



CERCARIAL BEHAVIOR DETERMINES RISK OF PREDATION

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KEY WORDS ABSTRACT

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The potential for local biodiversity to affect transmission success of parasites has been shown to be particularly important in trematodes, where non-host organisms can feed on and ‘dilute’ free-living infective stages (cercariae). Earlier studies have analyzed the effects of various predators on transmission stages of single trematode species, but not how cercariae of different species react to predation pressure. Here, we tested whether cercariae with different host-searching movement patterns show varying susceptibility to predation by non-host species with different feeding habits. For this study, we performed a set of predation experiments with 6 species of trematode cercariae (*Coitocaecum parvum*, *Maritrema poulini*, *Apatemon* sp., *Telogaster opisthorchis*, Plagiorchioid sp. I, and Aporocotylid sp. II) that represent 2 groups of host-searching behavior, free-swimming vs. bottom-dwelling, and 2 predators (*Sphaerium* sp., *Physa acuta*) with distinct feeding modes, a filter feeder and a grazer. Our results show that cercarial susceptibility to predation is highly dependent on the interspecific interaction between dispersal behavior of cercariae and feeding behavior of non-host organisms: Filter feeders only diluted free-swimming cercarial stages, not bottom-dwelling ones; grazers on the other hand, had no effect on free-swimming cercariae but reduced bottom-dwelling cercariae in 1 trematode species. Our findings give further support to the hypothesis that the transmission dynamics of trematodes do not simply depend on local biodiversity but rather on the species-specific interactions between parasite transmission stages and free-living organisms in the ecosystem. This has important implications for disease dynamics in ecological communities (e.g., the parasites’ infection success), and for ecosystem energetics, as cercariae constitute important food items.

The complex life cycles of parasites often consist of series of challenging transmission events from 1 host to the next (Poulin and Lagrue, 2015). Trematodes overcome 1 of these transmission events by multiplying asexually in their first intermediate mollusc host, producing numerous genetically identical dispersal stages to increase the odds that at least a few individuals will reach their next target host (Poulin, 2011). Typically released into the aquatic environment in large numbers, these dispersal stages (cercariae) form an important component of the zooplankton community (Morley, 2012), and their production contributes significantly to the total biomass and energy flow in aquatic ecosystems (Kuris et al., 2008; Preston et al., 2013; Soldánová et al., 2016). In their search for an appropriate host to infect, cercariae themselves serve as prey for a wide variety of non-host organisms, such as insects, cnidarians, bivalves, crustaceans, juvenile fish, or even other zooplankton organisms (e.g., Mouritsen and Poulin, 2003; Schotthoefer et al., 2007; Thieltges et al., 2008a, 2008b; Welsh et al., 2014; Mironova et al., 2019). Consequently, predation is a central selective factor in the success of cercarial transmission and the chances of encountering and infecting a suitable target host

(Johnson et al., 2010; Thieltges et al., 2013; Gopko et al., 2017), with a variety of factors influencing these interactions, ranging from parasite density and the presence of alternative prey items (Welsh et al., 2017) to host and predator size and predator foraging mode (Orlofske et al., 2012, 2015). Overall, this removal of parasite dispersal stages from the ecosystem is considered an important dilution effect that structures parasite communities in ecosystems, and recent studies have highlighted its potentially strong impact on the population dynamics of parasites (Thieltges et al., 2008a; Welsh et al., 2014, 2017), especially under changing climate conditions (Goedknecht et al., 2015). Studying these fundamental processes in detail is, therefore, a central prerequisite for our understanding of the dynamics of parasite communities and disease ecology.

Previous studies have predominantly tested the effects of different predators on cercariae of a single model species. However, since cercariae come in all shapes and sizes (see Morley, 2012), and their host-finding behavior and orientation in the water column are highly species-specific and depend on the target host (Combes et al., 1994), we do not expect them to be equally

likely to be preyed on by the same predators. On the contrary, we hypothesize that trematode cercariae with different host-searching movement patterns will show different susceptibility to predation by species with different feeding habits, comparable to the effects of cercarial size (Orlofske et al., 2015). Here, we sought to test this hypothesis via a set of feeding experiments with cercariae representing 6 species of trematode released by the New Zealand mud snail *Potamopyrgus antipodarum* and 2 predators with distinct feeding modes, a filter feeder and a grazer.

