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Yi-Te Lai

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EASTERN FINLAND

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ABSTRACT

Parasites are important selective agents in nature, not only because of their exploitation of hosts but also because they can impose important ecological and evolutionary pressure on their hosts. Parasites can cause physiological and behavioral responses on their hosts. According to Hamilton and Zuk's 'parasite-mediated sexual selection hypothesis', parasitic infection has a negative impact on phenotypic traits such as sexual ornaments and displays because the expression of particular male traits is associated with resistance to parasites. Therefore, females should be able to use these traits as an indicator of heritable parasite resistance for their offspring. Moreover, the 'handicap hypothesis' predicts that the costly expression of male sexual displays signals reliably a male's capacity to tolerate and fight against parasite infections. Therefore, the evolution of parasite-mediated sexual selection, especially in mate choice, can be expected. In addition to their impact on female mate choice, parasites are expected to modify the behavioural competitive ability of the males and thus play an important role in intra-sexual selection. However, existing evidence for the effect of parasite infections on intra-sexual selection is contradictory and may vary depending on the parasites and hosts that are studied, the mating systems and the parasites' virulence.

The European minnow (*Phoxinus phoxinus*) is a cyprinid fish and a good model species for sexual selection studies, as it exhibits spectacular sexual dimorphism and has intense inter- and intra-sexual selection during the reproductive period. Although the minnows harbor a number of parasite species in different body regions, the comprehensive effects of parasitism on the minnow biology have been rarely studied. In 2008, we found a minnow population in the Joensuu area of Eastern Finland, that was parasitized by *Philometra ovata* — a body cavity dwelling (BCD) nematode. Despite the relatively large size (80-120 mm) of *P. ovata*, their influences on hosts have rarely been reported. Thus, the aim of this study was to

comprehensively understand the impact of *P. ovata* on the host minnow's fitness, including swimming performance, body condition, relative gonad size, survival, and sexual selection. Moreover, a non-invasive diagnostic method for the infection status of BCD *P. ovata* was developed during this study.

In the present dissertation, I first explored seasonal occurrence and infection ecology of *P. ovata*. I found that *P. ovata* in minnows in Finland have a regular annual life cycle, and its yearly evacuation period may not be as regular as it has been documented in more southern parts of Europe. Secondly, in contrast to the expectations, my results indicate that infection of BCD *P. ovata* may not significantly impair survival, swimming performance and measured fitness-related traits of host minnows. The intensity and abundance of BCD *P. ovata* was not associated with condition or relative gonad size of minnows. In addition, the sperm quality in male minnows was not affected by the abundance of BCD *P. ovata*. However, the hematocrit was significantly negatively correlated with the abundance of BCD *P. ovata* in male minnows. Furthermore, evacuation of BCD *P. ovata* may cause physical damages to host minnows both internally and externally, and potentially raise the mortality of infected minnows.

Thirdly, the present study shows that male ornamentation in minnows acts as an honest signal of swimming performance, body condition and gonad size. Although previous studies have shown that female minnows have a clear behavioural preference towards colourful males when only visual cues are present, our study indicates that females prefer odours of males with a bright red (i.e. highly saturated) belly colouration when males are familiar to females, while showing no preference when males are unfamiliar to females. Accordingly, it seems likely that male odours *per se* in minnows are uninformative in terms of male body quality, but may be mainly used in individual recognition. Therefore, in natural conditions, female minnows may base their mating decisions largely on visual cues.

Furthermore, my study shows that the saturation of abdominal redness is negatively associated with abundance of

BCD *P. ovata* in male minnows, but such an effect was not demonstrated consistently. Finally, my results suggest that BCD *P. ovata* does not impact sexual selection among host minnows. Female minnows do not show behavioural preference between the odours of unfamiliar males infected with BCD *P. ovata* from those that are non-infected. Also, infected males had similar behavioural sexual competitiveness (dominance status and courtship performance) as non-infected males, i.e. infected males are similarly competitive as non-infected rivals.

In conclusion, the interaction of *P. ovata* and minnows provides a partly unexpected empirical example of parasites having little impacts on their hosts. This study includes not only a two-year survey of life history and infection ecology of the parasite, but also provides a comprehensive perspective on parasite-mediated sexual selection with the impact of parasites on hosts' mate choice.

Universal Decimal Classification: 57.081, 57.084, 575.21, 576.89, 591.69, 595.132, 597.551.2

CAB Thesaurus: Cyprinidae; Phoxinus phoxinus; parasites; animal parasitic nematodes; Philometra ovata; host parasite relationships; diagnostic techniques; phenotypes; colour patterns; males; swimming; body condition; odours; gonads; spermatozoa; haematocrit; mating preferences; mating behaviour; sexual selection; females; life cycle; infection; seasonality; seasonal abundance

Yleinen suomalainen asiasanasto: parasitologia; loiset; sukkulamadot; särkikalat; mutu; diagnostiikka; fenotyyppi; lisääntymiskäyttäytyminen; sukupuolivalinta; elinkaari; infektiot; kausivaihtelut

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referred to by the Roman numerals I-VI.

I Lai Y-T, Taskinen J, Kekäläinen J and Kortet R. Life cycle and infection ecology of *Philometra ovata* (Nematoda) in a wild European minnow (*Phoxinus phoxinus*) population. Submitted manuscript.

II Lai Y-T, Taskinen J, Kekäläinen J and Kortet R. Non-invasive diagnosis for *Philometra ovata* (Nematoda) infection in the common minnow *Phoxinus phoxinus*. *Parasitology Research 111: 2411-2418, 2012.*

III Kekäläinen J, Leppänen, H-R, Huuskonen, H, Lai Y-T, Valkama, H and Taskinen J. The information content of odour, colour and tactile cues in the mate choice of minnows. *Behaviour 148: 909-925, 2011.*

IV Lai Y-T, Kekäläinen J and Kortet R. Parasitized but not paralyzed: body cavity dwelling *Philometra ovata* does not impair behavioural sexual competitiveness or odour attractiveness of male European minnows (*Phoxinus phoxinus*). Submitted manuscript.

V Lai Y-T, Kekäläinen J and Kortet R. Male ornamentation in the European minnow (*Phoxinus phoxinus*) signals swimming performance. *Ethology 119: 1077-1085, 2013.*

VI Lai Y-T, Kekäläinen J and Kortet R. Large worm, little harm: parasitic infection of body cavity dwelling *Philometra ovata* (Nematoda) may not impair swimming performance of male European minnows (*Phoxinus phoxinus*). Submitted manuscript.

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AUTHOR'S CONTRIBUTION

The present author was responsible for major planning in articles I, II, IV and VI, and for cooperative planning in article V. The author was responsible for major data collecting, analyzing, and manuscript writing in all articles except article III, in which the author was responsible for minor data collecting and relevant analysis.

Contents

1 Introduction

Parasites have been suggested to play a key role in evolution, such as in sexual reproduction (e.g. Hamilton et al., 1990; Lively, 2010), ecological speciation (e.g. Karvonen & Seehausen, 2012), animal personalities (Kortet et al., 2010), and even language diversification and cultural evolution (Fincher & Thornhill, 2008). Since parasites are organisms that take their resources from other organisms, they often act as important ecological and evolutionary selective agents for their hosts. Although parasites usually utilize host energy resources, their deleterious effects are far beyond host nutrition, including various pathological (e.g. Dezfuli et al., 2007), physiological (Shaw et al., 2009) and behavioural effects (e.g. Barber et al., 2000; Kekäläinen et al., 2014). Thus, it is likely that parasites ultimately impact the reproductive fitness of the host and, eventually, sexual selection.

Sexual selection, a mode of natural selection in which some individuals that are better at securing mates have higher reproductive fitness in a population, has been considered a key evolutionary process since Darwin proposed the theory in 1871. Sexual selection consists of two broad mechanisms. Firstly, it can involve competition between individuals of the same sex (usually males), who fight against each other for mating opportunities with females (intra-sexual selection or male-male competition). Secondly, sexual selection acts via mate choice behaviour in which members of one (or both) sexes selectively mate with members of the other (i.e. inter-sexual selection or female choice; reviewed by Andersson, 1994; Beltran-Bech & Richard, 2014). Due to the fact that both sexual selection mechanisms commonly occur in the same species, the causation of performance in mating system and mating behavior can be complicated (reviewed by Berglund et al., 1996).

Elaborated secondary sexual characters, such as the peacock's tail, have often been assumed to evolve as a response

to both inter- and intra-sexual selection. With these phenotypic characters, the choosy sex is able to evaluate the condition and state of candidate individuals, while members in the opposite sex are able to demonstrate their condition and state to their rivals before physical conflicts. Since the reproductive success of males is commonly limited by access to females in general, females tend to be in the choosy role in most mating systems, and sexually selected traits are likely to be found in males more often than in females (reviewed by Andersson, 1994; but see Nordeide et al., 2013). It has been suggested that these extravagant phenotypic characters may be able to be maintained only by high-quality individuals because the relative cost of producing these signals would be lower in these individuals (handicap principle: Zahavi, 1975). In addition, such secondary sexual characters are generally presumed to increase the male's fitness, as well as his reproductive success, and this association has been demonstrated in many empirical studies. For example, in the three-spined stickleback (*Gasterosteus aculeatus*), the red breeding colouration of males acts as a signal in inter-sexual selection because females choose their mates based on the intensity of the colouration (Milinski & Bakker, 1990). In addition, the intensity of the red colouration is linked to individual condition, thus it also signals male physiological competitive ability and dominance status related to intra-sexual selection among sticklebacks (Milinski & Bakker, 1990). Red, orange and yellow ornaments of birds and fish usually consist of carotenoids (Aquilera & Amat, 2007), pigments that animals are unable to synthesize. Animals must, therefore, acquire their carotenoids from their food (e.g., Fox, 1976; Grether et al., 1999). Carotenoid ornaments are considered energetically costly to obtain and act as honest and reliable signals for the quality of males (Hill et al., 2002; McGraw & Ardia, 2003).

Since Hamilton & Zuk (1982) suggested that parasites may play an important role in sexual selection in their hosts, a wealth of research has been dedicated to understand the effects of parasites on mate choice (reviewed by Barber, 2002; Moore, 2002; Kortet, 2003). According to Hamilton and Zuk's parasite-

mediated sexual selection hypothesis, parasitic infection has a negative impact on phenotypic traits, such as sexual ornaments and displays, because the expression of particular male traits is associated with resistance to parasites. Therefore, females are able to use these traits as indicators of heritable parasite resistance for their offspring (Hamilton & Zuk, 1982). The handicap principle applied to parasite resistance also indicates that the expression of costly male sexual displays informs reliably the male's capacity to fight against parasite infection. In this case, sexual ornaments provide cues reflecting individual condition in a population infected with parasites (Kortet & Taskinen, 2004; Kortet et al., 2004b; reviewed by Beltran-Bech & Richard, 2014). On the other hand, according to the contagion indicator hypothesis, male secondary sexual characters are associated with the intensity of transmittable parasites (Able, 1996). Thus, by discriminating against heavily infected males in mate choice, females can also avoid sexually or vertically transmitted parasites (Able, 1996; Hillgarth, 1996). Taken together, based on the suggestion of honest sexual ornamentation for parasitic infection level, the evolution of parasite-mediated sexual selection, especially in relation to mate choice, can be expected.

