Infection ecology of *Philometra ovata* (Nematoda: Philometridae) in a wild European minnow (*Phoxinus phoxinus*) population in Finland

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SUMMARY

Seasonal life cycle of body cavity dwelling (BCD) *Philometra ovata* (Nematoda: Philometridae) has been reported in southern and central European countries, but its swim bladder dwelling (SBD) stage and northern populations have remained unstudied. In this study, we investigated the seasonal life cycle and infection ecology of *P. ovata* in both swim bladder and body cavity in the European minnow (*Phoxinus phoxinus*) in Finland. The larval *P. ovata* infected the swim bladder of minnows mainly in August. Female SBD *P. ovata* emigrated to body cavity mostly in September, grew to their full size by the end of the next June, and evacuated from minnows in July. In addition, female SBD *P. ovata* retarding their development and staying in swim bladder were found commonly in minnows, thus the mean monthly prevalence (6·7 ± 3·9%) and mean intensity (1·4 ± 0·8) of BCD *P. ovata* was lower than that of SBD *P. ovata* (37·8 ± 15·1% and 2·0 ± 1·5, respectively). Finally, despite the large size of BCD individuals, infection of *P. ovata* did not impair body condition and relative gonad size of minnows, but increased the mortality and caused physical damages in their hosts during the evacuation period.

Key words: *Philometra ovata*, European minnow, *Phoxinus phoxinus*, body cavity, swim bladder, parasitic infection, prevalence, intensity.

INTRODUCTION

Philometra ovata (Zeder, 1803) (=*Philometra abdominalis* Nybelin, 1928) is a parasitic nematode dwelling in the body cavity of its definitive host, fish of genera *Gobio*, *Phoxinus* and *Leuciscus* (Molnár, 1967; Moravec, 1977*a*, 2004; Keskin, 1988; Innal and Keskin, 2005; Saraiva *et al*. 2008). This species, as other *Philometra* nematodes living in the temperate zone, has been noted to have a regular, yearly seasonal life cycle (Molnár, 1967; Moravec, 1977*a*). In the early summer, first stage larvae of *P. ovata* are eaten by the intermediate hosts, copepods, where larvae reach their infective stage (Moravec, 1977*b*). Infective larvae of *P. ovata* are trophically transmitted to the definitive fish host when the parasitized copepod is preyed on by the aforementioned cyprinid fish (Molnár, 1967; Moravec, 1977*a*, *b*). In the definitive host, juvenile *P. ovata* first penetrate the intestine wall of the host fish, then migrate and inhabit under the serose cover of the swim bladder, where both male and female larvae sexually mature and mate (Molnár, 1967; Moravec, 1977*a*). After mating, while males remain in the swim bladder, gravid females with eggs in their uterus migrate from the swim bladder to the body cavity of the fish host. Mated female *P. ovata* are able to continue growing to their final size (which can be more than 70 mm long) only in the body cavity of the host. Females attain a fully developed uterus with great numbers of free first-stage larvae by the end of the next spring or early summer. At a particular time of the year, such as from late May to the end of June in central Europe (Molnár, 1967), *P. ovata* females leave the body cavity of the host by penetrating the tissues around the anus of the host. The worms rupture immediately due to the hypotonic effect of the surrounding water and release the first-stage larvae in to the water (Molnár, 1967; Moravec, 1977*a*).

In addition to southern and central European countries (e.g. Molnár, 1967; Moravec, 1977*a*; Innal and Keskin, 2005; Saraiva *et al*. 2008), *P. ovata* has been recorded in Finland in the European minnow (*Phoxinus phoxinus*) (Kekäläinen *et al*. 2011; Lai *et al*. 2012). During the breeding season, male minnows have intensive intra-sexual competition, and develop clearly visible breeding ornamentation, such as dark lateral colouration, bright red abdominal colouration and breeding tubercles on the head (Müller and Ward, 1995; Jacob *et al*. 2009; Kekäläinen *et al*. 2010, 2011). The male ornamentation in minnows has been demonstrated to signal dominance status (Jacob

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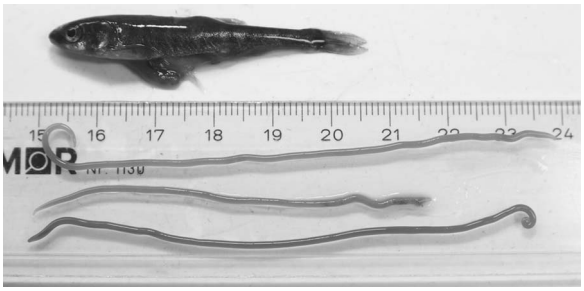

Fig. 1. A male minnow parasitized by three BCD *P. ovata*.

et al. 2009; Kekäläinen *et al*. 2010), low parasite load (Kekäläinen *et al*. 2011), genetic heterozygosity (Müller and Ward, 1995), fitness-related traits (Lai *et al*. 2013) and performance in anaerobic burst swimming (Lai *et al*. 2013). It has been shown that the occurrence of body cavity dwelling (BCD) *P. ovata* is associated with a decrease of male ornamentation in minnows (Kekäläinen *et al*. 2011). In addition, it has been reported that BCD *P. ovata* mainly locates among the gonads in minnows (Moravec, 1977*a*), and thus is likely to cause parasitic castration (Moravec, 2006). Interestingly, European minnows are smaller in body length (mostly <9 cm, see Fig. 1) than most other host species, such as European Chub, *Leuciscus cephalus* (Innal and Keskin, 2005) and *Gobio lozanoi* (Saraiva *et al*. 2008). It is thus possible that the fitness impact of BCD *P. ovata* may be more severe on minnows than on other host species.

Although the interaction and impact of BCD *P. ovata* on minnows has been noted (Kekäläinen *et al*. 2011), the ecology of *P. ovata* infection in minnows has only been studied in central Europe (Moravec, 1977*a*) and never in Finland (but see Lai *et al*. 2012). In other words, our knowledge on the seasonality and ecology of *P. ovata* infection in minnows has been limited, especially the infection ecology of swim bladder dwelling (SBD) *P. ovata*, which has never been studied. Therefore, by monthly monitoring of minnow population in Eastern Finland, as well as observing lab-housed minnows with/without BCD *P. ovata* for almost 1 year, we aimed to investigate the seasonality of infection and characteristics in both SBD *P. ovata* and BCD *P. ovata* in the European minnows.

