

Factors affecting trematode infection rates in freshwater mussels

Tomasz Müller · Marcin Czarnoleski ·
Anna Maria Labecka · Anna Cichy ·
Katarzyna Zajac · Dominika Dragoz-Kluska

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Abstract Mussels are intermediate hosts of digenean trematodes, but determinants of these infections remain unknown. To address this problem, we collected duck mussels *Anodonta anatina* in eighteen lakes from northeastern Poland and examined how mussel age, sex, and the encrustation with zebra mussels *Dreissena polymorpha* and environmental conditions in lakes influenced infection rates. We also assessed parasitic preferences to host gonads and hepatopancreas and the impact of parasites on female fertility. Mussels were infected with *Rhipidocotyle campanula* and *Phyllodistomum* sp. Infection rates were higher in older and female mussels but were unrelated to the biomass of encrusting *D. polymorpha* and the trophy, thermal conditions, and Ca^{2+} availability. Parasites occupied gonads more often than

hepatopancreas. Infected females were less likely to carry glochidia and incubated fewer glochidia. We suggest that the risk of infection by digenean trematodes increases with the amount of water processed by filter-feeding hosts and/or that parasites actively seek hosts which can provide them with abundant resources. This mechanism explains why parasites more often occupied older and female mussels and targeted their gonads. Future research on trematode-mussel interactions should integrate knowledge on different elements of the complex trematode life cycles, including effects of higher-order hosts such as fish.

Keywords Unionidae · Digenea · Glochidia · Life history · Physical environment · Life cycle

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T. Müller (✉) · K. Zajac
Institute of Nature Conservation, Polish Academy of Sciences, Mickiewicza 33, 31-120 Kraków, Poland
e-mail: tomasz.muller@uj.edu.pl

Present Address:
M. Czarnoleski · A. M. Labecka · D. Dragoz-Kluska
Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland
e-mail: marcin.czarnoleski@uj.edu.pl

Introduction

Parasitic platyhelminths evolved into three groups characterized by distinct life strategies, namely the

A. M. Labecka
Department of General Zoology, Faculty of Biology,
University of Szczecin, Felczaka 3c, 71-415 Szczecin,
Poland

A. Cichy
Department of Invertebrate Zoology, Faculty of Biology
and Environment Protection, Nicolaus Copernicus
University in Toruń, Lwowska 1, 87-100 Toruń, Poland

Monogenea, the Cestoda (tapeworms and allies), and the Trematoda, which include the major group of Digenea and the minor group of Aspidogastrea (Cribb et al., 2001, 2002). Monogeneans are external parasites that lack intermediate hosts and asexual reproduction, and consequently, their infections are restricted to hosts from water environments, mainly bony fishes. The intestinal parasitism of cestodes and trematodes allows them to exploit a wide range of hosts from aquatic and terrestrial environments (Cribb et al., 2002). In contrast to monogeneans, digenean trematodes evolved complex life cycles which typically involve larvae that reproduce asexually in one or more intermediate invertebrate or vertebrate hosts and an adult stage that reproduces sexually in a definite vertebrate host. The asexual reproduction of larvae was postulated to play a major role in the origin of the spectacular diversity of digenean trematodes (Cribb et al., 2001, 2002; Esch et al., 2002; Poulin & Cribb, 2002).

Transmission via multiple hosts makes digenean trematodes important players in multitrophic interactions (Mouritsen & Poulin, 2005). Although the role of digenean trematodes in the ecology and evolution of freshwater communities has been addressed by many studies of gastropods (Jokela & Lively, 1995; Sorensen & Minchella, 2001; Hechinger & Lafferty, 2005), interactions between digenean trematodes and their bivalve hosts remain relatively poorly understood. We now know that bivalves are used as intermediate hosts by several families of digenean trematodes (Overstreet & Curran, 2002; Campbell, 2008; Grizzle & Brunner, 2009). For example, swan mussels (*Anodonta cygnea*) serve as the intermediate host of the digenean trematode *Phyllodistomum elongatum* (Gorgoderidae), and zebra mussels (*Dreissena polymorpha*) are the intermediate host of *Phyllodistomum folium* (Orecchia et al., 1975; Laruelle et al., 2002). The life cycle of these parasites is completed in Cyprinidae fishes that serve as definite hosts (Orecchia et al., 1975; Peribanez et al., 2006, 2011). Our study organisms, duck mussels (*Anodonta anatina*), are the first intermediate host of the digenean trematode *Rhipidocotyle campanula* (Bucephalidae), which further transmits through the next intermediate host, the common roach (*Rutilus rutilus*), before entering its definite hosts, either the perch (*Perca fluviatilis*) or the zander (*Stizostedion lucioperca*) (Taskinen et al., 1991; Gibson et al., 1992). Fragmentary evidence suggests that digenean trematodes may

affect physiology (Jokela et al., 1993), growth (Taskinen, 1998), and reproductive ability (Taskinen & Valtonen, 1995) of their bivalve hosts. Similarly, aspidogastrea trematodes were observed to negatively impact physiology and growth of their mussel hosts (Gangloff et al., 2008). The selective pressure of digenean trematodes has only occasionally been considered in research on bivalve life history (Haukioja & Hakala, 1978; Jokela & Mutikainen, 1995, but see Jokela et al., 2005). Even less is known about the biotic and abiotic factors that determine infection rates in populations of these mollusks.