For this investigation, we collected snails (*P. antipodarum*) along the shoreline of Tomahawk Lagoon (South Island, New Zealand; 45°54'06.0"S, 170°33'02.2"E). For identification of patent trematode infections, snails were separated into individual wells of 24-well plates filled with 1 ml of filtered lake water and incubated for 48 hr at 20 C under constant light to induce shedding of trematode cercariae. Wells were checked daily for newly emerged cercariae. Cercariae were identified based on morphological features, using the keys of Winterbourn (1973), Hechinger (2012), and Presswell et al. (2014). We isolated snails infected with the following trematode species: *Coitocaeum parvum*, *Maritrema poulini*, *Apatemon* sp., *Telogaster opisthorchis*, Plagiorchioid sp. I, and Aporocotyloid sp. II. Infected snails were grouped according to their trematode species and kept in 5-L aquaria with aerated lake water and macrophytes (*Myriophyllum triphyllum* and *Eloдея canadensis*) for food at a constant temperature (20 C) under a 12 hr/12 hr light/dark photoperiod until the beginning of the experiments. The selected trematode species represent 2 groups of cercarial host-searching behavior: Fish-infecting cercariae (*T. opisthorchis*, *Apatemon* sp., Aporocotyloid sp. II) are good swimmers that move up and down in the water column in search of a suitable target host; cercariae of trematodes that target benthic arthropods (*C. parvum*, *M. poulini*, and Plagiorchioid sp. I) either crawl on the sediment surface or stay very close to it (see Selbach and Poulin, 2018).

We selected 2 potential predator species with different feeding behaviors: a filter feeder, the clam *Sphaerium* sp., and a grazer, the freshwater snail *Physa acuta*. Both species occur in sympatry and high densities with our snail–trematode system and were therefore likely to encounter the cercariae (Lagruе and Poulin, 2015a, 2015b). All predators were collected at the same sampling localities where snails were obtained. In the laboratory, individuals were sorted by species and kept in aquaria with filtered aerated lake water at a constant temperature (20 C) under a 12 hr/12 hr light/dark photoperiod for 24 hr to acclimatize before the beginning of the feeding experiments. Predator species were fasted for 48 hr prior to their use in experimental trials.

To obtain cercariae for the predation experiments, their emergence from infected snails was induced with a 2 hr incubation as described above. Since cercarial activity decreases over time, all cercariae used in the experiments were of the same 'age' (<2 hr). Active cercariae were haphazardly selected and individually transferred with a pipette to wells of 12-well plates containing 4 ml of filtered lake water. Compared to larger snail host species, such as *Lymnaea stagnalis*, which can release thousands of cercariae in a short amount of time (Soldánová et al., 2016), the small-bodied *P. antipodarum* often only release a few cercariae per day. Since specific shedding rates depend on the size of the individual cercariae, with smaller trematodes emerging in higher numbers (Rosenkranz et al., 2018), we chose the numbers of larvae in the feeding trials accordingly: For small-bodied cercariae

(*M. poulini*, *Apatemon* sp., Aporocotyloid sp. II), we used 10 individuals per trial; for larger cercariae, we used 5 (*T. opisthorchis*, Plagiorchioid sp. I) and 2 specimens (*Coitocaeum parvum*) per trial. Cercariae could move freely within the water column in the individual wells (diameter: 2.4 cm). To each well, we either added 1 of the 2 predators or no predator for control groups. Only predators of similar size were used in the experiment (*Sphaerium* sp., length 4.9 ± 0.4 mm; *Ph. acuta*, length 5.5 ± 0.6 mm). All well plates were then placed in an illuminated climate chamber at 20 C for 2 hr for the feeding experiment. After this time, the predators were carefully removed from the well, checked for any attached cercariae, and the remaining cercariae in each well were counted under a stereomicroscope. The experiment was performed in multiple (8) batches with equal numbers of replicate wells assigned to each treatment (*Sphaerium* sp., *Ph. acuta*, control) in each batch. All experiments were performed at the same time of day to avoid confounding effects. Treatments with inactive predators (i.e., snails that had not moved and clams that had not opened) were discarded, which resulted in slightly different numbers between treatments used in the analysis. Statistical analyses were performed with GraphPad Prism (v.5) to test for differences between the number of cercariae in the control groups vs. the groups with *Sphaerium* sp. and *Ph. acuta*, respectively. Since data were not normally distributed, median numbers were compared between groups (predator vs. control) using the non-parametric Mann–Whitney test.