MATERIALS AND METHODS

Monthly field survey in 2010 and 2011

From April to November (i.e. non-ice-covered period) in 2010 and 2011, we caught minnows monthly from brook Kuusoja (62° 48′N, 30° 1′E) in Eastern Finland by dip net and minnow traps (Promar, Gardena, California, USA). After catching, minnows were transported to the laboratory in the University of Eastern Finland and euthanized with an overdose of tricaine methanesulfonate (MS-222, Sigma®, Sigma Chemical Co., St. Louis, Missouri, USA). We measured the total length (L_T), body mass (W_M, not including the biomass of all BCD *P. ovata* in infected fish) and gonad weight (W_G) and determined the sex of each minnow by dissection. The condition factor (K) of each collected minnow was calculated using the equations: $10^6 \times W_M/L_T^b$, where b is the slope of a regression of $\log_{10}$ (W_M) on $\log_{10}$ (L_T) of all examined minnows (Bolger and Connolly, 1989), and gonadosomatic index (GI) as $W_G/W_M \times 100$ (Erickson *et al*. 1985).

In dissection, we also recorded the infection status and number of BCD *P. ovata* in each minnow, as well as the body length ($L_{P.o.}$), body weight ($W_{P.o.}$) and the development status of offspring (egg and larva) in the uterus of each worm. In addition, by squeezing the swim bladder of minnows between two glass plates, we also recorded the number of SBD *P. ovata* with a dissecting microscope. Moreover, from October 2010, we determined the sex (male, female or larva) of SBD *P. ovata* with the copulation spicules in male worms and uterus filled with eggs in female worms (Molnár, 1967), and thus we were able to distinguish the respective number of female, male and larval SBD *P. ovata* in minnows.

In total, we caught 1027 minnows (327 females, 181 males and 519 immature fish), an average of 86 individuals collected per month (range 67–112) in the field survey. No minnows were collected in November 2010 or April 2011, despite the fact that Brook Kuusoja was ice-opened, and no collecting was conducted in September of either year due to technical reasons. Monthly numbers, sizes and measured parameters of female, male and immature minnows caught are given in Fig. 2.

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

In May 2011, we collected 51 minnows that were found to be infected by BCD *P. ovata* with the highly reliable non-invasive diagnosis (Lai *et al*. 2012) from brook Kuusoja. Another 45 fish that were not infected by BCD *P. ovata* were collected in Kuusoja as the control group. In the lab, minnows were marked with group-specific (i.e. group of infected or non-infected fish) dorsal fin clips for the later identification of infection status. All 96 collected minnows were then mixed and housed in a 45 L aquarium with continuous flow of 12 °C water under a natural photoperiod, and were automatically fed daily with commercial fish food (Biomar®, Aqualife, Denmark).

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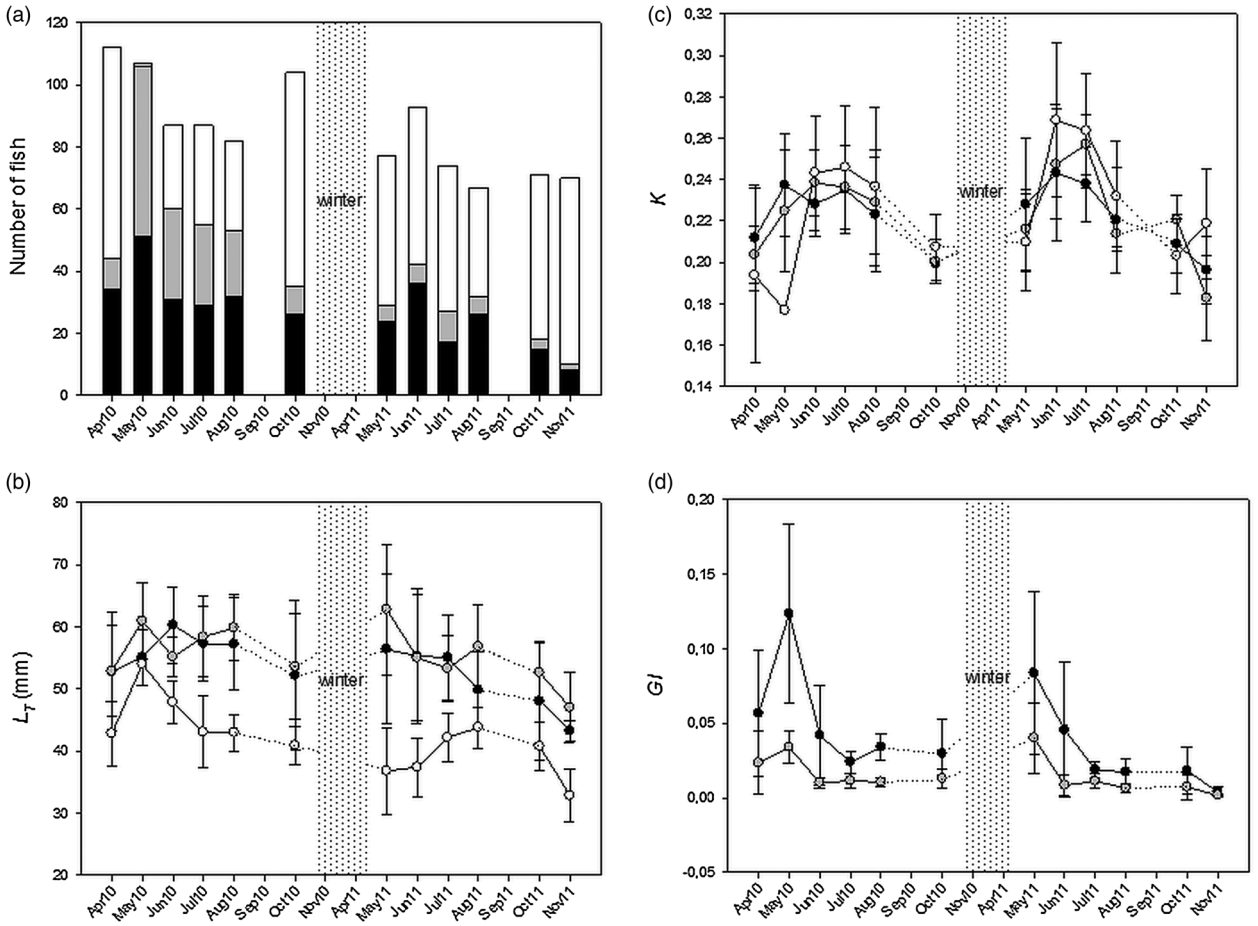

Fig. 2. Number (A), total length (B), condition factor (C) and gonadosomatic index (D) (mean ± S.D.) of female, male and immature (black, grey and white bar or circle, respectively) minnows collected monthly in Brook Kuusoja from April to November in 2010 and 2011. Dotted lines indicate the presumed pattern in non-surveyed months.

In June and July 2011, lab-housed minnows were monitored daily for the evacuation of gravid BCD *P. ovata*. The mortality of infected and non-infected minnows was recorded to understand if the penetration due to the evacuation of *P. ovata* leads to a higher acute mortality in minnows.