An understanding of the distribution and biological impacts of digenean trematodes on ecosystems requires consideration of a wide array of conditions that affect the life cycle dynamics of these parasites. Our study aims to identify the factors that shape the abundance of digenean trematodes in duck mussels. We studied duck mussels in eighteen lakes, which differed in their physicochemical conditions and in the intensity with which duck mussels were encrusted by zebra mussels. This large-scale comparative approach allowed us to test our hypotheses in a gradient of environmental conditions. First, we assessed how mussel age, sex, and zebra mussel overgrowth were correlated with infection rates. Infection by free-swimming trematode larvae (miracidium) should be correlated with the volume of water processed by a mussel host (Taskinen & Valtonen, 1995). We therefore expected to observe an increase in the prevalence of parasites in older mussels. Additionally, given that reproduction imposes more physiological costs on unionid females than on males (Heino & Kaitala, 1996), we expected higher infection rates in females. We then examined whether parasites occupied the gonads and hepatopancreases of their hosts with equal frequency and what type of damage they caused to the host tissue. We also quantified changes in mussel fertility associated with infections. Finally, we investigated whether environmental conditions in the lakes can explain differences in the abundance of parasites among mussel populations. Earlier studies suggest that parasitism among mussels might attain its highest rates in lentic habitats and nutrient-enriched streams (Humes & Jamnback, 1950; Fuller, 1974), but there were no studies directly investigating the links between physicochemical parameters and parasitism. Given the scarcity of data, we generally hypothesized that eutrophic waters with low oxygen supply would worsen the physiological



Fig. 1 Duck mussels, *A. anatina*, were studied in 18 lakes in northeastern Poland: Bartąg (Bg), Bartężek (Bk), Gant (Gt), Guzianka Wielka (Gw), Harsz Wielki (Hw), Kiełpińskie (Kl), Kiersztanowskie (Kr), Kierzlińskie (Kz), Kukowino (Ko),

Leleskie (Le), Łąkorz (Lz), Łaśmiady (Ly), Majcz Wielki (Mw), Mokre (Mo), Rydzówka (Ra), Sarąg (Sg), Sunowo (So), and Szymbarskie (Se)

state of duck mussels and result in an increased susceptibility to infections. Moreover, more productive lakes should support larger populations of fishes, which serve as intermediate and final hosts of digenean trematodes, increasing this way the exposure of duck mussels to infections by miracidia.

Methods

Abiotic conditions in lakes

We conducted our study in 18 duck mussel populations from dimictic lakes in northeastern Poland (Fig. 1). Each lake belongs to a separate drainage basin (Jańczak, 1999). We characterized the environmental conditions in these lakes using 15 physico-chemical parameters (Table 1). The parameters were measured between 2003 and 2007 during the annual water quality monitoring program of the Regional Inspectorate of Environmental Protection in Olsztyn, Poland (ISO/IEC 17025 standards). To analyze the relationships among the studied environmental parameters and to reduce their number in further statistical analyses, we performed a factor analysis (with bi-quartimax normalized rotation) on them (Statistica 10, Statsoft, Poland). If necessary, parameters were transformed by natural logarithms prior to the analysis to attain normality (Table 1).

Mussel collection and shell measurements

Approximately, 200 *A. anatina* mussels were collected in mid-September 2008 and 2009 in the littoral zone of each lake by SCUBA divers and by hand. At this time of the year, glochidia are typically fully developed in females of this bradyctictic species (Piechocki, 1969; Haukioja & Hakala, 1978). The mussels were transported alive to the Hydrobiological Station of the Polish Academy of Sciences in Mikołajki. Encrusting zebra mussels, *D. polymorpha*, were detached from *A. anatina* shells and weighed to the nearest 0.01 g on an electronic balance. The shells of *A. anatina* were separated from the soft body by cutting the anterior and posterior adductor muscles. The length, width, and height of each valve were measured to the nearest 0.01 mm with a Vernier caliper. The measurements of each dimension were averaged. A geometric three-dimensional mean was used as an index of body size (Czarneński et al., 2003). Mussel ages were assessed based on the annual ring deposition in each shell according to the methods of Haukioja & Hakala (1978).

