

# Trematode cercariae as prey for zooplankton: effect on fitness traits of predators

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## Research Article

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### Abstract

Removal of parasite free-living stages by predators has previously been suggested an important factor controlling parasite transmission in aquatic habitats. Experimental studies of zooplankton predation on macroparasite larvae are, however, scarce. We tested whether trematode cercariae, which are often numerous in shallow waters, are suitable prey for syntopic zooplankters. Feeding rates and survival of freshwater cyclopoids (*Megacyclops viridis*, *Macrocyclus distinctus*), calanoids (*Arctodiaptomus paulseni*), cladocerans (*Sida crystallina*) and rotifers *Asplanchna* spp., fed with cercariae of *Diplostomum pseudospathaceum*, a common fish trematode, were studied. In additional long-term experiments, we studied reproduction of cyclopoids fed with cercariae. All tested zooplankton species consumed cercariae. The highest feeding rates were observed for cyclopoids ( $33 \pm 12$  cercariae ind<sup>-1</sup> h<sup>-1</sup>), which actively reproduced (up to one egg clutch day<sup>-1</sup>) when fed *ad libitum* with cercariae. Their reproductive characteristics did not change significantly with time, indicating that cercariae supported cyclopoids' dietary needs. Mortality of rotifers and cladocerans was high (25–28% individuals) when exposed to cercariae in contrast to cyclopoids and calanoids (<2%). Cercariae clogged the filtration apparatus of cladocerans and caused internal injuries in predatory rotifers, which ingested cercariae. Observed trophic links between common freshwater zooplankters and cercariae may significantly influence food webs and parasite transmission in lentic ecosystems.

## Introduction

Though parasites may constitute a substantial part of total biodiversity and biomass in aquatic ecosystems (Hudson *et al.*, 2006; Kuris *et al.*, 2008; Preston *et al.*, 2013), they are traditionally ignored when studying food-webs. In the last decades, it has been shown that parasite–predator links are very abundant (36–44% of observed trophic links) in aquatic food webs (Lafferty *et al.*, 2006; Amundsen *et al.*, 2009). A large share of these links is formed by the concomitant predation when parasites are consumed together with their hosts (Johnson *et al.*, 2010). However, direct feeding on parasite free-living stages is also common in aquatic ecosystems (Lafferty *et al.*, 2008; Morley, 2012). Thus, almost all groups of benthic organisms (e.g. carnivorous plants, cnidarians, turbellarians, oligochaetes, bryozoans, mollusks, crustaceans, insect larvae, fishes) were reported to consume free-living stages of parasites (reviewed in Thieltges *et al.*, 2008a; Johnson *et al.*, 2010; Orlofske *et al.*, 2015; Gopko *et al.*, 2017a).

The role of zooplankters as predators of parasites is much less studied. Many zooplankters feed on nano- and microparticles and were found to effectively clean water of various pathogenic bacteria (*Escherichia coli*, fecal coliforms, streptococci), cysts of parasitic protists (*Giardia*) and zoospores of amphibian chytrid fungus (reviewed in Buck *et al.*, 2011; Hamilton *et al.*, 2012; Jasper *et al.*, 2013; Schmeller *et al.*, 2014). Some zooplankters (copepods) were reported to consume macroparasites and vectors, for example, juvenile nematodes (Achinelly *et al.*, 2003) and mosquito larvae (reviewed in Marten and Reid, 2007).

The information about predation of zooplankton on trematode free-living stages (miracidia and cercariae) is still scarce though trematode larvae, especially cercariae, are often numerous in plankton (Morley, 2012). The abundance of cercariae can reach up to 300 ind. L<sup>-1</sup> in still freshwater habitats (Morley, 2012) and their annual production in estuaries can constitute 10–43 kg ha<sup>-1</sup> year<sup>-1</sup> (Kuris *et al.*, 2008). On average, a single infected *Lymnaea stagnalis* snail can release more than 37 000 cercariae of the present study species, *Diplostomum pseudospathaceum*, daily (Karvonen *et al.*, 2004a). The smallest trematode free-living stages are <200 µm in size and therefore can serve as prey for many planktonic crustaceans (e.g. raptorial cyclopoids and cladocerans), which are voracious predators of similar-sized motile food objects (Cummins *et al.*, 1969; Kerfoot, 1977; Brandl, 1998).

However, to our knowledge, there is only one study (Christensen *et al.*, 1977) on feeding of zooplankton (*Daphnia pulex*) on miracidia (the first free-living stage of trematodes) and just a few focused on consumption of cercariae (Christensen, 1979; Tokobaev *et al.*, 1979; Christensen *et al.*, 1980; Intapan *et al.*, 1992; Schotthoefter *et al.*, 2007). For instance, cercariae

of different trematode species are ingested by the predatory rotifer (*Eosphora ehrenbergi*) (Tokobaev et al., 1979). Reported estimates of consumption of cercariae by planktonic crustaceans vary from a considerable effect (Christensen, 1979; Christensen et al., 1980; Intapan et al., 1992) to the absence of a feeding response (Schotthoefer et al., 2007). Moreover, the influence of cercarial prey on the fitness traits of predators is still unknown. Cercariae contain glycogen and lipid stocks (Thieltges et al., 2008b; Morley, 2012) that make them suitable food for zooplankters. In still waters, dense ‘clouds’ of cercariae are formed around infected freshwater snails and mussels, (Lyholt and Buchmann, 1996; Taskinen, 1998; Karvonen et al., 2004a; Morley, 2012), the typical first intermediate hosts of trematodes. Such cercarial aggregations could be easily detected and consumed by planktonic predators living in the same shallow water habitats, especially by selective-feeders, which effectively search the attractive prey.

The key role of zooplankton in aquatic food webs and high biomass and nutritional value of trematode cercariae (Thieltges et al., 2008b; Preston et al., 2013) suggest that these trophic links could be important. Removal of free-living stages of parasites by predators can strongly reduce parasite transmission in aquatic ecosystems resulting in decreased infection intensities and prevalences in host organisms (Schotthoefer et al., 2007; Orlofske et al., 2012; Gopko et al., 2017a).

The present study aims to assess the role of trematode cercariae as a food source for syntopic (sharing the habitat with mollusks producing cercariae) zooplankton organisms. We experimentally tested (1) the ability of rotifers, cladocerans and copepods to consume cercariae of the common fish trematode (eye fluke, *D. pseudospathaceum*) and (2) the effect of cercarial diet on the fitness-related traits of planktonic predators. Our hypotheses were (1) that certain freshwater zooplankters can consume/eliminate trematode cercariae and (2) that cercariae can serve as nutritious food objects, supporting their growth and reproduction.

