



Microinvertebrate consumption rates of *Fasciola hepatica* miracidia are not affected by alternate food

Daniel McDowell^{a,*}, Sarah E. Perkins^a, Frank Van Veen^b, Joanne Lello^a

^a School of Biosciences, Cardiff University, Cardiff, UK

^b Penryn Campus, University of Exeter, Cornwall, UK

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ABSTRACT

The liver fluke (*Fasciola hepatica*) is a significant parasite of the global livestock industry, leading to negative economic and animal welfare impacts. Control of *F. hepatica* is becoming increasingly difficult as many liver fluke populations are developing resistance to commonly used anthelmintics. Additional or alternate control methods are, therefore, required. Microinvertebrates such as those of the order Cyclopoida and subclass Ostracoda are common organisms found in the same aquatic habitats as *F. hepatica*'s intermediate snail host. We explore whether these microinvertebrates are effective predators of *F. hepatica* miracidia. We experimentally determined a) the consumption rates of miracidia by the two microinvertebrate groups, b) the form of functional feeding response displayed by each and c) whether inclusion of an alternate food source altered miracidial predation patterns. We find that cyclopoids and ostracods feed on miracidia and that where a statistically supported fit was found, the functional feeding response for both microinvertebrates was type II. Further, miracidial consumption by either microinvertebrate did not decline significantly in the presence of alternate prey. Our results suggest that cyclopoids and ostracods are both effective predators of *F. hepatica* and therefore have the potential as *F. hepatica* biocontrol agents. An important next step will be to explore what impact such predation has on the infection dynamics of the adult fluke in the definitive host.

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1. Introduction

The liver fluke, *Fasciola hepatica*, is a trematode of global importance in livestock, and the causative agent of fasciolosis, a neglected zoonotic disease in many Low- and Middle-Income Countries such as Bolivia and Peru, with around 180 million people at risk of infection (Caravedo and Cabada, 2020; Cwiklinski et al., 2016). Infections of *F. hepatica*, in livestock, are associated with a cost of €970 million per year in Europe (Charlier et al., 2014), due to reduced meat and dairy yields and, in extreme cases, host death (Skuce and Zadoks, 2013). Globally, *F. hepatica* is showing increasing resistance to the anthelmintics which target the fluke in the definitive host (Ceballos et al., 2019; Hassell and Chapman, 2012; Kamaludeen et al., 2019; Novobilský et al., 2012), making the development of additional control methods essential.

The liver fluke has a complex life cycle, involving a definitive host, an amphibious molluscan intermediate host and two motile free-living stages. The first motile stage, miracidia, are short lived (24 h) and hatch from eggs shed in the definitive host faeces

(Andrews et al., 2021). Upon emergence they seek out and infect an intermediate snail host (mainly *Galba truncatula* in the UK) in shallow, aquatic environments (Andrews et al., 2021). Asexual replication occurs within the snail through sporocysts and rediae before the release of the second motile stage, cercariae. Cercariae emerge from the snail and swim to vegetation, where they encyst as metacercariae and may then be consumed when the definitive host grazes the vegetation (Andrews et al., 2021).

Current chemotherapeutic treatments for *F. hepatica* only target the fluke within the definitive host, which can leave a reservoir of parasites within the environment (Kaplan, 2001), leading to reinfection. Further, practices such as draining land can remove the habitat of *G. truncatula*, and therefore *F. hepatica*, however, draining land is non-host specific and likely removes the habitat for a variety of other aquatic invertebrates. For parasites with complex life cycles, an understanding of the association between the free-living environmental stages (i.e. miracidia/Cercariae) and other organisms could be key to environmentally friendly control solutions (e.g. biocontrol), for example, by identifying natural predators of these parasite life stages (Muñiz-Pareja and Iturbe-Espinoza, 2018).

* Corresponding author.

E-mail address: mcdowellj@cardiff.ac.uk (D. McDowell).

Natural predators of the fluke intermediate stages could provide an ecological alternative, or supplement, to the current chemical control of *F. hepatica*. Although the consumption of other trematode cercarial stages has been widely studied (Koprivnikar et al., 2023; Mironova et al., 2019; Orlofske et al., 2012, 2015; Welsh et al., 2017), studies on the consumption of miracidia are much less common and for *F. hepatica* only the consumption by *Daphnia* sp. and *Chaetogaster limnaei* has been explored. (Christensen et al., 1977; Muñiz-Pareja and Iturbe-Espinoza, 2018). Consumption of miracidia by microinvertebrates could reduce or prevent infection of the intermediate host, preventing the substantial replicative phase within the snail and the subsequent formation of the long-lived and resistant metacercarial life stage. Liver fluke and their intermediate hosts are found in habitats that support a diverse community of freshwater invertebrates. Microinvertebrates, such as cladocerans, cyclopoids and ostracods, are often found within these shallow, transient, aquatic habitats, where they can occur in high abundance (Smith et al., 2015; Thackeray and Beisner, 2024). The filter feeding microinvertebrate *Daphnia pulex* was observed to consume *F. hepatica* miracidia (Christensen et al., 1977). *Daphnia pulex* are, however, passive filter feeders and consumption by predatory microinvertebrates has not been explored but could result in faster and / or more effective removal of the miracidia from a water body.