Sex identification

Mussel sex was determined under a light microscope (Eclipse E200, Nikon, Japan) by an examination of gonad fluid. Individuals with oocytes in ovaries were

Table 1 Two factors were extracted by the factor analysis (biquartimax normalized rotation method) from the data on the physicochemical conditions in 18 Polish lakes (Fig. 1)

Physicochemical parameters	Unit	Factor 1	Factor 2
Mean oxygen saturation of hypolimnion _{in}	[%]	-0.68	0.09
Biochemical oxygen demand _{in} (summer, surface)	[mgO ₂ /dm ³]	0.51	0.04
Biochemical oxygen demand _{in} (summer, bottom)	[mgO ₂ /dm ³]	0.70	0.27
Phosphates _{in} (summer, bottom)	[mgP/dm ³]	0.92	-0.12
Total phosphorus _{in} (summer, bottom)	[mgP/dm ³]	0.93	-0.16
Total phosphorus _{in} (summer, surface)	[mgP/dm ³]	0.76	0.08
Ammonium nitrogen _{in} (summer, bottom)	[mgN/dm ³]	0.82	-0.33
Total nitrogen _{in} (spring/summer, surface average)	[mgN/dm ³]	0.49	0.50
Chlorophyll _{in} (spring/summer, surface average)	[mg/m ³]	0.74	0.30
Secchi disk _{in} (spring, summer)	[m]	-0.80	-0.24
Maximal depth _{in}	[m]	-0.61	0.22
Water quality index	1.33–3.29	0.87	0.24
Ca ²⁺ (spring, surface)	[mgC/dm ³]	-0.27	0.77
pH	— ln ₁₀ [H ₃ O ⁺]	0.54	0.40
Temperature (summer, at 3-m depth)	[C°]	-0.49	-0.77
Proportion of explained variance		0.49	0.14

The value of the coefficients (factor loadings) reveals the impact of the environmental parameters on the factor values. High values of Factor 1 reflect high eutrophication levels (trophy index). High values of Factor 2 reflect high Ca²⁺ levels and low water temperatures in the lakes (Ca²⁺/temperature index)

classified as females, whereas individuals with spermatozoa and/or spermatid morulae in testes were classified as males (Heard, 1975; Kotrla, 1989). Mussels with both oogenetic and spermatogenetic cells were classified as hermaphrodites (van der Schalie, 1970). The sex identification of individuals in the undifferentiated or spawning stage and/or with gonad tissues damaged by trematode larvae was performed with histological methods. Samples of

gonads collected from such mussels were fixed in 6% buffered neutral formalin for 2 weeks and dehydrated in graded ethanol (70, 80, 90, and 96%) series and a butyl alcohol. After clearing in chloroform, the samples were embedded in paraffin wax. Serial 5- μ m-thick sections were cut with a motorized rotary microtome (RM 2155, Leica, Germany), stained progressively with Ehrlich's hematoxylin (Carl Roth, Germany), counterstained with water eosin Y (Fluka, Germany), cleared in ST Ultra (Leica), and mounted with CV Ultra (Leica). The gonad cross sections were examined for the presence of oocytes in ovaries and spermatozoa and/or spermatid morulae in testes under a light microscope (Eclipse 80i, Nikon) equipped with a digital camera (Nikon) and NIS Elements analysis software (Nikon).

Glochidia number assessment

The outer demibranches of mussel gills carrying larvae were cut from the visceral mass and fixed in 70% ethanol. The assessment of glochidia number—our measure of female fecundity—was performed on mussel subsamples. From each population and each age class, we randomly sampled two gravid females not infected by trematode larvae (in some cases, only 1 female was available), and one gravid female that was infected by trematode larvae (if available). To separate the glochidia from the gill tissue, the outer demibranches were dissected, fragmented with a scalpel, and placed in a plastic beaker filled with 200 ml of tap water. The mixture was then stirred for 7 min with an electric stirrer. Next, the beaker was filled with water up to 1,000 ml and placed on a magnetic stirrer to create a homogenous suspension of glochidia in the water column. Eight 1 ml samples of glochidia suspension were taken with an Acura[®] 810 (Socorex Isba SA, Switzerland) adjustable micropipette placed near the beaker wall, halfway down the water column. The samples were placed in wells of cell culture plates (BD Falcon[™]) and photographed under a stereoscopic microscope (SZ CTV, Olympus, Japan) equipped with PixeLINK ver. 4.82 camera and Lucia Measurement image software (Lim Laboratory Imaging, Czech Republic). The pictures were used to count glochidia in the samples, and this information was then used to assess the total number of glochidia in the 1,000 ml volume of glochidia suspension, which

translated into the number of glochidia brooded by an individual female.

Parasitological analyses

Larval stages of digenetic trematodes were examined under a light microscope (Eclipse E200, Nikon) in the gonads and hepatopancreas of dissected mussels. Trematode species were identified on the basis of live cercariae morphology (Orecchia et al., 1975; Baturo, 1977; Richardson, 1990; Taskinen et al., 1991). To determine the damage inflicted on the gonadal tissue by trematode larvae, organs harboring sporocysts and/or cercariae were fixed in 6% buffered neutral formalin, and histological slides were prepared following the procedure used in sex identification.