## Materials and methods

### Study objects

Experiments were conducted at the Konnevesi research station (University of Jyväskylä, Finland) in summer 2016. We used cercariae of the eye fluke, *D. pseudospathaceum* for our study, because this parasite is very common in lymnetic systems of temperate and boreal zones and can hamper fish farming (Shigin, 1980; Karvonen et al., 2006). *Diplostomum pseudospathaceum* infects mollusks (first intermediate host), many fishes (second intermediate host) and fish-eating birds (definitive host) (Valtonen and Gibson, 1997; Karvonen et al., 2006). It decreases host fitness by reducing vision in fish (Owen et al., 1993; Karvonen et al., 2004b) and manipulates host behaviour to increase parasite transmission (Seppälä et al., 2004; Mikheev et al., 2010; Gopko et al., 2015, 2017b). In addition, cercariae of *D. pseudospathaceum* have different size, morphology and behaviour than most that have been used in previous feeding experimental studies (Christensen, 1979; Tokobaev et al., 1979; Christensen et al., 1980; Intapan et al., 1992; Schotthoefer et al., 2007).

Cercariae of *D. pseudospathaceum* were obtained from the infected pond snails *Lymnaea stagnalis* collected from the shallow nearshore habitats of Lake Konnevesi. Between the experiments, snails were stored in the dark at 4 °C. To induce cercariae shedding, they were placed under bright light at 18 °C for 2 h before each experiment. Zooplankton was collected from the same site (depth 0.5–3 m) as snails. Copepods and cladocerans were collected with a plastic bucket (to avoid their damage) and rotifers

with a small Epstein plankton net (70- $\mu$ m mesh). In the laboratory, we picked out individuals of several common planktonic species with a pipette (rotifers *Asplanchna* spp., cyclopoids *Megacyclops viridis*, *Macrocyclops distinctus*, calanoids *Arctodiaptomus paulseni*, cladocerans *Sida crystallina*). Before the experiments, zooplankters were acclimated to the laboratory conditions (18 °C, 20:4 L:D light) in 250-mL beakers filled with filtered (50- $\mu$ m mesh) lake water containing natural microplankton for 24–48 h. All zooplankters tested in each experiment were from the same sample.

### Short-term feeding experiments

The consumption of trematode cercariae (*D. pseudospathaceum*) by the predators (*Asplanchna* spp., *M. distinctus*, *M. viridis*, *A. paulseni*, *S. crystallina*) was assessed in incubation experiments which lasted for 5 h (Table 1). Since prey removal is often higher in the first hours of incubation (Conover, 1978) we additionally estimated grazing effect after the first 2 h of 5-h incubation experiments and in separate feeding experiments lasted for 2 h (only with copepods, which fed most intensively). Two species of cyclopoids (*M. distinctus*, *M. viridis*) were tested together because it was not possible to identify them to the species level before fixation in the end of the experiment. The number of *M. distinctus* was about twice that of *M. viridis*. Experiments with each planktonic predator were repeated twice on different dates, except for *S. crystallina* (only one experiment was conducted).

In predation treatments, zooplankters were placed in the beakers with 100 mL of filtered (pore size 1  $\mu$ m) lake water and acclimated without food for 4–7 h. Each beaker contained 10–19 crustaceans or 35–40 rotifers depending on the experiment (Table 1). Control treatments (without zooplankters) were used to assess changes in cercariae numbers unrelated to predation. There were five control and five ‘predation’ treatment replicates in all experiments. The only exception was one of the experiments with *Asplanchna* spp. where there were only three ‘predation’ replicates.

Cercariae of *D. pseudospathaceum* freshly produced (in 2 h before the experiment) from 4–5 snails were mixed and added in equal volume (20–25 mL) to all replicates in control and predation treatments. The age of cercariae was less than 2 h at the beginning of experiments and no more than 4 and 7 h at the end (depending on incubation time). Mean initial concentration of cercariae in different experiments varied from 6 to 28 cercariae mL<sup>-1</sup> (Table 1). Such high concentrations of cercariae were used to simulate natural density of cercariae in the vicinity of infected snails (Morley, 2012). We estimated the concentration of cercariae at the start/end of the incubation (in case of copepods also after the first 2 h of 5-h incubation) from three 2-mL samples taken from each beaker after gentle mixing. Averages from these three samples were used in the subsequent statistical analysis. Cercariae were counted in a Bogorov zooplankton counting chamber under a dissecting microscope (28 $\times$  magnification) within 2 h after sampling to avoid a decrease in cercariae number due to the natural mortality. The effect of predation was evaluated by the comparison of changes in cercariae numbers in control/predation treatments at the beginning and the end of the experiment using repeated-measures ANOVA. Clearance and ingestion rates were calculated according to Frost (1972) and Conover (1978).

Mortality of zooplankters used in the experiments was assessed at the end of the incubation. Planktonic crustaceans were measured after fixation with buffered formaldehyde (2% final concentration), while rotifers were measured *in vivo* (Table 1). Sizes of *D. pseudospathaceum* cercariae (body length with furca) were also measured *in vivo* and varied from 375 to 550  $\mu$ m (mean  $\pm$  s.d. = 454  $\pm$  60  $\mu$ m, *N* = 50).

**Table 1.** The design and results of incubation experiments with planktonic predators feeding on *Diplostomum pseudospathaceum* cercariae

Exp.	Predators	Mean sizes of predators (±s.d., µm) <sup>a</sup>	N (ind.)	Time (h)	Control treatment		Predation treatment		Predation effect <sup>b</sup>
					C start	C final	C start	C final	
1	Calanoids <i>Arctodiaptomus paulseni</i>	993 ± 43	10	2	24 ± 2	23 ± 2	28 ± 3	22 ± 4	ns
2		964 ± 40	18	2 <sup>c</sup> 5	6 ± 1 6 ± 1	6 ± 1 6 ± 1	6 ± 0.3	4 ± 1 4 ± 1	$P = 0.028$ , $F_{1,8} = 7.17$ 2 h; ns
3	Cyclopoids <i>Macrocyclus distinctus</i> and <i>Megacyclus viridis</i>	1132 ± 70 and 1258 ± 139, respectively	10	2	24 ± 2	23 ± 2	23 ± 2	17 ± 2	$P = 0.009$ , $F_{1,8} = 11.74$
4		1094 ± 57 and 1285 ± 130, respectively	16	2 5	12 ± 2 12 ± 1	11 ± 1 12 ± 1	11 ± 1	4 ± 1 2 ± 1	$P < 0.001$ , $F_{2,16} = 26.44$
5	Cladocerans <i>Sida crystallina</i>	2391 ± 201	18–19	5	10 ± 1	11 ± 1	12 ± 0.3	5 ± 3	$P = 0.002$ , $F_{1,7} = 24.87$
6	Rotifers <i>Asplanchna</i> spp.	No measurements	40	5	10 ± 1	11 ± 1	12 ± 1.8	10 ± 2	$P = 0.009$ , $F_{1,6} = 14.48$
7		1255 ± 281	35	5	17 ± 1	18 ± 1	18 ± 1	17 ± 2	ns

