, 1937 (Copepoda, Ergasilidae). Syst. Parasitol. 20: 165-172.

Christian, J. J. & Davies, D. E. 1964. Endocrines, behaviour and population. Science 146: 1550-1560.

Chrousos, G. P. & Gold, P. W. 1992. The concepts of stress and stress system disorders. Overwiew of physical and behavioural homeostasis. J. Am. Med. Assoc. 267: 1244-1252.

Combes, C. 1991. Evolution of parasite life cycles. In: Toft, C. A., Aeschlimann, A. & Bolis, L. (eds), Parasite-Host Associations: Coexistence of Conflict?: 62-82. Oxford Univ. Press, Oxford.

Van Damme, P. A. & Ollevier, F. 1994. Infection dynamics of Lernaeocera lusci on sand goby Pomatoschistus minutus in the Oosterschelde (The Netherlands). Dis. Aquat. Org. 19: 83-87.

Davelos, A. L., Alexander, H. M. & Slade, N. A. 1996. Ecological genetic interactions between a clonal host plant (Spartina pectinata) and associated rust fungi (Puccinia seymouriana and Puccinia sparganioides). Oecologia 105: 205-213.

Davey, J. T. & Gee, J. M. 1976. The occurrence of Mytilicola intestinalis Steuer, an intestinal copepod parasite of Mytilus, in the south-west of England. J. Mar. Biol. Assoc. U.K. 56: 85-94.

Davids, C., Holtslag, J. & Dimock, R.V. JR 1988. Competitive exclusion, harem behaviour and host specificity of the water mite Unionicola ypsilophora (Hydrachnellae, Acari) inhabiting Anodonta cygnea (Unionidae). Int. Rev. gesamt. Hydrobiol. 73: 651-657.

Davis, K. B., Griffin, B. R. & Gray W. L. 2002. Effect of handling stress on susceptibility of channel catfish Ictalurus punctatus to Ichthyophthirius multifiliis and channel catfish virus infection. Aquaculture 214: 1-4.

Deerenberg, C., Apanius, V., Daan, S. & Bos, N. 1997. Reproductive effort decreases antibody responsiveness. Proc. R. Soc. Lond. B 264: 1021-1029.

Dimock, R. V. JR & Davids, C. 1985. Spectral sensitivity and photo-behaviour of the water mite genus Unionicola. J. Exp. Biol. 119: 349-363.

Drobney, R. D., Train, C. T. & Fredricson, L. H. 1983. Dynamics of the platyhelminth fauna of wood ducks in relation to food habits and reproductive state. J. Parasitol. 69: 375-380.

Dudgeon, D. & Morton, B. 1983. The population dynamics and sexual strategy of Anodonta woodiana (Bivalve: Unionacea) in Plover Cove Reservoir, Hong Kong. J. Zool. 201: 161–183.

Dufva, R. 1996. Sympatric and allopatric combinations of hen fleas and great tits: a test of the local adaptation hypothesis. J. Evol. Biol. 9: 505-510.

Dybdahl, M. F. & Storfer, A. 2003. Parasite local adaptation: Red Queen versus Suicide King. Trends Ecol. Evol. 18: 523-530.

Ebert, D. 1994. Virulence and local adaptation of a horizontally transmitted parasite. Science 265: 1084-1086.

Ebert, D. & Hamilton, W. D. 1996. Sex against virulence: the coevolution of parasitic diseases. Tree 11: 79-82.

Ellis, A. E. 1978. British freshwater bivalve Mollusca. Synopsis of the British Fauna 11. 109 p., Academic Press, London.

40

Ennos, R. A. & McConnel, K. C. 1995. Using genetic markers to investigate natural selection in fungal populations. Can. J. Bot. 73(S1): S302-S310.

Festa-Bianchet, M. 1989. Individual differences, parasites, and the cost of reproduction for bighorn ewes (Ovis canadensis). J. Anim. Ecol. 58: 785-796.

