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

Year: 2019

Version: Submitted version (Preprint)

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Please cite the original version:

Mironova, E., Gopko, M., Pasternak, A., Mikheev, V., & Taskinen, J. (2019). Trematode cercariae as prey for zooplankton : effect on fitness traits of predators. *Parasitology*, 146(1), 105-111.
<https://doi.org/10.1017/S0031182018000963>

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

Short running title: "Removal of trematode larvae by zooplankters"

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SUMMARY

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 ± 12 cercariae $\text{ind}^{-1} \text{h}^{-1}$), which actively reproduced (up to one egg clutch day^{-1}) 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.

Keywords: *Diplostomum*, eye fluke, parasite transmission, freshwater ecosystem, food web, reproduction, mortality, cladocerans, copepods, rotifers.

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 Jasper *et al.* 2013; Buck *et al.* 2011; Hamilton *et al.* 2012; 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⁻¹ in still freshwater habitats (Morley, 2012) and their annual production in estuaries can constitute 10–43 kg ha⁻¹ y⁻¹ (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 less than 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 (Tokobaev *et al.* 1979;

Christensen, 1979; Christensen *et al.* 1980; Intapan *et al.* 1992; Schotthoefer *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 molluscs 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, *Diplostomum 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.*

pseudopathaceum 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). *D. pseudopathaceum* 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. pseudopathaceum* have different size, morphology and behaviour than most that have been used in previous feeding experimental studies (Tokobaev *et al.* 1979; Christensen, 1979; Christensen *et al.* 1980; Intapan *et al.* 1992; Schotthoefer *et al.* 2007).

Cercariae of *D. pseudopathaceum* 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 hours 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-µm mesh). In the laboratory, we picked out individuals of several common planktonic species with a pipette (rotifers *Asplanchna* spp., cyclopoids *Megacyclops viridis*, *Macrocyclus 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-µm mesh) lake water containing natural microplankton for 24-48 hours. All zooplankters tested in each experiment were from the same sample.

Short-term feeding experiments

The consumption of trematode cercariae (*D. pseudopathaceum*) 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 two hours of 5-h incubation experiments and in separate feeding experiments lasted for two hours (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 μm) 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 5 control and 5 '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 two hours 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 two hours at the beginning of experiments and no more than four or seven hours at the end (depending on incubation time). Mean initial concentration of cercariae in different experiments varied from 6 to 28 cercariae mL^{-1} (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 two hours of 5-h incubation) from three 2-mL samples taken from the 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 two hours after sampling to avoid 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 (1982) 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 μm (mean \pm SD = 454 \pm 60 μm , N = 50).

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 mL^{-1} (average \pm SD = 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 hours. 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 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 was used for statistical analysis (R Core Team, 2017). To compare coefficients of variation (CV) of clutch and interclutch periods in *M. distinctus* and *M. viridis*, we used an asymptotic test for the equality of coefficients of variation from k populations' (Feltz and Miller, 1996) from the 'cvequality' R package (Marwick and Krishnamoorthy, 2016). The 'ggplot2' package (Wickham, 2010)