Mean sizes of zooplankters, their numbers per beaker ( $N$ , ind./120–125 mL), mean concentration of cercariae (cercariae  $\text{mL}^{-1} \pm \text{s.d.}$ ) in the control and predation treatments at the start and end of the experiment (C start, C final) and incubation times for each experiment are presented. There were five control (without zooplankters) and five predation treatment replicates in all experiments, except the exp.6 with *Asplanchna* spp. (three predation replicates). Predation effect was studied as a change in cercariae number in control vs. predation treatment at the beginning and the end of the experiment (effect of time × treatment interaction). More detailed data are presented in the Supplementary Table S1.

<sup>a</sup>Copepod prosome lengths and cladoceran body lengths were measured after fixation; rotifer body lengths (in the stretched state) were measured *in vivo*.

<sup>b</sup>Results of repeated-measures ANOVA. ns – non-significant effect ( $P > 0.05$ ) of time × treatment interaction.

<sup>c</sup>Cercariae were counted also after the first 2 h of 5-h incubation.

### Testing the effect of cercariae on fitness traits of predators

#### Cyclopoids *M. distinctus* and *M. viridis*

Since *M. distinctus* and *M. viridis* demonstrated low mortality and significant removal of cercariae in incubation experiments they were chosen as objects for the additional long-term experiments. In the experiments, we tested how feeding on cercariae during 9–17 days affects the survival and reproduction in these cyclopoids. We used adult *M. distinctus* and *M. viridis* females (18 and 11 ind. respectively, identified at the end of the experiment). All females were at the similar spawning stage (after formation of egg clutches). In order to achieve this, females with egg clutches were sorted from the stock culture. After hatching of nauplii and formation of the next egg clutch these females were placed individually in beakers with 50 mL of filtered (pore size 1 µm) lake water and used in the experiment. They were maintained at 18 °C, 20:4 L:D light and fed only with the *D. pseudospathaceum* cercariae. Concentrations of cercariae varied from 8 to 13 cercariae  $\text{mL}^{-1}$  (average ± s.d. =  $9 \pm 3$ ) from day to day but were similar in all beakers each day. Cercariae were produced every day by several (4–5) infected *L. stagnalis* snails placed in filtered (pore size 1 µm) water for 2 h. Before the addition of fresh cercariae, the water from each experimental beaker was removed by the pipette after gentle mixing (about 10 mL was left) and replenished by fresh food medium to restore the initial water volume. Each female was checked twice a day to monitor survival, egg formation and hatching. Numbers of hatched nauplii were counted by inspecting the whole water volume (50 mL) in a Bogorov chamber. To avoid cannibalism, the females were separated from nauplii immediately after hatching and placed in new beakers with cercariae. The nauplii hatched from the first clutch were not counted since their number can be more strongly affected by the female feeding before the start of an experiment than by experimental feeding conditions. The durations of clutch periods (=embryonic development time, from egg clutch emergence to nauplii hatching) and interclutch periods (time between two clutches) were calculated for each female throughout the

experiment. At the end of experiments, all females were preserved with the formaldehyde solution (2% final concentration) for species identification and measurements of prosome length).

The STATISTICA 10 (StatSoft Inc., 2011) and R software were used for statistical analysis (R Core Team, 2017). To compare coefficients of variation (CV) of the clutch and interclutch periods in *M. distinctus* and *M. viridis*, we used an asymptotic test for the equality of CV from  $k$  populations' (Feltz and Miller, 1996) from the 'cvequality' R package (Marwick and Krishnamoorthy, 2016). The 'ggplot2' package (Wickham, 2009) was used for graphical presentation of the data.

#### Rotifers *Asplanchna* spp.

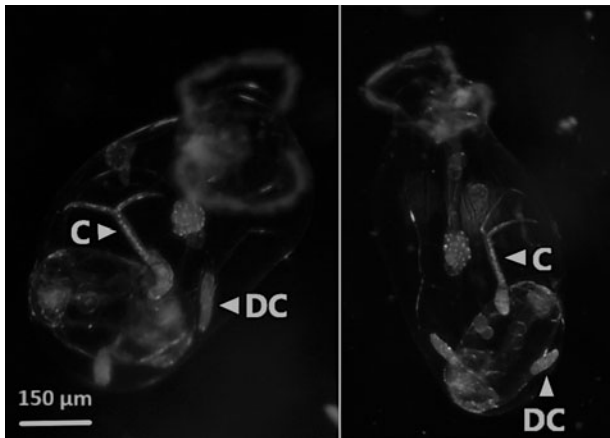
Since microscopical observations of rotifers *Asplanchna* spp. at the end of the grazing experiments indicated that some large individuals ingested cercariae and then died we asked if cercariae can influence size structure of populations of this predatory rotifer. To answer this question we measured sizes of rotifers, which survived after exposure with cercariae (mean initial concentration  $18 \pm 1$  cercariae  $\text{mL}^{-1}$ ) in the grazing experiment (38 ind., five beakers) and rotifers of the control group (54 ind., five beakers), exposed in filtered (pore size 1 µm) lake water without cercariae. Measurements were done *in vivo* (in the stretched state) under the microscope and then mean sizes were compared using *t*-test. The rotifers of both groups were from the same sample and acclimated and exposed (during 24 h) in similar conditions, but the control group was tested a day later (after the end of grazing experiment). Although the mean size of rotifers was not estimated at the beginning of the grazing experiment we supposed that it did not differ from the mean size of rotifers in the control group.

## Results

### Zooplankton feeding on cercariae

Cyclopoids *M. distinctus*, *M. viridis* and cladocerans *S. crystallina* significantly reduced the number of cercariae during all





**Fig. 1.** The rotifer *Asplanchna priodonta* with ingested *Diplostomum pseudospathaceum* cercariae inside its body, which are indicated by arrows (C – recently ingested, DC – partially digested, without furca).

incubation experiments, i.e. time  $\times$  treatment interaction was significant ( $P < 0.05$  in all cases, Table 1, Supplementary Table S1). The effects of other tested zooplankters on cercariae numbers were significant only in some of the experiments. Rotifers *Asplanchna* spp. did not affect cercariae abundance in one experiment but showed a significant reduction effect ( $P = 0.009$ , Table 1, Supplementary Table S1) in another one. Calanoids *A. paulseni* removed cercariae ( $P = 0.028$ ) in the first 2 h of one experiment, but their effect was not significant during the next 3 h of incubation and in the second experiment (Table 1, Supplementary Table S1).