Fisher, G. R., Dimock, R. V. Jr & Kuhn, R. E. 2000. The symbiotic water mite Unionicola formosa (Acari: Unionicolidae) ingests mucus and tissue of its molluscan host. J. Parasitol. 86: 1254-1258.

Fisher, W. S. & DiNuzzo, A. R. 1991. Agglutination of bacteria and erythrocytes by serum from six species of marine molluscs. J. Invertebr. Pathol. 52: 380-388.

Folstad, I. & Karter, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. Am. Nat. 139: 603-622.

Folstad, I., Nilssen, A. C., Halvorsen, O. & Andersen, J. 1989. Why do male reindeer (Rangifer t. tarandus) have higher abundance of second and third instar larvae of Hypoderma tarandi than females? Oikos 55: 87-92.

Frank, S. A. 1993. Evolution of host-parasite diversity. Evolution 47: 1721-1732.

Fry, J. D. 1990. Trade-offs in fitness on different hosts: evidence from a selection experiment with a phytophagous mite. Am. Nat. 136: 569-580.

Gandon, S. & Van Zandt, P. A. 1998. Local adaptation and host-parasite interactions. Tree 13: 214-216.

Gandon, S., Capowiez, Y., Ddubois, Y., Michalakis, Y. & Olivieri, I. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. Proc. R. Soc. Lond. B Biol. Sci. 263: 1003-1009.

Gee, J. M. & Davey, J. T. 1986. Experimental studies on the infestation of Mytilus edulis (L.) by Mytilicola intestinalis Steuer (Copepoda, Cyclopoida). Journal du Conceil, Conceil International pour 1'Exploration de la Mer 42: 265-271.

Gledhill, T. 1985. A new species of water-mite, Unionicola (Pentatax) macani (Unionicolidae, Hydrachnellae, Acari), from the mantle cavity of the prosobranch mollusc Lanistes ovum Peters in Nigeria, with remarks on some aspects of host/parasite relationship between unionicolids and molluscs. Arch. Hydrobiol. 104: 77-92.

De Goeij, P. & Luttikhuizen, P. 1998. Deep-burying reduces growth in intertidal bivalves: field and mesocosm experiments with Macoma balthica. J. Exp. Mar. Biol. Ecol. 228: 327-337.

Gustafsson, L., Nordling, D., Andersson, M. S., Sheldon, B. C. & Qvarnström, A. 1994. Infectious diseases, reproductive effort and the cost of reproduction in birds. Philos. Trans. R. Soc. Lond. B 346: 323-331.

Hafner, M., Sudman, P., Villablanca, F., Spradling, T., Demastes, J. & Nadler. S. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. Science 265: 1087-1090.

Hamilton, W. D., Axelrod, R. & Tanese, R. 1990. Sexual reproduction as an adaptation to resist parasites (a review). Proc. Natl. Acad. Sci. US. 87: 3566-3573.

Hart, B. L. 1994. Behavioural defence against parasites: interaction with parasite invasiveness. Parasitology 109: 139-151.

Haukioja, E. & Hakala, T. 1978a. Life-history evolution in Anodonta piscinalis (Mollusca, Pelecypoda). Oecologia 35: 253-266.

Haukioja, E. & Hakala, T. 1978b. Measuring growth from shell rings in populations of Anodonta piscinalis (Pelecypoda, Unionidae). Ann. Zool. Fennici 15: 60-65.

Hine, P. M. 1999. The inter-relationship of bivalve haemocytes. Fish Shellfish Immunol. 9: 367-385.

Hine, P. M., Diggles, B. K., Parsons, M. J. D., Pringle, A. & Bull, B. 2002. The effects of stressors on the dynamics of Bonamia exitiosus Hine, Cochennec-Laureau & Berthe, infections in flat oysters Ostrea chilensis (Philippi). J. Fish Dis. 25: 545-554.

Hockley, A. R. 1951. On the biology of Mytilicola intestinalis (Steuer). J. Mar. Biol. Assoc. U.K. 30: 223-232.

Hoffmann, A. A. & Hercus M. J. 2000. Environmental stress as an evolutionary force. Bioscience 50: 217-226.