The filter-feeding clam *Sphaerium* sp. significantly reduced the numbers of all free-swimming species by 36 to 66% (all $P < 0.001$), but its presence in a well had no effect on the abundance of bottom-dwelling cercariae (Fig. 1A). Only a small number of cercariae of Plagiorchioid sp. I was available, which only allowed a limited number of replicates for this species, and no statistical test was performed for this group. The grazing snail *Ph. acuta* only showed a significant reduction of cercarial numbers for the bottom-dwelling *C. parvum* (Fig. 1B). A shortage of cercariae prevented any test of the impact of this predator on cercariae of Plagiorchioid sp. I.

In diverse communities, transmission between hosts can be a challenging event in the life cycle of parasites, with the presence of multiple non-host organisms reducing the chance that infective stages reach their target host (Thieltges et al., 2008a, 2008b). Our results indicate for the first time that cercarial susceptibility of different trematode species to predation is not only highly dependent on the feeding behavior of non-host organisms, but it is also dependent on the dispersal behavior of cercariae. In our study, filter feeders only diluted free-swimming cercariae, not bottom-dwelling ones, whereas grazers had no effect on free-swimming cercariae but reduced the numbers of 1 species with bottom-dwelling cercariae. The difference in susceptibility to predation between the bottom-dwelling cercariae of *M. poulini* and *C. parvum* is due to their species-specific movement patterns, which we could observe under the stereomicroscope. The small cercariae of *M. poulini* cover the ground in small horizontal loops and are simply pushed aside by the grazing snail. *Coitocaeum parvum* cercariae, on the other hand, attach to the substrate with their short sucker-like tail and move in a crawling motion (Selbach and Poulin, 2018), where they can get 'run-over' and scraped off by the grazing mollusc. However, the generality of the effects of grazers on bottom-dwelling trematode transmission stages, including metacercariae (see Vielma et al., 2019), remains

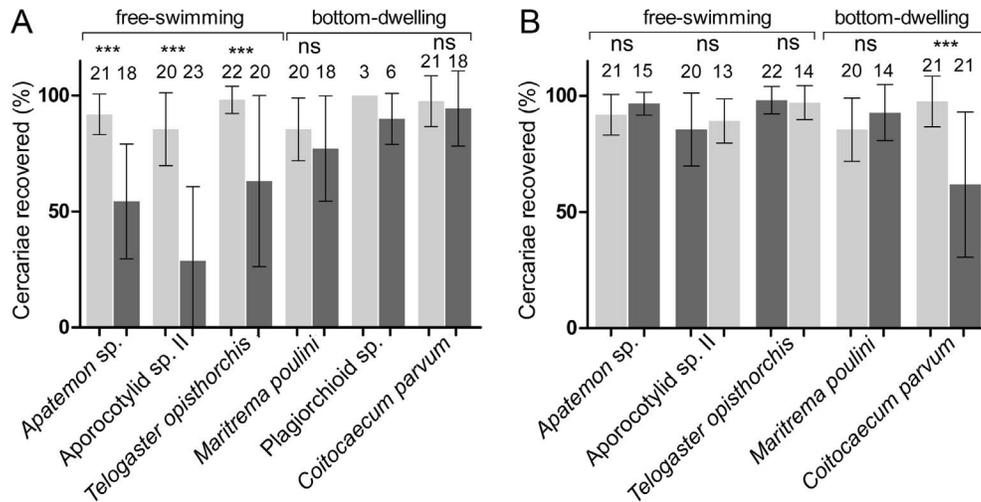


Figure 1. Percentage of cercariae remaining after the 2 hr predation experiments with 2 types of molluscan predators: (A) *Sphaerium* sp., a filter feeder, and (B) *Physa acuta*, a grazer. Dark bars show treatments with predators, and light bars are controls without predator; error bars show SD. Trematode cercariae are grouped as either free-swimming or bottom-dwelling types. Numbers above bars show the number of replicates for each treatment; asterisks above bars denote statistically significant differences between medians of control vs. predator treatments (*** $P < 0.001$, ns = not significant; Mann–Whitney test).