Hypothesis testing

Predictors of infection rates

All statistical analyses were performed in Statistica 10 (StatSoft, Poland). First, we identified the predictors of infection rates in mussels. We classified mussels as being infected if our histological analyses of gonads and hepatopancreas revealed the presence of trematode larvae in either organ (we did not differentiate between trematode species in this analysis). We examined these data with a generalized linear model (GLM) with a binomial distribution and logit link function. The model considered population and sex as grouping predictors and mussel age (years) and the total mass of overgrowing zebra mussels (our index of overgrowth) as numerical predictors. We excluded hermaphrodites from this analysis. The mass of zebra mussels was transformed by natural logarithms after adding 0.01 because some *A. anatina* were not overgrown by zebra mussels. The infection level estimates for lakes (hereafter the infection level) represent values that would be expected if the lakes were uniform with respect to the sex ratio, age structure, and overgrowth intensity. These values were further used in the analysis of partial correlations to test whether cross-population differences in the infection rates correlated with environmental conditions in the lakes.

Our factor analysis of lake environmental parameters revealed two major factors, which together explained 63% variance in the environmental data

(Table 1). Factor 1 was primarily determined by parameters closely related to the lake trophic status (hereafter trophy index). Factor 1 was positively correlated with the measures of biochemical oxygen demand, the amount of phosphates and total phosphorous, the amount of ammonium nitrogen, and the chlorophyll concentration and negatively correlated with Secchi disk visibility, maximal lake depth, and hypolimnetic oxygen saturation. Factor 2 was positively correlated with the Ca^{2+} and negatively correlated with the water temperature (hereafter Ca^{2+} /temperature index). To test whether conditions captured by the two factors were linked to the infection level in the populations, we performed an analysis of partial correlations between the infection level index and the indices of trophy and Ca^{2+} /temperature.

Distribution of trematode larvae in organs

We tested whether trematode larvae preferentially occupied gonads or the hepatopancreas. The analysis involved only infected mussels. Given the scarcity of data on infected mussels, we analyzed the data on both sexes and hermaphrodites pooled together. Based on our histological examination of organs, we assigned each infected mussel with a status value of -1 (parasites in hepatopancreas only), 0 (parasites in gonads and hepatopancreas), or 1 (parasites in gonads only)—hereafter known as the organ infection index. We calculated the mean values of the organ infection index in each population. Using Student's *t* test, we compared these values against the zero value. Under this scenario, a rejection of the null hypothesis indicates that the infection rates in the populations are biased toward one organ or the other. Retention of the null hypothesis indicates that infections are randomly distributed between the two organs. We first analyzed mussels infected with all trematodes pooled together and then conducted separate analyses on mussels infected with a given trematode.

Effects of infection on glochidia production

We tested whether the infection with trematode larvae affects the production of glochidia by females. In the first analysis, we used the GLM to examine whether the proportion of females with glochidia differed between the infected and uninfected females. The model considered population and infection status as

grouping factors and age as a numerical covariate. The second analysis included only females that incubated glochidia. We used the General Linear Model to examine whether glochidia abundance differed between the infected and uninfected females. The model considered populations as a random grouping factor, infection status as a fixed grouping factor, body size index as a numeric covariate, and the interaction between population and the body size index. Note that significance of this interaction would indicate that the scaling between female size and glochidia number differed among populations. Prior to this analysis, the data on glochidia number were transformed with natural logarithms.

Results

Histological and parasitological analyses

We collected a total of 3,535 mussels (2,111 females, 1,280 males, and 47 hermaphrodites; Online Resource). We did not identify the sex of 97 individuals that had already totally spawned or with gonads which were completely damaged by parasites. In total, 7.5% of the mussels were infected with trematode larvae. We classified trematodes to the species as *Rhipidocotyle campanula* or *Phyllodistomum* sp. (Online Resource). The infection rates ranged from 0.5% in Lake Rydzówka to 27.0% in Lake Gant (Online Resource).

The gonads of parasite-free mussels were filled solely with reproductive follicles containing the reproductive cells of the host, whereas infected individuals also had a fraction of follicles housing parasitic larvae at different developmental stages (Fig. 2A, B, D). The majority of male and female gonads of *A. anatina* were found in the final phase of the reproductive cycle. In males, spermatogenic follicles contained spermatid morulae and/or unspawned spermatozoa (Fig. 2B). In females, the ovaries were filled with degenerating unspawned oocytes (Fig. 2D). In heavily infected hosts, the reproductive follicles almost exclusively contained parasitic larvae (Fig. 2A). Serial cross sections of parasitized follicles revealed digenean trematode larvae in the same developmental stage or in different larval stages, such as germ balls and immature and mature cercariae (Fig. 2A, B, D). Cercariae in a wide range of

developmental stages were commonly found within sporocyst lumina and most frequently directly inside the reproductive follicles (when the sporocysts burst open) (Fig. 2B). The wall of the reproductive follicles in infected hosts was thicker than that in healthy individuals due to muscle cells proliferation in the membrane of reproductive follicles (Fig. 2A). Invading hemocytes were also visible in mussel testes and ovaries (Fig. 2C). A few solitary digenean trematode larvae were found between hemocel spaces. The proliferation of parasitic larvae caused substantial damage to vast areas of the host reproductive tissues (Fig. 2A).