Humphrey, S. R., Courtney, C. H. & Forrester, D. J. 1978. Community ecology of the helminth parasites of the brown pelican. Wilson Bull. 90: 587-598.

Huz, Rl., Lastra, M., & Lopez, J. 2002. The influence of sediment grain size on burrowing, growth and metabolism of Donax trunculus L. (Bivalvia: Donacidae). J. Sea Res. 47: 85-95.

Ilmonen, P., Taarna, T. & Hasselquist, D. 2000. Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. Proc. R. Soc. Lond. B 267: 665-670.

Imhoof, B. & Schmid-Hempel, P. 1998. Patterns of local adaptation of a protozoan parasite to its bumblebee host. Oikos 82: 59-65.

Jirka, K. J. & Neves, R. J. 1992. Reproductive biology of four species of freshwater mussels (Mollusca: Unionidae) in the New River, Virginia and West Virginia. J. Freshwat. Ecol. 7: 35-44.

Jokela, J. 1996. Within-season reproductive and somatic energy allocation in a freshwater clam, Anodonta piscinalis. Oecologia 105: 167-174.

Jokela, J. & Lively, C. M. 1995a. Spatial variation in infection by digenetic trematodes in a population of freshwater snails (Potamopyrgus antipodarum). Oecologia 103: 509-517.

Jokela, J. & Lively, C. M. 1995b. Parasites, sex and early reproduction in a mixed population of freshwater snails. Evolution 49: 1268-1271.

Jokela, J. & Mutikainen, P. 1995a. Effect of size-dependent muskrat (Ondatra zibethica) predation on the spatial distribution of a freshwater clam, Anodonta piscinalis Nilsson (Unionidae, Bivalvia). Can. J. Zool. 73: 1085-1094.

Jokela, J. & Mutikainen, P. 1995b. Phenotypic plasticity and priority rules for energy allocation in freshwater clam: a field experiment. Oecologia 104: 122-132.

Jokela, J., Valtonen, E. T. & Lappalainen, M. 1991. Development of glochidia of Anodonta piscinalis and their infection of fish in a small lake in northern Finland. Arch. Hydrobiol. 120: 345–356.

Jokela, J., Uotila, L. & Taskinen, J. 1993. Effect of the castrating trematode parasite Rhipidocotyle fennica on energy allocation of freshwater clam Anodonta piscinalis. Funct. Ecol. 7: 332-338.

Jokela, J., Schmid-Hempel, P. & Rigby, M. C. 2000. Dr. Pangloss restrained by the Red Queen - steps towards a unified defence. Oikos 89: 267-274.

Jones, R. K. H. 1978. Parasitism by Unionicola spp. larvae on chironomids. Hydrobiologia 60: 81-87.

Kaltz, O., Gandon, S., Michalakis, Y. & Shykoff, J. A. 1999. Local maladaptation in the anther-smut fungus Microbotryum violaceum to its host plant Silene latifolia: evidence from a cross-inoculation experiment. Evolution 53: 395-407.

Klasing, K. C., Johnstone, B. J. & Benson, B. N. 1991. Implications of an immune response on growth and nutrient requirements of chicks. In: Haresing, W. & Cole, D. J. A. (eds), Recent advances in animal nutrition: 135-146. Butterworth Heinemann, Stoneham.

Kostarev, G. F. 1977. Bream and pike–perch infected by Paraergasilus rylovi, Markewitsch 1937. Biologiya Vnutrennikh Vod. Informatsionnyj Bjulletin 36: 76-77. (In Russian).

Krist, A. C., Lively, C. M., Levri, E. P. & Jokela, J. 2000. Spatial variation in susceptibility to infection in a snail-trematode interaction. Parasitology 121: 395-401.

Krist, A. C., Jokela, J., Wiehn, J. & Lively, C. M. 2004. Effects of host condition on susceptibility to infection, parasite developmental rate, and parasite transmission in a snail-trematode interaction J. Evol. Biol. 17: 33-40.