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 ± 1 cercariae mL^{-1}) in the grazing experiment (38 ind., 5 beakers) and rotifers of the control group (54 ind., 5 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 the 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 incubation experiments, i.e. time*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 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 two hours 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 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 1.5-3.6 (mean±SD = 2.6±0.8) mL ind⁻¹ h⁻¹ for cyclopoids and 1.3-3.2 (mean±SD = 2.2±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 two hours 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±SD = 2.9±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±SD = 33±12 cercariae ind⁻¹ h⁻¹), but were lower and similar to the mean ingestion rates of *S. crystallina* (14±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 hours. The mortality of rotifers exposed to cercariae (28% of 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 (±SD) 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±0.3 mm, $N = 38$ and 1±0.2 mm, $N = 54$, respectively). In addition, at the end of 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 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 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 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*, $P = 0.85$, $P = 0.39$ for *M. distinctus*, $P = 0.88$, $P = 0.06$ 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 SD = 89 \pm 22 nauplii, $N = 20$), than in *M. distinctus* females (19-69, mean \pm SD = 46 \pm 13 nauplii, $N = 33$), which were smaller in size (mean \pm SD = 1258 \pm 139 and 1132 \pm 70 μ m, respectively). The larger cyclopoid species (*M. viridis*) had a longer interclutch period (mean \pm SD = 50 \pm 22 h), than smaller *M. distinctus* (mean \pm SD = 24 \pm 23 h) (*t*-test, $t_{64} = -4.44$, $P < 0.001$), but had a shorter clutch period (mean \pm SD = 55 \pm 9 and 74 \pm 42 h, respectively) (*t*-test, $t_{68} = 2.27$, $P = 0.026$). As a result, the duration of whole cycle did not differ between these species ($P = 0.75$) and constituted 24-259 h (mean \pm SD = 97 \pm 5 h) and 72-144 h (mean \pm SD = 102 \pm 3 h) for *M. distinctus* and *M. viridis*, respectively. In both species, the duration of interclutch period was more variable ($CV = 0.959$ for *M. distinctus*, $CV = 0.437$ for *M. viridis*) than 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 evidences obtained in feeding experiment are still scarce and provide controversial information (Tokobaev *et al.* 1979; Intapan *et al.* 1992; Schotthoefler *et al.* 2007). Thus, some species of cladocerans (*S. crystallina*, *Daphnia pulex*, *D. longispina*), rotifers (*Eosphora ehrenbergi*) and cyclopoids (*Cyclops strenuous*, *Mesocyclops leuckarti*) have been reported to remove cercariae of different trematodes (*Schistosoma mansoni*, *Opisthorchis viverrini*, *Plagiorchis* sp., etc.) less than 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 (Schotthoefler *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 *Arctodiaptomus 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.* 2008*b*; 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 nearshore 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 (Orlofske *et al.* 2012; Thieltges *et al.* 2008a). 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; Schotthoefer *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 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, 1989; 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.* 2016). 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 ability of zooplankters to harvest cercariae and reproduce on a cercarial diet, supporting the view of important trophic interaction between zooplankton and parasite larvae. We assume that this can affect 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 is available at Figshare data repository (<https://figshare.com/s/b44fa55b0d78eabf9e65>).

ACKNOWLEDGEMENTS

We are very grateful to the technical staff of the Konnevesi research station (University of Jyväskylä, Finland) for their assistance.

FINANCIAL SUPPORT

The research was supported by the Academy of Finland (J. T., mobility grants 298911, 311033); the Ella and Georg Ehrnrooth Foundation (E. M. and M. G. mobility grants 2016 and 2017, respectively) and the Russian Foundation for Basic Research (V. M., grant 17-04-00247).

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.

CONFLICTS OF INTEREST

None

For Peer Review

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TABLES

Table 1. The design and results of incubation experiments with planktonic predators feeding on *D. pseudospathaceum* cercariae. Mean sizes of zooplankters, their numbers per beaker (N, ind./120-125 mL), mean concentration of cercariae (cercariae mL⁻¹±SD) in the control and predation treatments at the start and end of experiment (C start, C final) and incubation times for each experiment are presented. There were 5 control (without zooplankters) and 5 predation treatment replicates in all experiments, except of the exp.6 with *Asplanchna* spp. (3 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.

Exp.	Predators	Mean sizes of predators (±SD, µm) [†]	N (ind.)	Time (h)	Control treatment		Predation treatment		Predation effect
					C start	C final	C start	C final	
1	Calanoids <i>Arctodiaptomus</i>	993±43	10	2	24±2	23±2	28±3	22±4	ns
2	<i>paulseni</i>	964±40	18	2 [‡]	6±1	6±1	6±0.3	4±1	$P = 0.028, F_{1,8} = 7.17$ 2 h;
				5		6±1		4±1	ns
3	Cyclopoids <i>Macrocyclops distinctus</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	and <i>Megacyclops viridis</i>	1094±57 and 1285±130, respectively	16	2	12±2	11±1	11±1	4±1	$P < 0.001, F_{2,16} = 26.44$
				5		12±1		2±1	
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	no measurements	40	5	10±1	11±1	12±1.8	10±2	$P = 0.009, F_{1,6} = 14.48$
	Rotifers <i>Asplanchna</i> spp.							
7	1255±281	35	5	17±1	18±1	18±1	17±2	<i>ns</i>

† copepod prosome lengths and cladoceran body lengths were measured after fixation; rotifer body lengths (in the stretched state) were measured *in vivo*.