In addition to their influences on female mate choice, parasite infections can be expected to modify the behavioural competitive ability of the males and thus play an important role in intra-sexual selection (Schall & Dearing, 1987; Hamilton & Poulin, 1995; Maksimowich & Mathis, 2000; Klein, 2003). Studies supporting this hypothesis have shown that parasite infection can impair male behavioural competitiveness in intra-sexual selection (Schall & Dearing, 1987; Maksimowich & Mathis, 2000; Pelabon et al., 2005). Moreover, a male's success in intra-sexual competition seems to be connected with his immunocompetence (e.g. Rantala & Kortet, 2004; Väänänen et al., 2006). Other studies have, however, failed to find a connection between parasitism and male behavioral competiveness in intra-sexual selection (Berdoy et al., 1995; Hamilton & Poulin, 1995; Thomas et al., 1995; Barber, 2002), and in one case, male behavioural

competitiveness has been shown to be positively correlated with parasite infection status (Bull & Burzacott, 1993). Thus, existing evidence for the effects of parasite infections on male behavioural competitiveness in intra-sexual selection is contradictory and highly dependent on the species of parasite, host species, mating system and parasite virulence (see Møller, 1990; Moore, 2002; Beltran-Bech & Richard, 2014).

1.1 EUROPEAN MINNOW *PHOXINUS PHOXINUS*

1.1.1 Male ornamentation and sexual selection in minnows

The European minnow (*Phoxinus phoxinus*) is a cyprinid fish species with spectacular sexual dimorphism, and both inter- and intra-sexual selection occurs during the reproductive period (Figure 1).

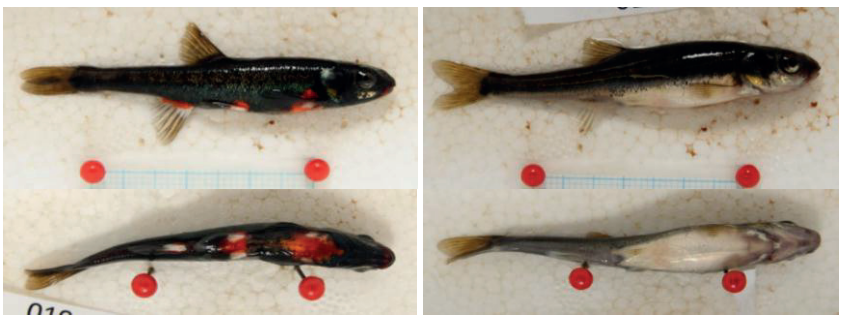

Figure 1. *Sexual dimorphism between male (left) and female (right) minnows during spawning season. The male develops clearly visible breeding ornamentation including metallic green flanks, white spots, red belly and head tubercles.*

During the breeding season, male minnows develop clearly visible breeding ornamentation, including dark lateral colouration, a bright red abdomen and lips, metallic green or blue flanks, and breeding tubercles on the head (Müller & Ward, 1995; Jacob et al., 2009; Kekäläinen et al., 2010b). Tubercles are small, colorless and horny epidermal structures that are common in many fish species (e.g. Kortet & Taskinen, 2004; Kekäläinen et al., 2010a). Despite the fact their functional

significance is not fully understood (Wiley & Collette, 1970; Wedekind et al., 2008), breeding tubercles in minnows may facilitate the maintenance of body contact between the mating partners, be used as weapons during male fights, or act as signals that provide information about male genetic quality and parasitic load through visual, tactile or hydrodynamic signals (Wiley & Collette, 1970; Müller & Ward, 1995; Jacob et al., 2009).

In the spawning season, male minnows constantly patrol oviposition sites and often form large male aggregations. Once a mature female enters a site, she will be approached and followed by a number of males. The female tests the size of gravel by butting it with her head, while males gather around and often butt the female. This could allow the female to estimate the development of a male's tubercles (Müller & Ward, 1995). In a smaller group, males may establish a dominance hierarchy and defend their territories by showing agonistic behaviour towards each other before females begin to spawn (Bless, 1992; Jacob et al., 2009). Male ornaments in minnows have been observed to correlate positively with body size, body condition, dominance status, genetic heterozygosity, and parasitic loads of individuals (Müller & Ward, 1995; Jacob et al., 2009; Kekäläinen et al., 2010b), thus they can be assumed to act as honest signals in the male-male competition.

In addition to being used for signaling between males, male minnows present their ornaments to females in head-down positions (Kekäläinen et al., 2010b). Females usually release their eggs only when at least one large (dominant) male is present (Müller & Ward, 1995; Jacob et al., 2009). Female minnows have a clear behavioural preference towards more ornamented and dominant males when only visual cues are present (Kekäläinen et al., 2010b). It is thus implied that, through female choice in the mating system, female minnows actively determine their spawning decision based on male ornaments.

1.1.2 Influence of parasite infection on sexual selection in minnows

Although it has been documented that minnows harbour a variety of parasites, including enormous numbers of *Diplostomum phoxini* metacercariae in the brain (Barber & Crompton, 1997a, b), *Macrolecithus papilliger* flukes in the intestine (Müller & Ward, 1995), and *Raphidascaris acus* larval nematodes in the liver and pancreas (Dezfuli et al., 2009), parasitic infections and their effects on minnows have been studied mostly in basic parasitology (e.g. Ballabeni & Ward, 1993; Barber & Huntingford, 1996; Barber & Crompton, 1997a, b), with less emphasis on the ecological or evolutionary impacts of the parasites on host minnows. For example, empirical tests of the parasite-mediated sexual selection hypothesis in minnows have been conducted only by Müller & Ward (1995). They showed that the number of *M. papilliger (*flukes) was negatively related to the redness of male abdominal ornamentation and positively related to the size of male tubercles, and that the number of *D. phoxini* (metacercariae) was positively related to the size of male tubercles. On the other hand, heterozygosity was positively related to redness and negatively related to the size of the tubercles. It was thus suggested that male minnows unable to develop exaggerated characters (i.e. a bright red abdomen) have an alternative strategy of emphasizing weapons that may improve their competitive ability in intra-sexual selection.

1.2 PARASITIC NEMATODE *PHILOMETRA OVATA*

1.2.1 Life history of *P. ovata*

Philometra ovata, frequently referred to by its junior synonym *Philometra abdominalis*, is a parasitic nematode dwelling in the body cavity of its definitive fish hosts, including the genera *Gobio*, *Phoxinus*, *Abramis*, *Rutilus* and *Leuciscus* (Molnár, 1966; Keskin, 1988; Moravec, 2004; Innal & Keskin, 2005) (Figure 2). According to the study by Molnár (1966) conducted in Hungary,

the life cycle of *P. ovata* shows a regular yearly life cycle with the variant of only a few days. In June, the first stage larvae of *P. ovata* are ingested by intermediate copepod hosts (e.g. *Acanthocyclops vernalis*, *Cyclops strenuus*, *Macrocyclops albidus* and *Megacyclops gigas*; Moravec, 1980), where the larvae penetrate into the host's haemocoel and molt to the 2nd larval stage after 3 to 4 days. The molt to 3rd larval stage occurs 5 to 7 days after infection, and then the larvae remain inside the exuviae of the cuticle of the 2nd molt and become infective to their definitive host (Moravec, 1980). Infective larvae of *P. ovata* are trophically transmitted to the definitive fish host when the parasitized copepods are preyed upon by the aforementioned cyprinid fish (Molnár, 1966; Moravec, 1977) in late June.

In the definitive fish host such as the bream (*Abramis brama*), larval *P. ovata* first penetrate through the intestine wall of the host fish, then migrate to the swim bladder living under the serose cover, where the larvae mature sexually then mate in July (Molnár, 1966). After mating, males stay in the swim bladder, while gravid females with eggs in their uterus emigrate from the swim bladder to the body cavity of the fish host in August. It is only in the body cavity of the fish host that gravid female *P. ovata* are able to grow to their final size (which can be more than 70 mm long) and attain a fully developed uterus with thousands of free first stage larvae by the end of the next spring and early summer. At a particular time of the year, such as from the end of May to the beginning of June in central Europe (Molnár, 1966), *P. ovata* females leave the body cavity of the host by penetrating its tissues around the anus, and rupture immediately due to the hypotonic effect of the surrounding water (Molnár, 1966; Keskin, 1988). The first stage larvae are released during the evacuation, and begin seesaw oscillating which in turn attracts copepod predator, thus starting the new life cycle for *P. ovata*.

Figure 2. *Several gravid Philometra ovata in the body cavity of the host minnow Phoxinus phoxinus.*

1.2.2 Infection ecology and influence of *P. ovata* on its host

Infection ecology of *P. ovata* on fish host has been surveyed only partially in previous studies. In a one year survey of European Chub (*Leuciscus cephalus*) from Turkey, the prevalence (proportion of parasitized individuals) of body cavity dwelling (BCD) *P. ovata* was shown to vary between 4.0 % and 20.0 %, and mean intensity (quantity of parasite individuals in infected host) was around 2.5 from May to July (Innal & Keskin, 2005). A one month survey of *Gobio lozanoi* in Spain found that the

prevalence of BCD *P. ovata* was over 50.0 %, and mean intensity was 7.4 ± 7.6 with a maximum of 30 individuals (Saraiva et al., 2008). In a survey from 2005 to 2008, the prevalence of BCD *P. ovata* among roach (*Rutilus rutilus*) from Lithuania was found to be less than 1.0 % (Rakauskas & Blaževičius, 2009). In contrast, the intensity of BCD *P. ovata* in bream and roach from Hungary was shown to be as high as 20–300 and 1–14, respectively (Molnár, 1966). Only BCD *P. ovata* had been surveyed in these previous studies, with the infection ecology of *P. ovata* in swim bladder dwelling (SBD) stage largely being ignored (but see Molnár, 1966).

Despite the relatively large size of BCD female worms in host fishes, the influence of *P. ovata* on host fitness has rarely been reported in previous studies. Saraiva et al. (2008) reported that *P. ovata* infection in the body cavity of *G. lozanoi* led to a severe abdominal swelling and seriously impaired the swimming ability of the host fish. The infection of BCD *P. ovata* is not guaranteed to result in harmful influences on host fish in spite of its large size. According to Saraiva et al. (2008), the intensity of BCD *P. ovata* was shown to be positively correlated with the body condition of *G. lozanoi*, while the prevalence of BCD *P. ovata* was positively correlated with relative gonad size of fishes. Although the life history of *P. ovata* has been surveyed mainly in definitive hosts, such as roach (Molnár, 1966) and bream (Moravec, 1986), its influence on these host fish has never been examined.

1.3 *P. OVATA* INFECTION IN MINNOWS & ITS POTENTIAL INFLUENCE ON HOST

In 2008, a minnow (*P. ovata*) population in the Joensuu area of Eastern Finland was found to be parasitized by BCD *P. ovata*. It is noteworthy that minnows are much smaller in body length (mostly < 9 cm) than all other fishes that serve as hosts for *P. ovata*, including roach, bream, European chub and *G. lozanoi*. Thus, the fitness impact of BCD *P. ovata* is likely to be more

severe in minnows than in other host species (Figure 3). Moreover, the relatively large size of BCD *P. ovata* is also expected play a role in parasite-mediated sexual selection in these minnows. Interestingly, the infection of *P. ovata* and its potential influences on the sexual selection and fitness of host minnows have never been studied.