From August 2011 to April 2012, we took ca. 20 minnows randomly from the aquarium bimonthly, and euthanized, measured and examined these fish as described above. After dissection, the infection status and number of BCD *P. ovata*, along with $L_{P.o.}$, $W_{P.o.}$, and development status of the offspring (egg and larva) in the uterus of each BCD *P. ovata* in each minnow was recorded. We also recorded the number of male, female and larval SBD *P. ovata*, respectively.

As five minnows died before August 2011, we had 91 minnows in the final analysis, of which 46 and 45 individuals were diagnosed as infected and non-infected by BCD *P. ovata* respectively in May 2011. From August 2011 to April 2012, we examined on average 18 (range: 11–21) individuals of these lab-housed minnows bimonthly. All the procedures were performed according to the license of the Finnish Animal Experimental Board (ESAVI/1906/04·10·03/2012).

Statistics

Monthly field survey in 2010 and 2011. The following analyses were conducted in SBD *P. ovata* and BCD *P. ovata*, respectively. The prevalence (proportion of infected individuals in the population) and intensity (number of parasites in an infected individual) of *P. ovata* infections, according to Bush *et al.* (1997), were calculated for female, male, mature (i.e. female+male), immature minnows and for the total (i.e. female+male+immature) minnows collected in every month. A paired *t*-test was used to examine the difference of monthly prevalence of *P. ovata* between female and male minnows and between mature and immature minnows. Due to the non-parametric data characteristics of intensity, Kruskal–Wallis test was used to examine the difference of overall intensity of *P. ovata* between male and female minnows and between mature and immature fish. In addition, to reveal the potential impact of *P. ovata* infection on

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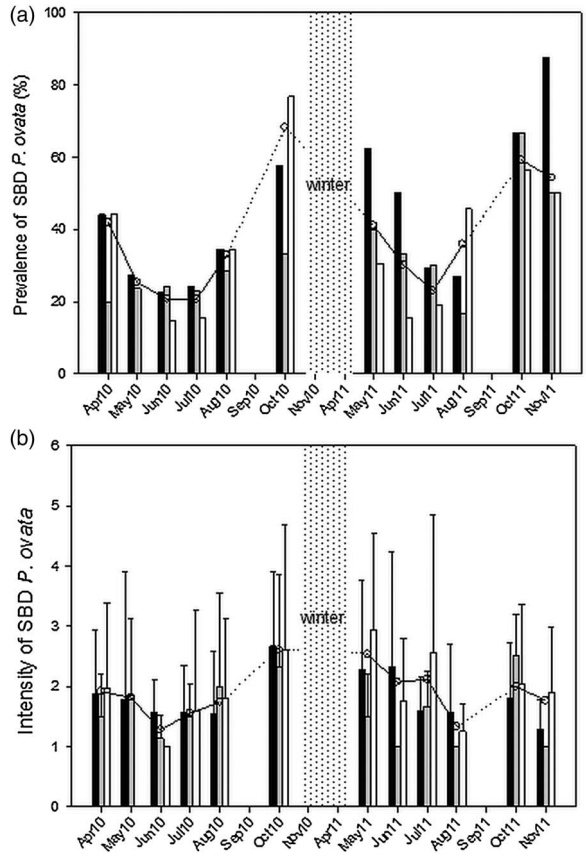

Fig. 3. Prevalence (A) and intensity (B) (mean ± S.D.) of SBD *P. ovata* in female, male, immature (black, grey and white bar, respectively) and in total (empty circle) minnows collected monthly in Brook Kuusoja from April to November in 2010 and 2011. Dotted lines indicate the presumed pattern in non-surveyed months. Numbers of female, male and immature minnows examined in each month can be referred in Fig. 2A.

the fitness of minnows, we tested the association between each fitness-related factor (L_T, K and GI, dependent variables) of minnows and intensity of *P. ovata* infection and, in particular, the sum $L_{P.O.}$ of BCD *P. ovata* (covariates) with a general linear model (GLM) analysis. The collecting month and fish sex were included as fixed factors in these analyses.

After October 2010, to investigate the dynamics of *P. ovata* larvae transmitting from intermediate hosts to the swim bladder of minnows, we conducted a one-way analysis of variance (ANOVA) to test the difference of proportion of larval SBD *P. ovata* among all SBD *P. ovata* (dependent variable) in all (female+male+immature) minnows between each month. To investigate the emigrating dynamics of female *P. ovata* from the swim bladder to the body cavity in minnows, and the dynamic of male *P. ovata* in the swim bladder, we also conducted two one-way ANOVAs for female and male SBD *P. ovata* among all SBD *P. ovata*, respectively, to test the difference of proportion of female and male SBD *P. ovata* (dependent variables) between the months. In addition, the monthly proportion of female and male SBD *P. ovata* in all minnows was analysed with a paired *t*-test to reveal if *P. ovata* has any sex-bias. The association between the developmental status of offspring (egg and larva, dependent variable) in uterus and the body size (length and weight, covariates) of BCD *P. ovata* was also tested with logistic regression.

Bimonthly survey of P. ovata *in lab-housed minnows in 2011 and 2012.* The prevalence and intensity of SBD *P. ovata* and BCD *P. ovata*, as well as the proportion of female, male and larval SBD *P. ovata*, were calculated, respectively, in each examining month. The proportion of female and male SBD *P. ovata* was analysed with a paired *t*-test to reveal if emigration of female *P. ovata* from swim bladder leads to a male-biased group in the swim bladder without a fresh infection of larvae. The dependence of the developmental status of offspring in uterus (egg or larvae) on the body length of BCD *P. ovata* in the survey was tested with logistic regression. Finally, the infection status of BCD *P. ovata* distinguished previously by non-invasive diagnosis in May 2011 in each minnow was compared with the infection status revealed by dissection under examination by Chi-Square (χ^2) test. Since the infection status of BCD *P. ovata* revealed by dissection in the survey belonged to the next annual cycle of *P. ovata* comparing to the infection status distinguished in May 2011, we were able to examine if the emigration of female *P. ovata* from the swim bladder is correlated between two sequential annual cycles in the analysis.