Predictors of infection rates

Our GLM analysis (Table 2) revealed that the infection rate generally increased with mussel age ($P < 0.002$) but was independent of the overgrowth by zebra mussels ($P = 0.50$). Controlling for the effects of mussel age, populations differed with respect to the fraction of infected individuals ($P < 0.001$; Fig. 3A), and females were infected more often than males ($P = 0.001$; Fig. 3B). The partial correlation analysis revealed that neither the trophy index nor the Ca^{2+} /temperature index (Table 1) explained the cross-population differences in infection rates ($r = -0.08$, $P = 0.74$ and $r = 0.26$, $P = 0.30$, respectively).

Distribution of trematode larvae in organs

The mean value of the organ infection index was positive in all three considered types of infection (all trematodes: 0.36, *R. campanula* 0.31, *Phyllodistomum* sp. 0.32; Fig. 4). Student's *t* test revealed that the organ infection indices calculated for all infected mussels (238 mussels, including individuals with unidentified trematode species) and for mussels infected with *R. campanula* (159 mussels) were both significantly larger than 0 ($t = 5.14$, $df = 17$, $P < 0.001$ and $t = 4.65$, $df = 13$, $P < 0.001$, respectively), indicating that in all infected mussels and in mussels infected with *R. campanula*, the trematode larvae occupied the gonads more frequently than the hepatopancreas. Although the mean value of the organ infection index calculated for the mussels infected with *Phyllodistomum* sp. (56 mussels) was also positive, its value was not significantly different from 0 ($t = 1.71$, $df = 12$, and $P = 0.11$).

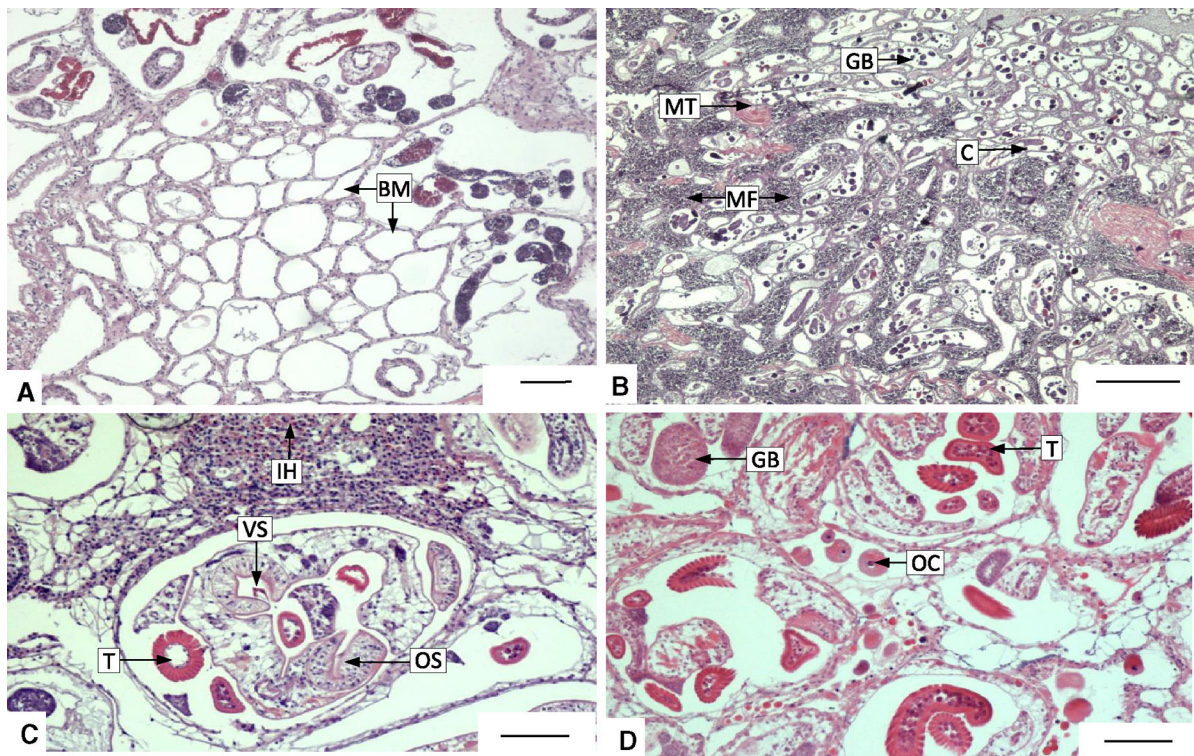


Fig. 2 Damage to the gonads of *A. anatina* caused by *R. campanula* (A–B) and *Phyllodistomum* sp. (C–D). **A** Reproductive follicles of duck mussels with thickened wall in the basement membrane of reproductive follicles (scale bar 100 µm). **B** Male follicles with spermatid morulae and unspawned spermatozoa. Follicles contain parasitic larvae in different developmental stages (scale bar 500 µm). **C** Invading hemocytes in mussel gonads (scale bar 100 µm). **D** Female

follicles with degenerating unspawned oocytes and with parasitic larvae in different developmental stages (scale bar 100 µm). *BM* basement membrane of follicle, *C* cercariae, *GB* germ balls, *IH* invading hemocytes, *MF* male reproductive follicles with sperm and/or spermatid morulae, *MT* muscle tissue, *OC* oocytes, *OS* oral sucker, *T* tail of cercariae, and *VS* ventral sucker