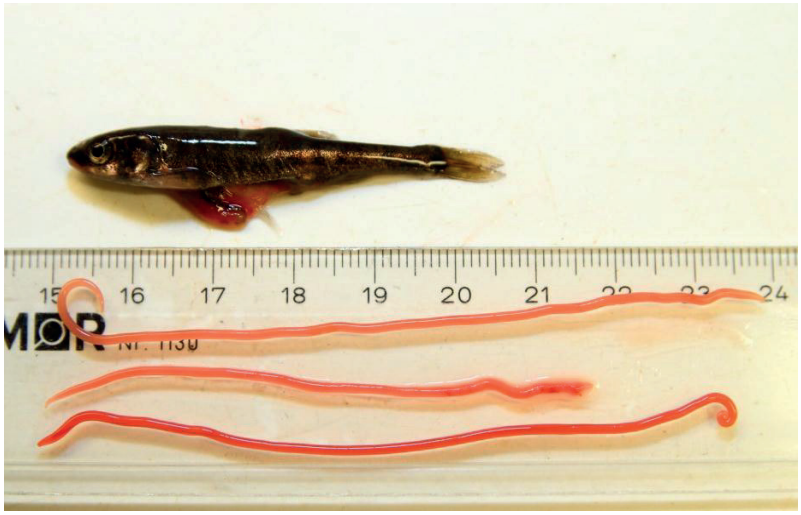

Figure 3. *A male minnow (Phoxinus phoxinus) parasitized by three body cavity dwelling Philometra ovata.*

1.4 AIMS OF THE STUDY

The specific aims of this study were to comprehensively understand the influences of *P. ovata* on condition, performance, survival, and more importantly, on sexual selection of its host minnows (*P. phoxinus*). More detailed aims are as follows:

1. To clarify the infection ecology of *P. ovata* and evaluate the potential fitness effects of the parasite in minnows in general. (I)
2. To examine the influences of *P. ovata* on condition, performance and survival in minnows. (I), (IV) & (VI)
3. To develop a novel non-invasive diagnosis for determining the infection status of BCD *P. ovata* in behavioral studies. (II)

4. To clarify the signaling content of male secondary sexual ornamentation. (III) & (V)
5. To demonstrate whether females perform olfactorial-based female choice, and the link between male odour and male ornamentation. (III)
6. To test the influences of BCD *P. ovata* on olfactorial-based female mate choice and male-male competition. (IV)

2 Materials and methods

2.1 STUDY AREA

Minnows were collected by dip net and minnow traps (Promar, Gardena, California, U.S.A.) from two brooks in Eastern Finland: Brook Kuusoja (62° 48′ N, 30° 1′ E) and Brook Uuronpuro (62° 51′ N, 29° 59′ E) in 2010-2013. In article (I) and (II), all the examined minnows were collected from Brook Kuusoja, while in the other articles male and female minnows were collected from Brook Kuusoja and Brook Uuronpuro, respectively. Collected minnows were immediately transported to the laboratory at the Joensuu campus of the University of Eastern Finland, where all the experiments were conducted.

2.2 LIFE HISTORY AND INFECTION ECOLOGY OF *P. OVATA* IN MINNOWS

2.2.1 Monthly survey in the field in 2010 and 2011 (I)

From April to November in 2010 and 2011, I caught minnows monthly from Brook Kuusoja during the period when the brook was not ice-covered. I determined the sex of every minnow and conducted the fitness-related trait analysis (see 2.4.5). The body mass of a minnow (W_M) was obtained by subtracting the biomass of all BCD *P. ovata* (the sum of body weight of each worm) from the total mass of the minnow.

I dissected the minnows and determined the infection status of *P. ovata*, the abundance of the parasite in the body cavity, as well as body length ($L_{P.o.}$), body weight ($W_{P.o.}$) and the development status of offspring (egg and larva) in the uterus of each BCD female worm. By squeezing the swim bladder of minnows between two glass plates, I also recorded the abundance of SBD *P. ovata* under a dissecting microscope. From

October 2010, I also determined the sex and developmental stage of SBD *P. ovata* (see Moravec, 1986) and thus distinguished the respective abundance of female, male, and larval (immature) SBD *P. ovata* in minnows.

2.2.2 Observation of *P. ovata* evacuation in summer 2011 and bimonthly survey of *P. ovata* in lab-housed minnows in 2011 and 2012 (I)

In May 2011, I collected 96 minnows from the Brook Kuusoja, in which 51 fish were distinguished as infected with BCD *P. ovata* using the highly reliable non-invasive diagnosis (II). These minnows were then transported to the laboratory and were marked with dorsal fin clips to note the infection status. Infected and non-infected fish were mixed and housed in a 45 L aquarium with continuous flow of 12° C water under a natural photoperiod. Fish were fed daily with commercial fish food (Biomar®, Aqualife, Denmark).

In June and July 2011, I observed infected minnows and recorded the evacuation period of female *P. ovata* from the body cavity of their hosts. I also recorded the mortality of infected and non-infected minnows to determine if the penetration damage due to the evacuation of *P. ovata* leads to a higher acute mortality.

From August 2011 to April 2012, I took ca. 20 minnows from the group bimonthly, euthanized them with an overdose of tricaine methanesulfonate (MS-222, Sigma®, Sigma Chemical Co., St. Louis, Missouri, U.S.A.) and dissected them. I measured L_T, W_M, and determined the sex of every minnow as well as measured their gonad weight (W_G). During the dissection, the infection status, abundance, $L_{P.o.}$, $W_{P.o.}$ and development status (egg or larva) of the offspring in the uterus of each BCD *P. ovata* was also recorded. By the same method mentioned in 2.2.1, I also recorded the abundance of male, female, and larval SBD *P. ovata*.

2.3 NON-INVASIVE DIAGNOSIS FOR BCD *P. OVATA* INFECTION

2.3.1 Development and evaluation of non-invasive diagnosis (II)

The noninvasive diagnoses were conducted in 2011 and 2012. In the laboratory, the observer gently and carefully held the minnow out of the water in an abdomen-upward position and visually inspected the ventral and lateral areas of the fish body. The outline of the abdominal surface of an uninfected minnow typically is smooth without any contingent protrusions or uplifts, while the lateral and ventral abdominal surface of an infected minnow usually possesses irregular or vermiform-shaped protrusions and uplifts as a consequence of the presence of BCD *P. ovata* (Figure 4). Moreover, these protrusions and uplifts in an infected minnow are often moving because of the movements of BCD *P. ovata*. Collectively, these clues became the determinant factors of an infection. Nearly half of the minnows (i.e., fish used in behavioral experiments of sexual selection and cultivation of lab-rearing offspring; N = 359) were examined carefully but quickly (less than 20 seconds out of water in the observer's hands) alive and without anesthetization, while others (i.e., fish collected for the parasite population dynamics study; N = 375) were examined after being anesthetized using 70 mg L^{-1} of MS-222. Thus, this study included examination of 734 minnows in total. After the non-invasive diagnoses, I euthanized the fish with an overdose of MS-222.

After euthanasia, I measured the total length and fresh mass of the minnows, and dissected them to determine the true infection status of BCD *P. ovata*. I recorded the number of BCD *P. ovata* (dead or alive) found in each minnow, as well as the $L_{P.o.}$, $W_{P.o.}$, and the reproductive status of each parasite. By comparing the diagnosed and true infections, I classified the results as follows: 1) true positive (diagnosed as infected when an individual was infected), 2) false positive (diagnosed as infected when an individual was not infected), 3) true negative (diagnosed as uninfected when an individual was uninfected), and 4) false negative (diagnosed as uninfected when an

individual was infected). With these results, I was able to assess the sensitivity, specificity, positive predictive power, negative predictive power, and the reliability of my noninvasive diagnosis method according to the definitions in previous studies (Baldessarini et al., 1983; Gjørup, 1997; see Table 1). I used a Chi-square analysis to test whether the results of my noninvasive diagnosis method differed statistically from the true infection results of dissected fish.

Figure 4. *The abdominal surface of a minnow (Phoxinus phoxinus) infected by several body cavity dwelling P. ovata. Arrows of different colours indicate the vermiform uplifts due to different individuals of P. ovata in the body cavity.*

Table 1. *Definition of terms used in the present study adapted from Baldessarini et al. (1983).*

Term	**Definition**
Reliability	(True positive + True negative) / All tests
Sensitivity	True positive / All truly infected
Specificity	True negative / All truly uninfected
Positive predictive power	True positive / All diagnosed as infected
Negative predictive power	True negative / All diagnosed as uninfected
Prevalence	All truly infected / All tests

To test the repeatability of the diagnostic method between observers, I collected minnows from Kuusoja in May 2012 and anesthetized them in the laboratory. For this purpose, a naïve

research assistant (inexperienced observer) was assigned to diagnose the minnows after a short introduction to the method. The anesthetizing and examining procedures were the same as those used in 2011. The repeatability test included an examination of 97 mature minnows (29 females and 68 males) by an experienced observer, while the inexperienced observer diagnosed 93 individuals (27 females and 66 males). As described above, I determined the sensitivity, specificity, positive predictive power, negative predictive power, and the reliability of the inexperienced and experienced observers' diagnoses, respectively. These results were compared using a Chi-square analysis.

2.4 BEHAVIOURAL TESTS AND MEASUREMENTS IN THE EUROPEAN MINNOWS

2.4.1 Housing and spawning induction of the minnows (III)-(VI)

In the laboratory, each male group (i.e. a group of males infected with BCD *P. ovata* and a group of non-infected males) was mixed with females and housed in a 45 L aquarium with continuous flow of 12° C water under a natural photoperiod. Fish were fed daily with commercial fish food (Biomar®, Aqualife, Denmark). After being held under these conditions for one week, I induced the breeding behaviour of the males by gradually increasing the water temperature to 18° C, and then added a layer of gravel (20-40 mm in diameter) to the bottom of the aquarium. Insertion of gravel into the warmed aquaria invariably triggered the spawning behaviour and the full appearance of breeding colouration of the minnows within a few minutes.

2.4.2 Olfactorial-based female mate choice between differently-ornamented males (III) or between males infected or non-infected with BCD *P. ovata* (IV)

Prior to the mate choice trials, two sexually active, size-matched males and one gravid female were placed into a glass aquarium (300L × 300H × 225W mm) containing 10 L of 18°C water and a layer of 20–40 mm gravel. One of the paired males had a highly saturated belly colouration, whereas the other male had a much paler colouration. Three sides of the aquarium were covered with brown cardboard to prevent the effect of outside visual stimuli on the behaviour of the fish. The experimental aquarium was illuminated from above with fluorescent lamps. Otherwise the room was dark to minimize the biasing effect of visual stimuli from the uncovered side of the aquarium. Before the test began, the fish were allowed to interact in the aquarium for 10 min. The aim of this procedure was to enable the female to gather information from the potential mates. The fish then were placed into the mate choice fluvarium (Hirvonen et al., 2000; Figure 5). (III)

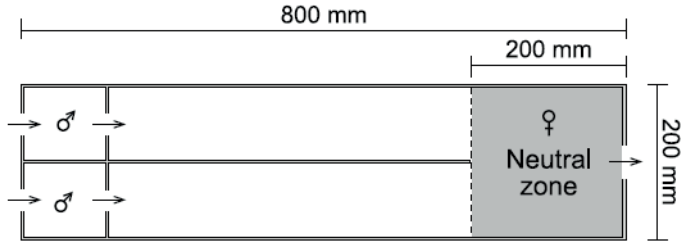

Figure 5. *A schematic illustration of the fluvarium used in the female mate choice experiments. The direction of water flow is indicated by arrows. Prior to the mate choice trials, females were isolated in a neutral zone (grey area) of the fluvarium for 3 min.*

During the mate choice trials, water flowed (100 ml/min) from the two male compartments of the fluvarium to the female compartment. All visual cues between the two males as well as between the males and the female were prevented. Also, all olfactory contacts between the males were excluded. Before each trial, the fish were allowed to habituate to the testing conditions

of the fluvarium for 3 min. During the habituating period, the female was isolated in the female compartment with a gate. At the beginning of each trial, the gate was carefully removed and then the female was allowed to move freely in the fluvarium throughout the 10 min experimental period. Female preference towards male odours was recorded by measuring the time the female spent on each male side in the fluvarium during each trial. To eliminate the potential side effects of the testing conditions on fish behaviour, the position of males (more ornamented and less ornamented in III, infected and non-infected in IV) was changed between the left and right compartments in each trial. (III) & (IV)

2.4.3 Behavioural competitiveness between non-infected male minnows and those infected with BCD *P. ovata* (IV)

In this experiment, an infected and non-infected male as well as a female minnow were simultaneously introduced into the experimental aquarium as described in the first paragraph of 2.4.2.