RESULTS

Monthly field survey of minnows in 2010 and 2011

Philometra ovata *in swim bladder.* Over the whole field survey, the monthly prevalence of SBD *P. ovata* infection varied between 20 and 70% with a regular annual cycle, in which the prevalence decreased in spring, stayed low in mid-summer, then increased in late summer and autumn (difference of SBD *P. ovata* prevalence between July (mid-summer) and October (autumn): in 2010, $\chi^2 = 143{\cdot}48$, $P < 0{\cdot}001$; in 2011, $\chi^2 = 52{\cdot}54$, $P < 0{\cdot}001$; Fig. 3A). Such a pattern of an annual seasonal cycle of SBD *P. ovata* infection was very clear in females and immature minnows, and also obvious in male minnows (Fig. 3A). In addition, the mean monthly prevalence of SBD *P. ovata* infection in minnows in our field survey was 37·8 ± 15·1%, while in female, male and immature minnows was 44·4 ± 19·8%, 32·5 ± 13·5% and 33·6 ± 20·9%, respectively. The mean monthly prevalence of SBD *P. ovata* in female minnows was significantly

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Table 1. GLM statistics for the association between fitness-related factors of minnows (L_T, K and GI; dependent variable) and intensity of SBD *P. ovata* in minnows, with fish sex and collecting month as fixed factors

Fitness-related factor	Source	Type III SS	D.F.	MS	F
L_T	Intensity of SBD *P. ovata*	4·855	1	4·855	0·120
	Sex	11942·017	2	5971·009	147·480***
	Month	3461·829	11	314·712	7·773***
	Error	15061·156	372	40·487	
	Collected total	40795·959	386		
K	Intensity of SBD *P. ovata*	$9·139 \times 10^{-7}$	1	$9·139 \times 10^{-7}$	0·001
	Sex	0·006	2	0·003	3·177*
	Month	0·076	11	0·007	8·040***
	Sex × month	0·030	21	0·001	1·690*
	Error	0·301	351	0·001	
	Collected total	0·463	386		
GI	Intensity of SBD *P. ovata*	$1·546 \times 10^{-5}$	1	$1·546 \times 10^{-5}$	0·036
	Sex	0·082	2	0·041	95·394***
	Month	0·062	11	0·006	13·199***
	Sex × month	0·051	21	0·002	5·736***
	Error	0·150	351	0·000	
	Collected total	0·464	386		

Non-significant interactions in each analysis ($P > 0·1$, $P > 0·3$, $P > 0·7$, respectively) were excluded from this final table. *: $P < 0·05$; **: $P < 0·01$; ***: $P < 0·001$.

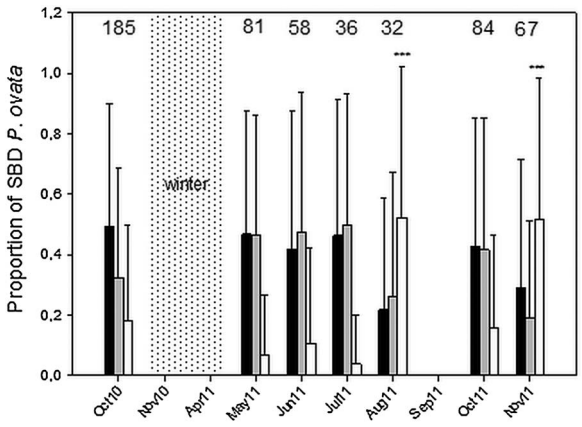

Fig. 4. Monthly proportion (mean ± S.D.) of female, male and larval SBD *P. ovata* (black, grey and white bar, respectively) in minnows from October 2010 to November 2011. The asterisks over the bar indicate the proportion of larval SBD *P. ovata* in the month was significantly ($P < 0·001$) higher than in other months. The number of SBD *P. ovata* examined in each month is indicated above.

higher than in males, while the mean monthly prevalence between mature and immature minnows was not significantly different (paired t-test: female and male, $t_{11} = 3·252$, $P = 0·008$; mature and immature, $t_{11} = -1·491$, $P = 0·164$, Fig. 3A). On the other hand, the mean intensity of SBD *P. ovata* infection in all infected minnows in our field survey was 2·0 ± 1·5 ($n = 387$), while in 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 (Fig. 3B). The seasonal cycle in intensity of SBD *P. ovata* infection (Fig. 3B) followed roughly the same pattern as the prevalence. In addition, differences in intensity of SBD *P. ovata* infection between female and male infected minnows, as well as between mature and immature infected fish were both non-significant (Kruskal–Wallis test: female and male, $\chi^2 = 0·756$, $P = 0·385$; mature and immature, $\chi^2 = 3·124$, $P = 0·077$, Fig. 3B). Taken together, it is thus indicated that female minnows have a higher possibility than male minnows to be infected by SBD *P. ovata*, but the numbers of SBD *P. ovata* in infected female and male minnows were similar.

GLM analysis showed that, despite the significant effects of the seasonality and sex of minnow on K and GI (i.e. K and GI of minnows increased in the spawning season, Fig. 2C and D), all the associations between each fitness-related factor (L_T, K and GI) of minnows and the intensity of SBD *P. ovata* infection were non-significant (Table 1). Thus, it is suggested that infection of SBD *P. ovata* may not retard the host growth, impair the host body condition or decrease the host relative gonad size in minnows.

The proportion of female and male SBD *P. ovata* was generally higher than that of larval SBD *P. ovata* (Fig. 4). The proportion of female SBD *P. ovata* from total SBD *P. ovata* was not different between months (ANOVA: $F_6 = 1·953$, $P = 0·073$), while proportion of male and larva SBD *P. ovata* were both significantly different between months (ANOVA: male, $F_6 = 2·744$, $P = 0·013$; larva, $F_6 = 10·489$, $P < 0·001$). However, *post hoc* tests showed that the proportion of larval SBD *P. ovata* was significantly higher only in August and November 2011 than in other surveying months (Fig. 4). On the other hand, the monthly proportion of female and male SBD *P. ovata* was not different (pair

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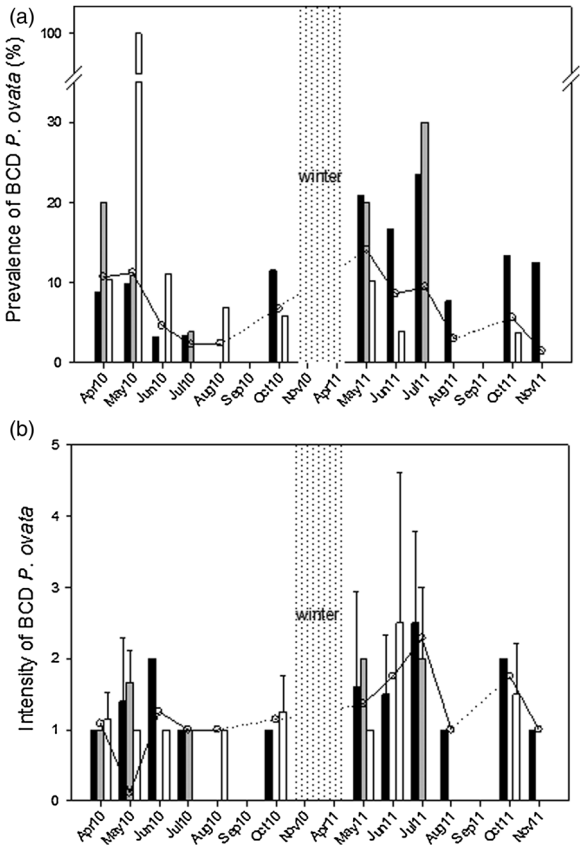

Fig. 5. Prevalence (A) and intensity (B) (mean ± S.D.) of BCD *P. ovata* in female, male, immature (black, grey and white bar, respectively) and in total (empty circle) minnows collected monthly in Brook Kuusoja from April to November in 2010 and 2011. Dotted lines indicate the presumed pattern in non-surveyed months. Numbers of female, male and immature minnows examined in each month can be referred in Fig. 2A.

t-test: female and male, $t_{244} = 1{\cdot}047$, $P = 0{\cdot}296$). Taken together, it is implied that the *P. ovata* population in our survey was not sex-biased. The monthly proportion of female *P. ovata* was stable in the swim bladder, while the proportion of larval *P. ovata* was variable between months, rising particularly in August, when fresh infections by larval *P. ovata* mainly occur.