‡ cercariae were counted also after the first two hours of 5-h incubation.

¶ results of repeated-measures ANOVA. *ns* - nonsignificant effect ($P > 0.05$) of time*treatment interaction.

FIGURE LEGENDS

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

Fig. 2. Reproductive characteristics of females of two cyclopoid species, fed exclusively with *D. pseudopathaceum* cercariae *ad libitum* during 17 days. The longevity[§] (h) of reproductive periods and the offspring numbers (nauplii) hatched from the each egg clutch[¶] 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.

† - data for 18 females (clutches I, II), 14 females (clutch III), 7 females (clutch IV).

‡ - data for 11 females (clutches I, II), 9 females (clutch III), 5 females (clutch IV).

§ - the longevity of periods may be overestimated since we monitored the reproductive status of females twice a day.

¶ - 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).

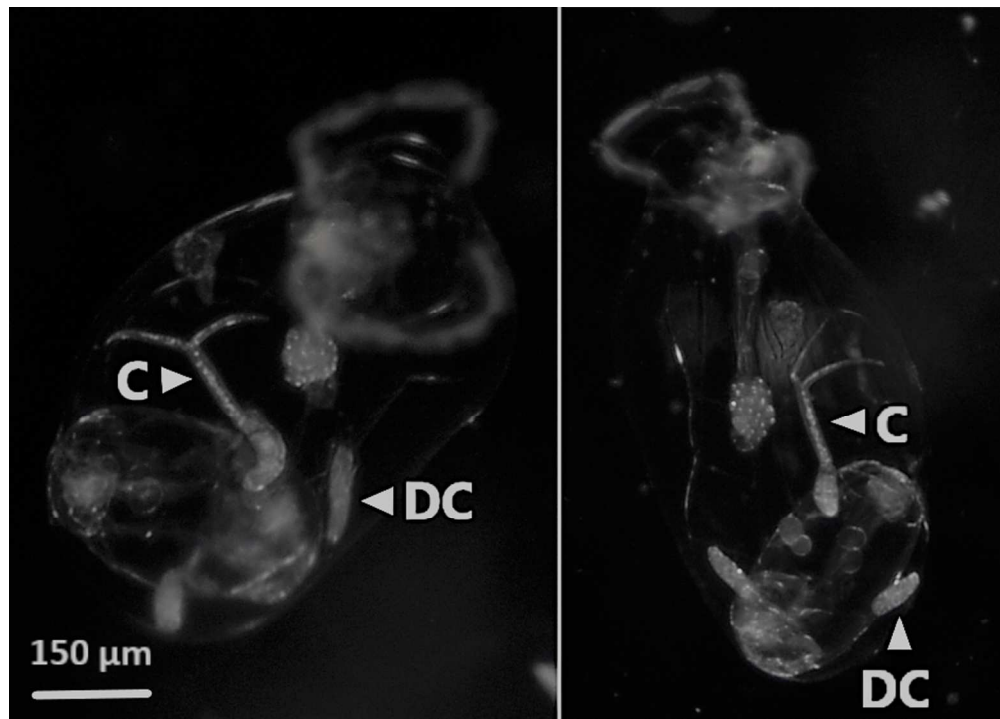


Fig. 1. The rotifer *Asplanchna priodonta* with ingested *D. pseudospathaceum* cercariae inside its body, which are indicated by arrows (C – recently ingested, DC – partially digested, without furca).

80x57mm (300 x 300 DPI)