In most cases, all three minnows stopped moving entirely for a period of time after being introduced into the aquarium. Therefore, a trial was started only when both the infected and non-infected males were actively swimming; the trial was terminated after both males had shown simultaneous activity for 30 minutes. The first 10 minutes of a trial was regarded as the acclimation period, so I recorded male behavior only for the last 20 minutes. During this period, I recorded the number of aggressive attacks of males toward each other and also recorded the number of courtship contacts of each male with the female. An aggressive attack was defined as a rapid dash towards the other male, which always ended with direct contact. Determining the number of courtship contacts was also based on physical contact. While the male-male attacks were often comprised long and aggressive chases of the rival male, the courtship contacts were more often gentle pokes to the belly or other parts of the female body (Kortet et al., 2004a). Unintentional contacts (e.g. contact during side by side swimming between males and between sexes) occurring were

not calculated. The number of aggressive attacks and courtship contacts was used as a measure of behavioural sexual competitiveness.

2.4.4 The association between male swimming performance, ornamentation (V) and infection with BCD *P. ovata* (VI)

The swimming performance of males was examined in an experimental swimming tube (Fig 6a), which is a modified version of the swimming tube system used in Kekäläinen et al. (2010a). The water temperature in the experiment (18°C) was the same as that of the maintenance aquaria. Before each trial, the swimming tube was filled with water, and an experimental minnow was placed individually into the tube via the upper opening. When the minnow had calmed down and turned against the water current in the swimming tube, I gradually turned on tap 1 of the swimming tube, with the water current inside the tube reaching its final velocity (45-50 cm/s) within three seconds. The final velocity was similar to the natural maximum water velocities measured from the spawning site in Brook Kuusoja (where the average flow rate is much higher than in Brook Uuronpuro). Thus, the swimming endurance in this trial should represent biologically realistic differences in individual swimming performance. All the fish were allowed to swim against the water current in the swimming tube until they were regarded as exhausted (i.e. fish were held by the water current against a vertical mesh at the end of the tube and did not move within one minute). The fish were then immediately removed from the swimming tube via the lower opening of the tube. The swimming time of the fish was recorded and was regarded as a measure of swimming performance.

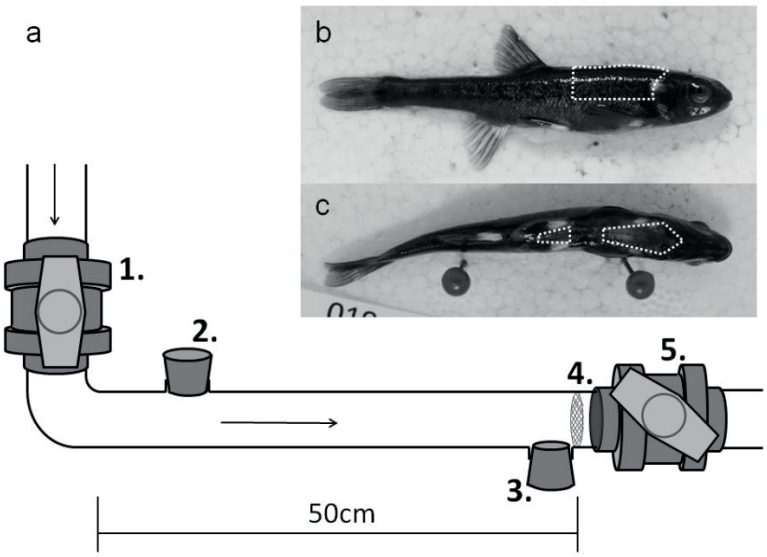

Figure 6. *Experimental tube system used to measure the swimming performance trials (a), and the skin areas for the colouration measurements of the lateral darkness (b) and abdominal redness (c) of male minnows. The swimming tube system (a) consisted of an aquarium pump (not shown), which lifted water up to a plastic container at a height of 180 cm from where the water flowed into the swimming tube (diameter 4 cm). The latter tap (5) was adjusted before the experiment and then fixed to keep the velocity of the water current constant through all trials, while the former tap (1) was completely turned on or off at the beginning and the end of every swimming trial, respectively. Experimental fish were placed into the swimming tube via the entrance (2). After a fish had been exhausted in the swimming trial and held against the net by the current (4), it was removed from the swimming tube via the exit (3).*

2.4.5 Colouration and fitness-related trait analyses (I)-(VI)

After the experiments aforementioned from 2.4.2 to 2.4.4, male minnows were euthanized with an overdose of MS-222. I immediately photographed each male laterally and ventrally in standardized lighting conditions and with white balance by using a Canon 350D digital camera. For colouration analysis, I measured the mean hue, saturation and lightness (HSL) values of both the lateral and abdominal skin of the males using Photoshop CS4 (Adobe, San José, CA, USA). The HSL values for lateral darkness were measured from the lateral skin area above the lateral line, and between the edge of the gill cover and the origin of the pelvic fin (Fig 6b). The HSL values for abdominal

redness was the average value of these colour coordinates in two standardized areas: 1) the elongated pentagon area from the end of left and right gill covers and between left and right pectoral fins, and 2) the rectangle area between pelvic fins (Fig 6c). (III)-(VI)

In the fitness-related trait analysis, I measured the total body length (L_T) and total weight (W) of the experimental minnows, and dissected the fish to measure their gonad weights (W_G). I also examined these males for infection with abdominal cavity dwelling *P. ovata* during dissection. It is noteworthy that the total weight of infected minnows included the biomass of all *P. ovata* in the body cavity. The condition factor (K) and the gonadosomatic index (GI) were calculated using the equations: $10^6 \times W/L_T^b$, where b is the slope of a regression of $\log_{10}(W)$ on $\log_{10}(L_T)$ of all tested males (Bolger & Connolly, 1989) and $W_G/W \times 100$ (Erickson et al., 1985), respectively. (I)-(VI)

2.4.6 Hematocrit and sperm quality analyses in infected and non-infected male minnows

In addition to the aforementioned traits, the hematocrit and the sperm motility of the males were also measured in 2012. During dissection, blood of every male minnow was collected from sinus venosus with a heparinized capillary tube (length: 32 mm, inner diameter: 0.5-0.6 mm). Because of the small blood volume, replicates from a single individual were impossible, and the entire blood volume was used in subsequent hematocrit determination. For hematocrit determination, capillary tubes were centrifuged at 11000 RPM for five minutes, and the hematocrit was analyzed using a microcapillary tube reader.

Sperm were obtained directly from the male gonads by pulverizing them in a 0.9 % NaCl solution. Sperm motility was measured using computer-assisted sperm analysis software (Integrated Semen Analysis System, ISAS v1: Proiser, Valencia, Spain) with a B/W CCD camera (capture rate 60 frames/s) and a negative phase contrast microscope (100 x magnification). Sperm motility analyses were performed by adding 0.1 µl of sperm dilution to Leja® 2-chamber (chamber height 20 µm, volume 6 µl) microscope slides (Leja, Nieuw-Vennep, The

Netherlands) and by activating sperm with 3 µl of Brook Kuusoja water. Sperm motility parameters were measured 10 s and 30 s after activation (three replicate measurements per male). Measured sperm quality parameters were: 1. straight line velocity (VSL); 2. curvilinear velocity (VCL); 3. average path velocity (VAP); 4 straightness of the swimming trajectory (STR); 5. linearity of the swimming trajectory (LIN); 6. amplitude of lateral head displacement (ALH); 7. beat cross frequency (BCF); 8. wobble (WOB); 9. the percentage of static (immobile) and 10. rapid sperm cells. Sperm viability was calculated as 1 – percentage of static cells in 30 s, while sperm velocity was represented by VAP in 30 s.

2.5 ETHICS OF THE STUDIES

The experiments and procedures were performed according to the license of the Finnish Animal Experimental Board (ESLH-2008-03722/Ym23) and (ESAVI/1906/04.10.03/2012).

3 Results and discussion

3.1 LIFE HISTORY AND INFECTION ECOLOGY OF *P. OVATA* IN MINNOWS

3.1.1 Life history of *P. ovata* in minnows (I)

Based on the prevalence and body size data (Figure 3, 5 and 6 in article I) and proportion of larval SBD *P. ovata* (Figure 4 in article I), *P. ovata* in minnows in Eastern Finland has a regular annual life cycle, as described earlier in other populations (Molnár, 1966; Moravec, 1986). I found that new generation *P. ovata* infect the minnows and settle to the swim bladder mainly in August. Before October, female *P. ovata* emigrate to the body cavity, where they grow and reach their full size by the end of next June, and evacuate from the body cavity of minnows in July or the beginning of August. Based on the delayed fresh infection and settling of larval *P. ovata* in the swim bladder, and evacuation of gravid *P. ovata*, the life cycle dynamics reported for *P. ovata* from this study is generally delayed by over one month compared to what has been reported for this parasite species in Hungary (Molnár, 1966). I suggest that the colder climate in Finland results in slower development of BCD *P. ovata* in minnows before evacuation, and consequently postpones the event of annual cycle in this minnow population.

In addition, the yearly evacuation period of *P. ovata* from minnows may not be as regular as in those populations parasitizing bream and roach in Hungary, where "*P. ovata* runs a regular yearly life-cycle adjusted even to period of days" (Molnár, 1966), according to the varied prevalence of BCD *P. ovata* in summer between 2010 and 2011 (Figure 5 in article I) and their variant body size in July and August (Figure 6 in article I). Interestingly, my observation of lab-housed minnows in June and July 2011 showed that the evacuation of BCD *P. ovata* in this minnow population occurred several times throughout July, which was a longer time period than in a few

days as previously described. I suggest that less stable temperatures in late spring and early summer in Finland may be the reason for the more variant evacuation time in this yearly cycle.