Philometra ovata *in body cavity*. As in SBD *P. ovata*, the prevalence of BCD *P. ovata* in our field survey also showed a regular annual pattern, in which the prevalence decreased from May to August then mildly increased in autumn, and was always less than 14% (difference of BCD *P. ovata* prevalence between July (mid-summer) and October (autumn): in 2010, $\chi^2 = 9{\cdot}09$, $P < 0{\cdot}005$; in 2011, $\chi^2 = 1{\cdot}21$, N.S.; Fig. 5A). Due to the different numbers of collected fish between sexes, the prevalence of BCD *P. ovata* in female, male and immature minnows was highly variable, and the pattern of prevalence in total minnows was merely observed in female minnows (Fig. 5A). The mean monthly prevalence of BCD *P. ovata* in our field survey was 6·7 ± 3·9%, while in female, male and immature minnows was 10·9 ± 6·8%, 7·1 ± 10·1% and 12·7 ± 26·6%, respectively. In addition, the mean monthly prevalence of BCD *P. ovata* between female and male minnows, as well as between mature and immature fish, were not significantly different (pair *t*-test: female and male, $t_{11} = 1{\cdot}577$, $P = 0{\cdot}143$; mature and immature, $t_{11} = -0{\cdot}281$, $P = 0{\cdot}784$). The seasonal changes in the intensity of BCD *P. ovata* in all minnows, as well as in female, male and immature minnows roughly followed the pattern observed for the prevalence of BCD *P. ovata* infection (Fig. 5B). The mean intensity of BCD *P. ovata* in all infected minnows in our field survey was 1·4 ± 0·8 ($n = 68$), while in female, male and immature minnows was 1·6 ± 0·9 ($n = 31$), 1·6 ± 0·7 ($n = 12$) and 1·2 ± 0·7 ($n = 25$), respectively. In addition, intensity of BCD *P. ovata* infection between female and male infected minnows as well as between mature and immature infected fish were not significantly different (Kruskal–Wallis test: female and male, $\chi^2 = 0{\cdot}480$, $P = 0{\cdot}488$; mature and immature, $\chi^2 = 3{\cdot}395$, $P = 0{\cdot}065$; Fig. 5B). Taken together, it is indicated that female and male minnows have similar possibility to be burdened by BCD *P. ovata*, and the numbers of BCD *P. ovata* between infected female and male minnows are similar.

Similarly, despite the significant effects of the seasonality and sex of minnows on *K* and *GI* (Fig. 2C and D), GLM analysis showed that all the associations between each fitness-related factor (L_T, *K* and *GI*) of minnows and the intensity, as well as sum $L_{P.O.}$ of BCD *P. ovata*, were non-significant (Table 2). Thus, it is indicated that BCD *P. ovata* may not retard host growth, impair host body condition or decrease host relative gonad size in minnows.

In total, we found 98 BCD *P. ovata* in minnows collected in our field survey in 2010 and 2011. The body length and body weight of these BCD *P. ovata* obviously showed a regular pattern of annual cycle, in which the length and weight of BCD *P. ovata* increased from autumn to spring, reached their maximum in June, then dropped with higher variance in July and August supposedly due to the evacuation (Fig. 6). On the other hand, only one BCD *P. ovata* (40 mm) in Aug 2011 was found with larvae in the uterus among 98 individuals, and thus no correlation was observed between development status of offspring in the uterus and body size of BCD *P. ovata*.

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

Observation in June and July 2011. In the 2 months of observation, five minnows that were

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Table 2. GLM statistics for the association between fitness-related factors of minnows (L_T, K and GI, dependent variable) and intensity and sum length of BCD *P. ovata* (covariate) in minnows, with fish sex and collecting month included as fixed factors

Fitness-related factor	Source	Type III SS	D.F.	MS	F
L_T	Intensity of BCD *P. ovata*	0·002	1	0·002	0·000
	Sex	1766·718	2	883·359	18·839***
	Month	1274·503	11	115·864	2·471*
	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
	Sex	1569·058	2	784·529	17·231***
	Month	1026·630	11	93·330	2·050*
	Error	2413·058	53	45·529	
	Collected total	6207·529	67		
K	Intensity of BCD *P. ovata*	0·000	1	0·000	0·194
	Sex	0·003	2	0·002	1·493
	Month	0·020	11	0·002	1·569
	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
	Sex	0·003	2	0·002	1·466
	Month	0·020	11	0·002	1·561
	Error	0·061	53	0·001	
	Collected total	0·094	67		
GI	Intensity of BCD *P. ovata*	0·002	1	0·002	2·120
	Sex	0·052	2	0·026	28·448***
	Month	0·028	11	0·003	2·821**
	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
	Sex	0·054	2	0·027	29·849***
	Month	0·031	11	0·003	3·145**
	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 this final table. *: $P < 0·05$; **: $P < 0·01$; ***: $P < 0·001$.

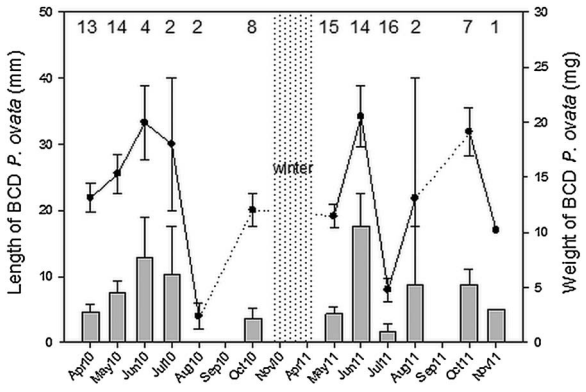

Fig. 6. Length and weight (circle referred to left axis and bar referred to right axis, respectively; mean ± S.D.) of BCD *P. ovata* in minnows collected monthly in Brook Kuusoja from April to November in 2010 and 2011. Dotted lines indicate the presumed pattern in non-surveyed months. The number of BCD *P. ovata* examined in each month is indicated above.

diagnosed as infected with BCD *P. ovata* died. During an autopsy, three of the five minnows were found to still contain BCD *P. ovata*. Furthermore, plenty of physical damages, including obvious skin necrosis around the anus, intensive adhesion among mesentery and organ membranes in the posterior body cavity, great areas of bruises under the body surface in the posterior abdomen and around the anus, as well as bruises in the abdominal body muscle near the anus and in the basal muscle of the anal fin, were found externally and internally in these dead minnows. We even observed a hole through the body muscle near the anus in one of the five minnows. Therefore, we highly suspected that these damages and death were caused by BCD *P. ovata* in evacuation. Taken together, since all the five minnows that died in the laboratory were diagnosed as infected, the mortality of 51 minnows infected with BCD *P. ovata* was 9·8%. When compared to the mortality (0·0%) of non-infected minnows ($n = 45$), it is implied that the mortality of minnows infected with BCD *P. ovata* may be increased because of the evacuation of BCD *P. ovata*.