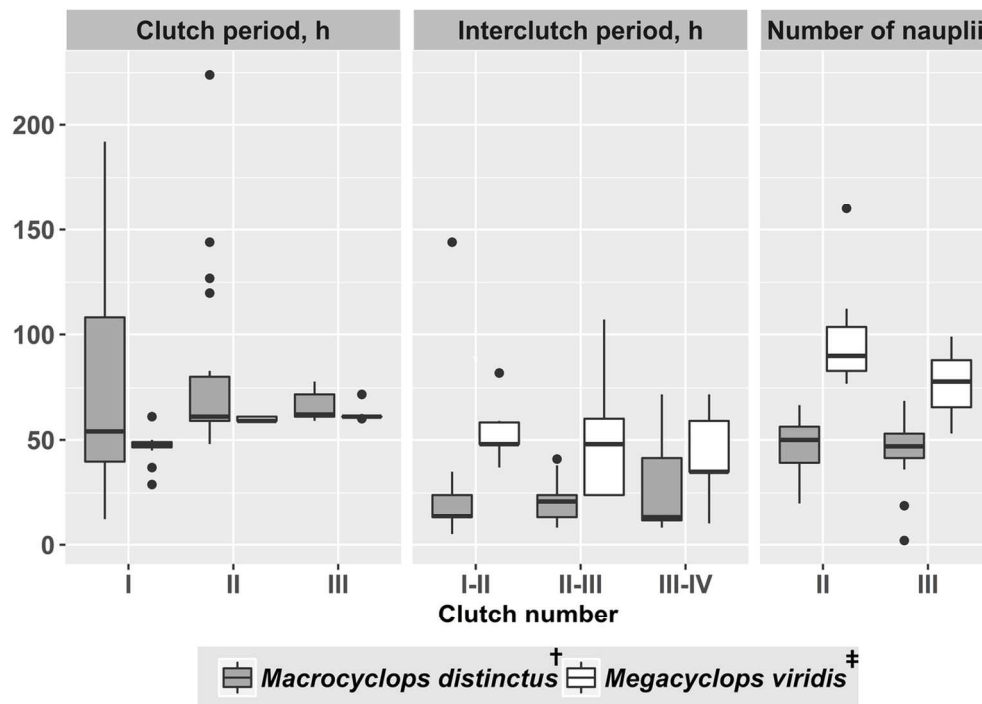


Fig. 2. Reproductive characteristics of females of two cyclopid species, fed exclusively with *D. pseudopathaceum* cercariae *ad libitum* during 17 days. The longevity^s (h) of reproductive periods and the offspring numbers (nauplii) hatched from the each egg clutchⁿ are shown. Clutch period is the period 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.!! † † - data for 18 females (clutches I, II), 14 females (clutch III), 7 females (clutch IV). !! † ‡ - data for 11 females (clutches I, II), 9 females (clutch III), 5 females (clutch IV).!! † § - the longevity of periods may be overestimated since we monitored the reproductive status of females twice a day.!! † ¶ - 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). !! †

119x86mm (300 x 300 DPI)

Supplementary table S1. Feeding experiments with different zooplankters and cercariae of *Diplostomum*