3.1.2 Infection ecology of *P. ovata* in minnows (I)

Over the whole field survey, the monthly prevalence of SBD *P. ovata* infection varied between 20% and 70% (Figure 3 in article I). The mean monthly prevalence of SBD *P. ovata* infection in minnows in our field survey was 37.8 ± 15.1%. The mean monthly prevalence of SBD *P. ovata* among female, male and immature minnows was 44.4 ± 19.8%, 32.5 ± 13.5% and 33.6 ± 20.9%, respectively. The monthly prevalence of SBD *P. ovata* in female minnows was significantly higher than in males, while the monthly prevalence between mature and immature minnows was not significantly different (Figure 3 in article I). On the other hand, the mean intensity of SBD *P. ovata* infection among all infected minnows in our field survey was 2.0 ± 1.5 (n = 387), while the mean intensity of these worms among female, male and immature infected minnows was 1.9 ± 1.4 (n = 130), 1.7 ± 1.0 (n = 47) and 2.1 ± 1.6 (n = 210), respectively (Figure 3 in article I). In addition, differences in intensity of SBD *P. ovata* infections between female and male minnows, as well as between mature and immature fish, were both not significant (Figure 3 in article I).

The monthly prevalence of BCD *P. ovata* in our field survey was always less than 14% (May 2011; Figure 5 in article I). The mean monthly prevalence of BCD *P. ovata* in minnows of our field survey was 6.7 ± 3.9%, while the mean prevalence among female, male and immature minnows was 10.9 ± 6.8%, 7.1 ± 10.1% and 12.7 ± 26.6%, respectively. The mean monthly prevalence of BCD *P. ovata* between female and male minnows, as well as between mature and immature fish, was not significantly different. The mean intensity of BCD *P. ovata* in all infected minnows in our field survey was 1.4 ± 0.8 (n = 68); among female, male, and immature minnows the mean intensity values were 1.6 ± 0.9 (n = 31), 1.6 ± 0.7 (n = 12) and 1.2 ± 0.7 (n = 25), respectively. The mean intensity of BCD *P. ovata* infection

between female and male minnows as well as between mature and immature fish was not significantly different (Figure 5 in article I).

Since the transmission of *P. ovata* relies on minnows preying on the infected intermediate hosts, it is likely that larger minnows may feed on more intermediate hosts of *P. ovata*. In our field collected minnows, females have a higher prevalence but a similar intensity of SBD *P. ovata* when compared to males. Due to the fact that many females were larger than males in our field collected minnows, it is reasonable that female minnows on average fed on more of the intermediate hosts of *P. ovata* than males did in our field survey, thus resulting in a higher prevalence of SBD *P. ovata* in female minnows. It is also likely that female minnows may naturally spend more time feeding than males do to maintain the energetic requirements of the eggs, and thus females are more likely to be infected by SBD *P. ovata* through feeding on more intermediate hosts of *P. ovata*. Although female minnows reported a higher prevalence of SBD *P. ovata*, there was no significant difference in the prevalence and intensity of BCD *P. ovata* among female and male minnows.

3.2 INFLUENCE OF *P. OVATA* INFECTION ON HOST FITNESS IN MINNOWS

3.2.1 Influence of *P. ovata* infection on body condition in minnows (I), (IV) & (VI)

According to my results in article (I), the intensity and the summed total length of BCD parasites was not associated with the condition factor of minnows (Table 2). This result is supported by the non-significant association between condition factor and abundance of BCD *P. ovata* in male minnows from article (VI). However, a significant positive association between condition factor and abundance of BCD *P. ovata* in male minnows was detected in article (IV). Intensity of BCD *P. ovata* has also been found to be positively correlated with condition factor among *G. lozanoi* (Saraiva et al., 2008), which suggests that

higher parasite intensity can be expected in fish that have fed on a larger number of copepods (intermediate hosts of *P. ovata*).

Table 2. *General linear model statistics for the association between fitness-related factors of minnows (total length (L_T), condition factor (K) and gonadosomatic index (GI), dependent variables), intensity, and sum length of body cavity dwelling (BCD) Philometra ovata (covariates) in minnows, with fish sex and collecting month as fixed factors.*

Fitness-related factor	Source	Type III SS	df	MS	*F*	*P*
L_T	Intensity of BCD *P. ovata*	0.002	1	0.002	0.000	0.995
	Sex	1766.718	2	883.359	18.839	< 0.001
	Month	1274.503	11	115.864	2.471	0.014
	Error	2485.167	53	46.890		
	Collected total	6207.529	67			
L_T	Sum length of BCD *P. ovata*	72.111	1	72.111	1.584	0.214
	Sex	1569.058	2	784.529	17.231	< 0.001
	Month	1026.630	11	93.330	2.050	0.041
	Error	2413.058	53	45.529		
	Collected total	6207.529	67			
K	Intensity of BCD *P. ovata*	0.000	1	0.000	0.194	0.661
	Sex	0.003	2	0.002	1.493	0.234
	Month	0.020	11	0.002	1.569	0.135
	Error	0.061	53	0.001		
	Collected total	0.094	67			
K	Sum length of BCD *P. ovata*	0.000	1	0.000	0.214	0.616
	Sex	0.003	2	0.002	1.466	0.240
	Month	0.020	11	0.002	1.561	0.138
	Error	0.061	53	0.001		
	Collected total	0.094	67			
GI	Intensity of BCD *P. ovata*	0.002	1	0.002	2.120	0.151
	Sex	0.052	2	0.026	28.448	< 0.001
	Month	0.028	11	0.003	2.821	0.006
	Error	0.049	53	0.001		
	Collected total	0.126	67			
GI	Sum length of BCD *P. ovata*	0.003	1	0.003	3.055	0.086
	Sex	0.054	2	0.027	29.849	< 0.001
	Month	0.031	11	0.003	3.145	0.002
	Error	0.048	53	0.001		
	Collected total	0.126	67			

Non-significant interactions in each analysis (P = 0.4, P = 0.3, P = 0.09, P = 0.7, P = 0.08, P = 0.1, respectively) were excluded from the table.

Although the impairment of BCD *P. ovata* on body condition has not been found, BCD *P. ovata* may still cause other physical damages in host minnows. During the evacuating period, my observation in article (I) revealed that BCD *P. ovata* may

potentially increase the mortality of infected minnows. Physical damages, including obvious skin necrosis around the anus, intensive adhesion among mesentery and organ membranes in the posterior body cavity, a large area with bruises under the body surface in the posterior abdomen and around the anus, as well as bruises in the abdominal body muscles near the anus and in the basal muscles of the anal fin, were found externally and internally in these dead minnows, and are highly likely due to the evacuation of BCD *P. ovata*.

Taken together, my results indicate that, contrary to my original expectations, infection of BCD *P. ovata* may not impair the body condition of host minnows, but may still potentially increase mortality and cause physical damages during the evacuation period.

3.2.2 Influence of BCD *P. ovata* infection on gonad size and sperm quality in minnows (I), (IV) and (VI)

The intensity and the summed total length of BCD *P. ovata* was not associated with relative gonad size of the minnows (Table 2), which is also in agreement with the results from male minnows presented in article (IV) and (VI). In addition, the prevalence of BCD *P. ovata* was positively correlated with relative gonad size in *G. lozanoi* (Saraiva et al., 2008). These results suggest that higher prevalence of BCD *P. ovata* can be expected in larger (older) fish that have fed on a larger number of copepods that serve as intermediate hosts of *P. ovata*. If relative gonad size is a reliable indicator of reproductive fitness, the costs of BCD *P. ovata* on male minnows may be modest and compensable.

In addition, BCD *P. ovata* infection was not associated with male sperm quality. In previous studies in Arctic charr (*Salvelinus alpinus*), testes mass increased but the spermatocrit decreased with increasing parasite intensities (Figenschou et al., 2013), which suggests that parasite infections can affect male fertility. However, I found that neither sperm viability (i.e. percentage of mobile sperm in 30 s) nor sperm swimming velocity (VSP in 30 s) were affected by the abundance of BCD *P.*

ovata in male minnows (Table 3). Despite the relatively large size of BCD *P. ovata* and the fact that they dwell in the host's body cavity, they do not appear to impair the fertility and reproductive fitness of host minnows.

Table 3. *General linear model statistics for the association between each indices of sperm quality (i.e. sperm viability (1 – percentage of static sperm in 30 s) and sperm velocity (VAP of sperm in 30 s); dependent variables) and fitness-related traits ($PC_{Quality}$, covariate) in male minnows. Abundance of* P. ovata *was included as a fixed factor in the models.*

Index	Source	Type III SS	df	Mean square	*F*	*P*
Sperm viability	P. ovata abundance	1104.874	3	368.291	0.909	0.445
	$PC_{Quality}$	1778.688	1	1778.688	4.391	**0.042**
	Error	16608.040	41	405.075		
	Corrected total	19486.457	45			
Sperm velocity	P. ovata abundance	62.041	3	20.680	1.255	0.302
	$PC_{Quality}$	87.905	1	87.905	5.335	**0.026**
	Error	675.537	41	16.477		
	Corrected total	816.698	45			

The interactions between abundance of P. ovata *and the principal component in each analysis were all non-significant (P = 0.6) and thus were excluded from this final table.*

3.2.3 Influence of *P. ovata* infection on hematocrit in minnows

According to my results, the quality of the hematocrit was not correlated with body quality (i.e. total body length, condition factor and gonadosomatic index), but was significantly negatively correlated with the abundance of BCD *P. ovata* in male minnows (Table 4, Figure 7). On the other hand, hematocrit was not associated with $PC_{Darkness}$ and $PC_{Redness\_Tone}$, but was negatively correlated with $PC_{Redness\_Light}$ in minnows (Table 4, Figure 7). Taken together, these results indicate that the hematocrit is negatively influenced by the infection of BCD *P. ovata* in male minnows, and is also associated with the paleness of abdominal redness in male ornaments. This implies that BCD *P. ovata* may have a negative impact on the aerobic swimming performance of minnows. Moreover, although the diet of *P. ovata* has never been reported directly, this result may also indicate that *P. ovata* feed on blood or tissues in the body cavity of host fish, as indirectly suggested by Saraiva et al. (2008).

Table 4. *General linear model statistics for the association between hematocrit (dependent variable) and fitness-related traits ($PC_{Quality}$) and two male ornaments: lateral darkness ($PC_{Darkness}$) and abdominal redness ($PC_{Redness\_Tone}$ and $PC_{Redness\_Light}$) (covariates) in male minnows. Abundance of* P. ovata *was included as a fixed factor in the models.*

Source	Type III SS	df	Mean square	*F*	*P*
P. ovata abundance	0.158	4	0.039	2.811	**0.039**
$PC_{Quality}$	0.024	1	0.024	1.739	0.195
Error	0.533	38	0.014		
Corrected total	0.707	43			
P. ovata abundance	0.183	4	0.046	3.488	**0.021**
$PC_{Darkness}$	0.028	1	0.028	2.149	0.155
Error	0.327	5	0.013		
Corrected total	0.534	30			
P. ovata abundance	0.201	4	0.050	4.023	**0.012**
$PC_{Redness\_Tone}$	0.003	1	0.003	0.231	0.635
$PC_{Redness\_Light}$	0.056	1	0.056	4.476	**0.045**
Error	0.300	24	0.012		
Corrected total	0.534	30			

The interactions between the abundance of P. ovata *and principal components in the analyses were all non-significant (P = 0.1) and thus were excluded from this table.*

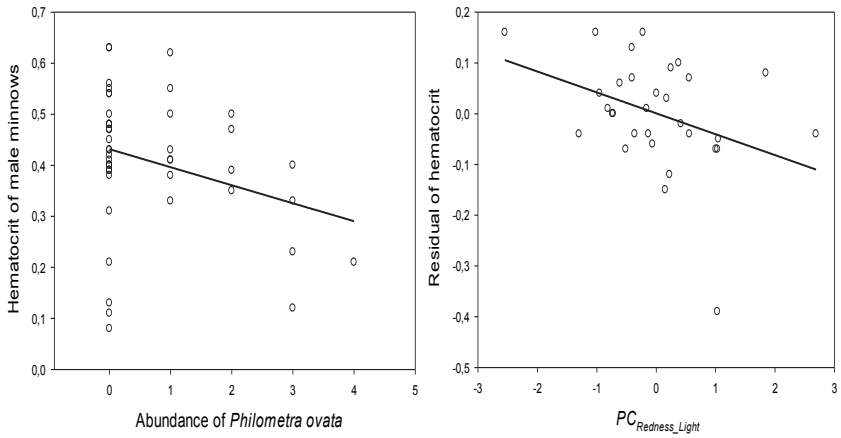

Figure 7. *The association between hematocrit and abundance of BCD* P. ovata *and lightness of abdominal redness in male minnows (*Phoxinus phoxinus*). Note that a higher score in* $PC_{Redness\_Light}$ *indicates paler abdominal colouration.*

3.3 NON-INVASIVE DIAGNOSIS OF *P. OVATA* IN MINNOWS

3.3.1 Performance and reliability of non-invasive diagnosis (II)

My results indicate that the present non-invasive visual diagnosing method for the detection of the infection of *P. ovata*

is practical and highly reliable (>95% reliability, Table 5). The slightly lower reliability in the non-anesthetized group was probably due to the larger size and thus thicker abdominal muscular layer and larger body cavity of the fish in this group, as well as the difficulty of working with non-anesthetized, continuously moving fish.