On July 1st, we observed the sign of evacuation, i.e. several pieces of white, soft, worm-like, floating lines with a great number of white particles in the aquarium, for the first time. Under the dissecting

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microscope, we found that these white particles in the aquarium were the first stage larvae of *P. ovata*, while the pieces of white floating lines were the broken and collapsed body parts of *P. ovata* after evacuating from the body cavity. Such a sign of evacuation was observed on the 1st, 4th, 6th, 11th, 14th and 22nd of July.

Bimonthly survey of P. ovata *in lab-housed minnows in 2011 and 2012:* P. ovata *in swim bladder*. In all 91 examined minnows, three out of four minnows were infected with SBD *P. ovata*, while the mean intensity of SBD *P. ovata* infection was higher than two worms per infected fish (Table 3). We found no larval SBD *P. ovata* in the examination. Furthermore, the proportion of female SBD *P. ovata* was significantly lower than that of males (proportion: female, $0{\cdot}2 \pm 0{\cdot}4$; male, $0{\cdot}8 \pm 0{\cdot}3$; pair *t*-test: $t_{68} = -7{\cdot}664$, $P < 0{\cdot}001$, Fig. 7). Therefore, it is obvious that the SBD *P. ovata* population in our examination was male-biased, which is likely due to the emigration of female *P. ovata* from the swim bladder without a fresh infection of new larval individuals.

Bimonthly survey of P. ovata *in lab-housed minnows in 2011 and 2012:* P. ovata *in body cavity*. In these 91 minnows (of which 46 and 45 individuals were diagnosed as infected and non-infected by BCD *P. ovata*, respectively, in May 2011), 51 individuals were found infected with BCD *P. ovata* under examination, and the intensity of BCD *P. ovata* in these 51 infected minnows was more than 1·5 (Table 3). In addition, 33 out of these 51 infected minnows were diagnosed as infected in May 2011 (Table 4). Thus, if a minnow was infected with BCD *P. ovata* in the previous year, the probability of being infected again for this individual was 71·7% (33/46), which was significantly higher than the prevalence of BCD *P. ovata* in the lab-housed group (50·6%; $\chi^2 = 8{\cdot}26$, $P < 0{\cdot}005$). In the other 40 individuals which were found to be non-infected with BCD *P. ovata* under examination, 27 individuals were diagnosed as non-infected in May 2011 (Table 4), Thus, if a minnow was non-infected with BCD *P. ovata* in the previous year, the probability of remaining non-infected for this individual was 60·0% (27/45), which was non-significantly different from the non-infection rate of BCD *P. ovata* in the lab-housed group (49·4%; Chi-square test: $\chi^2 = 2{\cdot}00$, $P > 0{\cdot}1$). Taken together, it is implied that the present infection status of BCD *P. ovata* was correlated with the infection status in the following cycle, i.e. the emigration of female *P. ovata* from swim bladder is correlated in sequential annual cycles.

Under examination, we found 81 BCD *P. ovata* from the 51 infected minnows. Among these 81 BCD *P. ovata*, 7 individuals were atrophied dead

Table 3. Prevalence and intensity (mean ± S.D.) of SBD and BCD *P. ovata* in lab-housed minnow *P. phoxinus* examined bimonthly from August 2011 to April 2012

		Examining month					
		Aug. 2011	Oct. 2011	Dec. 2011	Feb. 2012	Apr. 2012	Total
SBD *P. ovate*	Prevalence (%)	80·0	79·0	80·0	76·2	54·6	75·8
	Intensity (range)	1·9 ± 1·1 (1–5)	2·5 ± 2·1 (1–9)	2·6 ± 1·7 (1–6)	2·1 ± 1·4 (1–6)	1·5 ± 0·8 (1–3)	2·2 ± 1·5 (1–9)
BCD *P. ovata*	Total number (dead corpse)	20 (1)	16 (2)	16 (2)	17 (2)	12 (0)	81 (7)
	Prevalence	65·0%	42·1%	55·0%	52·4%	72·7%	56·0%
	Intensity (range)	1·6 ± 0·8 (1–3)	2·0 ± 0·9 (1–2)	1·5 ± 0·5 (1–2)	1·6 ± 1·2 (1–5)	1·4 ± 0·5 (1–2)	1·6 ± 0·8 (1–5)

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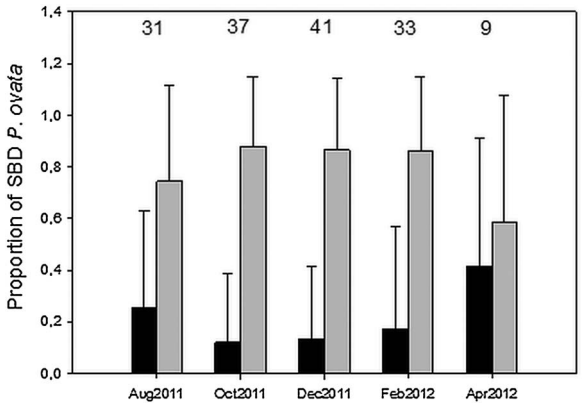

Fig. 7. Proportion (mean ± S.D.) of female and male (black and grey bar, respectively) SBD *P. ovata* in minnows examined in every 2 month from August 2011 to April 2012. The number of SBD *P. ovata* examined in each month is indicated above.

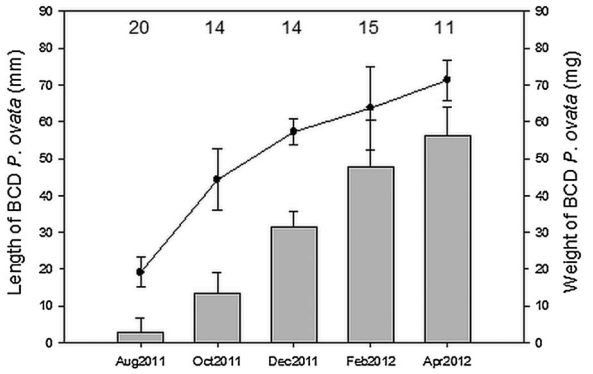

Fig. 8. Length and weight (circle and bar, respectively; mean ± S.D.) of BCD *P. ovata* in minnows examined in every 2 month from August 2011 to April 2012. The number of BCD *P. ovata* examined in each month is indicated above.