Experiment	glass №	Treatment	Concentration of cercariae (cercar			Water volu	Number of predators, i	
			Start	Final 1 (2 h	Final 2 (5 h		Initial	Final
1	1	Arctodiaptc	28.5	18.165	-	120	10	10
1	2	Arctodiaptc	29.835	17.5	-	120	10	10
1	3	Arctodiaptc	24	23.335	-	120	10	10
1	4	Arctodiaptc	31.835	23.5	-	120	10	10
1	5	Arctodiaptc	24.665	25.5	-	120	10	10
1	1	Control	25.665	24.5	-	120	0	0
1	2	Control	22.835	19.5	-	120	0	0
1	3	Control	27.335	23.835	-	120	0	0
1	4	Control	23.5	23	-	120	0	0
1	5	Control	22.835	25	-	120	0	0
2	1	Arctodiaptc	6	4.666667	1.666667	125	18	18
2	2	Arctodiaptc	6.166667	2.833333	4	125	18	18
2	3	Arctodiaptc	6.333333	4.666667	2.666667	125	18	18
2	4	Arctodiaptc	6	5.5	5	125	18	16
2	5	Arctodiaptc	6.833333	4.166667	4.333333	125	18	18
2	1	control	5.666667	6.666667	6.166667	125	0	0
2	2	control	6.666667	5.333333	3.666667	125	0	0
2	3	control	7.5	6.666667	5	125	0	0
2	4	control	4.25	4.166667	6.833333	125	0	0
2	5	control	4.75	5.333333	6.333333	125	0	0
3	1	Cyclopoids	25.66667	17.66667	-	120	10	10
3	2	Cyclopoids	25.5	15.66667	-	120	10	10
3	3	Cyclopoids	20.5	17.33333	-	120	10	9
3	4	Cyclopoids	20.83333	14.66667	-	120	10	10
3	5	Cyclopoids	23.33333	17.83333	-	120	10	10
3	1	Control	25.665	24.5	-	120	0	0
3	2	Control	22.835	19.5	-	120	0	0
3	3	Control	27.335	23.835	-	120	0	0
3	4	Control	23.5	23	-	120	0	0
3	5	Control	22.835	25	-	120	0	0
4	1	Cyclopoids	11.5	5.5	2.666667	120	16	15
4	2	Cyclopoids	10	4.5	3.833333	120	16	15
4	3	Cyclopoids	13.33333	5.333333	1.5	120	16	16
4	4	Cyclopoids	10.66667	3	1	120	16	16
4	5	Cyclopoids	9.833333	3	2	120	16	16
4	1	control	10.875	11.5	11.66667	120	0	0
4	2	control	9.375	12.83333	12.66667	120	0	0
4	3	control	14.5	10.83333	12	120	0	0
4	4	control	11.83333	9.666667	10.5	120	0	0
4	5	control	14	11.5	12.5	120	0	0
5	1	Sida crista	11.66667	-	8.833333	120	19	9
5	2	Sida crista	11.33333	-	5.166667	120	19	14
5	3	Sida crista	12	-	1.666667	120	19	15
5	4	Sida crista	11.33333	-	3.833333	120	18	13
5	5*	Sida crista	10.16667	-	10.83333	120	18	3
5	1	control	8.5	-	10.5	120	0	0
5	2	control	10	-	10.8	120	0	0
5	3	control	11	-	11.66667	120	0	0
5	4	control	11.16667	-	11.16667	120	0	0
5	5	control	11.16667	-	10.5	120	0	0
6	1	Asplanchni	10.83333	-	7.333333	120	40	no data
6	2	Asplanchni	13.83333	-	12	120	40	no data

6	3 Asplanchn:	10.66667 -	9.166667	120	40	no data
6	1 control	8.5 -	10.5	120	0	0
6	2 control	10 -	10.8	120	0	0
6	3 control	11 -	11.66667	120	0	0
6	4 control	11.16667 -	11.16667	120	0	0
6	5 control	11.16667 -	10.5	120	0	0
7	1 Asplanchn:	17.5 -	18.16667	125	35	24
7	2 Asplanchn:	19 -	14.16667	125	35	29
7	3 Asplanchn:	19.33333 -	15.83333	125	35	24
7	4 Asplanchn:	17 -	19.33333	125	35	26
7	5 Asplanchn:	18.5 -	17	125	35	23
7	1 control	17.66667 -	20.16667	125	0	0
7	2 control	17 -	16.16667	125	0	0
7	3 control	18.33333 -	17.83333	125	0	0
7	4 control	17 -	18.5	125	0	0
7	5 control	15.83333 -	18.16667	125	0	0

*was excluded from the analysis, because of high mortality of predators (>80% individuals c

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Supplementary table S2. Reproductive characteristics of females of two cyclopoid species, fed exclusively
 * - the nauplii hatched from the first clutch were not counted since their numbers could be more strongly