Lacoste, A., Malham, S. K. Cueff, A. & Poulet, S. A. 2001. Stress and sress-induced neuroendocrine changes increase the susceptibility of juvenile oysters (Crassostrea gigas) to Vibrio splendidus. Appl. Environ. Microbiol. 204: 1247-1255.

Lacoste, A., Malham, S. K., Célébart, F., Cueff, A. & Poulet, S. A. 2002. Stress-induced immune changes in the oyster Crassostrea gigas. Dev. Comp. Immunol. 26: 1-9.

Lafferty, K. D. & Kuris A. M. 1999. How environmental stress affects the impacts of parasites. Limnol. Oceanogr. 44: 925-931.

Lauckner, G. 1984. Impact of trematode parasitism on the fauna of a North Sea tidal flat. Helgol. Meeresunters. 37: 185-199.

Lauckner, G. 1986. Ecological effects of larval trematode infestation on littoral marine invertebrate populations. In: Howell, M. J. (ed), Parasitology - Quo Vadit? 741 p., Proceedings of the 6th International Congress of Parasitology, Brisbane.

Lehman, T. 1993. Ectoparasites: direct impact on host fitness. Parasitol. Today 9: 8-13.

Lewis, J. B. 1984. Comparative respiration in two species of freshwater unionid mussels (Bivalvia). Malacol. Rev.17: 101-102.

Lim, S. S. L. & Green, R. H. 1991. The relationship between parasite load, crawling behaviour, and growth rate of Macoma balthica (L.) (Mollusca, Pelecypoda) from Hudson Bay, Canada. Can. J. Zool. 69: 2202-2208.

Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature 328**:** 519-521.

Lively, C. M. 1996. Host-parasite coevolution and sex. Bioscience 46: 107-114.

Lively, C. M. 1999. Migration, virulence, and the geographic mosaic of adaptation by parasites. Am. Nat. 153: S34-S47.

Lively, C. M. & Jokela, J. 1996. Clinal variation for local adaptation in a host-parasite interaction. Proc. R. Soc. Lond. B 263: 891-897.

Lozano, G. A. 1991. Optimal foraging theory: a possible role for parasites. Oikos 60: 391-395.

Mackie, G. L. 1984. Bivalves. In: Tompa, A. S., Verdonk, N. H. & van den Biggelaar, J. A. M. (eds), The Mollusca. Vol. 7. Reproduction: 351-418. Academic Press, Orlando, Florida.

Manning, S., Woolhouse, M. & Ndamba, J. 1995. Geographic compatibility of the freshwater snail Bulinus globosus and shistosomes for the Zimbabwe Highveld. Int. J. Parasit. 25: 37-42.

Markewitsch, A. P. 1937. Copepoda parasitica of freshwaters of USSR. 259 p., Izdatelstvo Akademii Nauk Ukrainskoj SRR, Kiev. (In Russian).

Markewitsch, A. P. & Kusmorska, A. P. 1937. Paraergasilus rylovi Markewitsch, a new representative of parasitic copepods from the Caspian Sea. Naukovi Zapiski, Biologichnij Zbirnik 3: 381-397. (In Ukrainian, English summary).

McDonald, M. E. 1969. Annotated bibliography of helminthes of waterfowl. J. Parasitol. 68: 131-133.

McMahon, R. F. 1991. Mollusca: Bivalvia. In: Thorp, J. H. & Covich, A. P. (eds), The Ecology and Classification of North American Freshwater Invertebrates: 315-399. Academic Press, San Diego.

Mills, C. A. 1991. Reproduction and life history. In: Winfield, I. J. & Nelson, J. S. (eds.), Cyprinid fishes: systematics, biology and exploitation: 483-508. Chapman & Hall, London.

Mitchell, G. F., Goding, J. W. & Rickard, M. D. 1977. Studies on immune responses to larval cestodes in mice. Increased susceptibility of certain mouse strains and hypothymic mice to Taenia taeniaformis and analysis of passive transfer of resistance with serum. Aust. J. Exp. Biol. Med. Sci. 55: 165.