Table 2 Comparison of the infection rate with trematode larvae and the frequency of brooding females in populations of the duck mussel *Anodonta anatina* (inferential statistics of two Generalized Linear Models)

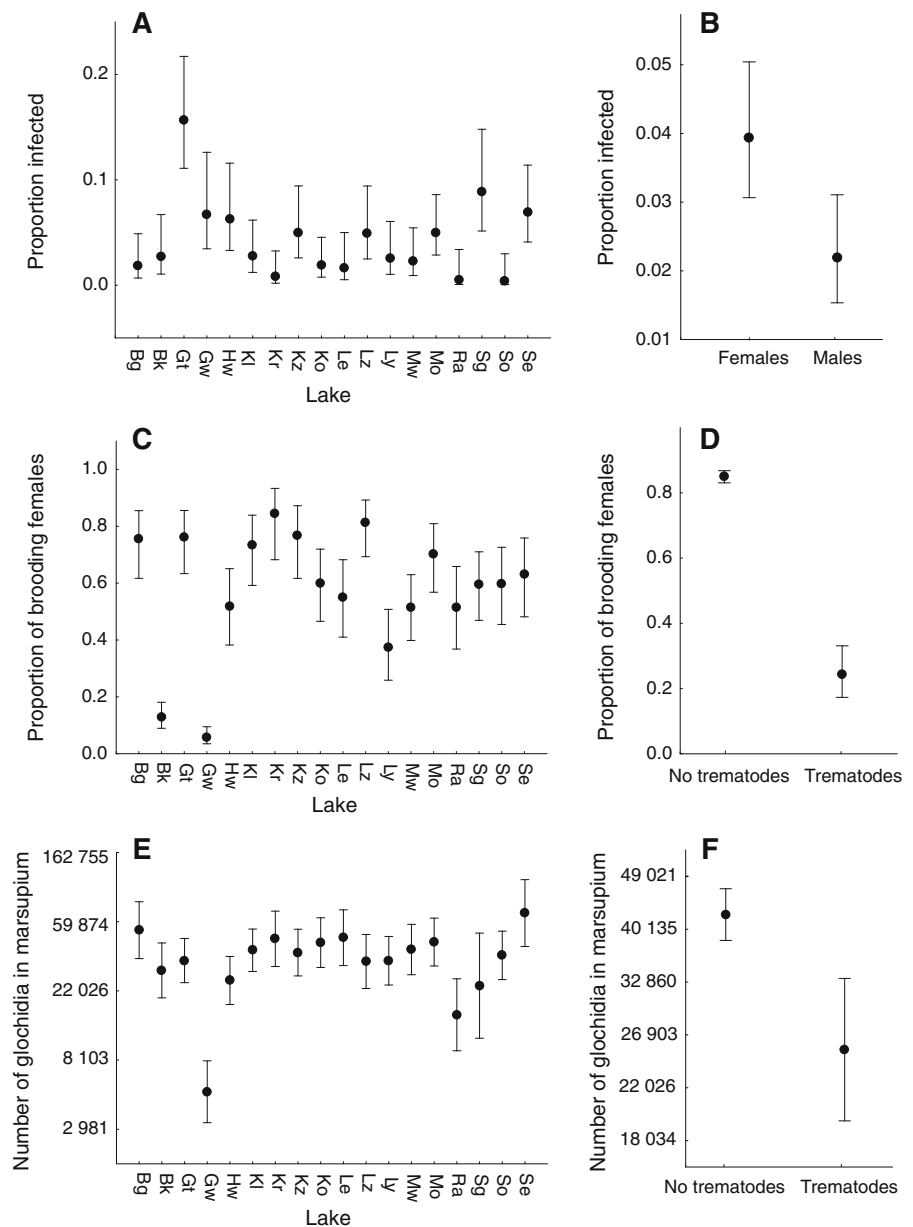
Dependent variable	Predictor	df	Wald statistics	<i>P</i>
Infection with trematodes	Population	17	83.66	<0.001
	Sex	1	10.66	0.001
	Age (covariate)	1	11.20	<0.002
	Mass of overgrowing zebra mussels (covariate)	1	0.46	0.50
Presence of glochidia	Population	17	345.44	<0.001
	Infection with trematodes	1	146.83	<0.001
	Age (covariate)	1	16.14	<0.001

Effects of infection on glochidia production

The GLM analysis of the presence of glochidia in females (Table 2) showed that the proportion of females which incubated glochidia increased across age classes ($P < 0.001$). A comparison of mussels at a given age demonstrated that the proportion of females incubating glochidia differed among the populations ($P < 0.001$; Fig. 3C) and was significantly higher in the uninfected females ($P < 0.001$; Fig. 3D).