The highest rates of the cercariae removal were observed for cyclopoids (mix of *M. distinctus* and *M. viridis*), which caused on the average 5-fold decrease in cercariae numbers in 5 h when compared with control (Table 1). The removal of cercariae by the cladocerans *S. crystallina* was less pronounced (average 2-fold decrease in 5 h), though the number of cladocerans in experimental beakers were slightly higher than that of cyclopoids (Table 1). Individual clearance rates constituted  $1.5\text{--}3.6$  (mean  $\pm$  s.d. =  $2.6 \pm 0.8$ ) mL ind $^{-1}$  h $^{-1}$  for cyclopoids and  $1.3\text{--}3.2$  (mean  $\pm$  s.d. =  $2.2 \pm 0.9$ ) mL ind $^{-1}$  h $^{-1}$  for *S. crystallina*. However, predation was discontinuous, thus the estimation of hourly clearance and ingestion rates is only approximate. Calanoids and cyclopoids (*M. distinctus* and *M. viridis* together) removed cercariae mainly during the first 2 h of incubation (on average 64–74% of all removed by cyclopoids cercariae). Therefore, clearance rates of cyclopoids calculated for this period (up to  $4.8$  mL ind $^{-1}$  h $^{-1}$ , mean  $\pm$  s.d. =  $2.9 \pm 1.2$ ) were higher than rates obtained for whole 5 h period. The ingestion rates of cyclopoids also were the highest when calculated for 2 h period (mean  $\pm$  s.d. =  $33 \pm 12$  cercariae ind $^{-1}$  h $^{-1}$ ), but were lower and similar to the mean ingestion rates of *S. crystallina* ( $14 \pm 3$  cercariae ind $^{-1}$  h $^{-1}$ ) when calculated for 5 h period.

### Effect of cercarial prey on zooplankton fitness traits

Consumption of cercariae affected their planktonic predators in different ways, e.g. supported reproduction of some species (cyclopoids *M. distinctus* and *M. viridis*) but increased mortality of other (rotifers *Asplanchna* spp., cladocerans *S. crystallina*). Microscopic observations of rotifers *Asplanchna* spp. at the end of the feeding experiments showed that some large individuals ingested 1–3 cercariae (Fig. 1) and died in the next 6 h. The mortality of rotifers exposed to cercariae (28% of the total number in 6 h) was significantly higher (Fisher's exact test,  $P < 0.001$ ) than in

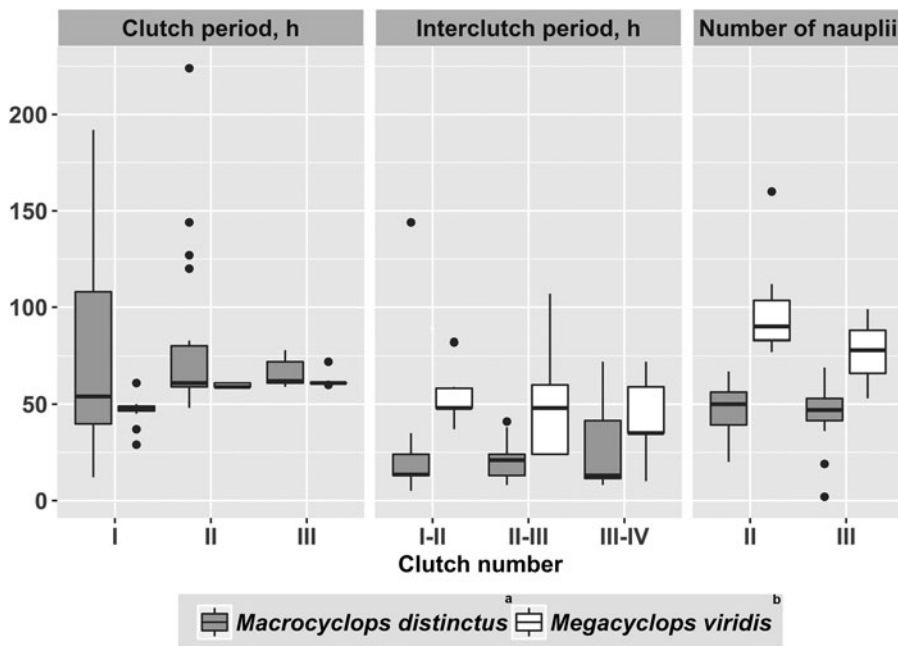
the control group (<3%). The additional experiment (24 h) showed that incubation with cercariae changed the size distribution of rotifers. The mean ( $\pm$ s.d.) size of rotifers was significantly lower in with-cercariae treatment ( $t$ -test,  $t_{90} = -3.96$ ,  $P = 0.033$ ) than in the control group ( $1.3 \pm 0.3$  mm,  $N = 38$  and  $1 \pm 0.2$  mm,  $N = 54$ , respectively). In addition, at the end of the experiment, large rotifers (>1.4 mm in size) constituted 13% of total abundance in the group with cercariae, while in the control group the share of this size class was 39%. It was not possible to obtain data about sizes of rotifers at the start of the experiment, but we suggest that size structure of both groups was similar, since they were randomly selected from the single plankton sample and maintained in similar conditions before the experiment. This difference in the size structure was possibly caused by the decrease in numbers of the largest rotifers (>1.4 mm in size), which ingested cercariae and were damaged by them as microscopic observations suggested. Cercariae kept moving inside the rotifers for a while after being ingested, often causing internal injuries of a predator by cercarial furcae. Small rotifers were not observed ingesting cercariae.

The mortality of cladocerans *S. crystallina* incubated with cercariae during 5 h was also high (25% of the initial number). Microscopic examination of the dead individuals showed that parts of cercariae clogged the filtration apparatus of the cladocerans. However, mortality of copepods (cyclopoids *M. distinctus*, *M. viridis* and calanoids *A. paulseni*) incubated with cercariae was low (<2%) and did not differ from the control vessels ( $P < 0.001$  in all cases).