The negative predictive power (the proportion of truly non-infected individuals over predicted non-infected individuals) of this non-invasive method was 95 % or higher (Table 5), which indicates that the "uninfected" diagnosis is highly dependable. In addition, the positive predictive power (the proportion of truly infected individuals over predicted infected individuals) of the diagnostic method was always higher than 96% (Table 5), which indicates that the accuracy of the diagnosis is high.

Table 5. *Distribution of results of detecting* P. ovata *infection from non-invasive diagnosis and dissection*

			Diagnosis of *P. ovata* infection in the examination		
			Infected	**Uninfected**	**Sum**
Detection of *P. ovata* infection in the dissection	**Anesthetized minnows**	**Infected**	15 Sen: 71.43% Ppp: 100.00%	6	21 *5.60%*
		Uninfected	0	354 Spe: 100.00% Npp: 98.33%	354
		Sum	15 *4.00%*	360	375
			Infected	**Uninfected**	**Sum**
	Non-anesthetized minnows	**Infected**	32 Sen: 65.30% Ppp: 96.97%	17	49 *13.65%*
		Uninfected	1	309 Spe: 99.68% Npp: 94.79%	310
		Sum	33 *9.19%*	326	359

The italicized numbers indicate the prevalence of body cavity dwelling P. ovata *assessed by non-invasive diagnosis (middle & bottom row) or by dissection (last column) in anesthetized or non-anesthetized groups. Npp: negative predictive power; Ppp: positive predictive power; Sen: sensitivity; Spe: specificity.*

3.3.2 Repeatability of non-invasive diagnosis (II)

In the repeatability test, the experienced and inexperienced observers' diagnostic results were found to be highly consistent (Table 4 in article II). The reliability of the experienced and inexperienced observers' diagnoses was 91.8% and 91.4%, respectively. The high reliability of the non-invasive diagnostic technique coupled with between-observer repeatability indicates that the non-invasive diagnosis described herein is a practical method for use in field conditions or other situations where anesthetization of the fish is not possible and/or rapid identification of BCD *P. ovata* infection status is needed. The non-invasive diagnosis method developed for this study was found to be a useful tool for reliable discrimination of infected and uninfected minnows by BCD *P. ovata* for later experimental studies. (IV, VI)

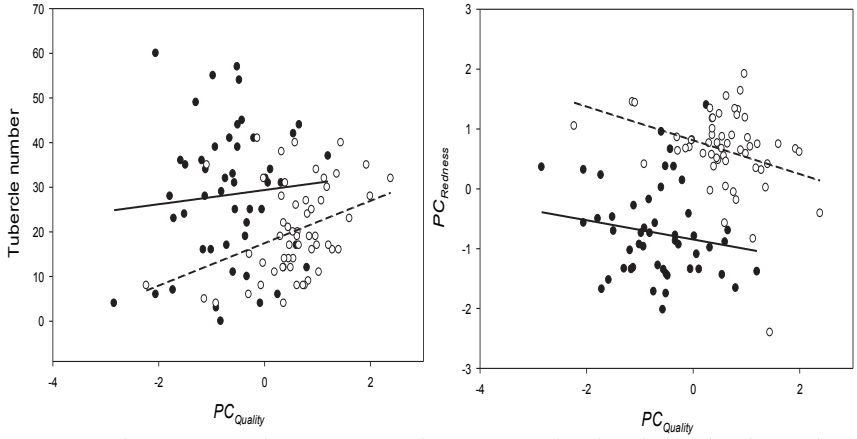

Figure 8. *The association between two male ornaments (i.e. breeding tubercle number and abdominal redness) and body quality in male minnows collected from the Brook Kuusoja population (filled circles, solid line) and the Brook Uuronpuro population (open circles, dashed line). Note that a higher score of* $PC_{Redness}$ *indicate paler abdominal redness.*

3.4 SEXUAL SELECTION IN MINNOWS

3.4.1 Male ornamentation as honest signals in minnows (III), (V) & (VI)

In the present study, male ornaments (i.e. breeding tubercle number and abdominal redness) were found to be positively correlated with male body quality (hematocrit, Table 4 and Figure 7; mainly body condition and relative gonad size, Figure 8; article III, V & VI). However, although both sperm viability and velocity were positively correlated with relative gonad size, but negatively with condition factor and body length (Table 3), they were not correlated with sexual ornaments in male minnows (Table 6, Figure 9). Thus, despite the positive association with relative gonad size, these two ornament traits may not be directly linked to the fertilization ability of males. In previous minnow studies, breeding tubercle number has been shown to be associated with body length, one of the fitness-related traits of male minnows (Müller & Ward, 1995). However, a more recent study found no association between these traits (Jacob et al., 2009). Previous studies of male minnows have shown that abdominal redness may signal several aspects of male quality, such as genetic heterozygosity and parasite resistance ability (Müller & Ward, 1995). In addition, higher breeding tubercle number and more intense belly colouration have been shown to signal the dominance status or courting activity of the males (Jacob et al., 2009; Kekäläinen et al., 2010b). Taken together, these ornaments may thus honestly signal body quality of male minnows under sexual selection.

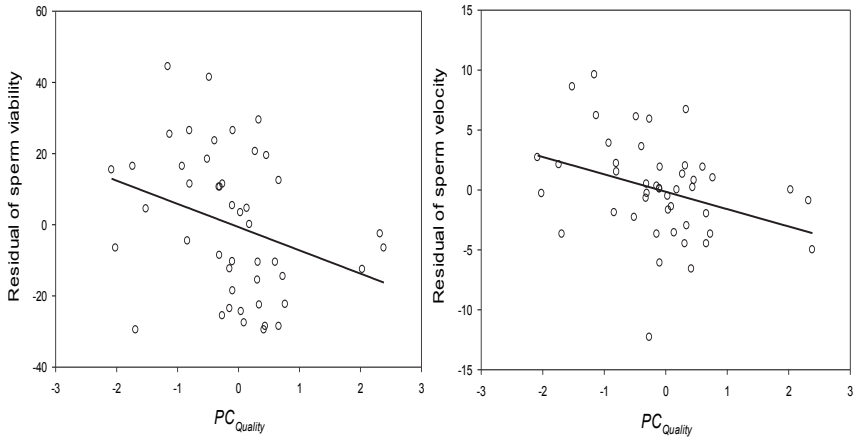

Figure 9. *The association between male body quality (*$PC_{Quality}$*) and residuals of sperm viability and sperm velocity respectively from abundance of BCD* P. ovata *in male minnows. Note that a higher score of* $PC_{Quality}$ *indicates larger body size and better body condition but relatively smaller gonads.*

Table 6. *General linear model statistics for the association between each index of sperm quality (i.e. sperm viability (1 – percentage of static sperm in 30 s) and sperm velocity (VAP of sperm in 30 s), dependent variable) and male ornaments (lateral darkness (*$PC_{Darkness}$*) and abdominal redness (*$PC_{Redness\_Tone}$ *and* $PC_{Redness\_Light}$*), covariates) in male minnows. Abundance of BCD* P. ovata *was used as a fixed factor in the models.*

Index	Source	Type III SS	df	Mean square	*F*	*P*
Sperm viability	*P. ovata* abundance	1070.035	3	356.678	0.734	0.542
	$PC_{Darkness}$	194.498	1	194.498	0.400	0.533
	Error	11177.991	2	486.000		
	Corrected total	12368.107	27			
Sperm velocity	*P. ovata* abundance	45.239	3	15.080	1.111	0.365
	$PC_{Darkness}$	12.554	1	12.554	0.925	0.346
	Error	312.186	23	13.573		
	Corrected total	379.957	27			
Sperm viability	*P. ovata* abundance	865.388	3	288.463	0.643	0.596
	$PC_{Redness\_Tone}$	1483.658	1	1483.658	3.306	0.083
	$PC_{Redness\_Light}$	234.396	1	234.396	0.522	0.477
	Error	9874.154	22	448.825		
	Corrected total	12368.107	27			
Sperm velocity	*P. ovata* abundance	58.928	3	19.643	1.440	0.258
	$PC_{Redness\_Tone}$	13.142	1	13.142	0.964	0.337
	$PC_{Redness\_Light}$	18.819	1	18.819	1.380	0.253
	Error	300.036	22	13.638		
	Corrected total	379.957	27			

The interactions between abundance of P. ovata *and principal components in the analyses were all non-significant (P = 0.1) and thus were excluded from the table.*

Furthermore, in addition to the association with body condition, my study showed that breeding tubercle number and abdominal redness of male minnows were also positively associated with swimming performance (Figure 10; article V and VI). Such correlation between male colour ornaments and locomotor performance has rarely, if ever, been demonstrated in other taxa. Moreover, this finding was detected in two independent minnow populations, which has also been rarely demonstrated (but see Nicoletto & Kodric-Brown, 1999). Nevertheless, I found that swimming performance was associated with body quality in both of my study populations (Figure 3 in article V & VI). Better body condition may be positively associated with the mass of body muscles, which may produce more power output during burst swimming. For example, body condition has been shown to positively affect swimming performance in Atlantic cod *Gadus morhua* (Martínez et al., 2003). However, the correlation between body condition and locomotor performance has not been found in other taxa, such as in lizards (McElroy et al., 2007). It is possible that relatively bigger gonads may produce more androgen and thus increase the mass of body musculature, heart mass, or muscle strength in fish (Thorarensen et al., 1996). However, in previous studies on locomotor performance of lizards, links between the androgen level, gonad size, and the sprint speed have remained unclear (Husak et al., 2006).

Conclusively, my study shows that male ornamentation (i.e. breeding tubercles and abdominal redness) in minnows act as honest signals for several aspects of male quality, such as swimming performance, body condition and gonad size, and should be highly informative to both males and females in sexual selection.