Table 4. Comparison between present infection status of BCD *P. ovata* examined in dissection and previous infection status detected in non-invasive diagnosis in May 2011

	Previous infection status detected in non-invasive diagnosis		
	Infected	Non-infected	Sum
Present infection status examined in dissection			
Infected	33	18	51
Non-infected	13	27	40
Sum	46	45	91

corpses and no measurement was conducted, thus we measured the body length and weight in 74 individuals in total. The length and weight of BCD *P. ovata* increased in each examining month (Fig. 8). None of these BCD *P. ovata* was found to have larvae in the uterus under examination, and thus again no correlation between development status of offspring in the uterus and body size of female parasite was observed.

DISCUSSION

To the best of our knowledge, this is the first study which includes the infection ecology of *P. ovata* dwelling not only in the body cavity but also in the swim bladder, and also includes the sex ratio of SBD *P. ovata*. According to the proportion of larval *P. ovata* and prevalence of SBD *P. ovata*, the fresh infection and settling of larval *P. ovata* in the swim bladder in our minnow population occurred mainly in August, which is included in the time period reported in Czech (Moravec, 1977*a*) but over 1 month later than it has been reported in Hungary (Molnár, 1967).

After infecting the swim bladder of minnows, it is implied that female *P. ovata* emigrate to the body cavity mainly in September, according to the raised prevalence and body size of BCD *P. ovata* in October. Nevertheless, the prevalence and intensity of SBD *P. ovata* was higher than BCD *P. ovata* in our field survey. The sex ratio of SBD *P. ovata* in our field survey indicated that the proportion of female SBD *P. ovata* was similar to that of SBD males in minnows, which implied that some female *P. ovata* stay in the swim bladder for reasons that remain unknown and were not able to emigrate to the body cavity to grow fully and eventually evacuate. These remaining female *P. ovata* in the swim bladder inevitably reduced the number of BCD *P. ovata*, and led to not only higher intensity but also higher prevalence of SBD *P. ovata* than BCD ones.

It has been suggested that in an obligatory host, all female SBD *P. ovata* are likely to reach maturity and start development when male worms are presented. On the contrary, in a facultative host, only some female *P. ovata* are able to complete the entire developmental cycle, while most female worms are remained in swim bladder and retarded in growth regardless of the number of young individuals in both sexes (Molnár, 1967). In our monthly survey, the proportion of females in SBD *P. ovata* has been constant and similar as males, and the prevalence of SBD *P. ovata* has been 3-to-4-folds higher than that of BCD ones as similar as the case in European chub (Molnár, 1967). In addition, the surveyed length and weight of BCD *P. ovata* increased constantly with constant variable before evacuation in both our field-collected minnows and the lab-housed ones. Taken together, it is indicated that a certain proportion of SBD female *P. ovata* in minnows were unlikely to emigrate freely to the body cavity anytime in the annual cycle but waited in the swim bladder until the end of the evacuating period, as documented in the facultative host

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European chub (Molnár, 1967). Therefore, although European minnow has been suggested as an obligatory host to *P. ovata* in the previous study in Czech (Molnár, 1967), it seems that minnow in the present study should be regarded as a facultative host to *P. ovata*.

After emigrating to the body cavity of minnows, female *P. ovata* grow to their full size from autumn to the next summer, and the eggs in uterus develop into first stage larvae nearly 1 month before the evacuation (Molnár, 1967; Moravec, 1977*a*). It has been observed that female BCD *P. ovata* were in various body sizes by evacuation (e.g. 50–60 mm in present study; 77–110 mm in Molnár, 1967; 70–95 mm in Moravec, 1977*a*; 44–70 mm in Innal and Keskin, 2005), which implies that the development of first stage larvae in uterus could occur in various sizes of female BCD *P. ovata* and is likely to be determined by environmental factors such as the water temperature (Moravec, 1977*a*).

According to the prevalence and body size of BCD *P. ovata*, it is indicated that *P. ovata* in minnows in Eastern Finland have a regular annual cycle, as other populations in previous studies have shown (Molnár, 1967; Moravec, 1977*a*). The prevalence of BCD *P. ovata* in minnows decreased from April/May to July or August and then mildly increased in autumn, while the body length and weight of these *P. ovata* increased from autumn to April/May, reached the peak in June, and then steeply decreased in July or August. Taken together, it is suggested that female BCD *P. ovata* grow from October and reach their full size by the end of the next June, and evacuate from the body cavity of minnows in July or the beginning of August at latest.

According to the varied prevalence of BCD *P. ovata* in summer between 2010 and 2011 and their variant body size in July and August, the yearly evacuation period of *P. ovata* in minnows may not be as regular as that population parasitizing in Hungary (Molnár, 1967). Instead, the evacuation time period and the regularity of *P. ovata* annual cycle in our minnow population are more similar to the case surveyed in Czech (Moravec, 1977*a*). Our observation of lab-housed minnows showed that the evacuation of BCD *P. ovata* in our minnow population occurred several times throughout July, which was a time period of similar length as previously described in Hungary from the end of May to the end of June (Molnár, 1967). However, the evacuation of *P. ovata* in July in Finland is later than the evacuation period from the surveyed population in Hungary (Molnár, 1967), but is included in the time range of evacuation from July to early September reported in Czech (Moravec, 1977*a*). As Moravec (1977*a*) suggested, it is likely that the lower temperature in late spring and early summer in Finland may be the reason for the variance of evacuation time in the yearly cycle.

After the evacuation of those BCD individuals, female *P. ovata* with retarded development that remained in the swim bladder may emigrate to the body cavity in the next developmental cycle. In our lab-housed minnows, the sex ratio of SBD *P. ovata*, which is supposedly unbiased according to our field survey, became male-biased from August, and the emigrated female *P. ovata* also occurred in the body cavity of minnows. Since lab-housed minnows were isolated from a fresh infection of *P. ovata*, and therefore a fresh infection of larvae from intermediate hosts is impossible, these small, newly emigrated female BCD *P. ovata* must be those retarded females which have remained in swim bladder. Therefore, it is implied that in the next developmental cycle after evacuation, females with retarded development that remained in swim bladder may have an opportunity again to emigrate from the swim bladder and then grow ordinarily even after being retarded. Since it has been evidenced that SBD *P. ovata* survives for 2 years and more in a host minnow (Moravec, 1977*a*), retarded females may at least have the second opportunity for emigration and further development in the body cavity.