Moore, J. 2002. Parasites and the Behavior of Animals. 315 p., Oxford Series in Ecology and Evolution. Oxford Univ. Press.

Mopper, S., Beck, M., Simberloff, D. & Stiling, P. 1995. Local adaptation and agents of selection in a mobile insect. Evolution 49: 810-815.

Mouritsen, K. N. 1997. Crawling behaviour in the bivalve Macoma balthica: The parasite-manipulation hypothesis revisited. Oikos 79: 513-520.

Murray, P. J. & Young R. A. 1992. Stress and immunological recognition in host-pathogen interactions. J. Bacteriol. 174: 4193-4196.

44

Mutikainen, P., Salonen, V., Puustinen, S. & Koskela, T. 2000. Local adaptation, resistance, and virulence in a hemiparasitic plant-host plant interaction. Evolution 54: 433-440.

Nagasawa, K. & Nagata, M. 1992. Effects of Pectenophilus ornatus (Copepoda) on the biomass of cultured Japanese scallop Patinopecten yessoensis. J. Parasitol. 78: 552-554.

Negus, C. L. 1966. A quantitative study of growth and production of unionid mussels in the river Thames at Reading. J. Anim. Ecol. 35: 513-532.

Nur, N. 1988. The concequences of brood size for breeding blue tits. III. Measuring the cost of reproduction; survival, future fecundity, and differential dispersal. Evolution 42: 351-362.

Økland, J. 1963. Notes of population density, age distribution, growth, and habitat of Anodonta piscinalis Nilss. (Moll. Lamellibr.) in a eutrophic Norweigian lake. Nytt Mag. Zool. 11: 19-43.

Oliver, L. M. & Fisher, W. S. 1995. Comparative form and function of oyster Crassostrea virginica hemocytes from Chesapeake Bay (Virginia) and Apalachicola Bay (Florida). Dis. Aquat. Org. 22: 217-225.

Oppliger, A., Christe, P. & Richner, H. 1996. Clutch size and malaria resistance. Nature 381: 565.

Oppliger, A., Vernet, R. & Baez, M. 1999. Parasite local maladaptation in the Canarian lizard Gallotia galloti (Reptilia: Lacertidae) parasitized by haemogregarian blood parasite. J. Evol. Biol. 12: 951-955.

Parker, M. A. 1989. Disease impact and local genetic diversity in the clonal plant Podophyllum peltatum. Evolution 43: 540-547.

Parker, M. A. 1991. Non-adaptive evolution of disease resistance in an annual legume. Evolution 45: 1209-1217.

Pekkarinen, M. 1992. Etelä-Suomen (Vantaanjoen) jokisimpukoilla kaksi poikuetta kesässä. (River mussels in the Vantaa River, southern Finland, can have two successive breedings per summer.). Luonnon Tutkija 96: 146–147. (In Finnish).

Pekkarinen, M. 1993. Reproduction and condition of unionid mussels in the Vantaa River, South Finland. Arch. Hydrobiol. 127: 357–375.

Pekkarinen, M. & Englund, P. M. 1995. Sizes of intramarsupial unionacean glochidia in Finland. Arch. Hydrobiol. 134: 379–391.

Peters, G., Faisal, M., Lang, T. & Ahmed, I. 1988. Stress caused by social interaction and its effect on susceptibility to Aeromonas hydrophila infection in rainbow trout Salmo gairdneri. Dis. Aquat. Org. 4: 83-89.

Pipe, R. 1990. Hydrolytic enzymes associated with granular haemocytes of the marine mussel Mytilus edulis. Histochem. J. 22: 595-603.

Pojmanska, T. 1984. An analysis of seasonality of incidence and maturation of some fish parasites, with regard to thermal factor. 1. General methods. Ergasilus sieboldi Nordmann, 1932. Acta Parasitol. Pol. 29: 217-228.

Poulin, R. 1996. Sexual inequalities in helminth infections: A cost of being a male? Am. Nat. 147: 287-295.

Poulin, R. 1998. Evolutionary ecology of parasites. From individuals to communities. 212 p., Chapman & Hall, London.