Cyclopoid	Female №	Clutch	peri	Sequence number of clutch	Female №	Number of	Sequence
Macrocyclus	1	192	I		Macrocyclus	1	67 II
Macrocyclus	2	61	I		Macrocyclus	2	50 II
Macrocyclus	3	48	I		Macrocyclus	3	47 II
Macrocyclus	4	120	I		Macrocyclus	4	38 II
Macrocyclus	5	120	I		Macrocyclus	5	37 II
Macrocyclus	6	125	I		Macrocyclus	6	50 II
Macrocyclus	7	12	I		Macrocyclus	7	54 II
Macrocyclus	8	48	I		Macrocyclus	8	61 II
Macrocyclus	9	34	I		Macrocyclus	9	54 II
Macrocyclus	10	48	I		Macrocyclus	10	47 II
Macrocyclus	11	72	I		Macrocyclus	11	38 II
Macrocyclus	12	132	I		Macrocyclus	12	59 II
Macrocyclus	13	60	I		Macrocyclus	13	43 II
Macrocyclus	14	48	I		Macrocyclus	14	57 II
Macrocyclus	15	13	I		Macrocyclus	15	29 II
Macrocyclus	16	37	I		Macrocyclus	16	51 II
Macrocyclus	17	37	I		Macrocyclus	17	60 II
Macrocyclus	18	61	I		Macrocyclus	18	20 II
Macrocyclus	1	66	II		Macrocyclus	1	53 III
Macrocyclus	2	53	II		Macrocyclus	2	54 III
Macrocyclus	3	48	II		Macrocyclus	3	47 III
Macrocyclus	4	59	II		Macrocyclus	7	43 III
Macrocyclus	5	60	II		Macrocyclus	8	69 III
Macrocyclus	6	61	II		Macrocyclus	9	43 III
Macrocyclus	7	127	II		Macrocyclus	10	19 III
Macrocyclus	8	48	II		Macrocyclus	11	47 III
Macrocyclus	9	72	II		Macrocyclus	12	53 III
Macrocyclus	10	120	II		Macrocyclus	13	42 III
Macrocyclus	11	59	II		Macrocyclus	14	41 III
Macrocyclus	12	59	II		Macrocyclus	15	53 III
Macrocyclus	13	67	II		Macrocyclus	16	2 III
Macrocyclus	14	224	II		Macrocyclus	17	53 III
Macrocyclus	15	144	II		Macrocyclus	18	36 III
Macrocyclus	16	83	II		Megacyclops	19	84 II
Macrocyclus	17	58	II		Megacyclops	20	82 II
Macrocyclus	18	61	II		Megacyclops	21	85 II
Macrocyclus	2	61	III		Megacyclops	22	106 II
Macrocyclus	7	72	III		Megacyclops	23	90 II
Macrocyclus	8	69	III		Megacyclops	24	80 II
Macrocyclus	9	59	III		Megacyclops	25	77 II
Macrocyclus	10	72	III		Megacyclops	26	112 II
Macrocyclus	11	61	III		Megacyclops	27	101 II
Macrocyclus	13	59	III		Megacyclops	28	92 II
Macrocyclus	15	78	III		Megacyclops	29	160 II
Macrocyclus	18	62	III		Megacyclops	20	66 III
Megacyclops	19	37	I		Megacyclops	22	53 III
Megacyclops	20	48	I		Megacyclops	23	98 III
Megacyclops	21	48	I		Megacyclops	24	78 III
Megacyclops	22	50	I		Megacyclops	25	99 III
Megacyclops	23	48	I		Megacyclops	26	78 III
Megacyclops	24	29	I		Megacyclops	27	88 III
Megacyclops	25	50	I		Megacyclops	28	82 III

Megacyclo	26	48 I	Megacyclo	29	63 III
Megacyclo	27	45 I			
Megacyclo	28	61 I			
Megacyclo	29	48 I			
Megacyclo	20	61 II			
Megacyclo	22	59 II			
Megacyclo	23	61 II			
Megacyclo	24	59 II			
Megacyclo	25	59 II			
Megacyclo	26	61 II			
Megacyclo	27	61 II			
Megacyclo	28	59 II			
Megacyclo	29	59 II			
Megacyclo	22	61 III			
Megacyclo	23	61 III			
Megacyclo	24	60 III			
Megacyclo	25	61 III			
Megacyclo	26	61 III			
Megacyclo	27	72 III			

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ively with *Diplostomum pseudospathaceum* cercariae *ad libitum* during 17 days. The longevity (h) of re-
/ affected by the female feeding before the experiment than by experimental feeding conditions (cercariae