to be tested for more trematode species. Additionally, we did not specifically design our study and test for the effect of cercarial size on susceptibility to predation. In the case of the clam, both large (*T. opisthorchis*) and small (*Apatemon* sp. and *Aporocotylid* sp. II) cercariae were diluted. It would be revealing to conduct future studies to test for interactions between body size and parasite removal rates by filter feeders that have been shown to be active predators, such as fish and larval damselflies (Orlofske et al., 2015).

Overall, the removal of trematode transmission stages is likely to have a strong parasite dilution effect in the studied ecosystem, especially given the high densities of both feeders that occur in sympatry with the host–parasite system (reaching several thousands per square meter; Lagrue and Poulin, 2015b). Our results lend further support to the hypothesis that the transmission dynamics of parasites do not so much depend on biodiversity per se, but rather on the species-specific interactions between transmission stages and non-host organisms, including alternative prey items, in the ecosystem (see Welsh et al., 2017 and references therein). Assessing the parasite dilution effects of biodiverse communities, and possible impacts on infection dynamics in an ecosystem should, therefore, take these species-specific interaction patterns into account.

This study was designed to test for the removal of cercariae from the water column under laboratory conditions to investigate whether different cercarial types show different susceptibility to predation. Based on our results, our hypothesis is supported. The extent to which this removal of transmission stages accounts for differences in the parasites' actual infection success, i.e., the successful encystment as metacercariae in the target host, under natural conditions remains to be tested, and the implications of the current results from small-scale laboratory experiments need to be interpreted with caution. However, recent studies have found similarly significant reductions of trematode transmission in larger experimental setups (e.g., Gopko et al., 2017; Welsh et al., 2017). More realistic mesocosm experiments, simulating

natural conditions (e.g., larger volumes of water, decoy organisms, alternative prey items, several predators in the same system) and species densities in the ecosystem, could answer this question for the present predator–parasite system in future studies. For example, the possible interactive effects on infection success of different trematode species of the presence of various predators and decoy organisms as well as hosts occurring in sympatry could be tested this way. Moreover, the effects of cercarial density on the removal rate of cercariae (see Welsh et al., 2017) would be particularly important for trematode species relying on a small-bodied intermediate host that can only provide the resources for the production of a limited number of dispersal stages. The species *M. poulini* and *C. parvum* would offer an ideal model system for this, since their cercariae target the same second intermediate host, the amphipod *Paracalliope fluviatilis*, and they show varying vulnerability to predation by grazers (see Friesen et al., 2017).

The role of parasites as food for non-host organisms has been increasingly recognized in recent years (Johnson et al., 2010; Thielges et al., 2013). Trematode cercariae appear to be regular components of the diet of many small-bodied organisms, in some cases even providing sufficient energy to sustain long-term predator survival and reproduction (Mironova et al., 2019). However, recent studies have shown that parasite biomass is not partitioned equally across the individual trematode taxa but is dominated by few individual species that can make up 80% of the cercarial mass released into the aquatic habitat (Rosenkranz et al., 2018). Accordingly, the extent to which this energy is available to predators largely depends on the feeding strategy of these organisms, and the type of cercariae that are present in a system (e.g., free-swimming or bottom-dwelling). For example, in a system dominated by trematodes with bottom-dwelling cercariae, filter feeders might simply not be able to access the energy provided by these parasites. Altogether, the complex and specific interactions between parasites and non-host organisms play

important roles in both food web and disease dynamics and are fundamental to our understanding of ecosystems.

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