The General Linear Model of female fecundity (Table 3) demonstrated that the number of brooded glochidia generally increased with female size ($P < 0.001$). At a given body size, females differed in the number of brooded glochidia among populations ($P < 0.001$; Fig. 3E). The size dependence of

Fig. 3 The proportion of *A. anatina* infected with trematode larvae differed among lakes (A), and females were more heavily infected than males (B). The proportion of brooding females differed among lakes (C), and brooding females were less frequent among mussels infected by trematode larvae (D). The number of glochidia carried by brooding females differed among lakes (E) and infected females brooded fewer glochidia than parasite-free females (for convenience data on glochidia number are shown after reverse transformation from natural logarithms). The mean values (\pm CI) were estimated by statistical models summarized in Table 2 (Fig. 3A–D) and Table 3 (Fig. 3E–F). The lake names are as given in Fig. 1



fertility differed among populations, as indicated by the significant interaction between population and body size index ($P < 0.001$). On average, females infected with trematode larvae incubated fewer glochidia than uninfected females ($P < 0.001$; Fig. 3F).

Discussion

The studied populations of duck mussels hosted larvae of digenean trematodes from two genera: *Rhipidocotyle*

and *Phyllodistomum*. One of the two parasites, *R. campanula*, has been frequently reported in duck mussels (Taskinen & Valtonen, 1995; Taskinen et al., 1997; Taskinen, 1998) and closely related species (Richardson, 1990), but the larvae of trematodes from the *Phyllodistomum* genus have only occasionally been found in this species of mussels (Taskinen et al., 1991). *Phyllodistomum* larvae have been reported in swan mussels *A. cygnea* (Orecchia et al., 1975), zebra mussels *D. polymorpha* (Laruelle et al., 2002), and North American unionids (Grizzle & Brunner, 2009).

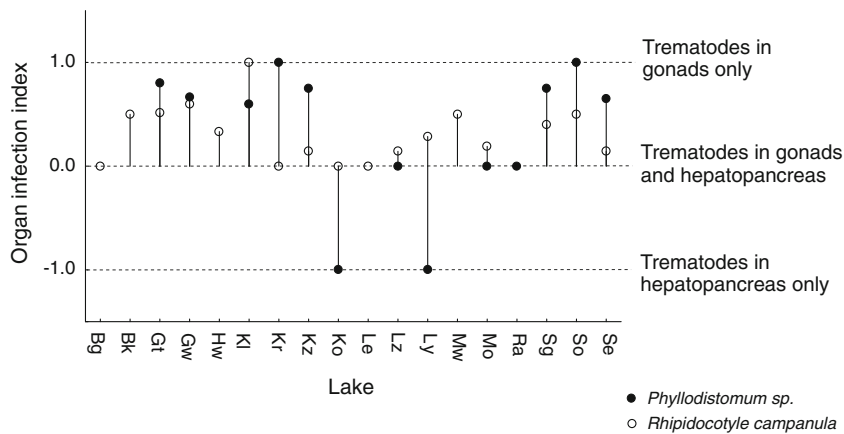


Fig. 4 Larvae of *R. campanula* were more often found in the gonads than in the hepatopancreas of *A. anatina*, but the larvae of *Phyllodistomum* sp. were evenly distributed between the two organs. To examine the distribution of trematodes in the organs, infected mussels were assigned an organ infection index: infection only in gonads (1), infection only in hepatopancreas

(−1), and infection in both organs (0). If a trematode occurs randomly in both organs, the mean value of the organ infection index in a population is equal to 0. If the mean value of the index deviates from 0, either the gonads (deviation toward 1) or the hepatopancreas (deviation toward −1) are more often infected. The lake names are as given in Fig. 1

Table 3 Comparison of the number of glochidia brooded by females in populations of *Anodonta anatina* mussels (inferential statistics of a General Linear Model)

Dependent variable	Predictor	df	Effect	F	P
Glochidia number	Population	17	Random	3.19	<0.001
	Infection with trematodes	1	Fixed	13.19	<0.001
	Body size index (covariate)	1	Fixed	71.11	<0.001
	Population × body size index	17	Random	3.10	<0.001
	Error	222			

Our results demonstrated that infections with trematode larvae became more prevalent with increasing mussel age and that female mussels were infected more often than males. Competition for food and oxygen with zebra mussels decreases the energy budgets of overgrown unionids (Patterson, 1997). Therefore, we expected that duck mussels encrusted by zebra mussels would have fewer resources to support immune defense, which should place them at a higher risk of infection. Although we observed that infections with trematodes involved the immunological reactions of hosts, we did not find evidence that overgrown mussels were more prone to infections.