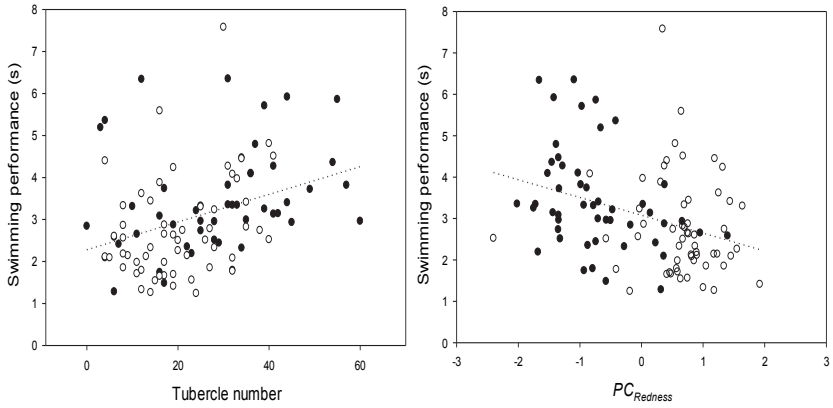

Figure 10. *The association between swimming performance and two male ornaments (i.e. breeding tubercle number and abdominal redness) in male minnows (*Phoxinus phoxinus*) collected from the Brook Kuusoja population (filled circles) and the Brook Uuronpuro population (open circles). Due to the insignificant difference of population means in GLM analysis, data from both minnow populations were combined. Note that a higher score of* $PC_{Redness}$ *indicate paler abdominal redness.*

3.4.2 Female mate choice in minnows (III)

When olfactorial-based female preference was tested in familiar males, I found that females preferred odours of males with a bright red (i.e. highly saturated) belly colouration. However, no significant preference for belly colouration was observed when unfamiliar males were tested. In addition, the swimming activity of females was significantly lower in the presence of unfamiliar male odours than when females were exposed to the odours of familiar males (article III). Accordingly, results of the present study indicated that female minnows may not be able to discriminate among males based only on olfactory cues. It seems likely that male odours may be uninformative to female minnows in terms of male body quality and are mainly used for individual recognition. Females probably learned the association between male ornamentation and odour during the pre-experimental contact period and later used this information when only odour signals were present. Nevertheless, previous studies have demonstrated that female minnows have a clear behavioural preference towards colourful males when only visual cues are present (Kekäläinen et al., 2010b), and they

usually release their eggs only when at least one large (dominant) male is present (Müller & Ward, 1995; Jacob et al., 2009). In natural conditions, it is likely that the female minnows determine their mating decisions by using multiple cues of males, such as breeding tubercle number and abdominal redness, or may use different cues in different situations or mating seasons.

3.5 *PHILOMETRA OVATA* MEDIATED SEXUAL SELECTION IN MINNOWS

3.5.1 The effect of BCD *P. ovata* infection on male ornamentation (III) & (VI)

In the field, the saturation of abdominal redness in male minnows was negatively associated with the abundance of BCD *P. ovata* (Figure 11; article III), which supports the predictions of parasite-mediated sexual selection models (e.g. Müller & Ward, 1995). The male ornamentation in minnows is shown as honest signalling through the association between parasite abundance and abdominal redness, and such an association may also imply the potential harmfulness of BCD *P. ovata* on host minnows. However, the effect of BCD *P. ovata* on male ornamentation was not detected in article VI, which suggested that infection of BCD *P. ovata* might not necessarily reduce male ornamental expression in minnows. The lack of association between body quality and abundance of BCD *P. ovata* in article VI implied that infection of BCD *P. ovata* may not be consistently harmful to the host fitness as was presumed in article III. When taking the preference of females on more ornamented males into account, it appears that female minnows prefer more ornamented males in mate choice because these ornamental traits signal male condition and dominance status honestly, and may also be regarded as indicator of heritable parasite resistance for offspring (Hamilton & Zuk, 1982).

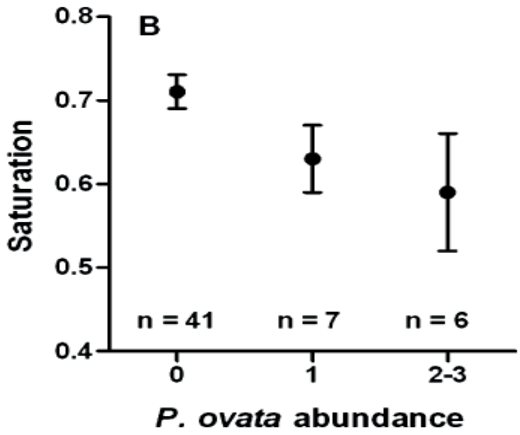

Figure 11. *Saturation values of belly colouration (mean ± SE) for different* P. ovata *abundance classes in male minnows collected from the wild.*

3.5.2 Influence of BCD *P. ovata* infection on female mate choice (IV)

In spite of being the first empirical study testing the effect of parasite infection on male odour attractiveness in fish (see review by Beltran-Bech & Richard, 2014), my data indicate that female minnows do not show a behavioural preference for the odours of infected and non-infected unfamiliar males when presented with a choice (Figure 12). Previous studies in rodents have shown that females can discriminate the odours of parasitized males and are more attracted to the odours of males that are unparasitized (e.g. Penn & Potts, 1998a; Kavaliers et al., 2003; Beltran-Bech & Richard, 2014). It has also been demonstrated that female rodents prefer the odours of unparasitized males because the odours of infected males lose their attractiveness. This is probably due to the change in metabolic by-products or androgen-dependent odorants, rather than the odours of parasitized males becoming repellant for females (reviewed by Penn & Potts, 1998a; Beltran-Bech & Richard, 2014). Studies in grain beetles *Tenebrio molitor* also demonstrated that females prefer the odours produced by healthy males over the males infected with the cestode *Hymenolepis diminuta* (Worden et al., 2000).

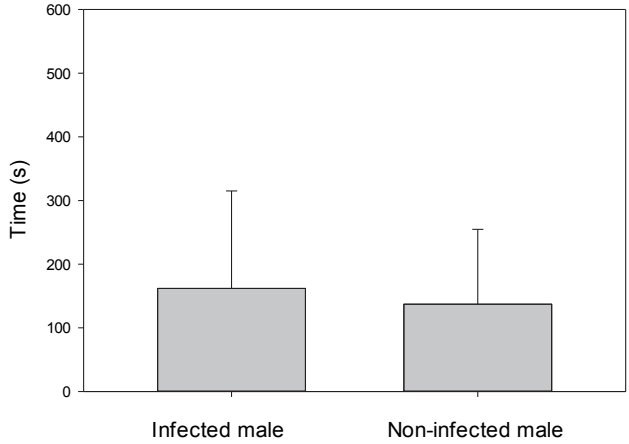

Figure 12. *Female preference time towards the odours of infected and non-infected male minnows (*Phoxinus phoxinus*).*

I propose two potential reasons to explain why I failed to replicate the outcomes of these earlier studies. First, it is possible that in minnows male odours do not provide information of infection status. Indeed, it is possible that male odours in minnows may be uninformative in terms of male body quality to females, and are mainly used in individual recognition (see article III). Like the experiment in article IV, in which males and females were completely unfamiliar to one another, females were unable to learn the association between other indicators (e.g. visual or behavioural: Kekäläinen et al., 2010b; article II; article III) of male infection status and individual body odours. As a consequence, females showed no behavioural preference between odours of infected and non-infected males in my study on minnows.

Alternatively, it is possible that the difference between odours of infected and non-infected males may be so minor that female minnows cannot distinguish between them. If female minnows are, however, able to distinguish odours of infected males and are more attracted by odours from non-infected males, as is the case for rodents (Penn & Potts, 1998a), then there might be a correlation between infection and fitness-related traits, i.e. relative gonad size and body condition. This is then

likely to be associated with odour-correlated androgen levels and metabolic by-products that are the main determinants of the attractiveness of male odours in female choice. However, body condition and relative gonad size were similar among infected and non-infected male minnows in my behavioural experiments (see articles IV and I), which implies that odour-correlated androgen levels or metabolic by-products in males are probably not affected by an infection with BCD *P. ovata*. In the end, changes in the odours of male minnows due to the infection of BCD *P. ovata* may be too minor to be distinguished by females.

3.5.3 Influence of BCD *P. ovata* infection on male-male competition (IV)

Despite the potential harmfulness of BCD *P. ovata* on its minnow hosts, my results demonstrated that infected males had similar behavioural sexual competitiveness (dominance status and courtship performance, Figure 13) as non-infected males (article IV). Although it is presumed that parasite infection should impair male competitiveness in intra-sexual selection, evidence for such effects has been contradictory (e.g. Schall & Dearing, 1987; Maksimowich & Mathis, 2000; Pelabon et al., 2005), and many studies, including the present one, have not found such effects (Berdoy et al., 1995; Hamilton & Poulin, 1995; Thomas et al., 1995; Barber, 2002).

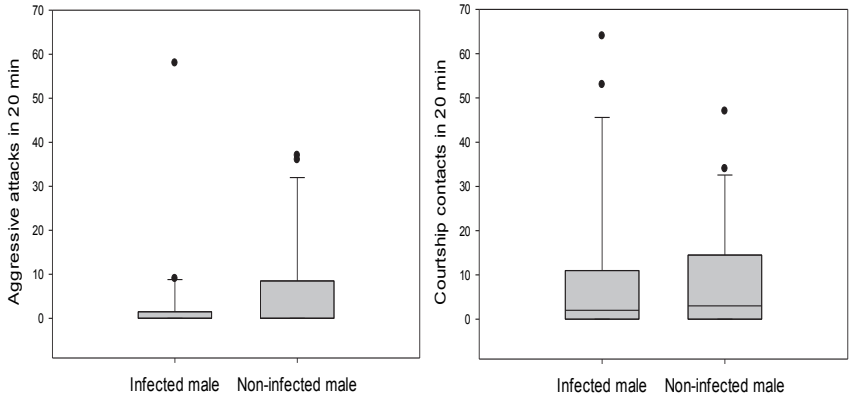

Figure 13. *Number of aggressive attacks and courtship contacts presented by infected and non-infected male minnows.*

In light of the parasite-induced host manipulation hypothesis (Barber et al., 2000), the non-significant effect of *P. ovata* on behavioural sexual competitiveness of male minnows may not necessarily be an unexpected finding. For trophically transmitted parasites, host manipulation and negative effects on host performance are expected when a parasite must move to a new host in its life cycle (e.g., Sprengel & Lüchtenberg, 1991; Barber et al., 2004; Médoc & Beisel, 2010). However, such negative effects on host behavioural performance tend to be weak or completely absent when a parasite has already successfully been transmitted to its definitive host (e.g. Umberger et al., 2013). This may be the case when BCD *P. ovata* is parasitizing the minnows. Moreover, parasite manipulation may specifically affect behavioural traits of the host which selectively benefits the parasite (Berdoy et al., 1995), and the impairment of sexual competitiveness (or odour attractiveness) of the hosts may not be one of them. Thus, I conclude that the impairment of behavioural sexual competitiveness by infection of BCD *P. ovata* in male minnows is unlikely.

3.5.4 Influence of BCD *P. ovata* infection on swimming performance of males (VI)

Contrary to my expectations, swimming performance of male minnows was not negatively affected by the infection of BCD *P. ovata* (Table 7), although it was correlated with male ornaments and male body quality as described in article V. Based on what is known about biological interactions between BCD *P. ovata* and minnow hosts, I propose several potential explanations for this seemingly counter-intuitive finding.