Price, P.W. 1980. Evolutionary biology of parasites. 237 p., Princeton Univ. Press, Princeton NJ.

Pruett, S. B., Ensley, D. K. & Crittenden P. L. 1993. The role of chemical-induced stress responses in immunosuppression: A review of quantitative associations and cause-effect relationships between chemical-induced stress responses and immunosuppression. J. Toxicol. Environ. Health 39: 163-192.

Pulkkinen, K. & Ebert, D. 2004. Host starvation decreases parasite load and mean host size in experimental populations. Ecology (in press).

Reznick, D. 1985. The cost of reproduction: An evaluation of the empirical evidence. Oikos 44: 257-267.

Richner, H., Christe, P. & Oppliger, A. 1995. Paternal investment affects prevalence of malaria. Proc. Natl. Acad. Sci. USA 92: 1192-1194.

Roberts, C. W., Satoskar, A. & Alexander, J. 1996. Sex steroids, pregnancy-associated hormones and immunity to parasitic infections. Parasitol. Today 12: 382-388.

Robson, E. M. & Williams, I. C. 1970. Relationship of some species of Digenea with the marine prosobranch Littorina littorea (L.) I. The occurrence of larval Digenea in L. littorea on the North Yorkshire Coast. J. Helminthol. 44: 153-168.

Roy, B. A. 1998. Differentiating the effects of origin and frequency in reciprocal transplant experiments used to test negative frequency-dependent selection hypotheses. Oecologia 115: 73-83.

Saarinen, M. & Taskinen, J. 2003. Burrowing and crawling behaviour of three species of Unionidae in Finland. J. Molluscan Stud. 69: 81-86.

Smith, I. M. & Cook, D. R. 1991. Water mites. In: Thorp, J. H. & Covich, A. P. (eds), The Ecology and Classification of North American Freshwater Invertebrates: 523-592. Academic Press, San Diego.

Smith, N. F. 2001. Spatial heterogeneity in recruitment of larval trematodes to snail intermediate host. Oecologia 127: 115-122.

Sousa, W. P. & Gleason, M. 1989. Does parasitic infection compromise host survival under extreme environmental conditions? The case for Cerithidea californica (Gastropoda: Prosobranchia). Oecologia 80: 456-464.

Sousa, W. P. & Grosholz, E. D. 1991. The influence of habitat structure on the transmission of parasites. In: Bell, A. A, McCoy, E. D. & Mushinsky, H. R. (eds), Habitat Structure: the Physical Arrangement of Objects in Space: 300-324. Chapman & Hall, London.

Sprung, M. 1991. Costs of reproduction: A study on metabolic requirements of gonads and fecundity of the bivalve Dreissena polymorpha. Malacologia 33: 63-70.

Strauss, S. Y. 1997. Lack of evidence for local adaptation to individual plant clones or site by a mobile specialist herbivore. Oecologia 110: 77-85.

Tankersley, R. A. & Dimock, R. V. J. 1993. The effect of larval brooding on the respiratory physiology of the freshwater unionid mussel Pyganodon cataracta. Am. Midl. Nat. 130: 146-163.

46

Taskinen, J. 1998. Influence of trematode parasitism on the growth of a bivalve host in the field. Int. J. Parasitol. 28: 599-602.

Taskinen, J. & Valtonen, E. T. 1995. Age-, size-, and sex-specific infection of Anodonta piscinalis (Bivalvia: Unionidae) with Rhipidocotyle fennica (Digenea: Bucephalidae) and its influence on host reproduction. Can. J. Zool. 73: 887-897.

Taskinen, J., Valtonen, E. T. & Gibson, D. I. 1991. Studies on bucephalid digeneans parasitising molluscs and fishes in Finland I. Ecological data and experimental studies. Syst. Parasitol. 19: 81-94.

Taskinen, J., Valtonen, E. T. & Mäkelä, T. 1994. Quantity of sporocysts and seasonality of two Rhipidocotyle species (Digenea: Bucephalidae) in Anodonta piscinalis (Mollusca: Bivalvia). Int. J. Parasitol. 24: 877-886.