Long-term experiments showed that cyclopoids *M. distinctus* and *M. viridis* successfully reproduced when fed with the cercarial monodiet. Many of the females (45 and 39% for *M. viridis* and *M. distinctus*, respectively) produced up to four egg clutches in 17 days (Fig. 2, Supplementary Table S2). The duration of the whole cycle (from clutch emergence to emergence of the next clutch) and numbers of nauplii hatched from one clutch did not change significantly with time for both species (ANOVA,  $P = 0.85$ ,  $0.39$  for *M. distinctus*;  $P = 0.88$ ,  $0.06$  for *M. viridis*, respectively, Supplementary Table S2). Numbers of nauplii were significantly higher ( $t$ -test,  $t_{51} = -8.67$ ,  $P < 0.001$ ) in *M. viridis* (53–160, mean  $\pm$  s.d. =  $89 \pm 22$  nauplii,  $N = 20$ ), than in *M. distinctus* females (19–69, mean  $\pm$  s.d. =  $46 \pm 13$  nauplii,  $N = 33$ ), which were smaller in size (mean  $\pm$  s.d. =  $1258 \pm 139$  and  $1132 \pm 70$   $\mu$ m, respectively). The larger cyclopoid species (*M. viridis*) had a longer interclutch period (mean  $\pm$  s.d. =  $50 \pm 22$  h), than smaller *M. distinctus* (mean  $\pm$  s.d. =  $24 \pm 23$  h) ( $t$ -test,  $t_{64} = -4.44$ ,  $P < 0.001$ ), but had a shorter clutch period (mean  $\pm$  s.d. =  $55 \pm 9$  and  $74 \pm 42$  h, respectively) ( $t$ -test,  $t_{68} = 2.27$ ,  $P = 0.026$ ). As a result, the duration of the whole cycle did not differ between these species ( $P = 0.75$ ) and constituted 24–259 h (mean  $\pm$  s.d. =  $97 \pm 5$  h) and 72–144 h (mean  $\pm$  s.d. =  $102 \pm 3$  h) for *M. distinctus* and *M. viridis*, respectively. In both species, the duration of the interclutch period was more variable (CV = 0.959 for *M. distinctus*, CV = 0.437 for *M. viridis*) than the duration of the clutch period (CV = 0.562 for *M. distinctus*, CV = 0.167 for *M. viridis*) (Fig. 2, Supplementary Table S2). These parameters were more variable in the smaller *M. distinctus*.

### Discussion

The results obtained indicated that not only benthic (reviewed in Johnson et al., 2010), but also planktonic organisms could prey on free-living stages of trematodes. Consumption of cercariae by zooplankters was detected previously using radioisotope methods (Christensen, 1979; Christensen et al., 1980), but direct evidence obtained in feeding experiment are still scarce and provide controversial information (Tokobaev et al., 1979; Intapan et al.,



**Fig. 2.** Reproductive characteristics of females of two cyclopoid species fed exclusively with *Diplostomum pseudospathaceum* cercariae *ad libitum* during 17 days. The longevity<sup>c</sup> (h) of reproductive periods and the offspring numbers (nauplii) hatched from each egg clutch<sup>d</sup> are shown. Clutch period is the period when female carries egg clutch until hatching of nauplii, interclutch – the period between two clutches. The ‘box’ represents the interquartile range (IQR) of the reproductive characteristics within groups with median (black line). Whiskers extend from the highest to lowest values within 1.5\*IQR. Suspected outliers, i.e. all observations lying outside 1.5\*IQR, are shown as dots. More detailed data are presented in the Supplementary Table S2. <sup>a</sup> – data for 18 females (clutches I, II), 14 females (clutch III), seven females (clutch IV). <sup>b</sup> – data for 11 females (clutches I, II), nine females (clutch III), five females (clutch IV). <sup>c</sup> – the longevity of periods may be overestimated since we monitored the reproductive status of females twice daily. <sup>d</sup> – the nauplii hatched from the first clutch were not counted since their numbers could be more strongly affected by the female feeding before the experiment than by experimental feeding conditions (cercarial diet).

1992; Schotthoefner *et al.*, 2007). Thus, some species of cladocerans (*S. crystallina*, *D. pulex*, *D. longispina*), rotifers (*E. ehrenbergi*) and cyclopoids (*Cyclops strenuous*, *Mesocyclops leuckarti*) have been reported to remove cercariae of different trematodes (*Schistosoma mansoni*, *Opisthorchis viverrini*, *Plagiorchis* sp., etc.) <0.5 mm in size (Tokobaev *et al.*, 1979; Christensen, 1979; Christensen *et al.*, 1980; Intapan *et al.*, 1992), but did not consume or demonstrated only low predation when fed with larger (about 1 mm, Orlofske *et al.*, 2015) *Ribeiroia ondatrae* cercariae (Schotthoefner *et al.*, 2007). In our feeding experiments, we used cercariae of *D. pseudospathaceum*, which are differed by size (about 450 µm in size) and morphology from cercariae that have been investigated previously; however, the swimming behaviour of *D. pseudospathaceum* (intermittent with positive phototactic orientation) is similar to some of these species (e.g. *S. mansoni*, *O. viverrini*) (Haas *et al.*, 1990; Haas, 1992). Our results indicated that several common freshwater planktonic organisms (cyclopoids *M. distinctus*, *M. viridis*, calanoids *A. paulseni*, cladocerans *S. crystallina*, predatory rotifers *Asplanchna* spp.) can consume cercariae of the trematode *D. pseudospathaceum*, which are often numerous in the nearshore lentic waters (Morley, 2012). Removal of cercariae was the highest in cyclopoids, lower in cladocerans and minimal in calanoids and rotifers.

To our knowledge, this is the first study focused on the effect of cercarial prey on the fitness-related traits of their predators. Many food objects ingested by zooplankters (e.g. cyanobacteria, green algae, some dinoflagellates) are toxic or nutritionally poor, and can suppress the growth, fecundity and predator-avoidance behaviour of the consumers (Bernardi and Giussani, 1990; Koski *et al.*, 1998; Turner, 2014; Lasley-Rasher *et al.*, 2016). The obtained results showed that the removal of cercariae by zooplankters does not necessarily mean their successful digestion, but can lead to increased mortality of predators. Cercariae are likely to clog the filtration apparatus of cladocerans and cause heavy internal injuries by the cercarial furca in predatory rotifers, thus leading to high mortality of both groups of predators (25–28% individuals in 5–6 h).