Table 7. *General linear model statistics for the association between swimming performance (dependent variable), abundance of* P. ovata *(covariate) and two male ornaments (covariates): lateral darkness (*$PC_{Darkness}$*) and abdominal redness (*$PC_{Redness\_Hue}$ *and* $PC_{Redness\_Saturation}$*) in male minnows (*Phoxinus phoxinus*).*

Source	Type III SS	df	Mean square	*F*	*P*
$PC_{Darkness}$	18.660	1	18.660	14.408	<0.001
P. ovata abundance	1.490	1	1.490	1.151	0.287
Error	82.888	64	1.295		
Corrected total	103.094	66			
$PC_{Redness\_Hue}$	14.935	1	14.935	11.035	0.001
P. ovata abundance	0.329	1	0.329	0.243	0.624
Error	86.614	64	1.353		
Corrected total	103.094	66			
$PC_{Redness\_Saturation}$	5.739	1	5.739	3.834	0.055
P. ovata abundance	1.779	1	1.779	1.188	0.280
Error	95.809	64	1.497		
Corrected total	103.094	66			
$PC_{Quality}$	9.940	1	9.940	6.944	0.011
P. ovata abundance	2.409	1	2.409	1.683	0.199
Error	91.609	64	1.431		
Corrected total	103.094	66			

The interactions between abundance of P. ovata *and each of the three principal components (*$PC_{Darkness}$, $PC_{Redness\_Hue}$ *and* $PC_{Redness\_Saturation}$*) and between male quality and abundance of* P. ovata *in the analysis were non-significant (P = 0.1) and thus were excluded from the table.*

Firstly, in light of the parasite-induced host manipulation hypothesis, the non-significant effect of *P. ovata* on swimming performance may in fact be fully expected. For trophically transmitted parasites, host manipulation and negative effects on host performance should be less severe or completely absent when parasites have already successfully parasitized their definitive hosts. For example, the fin-muscle embedding nematode *Philometroides paralichthydis*, which has been shown to influence the swimming ability of its definitive host (southern flounder *Paralichthys lethostigma*), merely impaired the velocity of swimming performance of small host individuals (Umberger et al., 2013). Since minnows are the definitive host of *P. ovata*, I would expect that the impairment of minnow swimming performance may be unnecessary and even detrimental for the BCD *P. ovata,* because it may increase the susceptibility of the host to predators and thus reduce the parasite's own fitness.

Secondly, even if parasite-induced fitness costs may result from non-adaptive pathological side-effects of *P. ovata* infections, it is not guaranteed that parasite-induced effects

would impair the swimming performance of host minnows. It has been observed that BCD *P. ovata* is commonly located in the gonads (Moravec, 1977), where they cause parasitic castration (Moravec, 2006), chronic inflammation, and necrosis of membranes in host fish (Saraiva et al., 2008). When dissecting infected minnows in my studies (article I, IV and VI), I also frequently found adhesion among the mesentery and organ membranes, which most likely resulted from *P. ovata*-induced inflammation and necrosis. To my knowledge, impacts of these non-muscular damages to locomotor performance have never been investigated. Although this damage can potentially be harmful for minnows, it is possible that this internal, organ-specific damage may not impair swimming performance, which mainly relies on muscular function and strength. Moreover, I was unable to find any evidence for *P. ovata*-induced abdominal swelling, which has previously been recorded to severely impair the swimming ability of the infected host *G. lozanoi* (Saraiva et al., 2008). I have found no evidence that BCD *P. ovata* impaired the male body quality in the relevant studies (article I, IV and VI) linked to the burst swimming performance in minnows (article V). It is entirely possible that *P. ovata*-induced pathological damage in minnows is restricted to the internal organs, and that negative effects on host swimming performance are less likely to occur.

Thirdly, despite the existence of *P. ovata*-induced pathological damages, specific features of the life cycle of *P. ovata* can partly explain the non-significant effects on swimming performance. The fitness costs of host fish infected by *P. ovata* can be expected to increase with the body size of *P. ovata*. Since gravid *P. ovata* typically evacuate from the minnows in July in Eastern Finland (article I), the corresponding fitness cost should reach its maximum at the end of June, i.e. just before the evacuation of *P. ovata*. However, in the experiment in article VI, I collected male minnows in late May and early June, i.e. nearly one month prior to the evacuation period, and the mean size of the measured *P. ovata* (29.8 ± 11.1 mm) was much smaller than the observed size of BCD *P. ovata* during the evacuation period in Eastern Finland (39.3 ± 12.9 mm, article I). It is thus possible that the negative

effects of BCD *P. ovata* on minnow swimming performance is negligible and can be observed only by the evacuation period.

Finally, indirect histological evidence has shown that BCD *P. ovata* may feed on host blood (Saraiva et al., 2008). Similar feeding habits have also been documented in some other abdominal cavity dwelling *Philometra* spp., such as *P. lateolabracis* (Hesp et al., 2002). My results have shown that the abundance of BCD *P. ovata* is negatively associated with hematocrit in male minnows, thus a BCD *P. ovata* infection should decrease the circulation efficiency of O_2 as well as impair the aerobic swimming performance of infected minnows. However, since I was testing burst swimming performance (which is mostly anaerobic) in the present study, such negative effects of infection may have gone undetected.

4 Conclusions

This PhD thesis aims to provide a comprehensive perspective in parasite-mediated sexual selection by including not only the impact of BCD *P. ovata* on its hosts (III-IV), but a two-year survey of the life history and infection ecology of the parasite (I). Moreover, a highly precise and reliable non-invasive diagnostic method for BCD *P. ovata* infection was developed during the project, which enabled reliable behavioural examinations between infected and non-infected minnows (II).

Based on the results of my studies, I can conclude that *P. ovata* has a regular annual life cycle in minnows, as observed earlier in other host species (I). However, events in the annual life cycle of *P. ovata* in the present minnow population, such as the fresh infection of the new generation and evacuation from host minnows, are generally postponed more than one month when compared to what has been reported previously (I). In addition, the yearly evacuation period of *P. ovata* in minnows may not be as regular as those individuals parasitizing bream and roach, as shown in previous studies. Based on my observations, the evacuation of BCD *P. ovata* in minnows occurred several times in July, implying that the evacuation occurred over a period of nearly one month. The evacuation period reported in this study was longer than previously described.

However, against theoretical expectations, my results indicated that infection by BCD *P. ovata* may not be harmful to host minnows. Instead of a negative influence, either no association (I & VI) or a positive association (IV) was found between the intensity of the BCD *P. ovata* infection and either the condition factor or relative gonad size of minnows. Also, sperm quality did not differ between infected and non-infected male minnows. However, the blood hematocrit was negatively correlated with the abundance of BCD *P. ovata*. Thus, it remains

possible that BCD *P. ovata* may impair the aerobic swimming performance of minnows, as that this is supposedly affected by blood hematocrit.

Although the impairment of BCD *P. ovata* on host body condition has never been found, this parasite may still cause other internal and external physical damages to host minnows and potentially raise the mortality of infected hosts (I). Overall, my results indicate that, despite the relatively large size of the parasite, infection of BCD *P. ovata* may be harmful to the fitness of host minnows only through decreasing blood hematocrit, potentially increasing the mortality, and causing physical damages during the evacuation period. BCD *P. ovata* infection s do not appear to influence physiological factors such as the condition factor, relative gonad size, and sperm quality.

My study suggests that male sexual ornamentation (i.e. breeding tubercles and abdominal redness) in minnows acts as an honest signal of several aspects of male quality. In addition to the body size, genetic heterozygosity, parasite resistance ability, dominance status and courting activity, male ornaments in minnows signal swimming performance (V & VI), body condition (III, V & VI), and gonad size (V & VI), and are likely to be highly informative to both males and females in sexual selection.

In previous female mate choice studies, female minnows usually release their eggs only when at least one large (dominant) male is present. It has also been shown that females have a clear behavioural preference towards colourful males when only visual cues are present. However, when olfactorial-based female preference was tested, females preferred the odours of males with a bright red (i.e. highly saturated) belly colouration only when those males were familiar to females (III). In addition, the swimming activity of females was significantly lower in the presence of unfamiliar male odours than when females were exposed to the odours of familiar males (III). Accordingly, it seems likely that male odours in minnows may be uninformative to females in terms of male body quality and are mainly used for individual recognition. In natural conditions, it is, therefore, probable that female minnows

determine their mating decisions using multiple cues of males. Females are most likely to use visual or tactile cues, such as abdominal redness and breeding tubercles, but may use other cues in different situations or mating seasons, since these ornaments imply different aspects of male body quality.

Interestingly, when BCD *P. ovata* is prevalent in minnows, these hosts are influenced only in certain aspects of sexual selection. Firstly, the saturation of abdominal redness was negatively associated with the abundance of BCD *P. ovata* in male minnows (III), which has been shown in previous studies with other parasites, and is predicted by the model of parasite-mediated sexual selection. However, an effect of BCD *P. ovata* on male ornamentation was not detected in this study (VI). Accordingly, despite the different environmental conditions (i.e. in the field versus lab) between studies III and VI in measuring male ornamentation, infection with BCD *P. ovata* does not consistently reduce male ornamental expression in minnows. Thus, it could be implied that there is no inevitable harm of BCD *P. ovata* to the fitness of host minnows. This is also supported by the fact that neither the intensity nor abundance of BCD *P. ovata* was found to be negatively associated with condition factor or relative gonad size in minnows (I, IV & VI).

Secondly, my data indicate that, in contrast to my predictions, female minnows do not show a behavioural preference for the odours of infected or non-infected males when the males were unfamiliar to the females. Such a result is reasonable, since male odours in minnows may be uninformative to females in terms of male body quality (III). However, since the body condition and relative gonad size was similar in infected and non-infected male minnows (I, IV & VI), one can assume that, if the mechanism of odour difference between infected and non-infected males in fish is similar to that in rodents, odour-correlated androgen levels or metabolic by-products in male minnows may not be affected by an infection with BCD *P. ovata*. It is possible that the difference between the odours of infected and non-infected males may be so negligible

that female minnows either cannot distinguish them or simply ignore them.

Thirdly, my results demonstrate that infected males show an exhibit patterns of behavioural sexual competitiveness (dominance status and courtship performance) observed among non-infected males (IV), indicating that BCD *P. ovata* infections may not determine male success during intra-sexual selection. The swimming performance of male minnows was also not negatively affected by the infection with BCD *P. ovata* (VI). Based on the life history and infection ecology of *P. ovata* in minnows, however, potential BCD *P. ovata* induced pathological effects (I, IV & VI) on swimming performance may unnecessarily be expected. Furthermore, I showed that the abundance of BCD *P. ovata* is negatively associated with blood hematocrit in male minnows. This indicates that BCD *P. ovata* infections may decrease the circulation efficiency of O_2 and thus impair the aerobic swimming performance of infected minnows. However, since I was testing burst swimming performance (which is mostly anaerobic) in the present study, the effects of infection on O_2 circulation efficiency may have gone undetected.

Overall, my results indicate that despite the relatively large size of BCD *P. ovata*, its negative impacts on minnows may be relatively minor: BCD *P. ovata* may decrease the hematocrit in male minnows, but not impair other fitness-related traits of its hosts, such as body condition, relative gonad size, or sperm quality. The interaction of *P. ovata* and minnows provides an interesting but unexpected case, in which host minnows are only slightly impacted by the parasite regarding sexual ornamentation, female mate choice and male-male competition.

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Yi-Te Lai

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