Taskinen, J., Mäkelä, T. & Valtonen, E. T. 1997. Exploitation of Anodonta piscinalis (Bivalvia) by trematodes: parasite tactics and host longevity. Ann. Zool. Fennici 34: 37-46.

Van Den Thillart, G. & De Vries, I. 1985. Excretion of volatile fatty acids by anoxic Mytilus edulis and Anodonta cygnea. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 80: 299-302.

Thomas, F. & Poulin, R. 1998. Manipulation of a mollusc by a trophically transmitted parasite: convergent evolution or phylogenetic inheritance? Parasitology 116: 431-43.

Tinsley, R. C. & Jackson, H. C. 1988. Pulsed transmission of Pseudodiplorchis americanus (Monogenea) between desert hosts (Scaphiopus couchii). Parasitology 97: 437-452.

Titar, V. M. & Chernogorenko, M. I. 1982. A new species of Paraergasilus Markewitsch, 1937 (Copepoda: Ergasilidae) from a freshwater bivalve mollusk Anodonta piscinalis Nils. (Mollusca: Unionidae). Parazity i parasitozy cheloveka i zivotnykh. Sbornik Nauchnykh Trudov: 214-218. Naukova Dumka, Kiev. (In Russian, English summary).

Tudorancea, C. 1969. Comparison of populations of Unio tumidus Philipsson from the complex of Crapina-Jijila marshes. Ekol. Polska, Ser. A. 17: 185–204.

Tuuha, H., Valtonen, E. T. & Taskinen, J. 1992. Ergasilid copepods as parasites of perch Perca fluviatilis and roach Rutilus rutilus in Central Finland: seasonality, maturity and environmental influence. J. Zool. 228: 405-422.

Valtonen, E. T., Tuuha, H. & Pugachev, O. N. 1993. Seasonal studies of the biology of Achtheres percarum in perch, Perca fluviatilis, from four Finnish lakes over a 3-year period. J. Fish Biol. 43: 621-632.

Väyrynen, T. Siddall, R., Valtonen E. T. & Taskinen, J. 2000. Patterns of trematode parasitism in lymnaeid snails from northern and central Finland. Ann. Zool. Fennici 37: 189-199.

Wahli, T., Verlhac, V., Gabaudan, J., Schüep, W. & Meier, W. 1998. Influence of combined vitamins C and E on non-specific immunity and disease resistance of rainbow trout, Onchorhynchus mykiss (Walbaum). J. Fish Dis. 231: 127-137.

Wakelin, D. 1984. Immunity to parasites. How parasitic infections are controlled. (2nd ed.) 204 p., Cambridge Univ. Press, Cambridge.

Watters, G. T., O'Dee, S. H. & Chordas, S. 2001. Patterns of vertical migration in freshwater mussels (Bivalvia: Unionoida). J. Freshwat. Ecol. 16: 541-549.

Webster, J. P. & Davies, C. M. 2001. Coevolution and compatibility in the snail-schistosome system. Parasitology 123: S41-S56.

Wedekind, C. & Jakobsen, P. J. 1998. Male biased susceptibility to helminth infection: an experimental test with a copepod. Oikos 81: 458-462.

Whittier, T. R., Paulsen, S. G., Larsen, D. P., Peterson, S. A., Herlihy, A. T. & Kaufmann P. R. 2002. Indicators of ecological stress and their extent in the population of northeastern lakes: A regional-scale assessment. Bioscience 52: 235-247.

Williams, J. A. & Esch, G. W. 1991. Infra- and component community dynamics in the pulmonate snail Helisoma anceps, with special emphasis on the hemiurid trematode Halipegus occidualis. J. Parasitol. 77: 246-253.

Zuk, M. 1988. Parasite load, body size, and age of wild-caught male field crickets (Orthoptera: Gryllidae): effects on sexual selection. Evolution 42: 969-976.

Zuk, M. 1990. Reproductive strategies and disease susceptibility: An evolutionary viewpoint. Parasitol. Today 6: 231-233.