number of clutch	Female №	Interclutch	Sequence numbers of clutches	Female №	Whole egg	
Macrocyclc	1	13	I-II	Macrocyclc	1	205
Macrocyclc	2	11	I-II	Macrocyclc	2	72
Macrocyclc	3	24	I-II	Macrocyclc	3	72
Macrocyclc	4	13	I-II	Macrocyclc	4	133
Macrocyclc	5	13	I-II	Macrocyclc	5	133
Macrocyclc	6	19	I-II	Macrocyclc	6	144
Macrocyclc	7	12	I-II	Macrocyclc	7	24
Macrocyclc	8	24	I-II	Macrocyclc	8	72
Macrocyclc	9	14	I-II	Macrocyclc	9	48
Macrocyclc	10	24	I-II	Macrocyclc	10	72
Macrocyclc	11	13	I-II	Macrocyclc	11	85
Macrocyclc	12	13	I-II	Macrocyclc	12	145
Macrocyclc	13	5	I-II	Macrocyclc	13	65
Macrocyclc	14	29	I-II	Macrocyclc	14	77
Macrocyclc	15	35	I-II	Macrocyclc	15	48
Macrocyclc	16	144	I-II	Macrocyclc	16	181
Macrocyclc	17	35	I-II	Macrocyclc	17	72
Macrocyclc	18	11	I-II	Macrocyclc	18	72
Macrocyclc	1	41	II-III	Macrocyclc	1	107
Macrocyclc	2	19	II-III	Macrocyclc	2	72
Macrocyclc	3	13	II-III	Macrocyclc	3	61
Macrocyclc	4	24	II-III	Macrocyclc	4	83
Macrocyclc	5	24	II-III	Macrocyclc	5	84
Macrocyclc	6	24	II-III	Macrocyclc	6	85
Macrocyclc	7	17	II-III	Macrocyclc	7	144
Macrocyclc	8	13	II-III	Macrocyclc	8	61
Macrocyclc	9	13	II-III	Macrocyclc	9	85
Macrocyclc	10	13	II-III	Macrocyclc	10	133
Macrocyclc	11	24	II-III	Macrocyclc	11	83
Macrocyclc	12	24	II-III	Macrocyclc	12	83
Macrocyclc	13	13	II-III	Macrocyclc	13	80
Macrocyclc	14	35	II-III	Macrocyclc	14	259
Macrocyclc	15	8	II-III	Macrocyclc	15	152
Macrocyclc	17	38	II-III	Macrocyclc	18	82
Macrocyclc	18	21	II-III	Macrocyclc	7	80
Macrocyclc	7	8	III-IV	Macrocyclc	8	93
Macrocyclc	8	24	III-IV	Macrocyclc	9	72
Macrocyclc	9	13	III-IV	Macrocyclc	10	83
Macrocyclc	10	11	III-IV	Macrocyclc	11	120
Macrocyclc	11	59	III-IV	Macrocyclc	13	131
Macrocyclc	13	72	III-IV	Macrocyclc	18	74
Macrocyclc	18	12	III-IV	Megacycloj	20	96
Megacycloj	20	48	I-II	Megacycloj	21	130
Megacycloj	21	82	I-II	Megacycloj	22	109
Megacycloj	22	59	I-II	Megacycloj	23	96
Megacycloj	23	48	I-II	Megacycloj	24	85
Megacycloj	24	56	I-II	Megacycloj	25	109
Megacycloj	25	59	I-II	Megacycloj	26	96
Megacycloj	26	48	I-II	Megacycloj	27	83
Megacycloj	27	38	I-II	Megacycloj	28	109
Megacycloj	28	48	I-II	Megacycloj	29	85
Megacycloj	29	37	I-II	Megacycloj	20	72

Megacycloj	20	107 II-III	Megacycloj	22	83
Megacycloj	22	24 II-III	Megacycloj	23	144
Megacycloj	23	83 II-III	Megacycloj	24	119
Megacycloj	24	60 II-III	Megacycloj	25	83
Megacycloj	25	24 II-III	Megacycloj	26	120
Megacycloj	26	59 II-III	Megacycloj	27	96
Megacycloj	27	35 II-III	Megacycloj	28	107
Megacycloj	28	48 II-III	Megacycloj	29	83
Megacycloj	29	24 II-III	Megacycloj	22	96
Megacycloj	22	35 III-IV	Megacycloj	23	120
Megacycloj	23	59 III-IV	Megacycloj	24	132
Megacycloj	24	72 III-IV	Megacycloj	25	96
Megacycloj	25	35 III-IV	Megacycloj	27	82
Megacycloj	27	10 III-IV			

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productive periods and the offspring numbers (nauplii) hatched from the each egg clutch* are shown. al diet).

Sequence numbers of clutches

I-II

I-II

I-II

I-II

I-II

I-II

I-II

I-II

I-II

I-II

I-II

I-II

I-II

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I-II

II-III

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II-III
II-III
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III-IV
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III-IV
III-IV
III-IV

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