In contrast, cyclopoids (*M. distinctus*, *M. viridis*), which can tear the soft prey into pieces (Brandl, 1998), survived well when fed with cercariae. They reproduced (up to four offspring generations in 17 days) when maintained exclusively on cercarial diet *ad libitum*. The average interclutch periods of these cyclopoids (1 and 2 days for *M. distinctus* and *M. viridis*, respectively) on

cercarial diet were similar to reported for these genera in food-rich conditions (Phong *et al.*, 2008). In addition, the average number of *M. viridis* offsprings hatched from one clutch was higher in our experiments than in literature (Phong *et al.*, 2008). Therefore, these results showed for the first time that cercariae can adequately satisfy dietary needs of their predators, e.g. cyclopoids. It is important to mention that feeding conditions in our experiments were similar to natural high-density cercarial ‘clouds’ and intensities of predation on cercariae may be lower in the presence of alternative prey (Welsh *et al.*, 2017). Therefore, additional experiments are needed to clarify whether zooplankters can consume cercariae selectively and detect cercarial ‘clouds’ produced by infected snails in natural conditions.

The trophic links between cercariae and planktonic predators can be an important energy pathway from benthic organisms (mollusk hosts of trematodes) to zooplankton through parasites’ free-living stages. It is now well established that inclusion of parasites in ecosystem models causes changes in food webs topology, e.g. increase the linkage density, food chain length, connectivity and nestedness (Hatcher *et al.*, 2012). The valuable role of cercariae in benthic food webs has been assumed previously (Thieltges *et al.*, 2008b; Morley, 2012); however, their impact on the energy transfer in plankton has not been studied yet. Since the production of both planktonic crustaceans and trematode cercariae is often high in the near-shore zone of lakes and ponds (Shuter and Ing, 1997; Lacroix *et al.*, 1999; Preston *et al.*, 2013), trophic relationships between these organisms could significantly contribute to the energy flow in food webs, at least, in shallow lentic ecosystems.

Our data on species-specific effects of cercariae on survival and reproduction of their predators suggest that parasites can influence the structure of planktonic communities. For example, the diet composed of *D. pseudospathaceum* cercariae enhanced mortality in some zooplankters (e.g. *Asplanchna* rotifers) and facilitated reproduction in others (copepods *M. distinctus*, *M. viridis*) in our experiments. We suppose that similar effects may occur in natural communities, leading to parasite-induced alterations in the relative abundance of certain zooplankters and their prey (nano- and microplankton) and predators (e.g. fish larvae) through trophic cascades. Our observations on predatory rotifers *Asplanchna* spp. showed that many of the largest individuals died soon after they ingested cercariae, while the small *Asplanchna* were not observed consuming cercariae at all. Exposure of rotifers

with cercariae led to a decrease of the largest size classes in the experimental group when compared with the control group (13 and 39% of total numbers, respectively). Thus, high abundance of cercariae in water may also lead to changes in the size structure in populations of planktonic organisms. However, this effect may be insignificant when cercariae are less abundant and there are other prey available because rotifers could avoid cercariae when having a food choice.

Another consequence of predation on infective stages of parasites is its negative effect on parasite transmission (Thieltges *et al.*, 2008a; Orlofske *et al.*, 2012). For example, aquatic cercaria-predators can remove a large share (up to 99%) of trematodes' free-living larvae and reduce infection intensities in target hosts by 30–100% (Christensen, 1979; Schotthoefler *et al.*, 2007; Orlofske *et al.*, 2012; Gopko *et al.*, 2017a). Our study indicated that cyclopoids can consume a substantial amount of *D. pseudospathaceum* cercariae, therefore their importance in the reduction of parasite transmission to fish hosts needs further investigation. Cyclopoids are common in coastal zones of lakes, where myriads of cercariae are produced by infected mollusks every day, therefore the removal of cercariae by these zooplankters could be substantial. We assume that in areas of high zooplankton densities fish may be more protected against infestation with cercariae.

Avoidance of parasites is an important factor structuring fish shoals and influencing fish habitat choice (Poulin and FitzGerald, 1989a; 1989b; Stumbo *et al.*, 2012; Mikheev *et al.*, 2013). Spatial distribution of small fish often correlates with high densities of zooplankton prey and is related to fish foraging needs, hydrography, etc. (George and Winfield, 2000; Höffle *et al.*, 2013; Swalethorp *et al.*, 2015). However, it is possible that fish aggregate with zooplankton also to avoid a parasitic threat. This assumption can be tested experimentally using non-planktivorous fishes. For the parasite, a release of cercariae close to zooplankton aggregations, which are attractive for fishes, can lead to the trade-off between the benefit of enhanced transmission and the cost of predation by zooplankters. Although the influence of predators' presence in the environment on the cercariae release by a snail host is still unknown, the significant effect of chemical cues from the target host was previously shown (Mouritsen, 2002; Lagrue *et al.*, 2017). Since trematodes have been reported to manipulate behaviour of their snail hosts in different ways, e.g. by changing morphology, habitat and feeding preferences of snails (Levri, 1999; Miura *et al.*, 2006; Kamiya and Poulin, 2012), they possibly can also regulate the production of cercariae depending on the presence/absence of the cercaria-predators in the environment.

Present results show the ability of zooplankters to harvest cercariae and reproduce on a cercarial diet, supporting the view of the important trophic interaction between zooplankton and parasite larvae. We assume that this can affect the functioning of aquatic food webs *via* direct consumption of cercariae and *via* indirect consequences on survival, reproduction and behaviour of other hosts in parasites' life cycles (mollusks, fishes, birds). Further investigations are needed to test these assumptions and provide more information about the role of direct predation on parasite larvae in modifying the structure and functioning of freshwater ecosystems.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0031182018000963>

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**Ethical standards.** The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