Interestingly, according to our examination on lab-housed minnows, minnows hosted with BCD *P. ovata* have a higher possibility to host BCD *P. ovata* again in the next annual cycle. This result indicates that female SBD *P. ovata* are likely to emigrate to the body cavity if the emigration already occurred in the previous year. We suggest that the emigrating possibility of SBD *P. ovata* is probably determined by the inheritable parasite resistance in host minnows. Thus, SBD *P. ovata* are more likely to develop and emigrate ordinarily to the body cavity in certain host individuals. On the other hand, since the swim bladder is not connected to the body cavity with a natural duct in minnows, it is also possible that female SBD *P. ovata* emigrate to the body cavity more easily because the emigrating route between swim bladder and body cavity was opened wider due to the emigration that occurred in the previous year.

The transmission of *P. ovata* relies on minnows preying on the infected intermediate hosts, and it is likely that larger minnows may feed on more intermediate hosts of *P. ovata*. It has also been reported in host *Gobio gobio* that older (larger) host fish has higher prevalence of BCD *P. ovata* infection than younger (smaller) individuals (Moravec, 1977*a*). In our field collected minnows, females have higher prevalence but similar intensity of SBD *P. ovata* than males. Due to the fact that a number of females were larger than males in our field collected minnows, it is reasonable that female minnows on average fed on more intermediate hosts of *P. ovata*

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than males did, which thus results 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 to obtain the energetic requirement for the eggs, and thus females take a higher risk to be infected by SBD *P. ovata* through feeding on more of the intermediate hosts of *P. ovata*. However, although female minnows have a higher prevalence of SBD *P. ovata*, no difference was found between female and male minnows in either the prevalence or intensity of BCD *P. ovata*.

Finally, despite the large size of the parasites, the impairment on fitness of minnows from BCD *P. ovata* infection may not be as considerable as presumed. Although *K* and *GI* of minnows increased in the spawning season, and were consequently associated with collecting month and sex, the intensity of infection and summed length of BCD *P. ovata* was not associated with *K* or *GI* of minnows, which demonstrates that BCD *P. ovata* do not impair relative gonad size or condition factor in minnows. Our results thus disagreed the previous observation that BCD *P. ovata* mainly locates among the gonads in minnows (Moravec, 1977*a*), and no parasitic castration (Moravec, 2006) was observed. In addition, the intensity of BCD *P. ovata* was shown to be positively correlated with the condition factor of host *G. lozanoi*, while the prevalence of BCD *P. ovata* was positively correlated with the relative gonad size of hosts (Saraiva *et al*. 2008). Accordingly, it has been implied that BCD *P. ovata* may not impair the condition factor and relative gonad size of the host. Nevertheless, although the impairment of BCD *P. ovata* on fitness-related traits has not been found, BCD *P. ovata* still potentially increased the mortality of infected minnows during the evacuation period, and caused apparent damages internally and externally in infected minnows in our observation, which has also been shown histologically in previous study (Saraiva *et al*. 2008). Taken together, it is indicated that infection of BCD *P. ovata* may be harmful to the fitness of minnows through potentially increased mortality and physical damages in evacuation period, but not impair fitness-related traits.

In conclusion, the regular pattern of yearly cycle and infection ecology of *P. ovata* in minnows in Eastern Finland is revealed with a survey of both the body cavity and swim bladder. The timing and length of yearly evacuation period of *P. ovata* in our minnow population is different from those of other populations, which is likely to be dependent on water temperature. Our study also reveals that minnows may merely be a facultative host rather than an obligatory host for *P. ovata* because a certain amount of female *P. ovata* retard their development and stay in the swim bladder until the end of the evacuating period. On the other hand, SBD *P. ovata* is likely to emigrate to the body cavity of minnows if the emigration had already occurred in the host individuals in the previous year. Finally, the evacuation may cause obvious injuries to hosts and potentially raise the mortality of infected minnows. Thus, infection of BCD *P. ovata*, despite its large size, may only be harmful to the fitness of minnows through potentially increased mortality and physical damages during the evacuation period, but not impair fitness-related traits as previously presumed.

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REFERENCES

Bolger, T. and Connolly, P. L. (1989). The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology* **34**, 171–182.

Bush, A. O. Lafferty, K. D., Lotz, J. M. and Shostak, A. W. (1997). Meet ecology on its own terms: Margolis *et al*. revisited. *The Journal of Parasitology* **83**, 575–583.

Erickson, D. L., Hightower, J. E. and Grossman, G. D. (1985). The relative gonadal index: an alternative index for quantification of reproductive condition. *Comparative Biochemistry and Physiology A* **18**, 117–120.

Innal, D. and Keskin, N. (2005). *Philometra ovata* (Zeder, 1803) (Philometridae) in European chub (*Leuciscus cephalus L.*, 1758) living in Çamkoru Lake (Çamlidere-Ankara). *Journal of Animal and Veterinary Advances* **4**, 959–961.

Jacob, A., Evanno, G., Renai, E., Sermier, R. and Wedekind, C. (2009). Male body size and breeding tubercles are both linked to intrasexual dominance and reproductive success in the minnow. *Animal Behaviour* **77**, 823–829.

Kekäläinen, J., Valkama, H., Huuskonen, H. and Taskinen, J. (2010). Multiple sexual ornamentation signals male quality and predicts female preference in minnows. *Ethology* **116**, 895–903.

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

Keskin, N. (1988). Türkiye'de *Leuciscus cephalus*'da (Tatlýsu kefali) *Philometra abdominalis* Nybelin, 1928 (Philometridae) olgusu. *Doða Türk Zool Dergisi* **12**, 70–74.

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

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

Molnár, K. (1967). Morphology and development of *Philometra abdominalis* Nybelin, 1928. *Acta Veterinaria Academiae Scientiarum Hungaricae* **17**, 293–300.

Moravec, F. (1977*a*). The life history of the nematode *Philometra abdominalis* in the Rokytka Brook, Czechoslovakia. *Acta Societatis Zoologicae Bohemoslovacae* **41**, 114–120.

Moravec, F. (1977*b*). The development of the nematode *Philometra abdominalis* Nyblin, 1928 in the intermediate host. *Folia Parasitologica* **24**, 237–245.
Moravec, F. (2004). The systematic status of *Philometra abdominalis* Nybelin, 1928 (Nematoda: Philometridae) (=a junior synonym of *P. ovata* (Zeder, 1803)). *Folia Parasitologica* **51**, 75–76.
Moravec, F. (2006). *Dracunculoid and Anguillicoloid Nematodes Parasitic in Vertebrates*. Academia, Prague, Czech Republic.
Müller, G. and Ward, P. I. (1995). Parasitism and heterozygosity influence the secondary sexual characters of the European minnow, *Phoxinus phoxinus* (L.) (Cyprinidae). *Ethology* **100**, 309–319.
Saraiva, A., Hermida, M., Costa, M. J., Maia, C., Reis, A. R., Cruz, C. and Valente, A. (2008). First record of *Philometra ovata* (Nematoda) infection in *Gobio lozanoi* in Portugal. *Journal of Fish biology* **73**, 2288–2292.

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