The increased abundance of trematode larvae among older mussels and females was previously observed by Taskinen & Valtonen (1995) in one population of duck mussels, but detection of the same pattern by our large-scale study suggests that the age and sex dependence of the infection rates may be a

widespread phenomenon in duck mussels and in other freshwater mussels and gastropods. Indeed, a link between trematode infections and the age and sex of the hosts has been found in other freshwater bivalves (Rantanen et al., 1998) and in aquatic gastropods (Jokela & Lively, 1995; Sorensen & Minchella, 2001; Graham, 2003). In filter-feeding mussels, the increase in the infection rates with age reflects the amount of water processed by the hosts in a lifespan (Taskinen & Valtonen, 1995). It remains unclear whether differential filtration rates also account for the female-biased infection rates. Alternatively, the female-biased infections may indicate tradeoffs in the allocation to reproduction and antiparasitic defense: offspring-brooding females spend more resources for reproduction than males (Heino & Kaitala, 1996), which should make females allocate fewer resources to other functions.

Development of trematode larvae likely depends on the amount of resources extracted from their hosts and

the timespan of infection. Looking from this angle at host-parasite interactions, an alternative class of models postulates that age- and sex-related differences in the infection rates of molluscan hosts reflect the preference of parasites for hosts which provide the best conditions for propagation (Jokela et al., 1993; Guralnick et al., 2004). The physical damage and resource extraction by parasites may negatively affect host longevity and thus the timespan of infection. These adverse effects can be minimized by the exploitation of resources not directly involved in host survival, such as the resources involved in host reproduction. In line with this expectation, we more often observed trematode larvae in the gonads than in the hepatopancreas of duck mussels. Previously, Taskinen et al. (1997) reported that the gonads of duck mussels in two Finnish lakes were more heavily parasitized by *R. campanula* and *R. fennica* than other organs, and Jokela et al. (1993) directly demonstrated that *R. fennica* primarily used the glycogen reserves of duck mussels stored for reproduction. Apparently, larval trematodes maximize their own fitness through preferences for hosts with the highest levels of resource allocation to reproduction. In indeterminately growing hosts such as many mollusks, the allocation of resources to reproduction increases with age and size (Czarnoleski & Kozłowski, 1998) and is higher in females (Heino & Kaitala, 1996).

Digenean trematodes are typically viewed as complete castrators of molluscan hosts (Kuris, 1974). Our histological analysis of duck mussels documented various levels of damage to the male and female reproductive organs caused by trematode larvae, though some infected mussels were still ready for reproduction and possessed reproductive cells in their gonads. The structural damage of gonads coupled with energetic costs of infections should negatively impact offspring production of the hosts, which explains why we observed fewer broods and glochidia among the infected females. These findings agree with earlier reports that the larvae of digenean trematodes reduced fecundity of duck mussels (Taskinen et al., 1997), other freshwater mussels (Rantanen et al., 1998) and gastropods (Sorensen & Minchella, 2001).

Taken together, our results suggest that the pressure exerted by digenean trematode larvae can significantly affect the reproductive output of duck mussels. The next important question is what factors differentiate the strength of this pressure among populations of

hosts. Thus far, we have demonstrated that host age and sex play a role in the infections, but apparently, these processes alone cannot account for the cross-population differences in the infection rates of duck mussels. We hypothesize that lake-specific environmental conditions may explain cross-population differences in the infection rates, but parameters related to lake trophy, thermal conditions, or Ca^{2+} availability did not appear to be good predictors of the infection rates in the studied populations of duck mussels. This finding was surprising because lake trophy should influence the abundance of fishes that are used by *Rhipidocotyle* and *Phyllodistomum* trematodes as higher-order hosts. Moreover, our analysis involved major environmental determinants of the energy budgets of mussels (Czarnoleski et al., 2010), which should affect resistance to parasitic infections. High values of our trophy index and low values of our Ca^{2+} /temperature index indicate lower supplies of oxygen and higher metabolic demands associated with the thermal sensitivity of physiology and costliness of shell formation. In addition, thermal conditions should directly affect the risk of encountering miracidia through the thermal dependence of filtration rates. Our results also contrast with the findings of experiments which demonstrated that the exposure of duck mussels to extreme environmental conditions (anoxic stress) in the laboratory increased their susceptibility to infections by copepods (Saarinen & Taskinen, 2005) and the virulence of trematode infections (Jokela et al., 2005).

Conclusion

The results of this study suggest that the trematodes *Rhipidocotyle campanula* and *Phyllodistomum* sp. play a significant role in freshwater communities by affecting the reproductive success of their intermediate hosts—duck mussels. Although our results demonstrated that the pressure posed by digenean trematode larvae is tightly linked to the age and sex of their hosts, a significant portion of the variation in the infection rates remained unexplained. Quite unexpectedly, environmental factors that affect the mussels' energy budgets and the abundance of higher-order hosts of digenean trematodes failed to explain this variance. A fuller understanding of the role digenean trematodes play in freshwater communities

may require the integration of information about all factors that affect the dynamics of parasite life cycles, including their demographic processes in intermediate and final hosts. Our knowledge of the interactions between unionidae mussels and their digenean trematode parasites may help protect these imperiled mollusks.

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