## References

- Achinelly MF, Micieli MV and García JJ (2003) Pre-parasitic juveniles of *Strelkovimermis spiculatus* Poinar and Camino, 1986 (Nematoda: Mermithidae) predated upon by freshwater copepods (Crustacea: Copepoda). *Nematology* 5, 885–888.
- Amundsen PA, Lafferty KD, Knudsen R, Primicerio R, Klemetsen A and Kuris AM (2009) Food web topology and parasites in the pelagic zone of a subarctic lake. *Journal of Animal Ecology* 78, 563–572.
- Bernardi R and Giussani G (1990) Are blue-green algae a suitable food for zooplankton? An overview. *Hydrobiologia* 200/201, 29–41.
- Brandl Z (1998) Feeding strategies of planktonic cyclopoids in lacustrine ecosystems. *Journal of Marine Systems* 15, 87–95.
- Buck JC, Truong L and Blaustein AR (2011) Predation by zooplankton on *Batrachochytrium dendrobatidis*: biological control of the deadly amphibian chytrid fungus? *Biodiversity and Conservation* 20, 3549–3553.
- Christensen NO (1979) *Schistosoma mansoni*: interference with cercarial host-finding by various aquatic organisms. *Journal of Helminthology* 53, 7–14.
- Christensen NO, Nansen P and Frandsen F (1977) Interference with *Fasciola hepatica* snail finding by various aquatic organisms. *Parasitology* 74, 285–290.
- Christensen NO, Frandsen F and Nansen P (1980) The interaction of some environmental factors influencing *Schistosoma mansoni* cercarial host-finding. *Journal of Helminthology* 54, 203–205.
- Conover RJ (1978) Transformation of organic matter. In Kinne (ed.), *Marine Ecology, Vol. 4, Dynamics*. New York, USA: Wiley, pp. 221–499.
- Cummins KW, Costa RR, Rowe RE, Moshiri GA, Scanlon RM and Zajdel RK (1969) Ecological energetics of a natural population of the predaceous zooplankter *Leptodora kindtii* Focke (Cladocera). *Oikos* 20, 189–223.
- Feltz CJ and Miller GE (1996) An asymptotic test for the equality of coefficients of variation from k populations. *Statistics in Medicine* 15, 647–658.
- Frost BW (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnology Oceanography* 17, 805–815.
- George DG and Winfield IJ (2000) Factors influencing the spatial distribution of zooplankton and fish in Loch Ness, UK. *Freshwater Biology* 43, 557–570.
- Gopko M, Mikheev VN and Taskinen J (2015) Changes in host behaviour caused by immature larvae of the eye fluke: evidence supporting the predation suppression hypothesis. *Behavioral Ecology and Sociobiology* 69, 1723–1730.
- Gopko M, Mironova E, Pasternak A, Mikheev V and Taskinen J (2017a) Freshwater mussels (*Anodonta anatina*) reduce transmission of a common fish trematode (eye fluke *Diplostomum pseudospathaceum*). *Parasitology* 144, 1971–1979.
- Gopko M, Mikheev VN and Taskinen J (2017b) Deterioration of basic components of the anti-predator behavior in fish harboring eye fluke larvae. *Behavioral Ecology and Sociobiology* 71, 68.
- Haas W (1992) Physiological analysis of cercarial behavior. *The Journal of Parasitology* 78, 243–255.
- Haas W, Granzer M and Brockelman CR (1990) *Opisthorchis viverrini*: finding and recognition of the fish host by the cercariae. *Experimental Parasitology* 71, 422–431.
- Hamilton PT, Richardson JML and Anholt BR (2012) *Daphnia* in tadpole mesocosms: trophic links and interactions with *Batrachochytrium dendrobatidis*. *Freshwater Biology* 57, 676–683.
- Hatcher MJ, Dick JTA and Dunn AM (2012) Diverse effects of parasites in ecosystems: linking interdependent processes. *Frontiers in Ecology and the Environment* 10, 186–194.
- Höffle H, Nash RDM, Falkenhaus T and Munk P (2013) Differences in vertical and horizontal distribution of fish larvae and zooplankton, related to hydrography. *Marine Biology Research* 9, 629–644.
- Hudson PG, Dobson AP and Lafferty KD (2006) Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology and Evolution* 21, 381–385.
- Intapan P, Kaewkes S and Maleewong W (1992) Control of *Opisthorchis viverrini* cercariae using the copepod *Mesocyclops leuckartii*. *Southeast Asian Journal of Tropical Medical Public Health* 23, 348–349.



- Jasper JT, Nguyen MT, Jones ZL, Ismail NS, Sedlak DL, Sharp JO, Luthy RG, Horne AJ and Nelson KL (2013) Unit process wetlands for removal of trace organic contaminants and pathogens from municipal wastewater effluents. *Environmental Engineering Science* **30**, 421–436.
- Johnson PTJ, Dobson A, Lafferty KD, Marcogliese DJ, Orlofske SA, Poulin R and Thieltges DW (2010) When parasites become prey: ecological and epidemiological significance of eating parasites. *Trends in Ecology and Evolution* **25**, 362–371.
- Kamiya T and Poulin R (2012) Parasite-induced behavioural changes to the trade-off between foraging and predator evasion in a marine snail. *Journal of Experimental Marine Biology and Ecology* **438**, 61–67.
- Karvonen A, Kirsi S, Hudson PJ and Valtonen ET (2004a) Patterns of cercarial production from *Diplostomum spathaceum*: terminal investment or bet hedging? *Parasitology* **129**, 87–92.
- Karvonen A, Seppälä O and Valtonen ET (2004b) Eye fluke-induced cataract formation in fish: quantitative analysis using an ophthalmological microscope. *Parasitology* **129**, 473–478.
- Karvonen A, Savolainen M, Seppälä O and Valtonen ET (2006) Dynamics of *Diplostomum spathaceum* infection in snail hosts at a fish farm. *Parasitology Research* **99**, 341–345.
- Kerfoot WC (1977) Implications of copepod predation. *Limnology and Oceanography* **22**, 316–325.
- Koski M, Breteler WK and Schogt N (1998) Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). *Marine Ecology Progress Series* **170**, 169–187.
- Kuris AM, Hechinger RF, Shaw JC, Whitney KL, Aguirre-Macedo L, Boch CA, Dobson AP, Dunham EJ, Fredensborg BL, Huspeni TC, Lorda J, Mababa L, Mancini FT, Mora AB, Pickering M, Talhouk NL, Torchin ME and Lafferty KD (2008) Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature* **454**, 515–518.
- Lacroix G, Lescher-Moutoué F and Bertolo A (1999) Biomass and production of plankton in shallow and deep lakes: are there general patterns? *Annales de Limnologie* **35**, 111–122.
- Lafferty KD, Dobson AP and Kuris AM (2006) Parasites dominate food web links. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 11211–11216.
- Lafferty KD, Allesina S, Arim M, Briggs CJ, De Leo G, Dobson AP, Dunne JA, Johnson PTJ, Kuris AM, Marcogliese DJ, Martinez ND, Memmott J, Marquet J, McLaughlin JP, Mordecai EA, Pascual M, Poulin R and Thieltges DW (2008) Parasites in food webs: the ultimate missing links. *Ecology Letters* **11**, 533–546.
- Lagrué C, Rinnevalli R and Poulin R (2017) Smelling the future: subtle life-history adjustments in response to environmental conditions and perceived transmission opportunities in a trematode. *Parasitology* **144**, 464–474.
- Lasley-Rasher RS, Nagel K, Angra A and Yen J (2016) Intoxicated copepods: ingesting toxic phytoplankton leads to risky behaviour. *Proceedings of the Royal Society of London B: Biological Sciences* **283**, 20160176.
- Levri EP (1999) Parasite-induced change in host behavior of a freshwater snail: parasitic manipulation or byproduct of infection? *Behavioral Ecology* **10**, 234–241.
- Lyholt HCK and Buchmann K (1996) *Diplostomum spathaceum*: effects of temperature and light on cercarial shedding and infection of rainbow trout. *Diseases of Aquatic Organisms* **25**, 169–173.
- Marwick B and Krishnamoorthy K (2016) cvequality: Tests for the Equality of Coefficients of Variation from Multiple Groups. R package version 0.1.1. Available at <https://CRAN.R-project.org/package=cvequality>.
- Marten GG and Reid JW (2007) Cyclopoid copepods. *Journal of the American Mosquito Control Association* **23**, 65–92.
- Mikheev VN, Pasternak AF, Taskinen J and Valtonen ET (2010) Parasite-induced aggression and impaired contest ability in a fish host. *Parasites and Vectors* **3**, 17.
- Mikheev VN, Pasternak AF, Taskinen J and Valtonen TE (2013) Grouping facilitates avoidance of parasites by fish. *Parasites and Vectors* **6**, 301.
- Miura O, Kuris AM, Torchin ME, Hechinger RF and Chiba S (2006) Parasites alter host phenotype and may create a new ecological niche for snail hosts. *Proceedings of the Royal Society of London B: Biological Sciences* **273**, 1323–1328.
- Morley N (2012) Cercariae (Platyhelminthes: Trematoda) as neglected components of zooplankton communities in freshwater habitats. *Hydrobiologia* **691**, 7–19.
- Mouritsen KN (2002) The *Hydrobia ulvae*-*Maritrema subdolum* association: influence of temperature, salinity, light, water-pressure and secondary host exudates on cercarial emergence and longevity. *Journal of Helminthology* **76**, 341–347.
- Orlofske SA, Jadin RC, Preston DL and Johnson PTJ (2012) Parasite transmission in complex communities: predators and alternative hosts alter pathogenic infections in amphibians. *Ecology* **93**, 1247–1253.
- Orlofske SA, Jadin RC and Johnson PTJ (2015) It's a predator-eat-parasite world: how characteristics of predator, parasite and environment affect consumption. *Oecologia* **178**, 537–547.
- Owen SF, Barber I and Hart PJB (1993) Low level infection by eye fluke, *Diplostomum* spp., affects the vision of three-spined sticklebacks, *Gasterosteus aculeatus*. *Journal of Fish Biology* **42**, 803–806.
- Phong TV, Nabuko T, Histohi K and Takagi M (2008) Comparative evaluation of fecundity and survivorship of six copepod (Copepoda: Cyclopoidae) species in relation to selection of candidates biological control agents against *Aedes aegypti*. *Journal of the American Mosquito Control Association* **24**, 61–69.
- Poulin R and FitzGerald GJ (1989a) Risk of parasitism and microhabitat selection in juvenile sticklebacks. *Canadian Journal of Zoology* **67**, 14–18.
- Poulin R and FitzGerald GJ (1989b). Shoaling as an anti-ectoparasite mechanism in juvenile sticklebacks (*Gasterosteus* spp.). *Behavioral Ecology and Sociobiology* **24**, 251–255.
- Preston DL, Orlofske SA, Lambden JP and Johnson PTJ (2013) Biomass and productivity of trematode parasites in pond ecosystems. *Journal of Animal Ecology* **82**, 509–517.
- R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at <https://www.R-project.org/>.
- Schmeller DS, Blooi M, Martel A, Garner TWJ, Fisher MC, Azemar F, Clare FC, Leclerc C, Jäger L, Guevara-Nieto M, Loyau A and Pasmans F (2014) Microscopic aquatic predators strongly affect infection dynamics of a globally emerged pathogen. *Current Biology* **24**, 176–180.
- Schotthoefer AM, Labak KM and Beasley VR (2007) *Ribeiroia ondatrae* cercariae are consumed by aquatic invertebrate predators. *Journal of Parasitology* **93**, 1240–1243.
- Seppälä O, Karvonen A and Valtonen ET (2004). Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke–fish interaction. *Animal Behavior* **68**, 257–263.
- Shigin A (1980) Trematodes of the genus *Diplostomum* in biocoenoses of the trout farm “Skhodnya”. *Trudy Gel' mintologicheskoi Laboratorii Gel' minty zhivotnykh i rastenii* **30**, 140–202. (in Russian).
- Shuter BJ and Ing KK (1997) Factors affecting the production of zooplankton in lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 359–377.
- StatSoft, Inc. (2011) STATISTICA (data analysis software system), version 10. Tulsa, OK, USA. Retrieved from <http://www.statsoft.com/>.
- Stumbo AD, James CT, Goater CP and Wisenden BD (2012) Shoaling as an antiparasite defence in minnows (*Pimephales promelas*) exposed to trematode cercariae. *Journal of Animal Ecology* **81**, 1319–1326.
- Swailethorp R, Malanski E, Agersted MD, Nielsen TG and Munk P (2015) Structuring of zooplankton and fish larvae assemblages in a freshwater-influenced Greenlandic fjord: influence from hydrography and prey availability. *Journal of Plankton Research*, **37**, 102–119.
- Taskinen J (1998) Cercarial production of the trematode *Rhipidocotyle fennica* in clams kept in the field. *Journal of Parasitology* **84**, 345–349.
- Thieltges DW, Jensen KT and Poulin R (2008a) The role of biotic factors in the transmission of free-living endohelminth stages. *Parasitology* **135**, 407–426.
- Thieltges DW, de Montaudouin X, Fredensborg B, Jensen KT, Koprivnikar J and Poulin R (2008b) Production of marine trematode cercariae: a potentially overlooked path of energy flow in benthic systems. *Marine Ecology Progress Series* **372**, 147–155.
- Tokobaev MM, Kutikova LA and Chibichenko NT (1979) The predatory rotifer, *Eosphora ehrenbergi*, a biological eliminator of cercariae in lake Issyk-Kul'. *Trudy Gel' mintologicheskoi Laboratorii Gel' minty zhivotnykh i rastenii* **29**, 146–149. (in Russian).
- Turner JT (2014) Planktonic marine copepods and harmful algae. *Harmful Algae* **32**, 81–93.
- Valtonen ET and Gibson DI (1997) Aspects of the biology of diplostomid metacercarial (Digenea) populations occurring in fishes in different localities of northern Finland. *Annales Zoologici Fennici* **34**, 47–59.
- Welsh JE, Liddell C, Van Der Meer J and Thieltges DW (2017) Parasites as prey: the effect of cercarial density and alternative prey on consumption of cercariae by four non-host species. *Parasitology* **144**, 1775–1782.
- Wickham H (2009) *ggplot2: Elegant Graphics for Data Analysis*. New York, USA: Springer-Verlag.