Potential miracidial feeders include cyclopoids, which are raptorial predators and shown to consume cercariae of trematodes of the genus *Diplostomum* (Mironova et al., 2020; Thackeray and Beisner, 2024), and ostracods which, though considered benthic detritivores, are motile, consuming organisms in the water column (Havel, 1993). Both are known to consume organisms of a similar size to *F. hepatica* miracidia (e.g. *Paraemicium caudatum*; Mironova et al., 2020; Shostak and Dick, 1986) and are often found within shallow, aquatic habitats where they can occur in high abundance (Smith et al., 2015; Thackeray and Beisner, 2024).

To determine the effectiveness of predators as a biocontrol agents, functional responses are often assessed (Delong and Uiterwaal, 2022; Islam et al., 2020). Functional responses detail the relationship between the number of prey consumed and the number of prey available, and can determine the stability of bio-control agent-pest/pathogen interactions (Khan, 2009). The shape of the functional response is dependent on a multitude of factors including prey mobility, temperature (Born-Torrijos et al., 2020), and the presence of multiple prey species (Elliott, 2004). In the case of multiple prey sources, when the density of one prey species becomes low the predator may preferentially switch to the alternative prey species, meaning that predation on the first prey species at low densities is lower than expected by chance (Prokopenko et al., 2023). The barnacle *Semibalanus balanoides* and the shrimp *Crangon crangon*, reduced consumption of cercariae when alternate prey was present. Conversely, consumption of cercariae was not shown to be affected by alternative prey for the crab, *Hemigrapsus takanoi*, the oyster *Crassostrea gigas* and the cyclopoid *Macrocyclus distimctus* (Mironova et al., 2020; Welsh et al., 2017). Here, we determined whether microinvertebrates from the subclass Ostracoda (*Heterocypris incongruens*) and order Cyclopoida, which are commonly found in liver fluke endemic habitat, predated *F. hepatica* miracidia. Specifically, we determined 1) their capacity to consume the miracidia, including assessment of the functional feeding responses, and 2) whether predation was maintained in the presence of alternate food (i.e. when both *F. hepatica* and *P. caudatum* were presented). We hypothesised that both Ostracoda and Cyclopoida would effectively predate *F. hepatica* miracidia, but that the raptorial cyclopoids would display a higher consumption rate than ostracods in both single and mixed feeding conditions due to a more active mode of hunting.

2. Methods

2.1. 2.1. Study organisms

Habitats identified as containing the intermediate host snail, *G. truncatula*, and contaminated with sheep and cattle faeces, were chosen at random for microinvertebrate sampling, from three farms in south Wales (Cardiff, Bridgend, Merthyr Tydfil). All three farms were confirmed by farmers to have sheep and cattle infected with *F. hepatica*. Water samples ($n = 16$) were collected in 50 ml plastic falcon tubes from aquatic habitats across the farms (puddles, poached land, drainage ditches). Densities of ostracods ranged from 0 to 193 individuals (mean = 32.75, SE = 3.64) and cyclopoids from 0 to 72 (mean = 11.99, SE = 2.35) per 50 ml. In the laboratory, ostracods (identified as *Heterocypris incongruens*) and cyclopoids were identified from these samples under a GX stereo microscope, collected using a 1 ml pipette with the end of the pipette tip cut off, allowing collection of microinvertebrates without damage. Ostracods and cyclopoids were then separated from one another and transferred into plastic boxes (1.46L) and maintained in sterilised dechlorinated water in a controlled temperature room at 20 ± 2 °C with a 16:8 light:dark regime and aeration, for 24 h without food. *F. hepatica* eggs were provided by the University of Liverpool from a liver fluke-definitive host (sheep) system. Eggs were stored at 4 ± 3 °C in dechlorinated, sterile water until required. The water was replaced every week to reduce the chance of fungal growth. A sample of eggs was transferred to a 3.5 cm diameter Petri dish with enough water to cover them. Eggs were then immediately incubated at 25 ± 1 °C for 2 weeks, in the dark, to stimulate embryonation. After 2 weeks, miracidia were visible within eggs, as detected by the presence of an eye spot under a GX stereo microscope, they were then placed under light to stimulate hatching and collected for experimentation. Experimentation was conducted within 2 h of miracidia hatching. The alternative food source *P. caudatum* were provided by the supplier (Blades Biological Ltd) in a hay-infused water solution. Upon delivery *P. caudatum* were stored at room temperature in hay infused water for no longer than two hours before storage in a controlled temperature room (20 ± 2 °C) for one day prior to the experiments.

All experiments were conducted at 20 ± 2 °C. First, we determined whether the predators consumed *F. hepatica* (2.2) then the duration at which the functional response experiments should take place (2.3), after which we conducted the functional response experiments to a single prey species (2.4), then to multiple prey species (2.5). A diagram showing the order of experiments is shown in the supplementary information (Fig. S1).

2.2. Consumption of *F. hepatica* miracidia

Ostracods ($n = 10$) and cyclopoids ($n = 10$), between 0.9 and 1.4 mm, taken from stock colonies after the 24-hour acclimatisation period were isolated in 2 cm³ single well plates containing 2 ml of sterile water at 20 ± 2 °C for a 24-hour starvation period. After the 24-hour starvation period, 15 miracidia were added to each well. After 45 min, we conducted a counting protocol whereby miracidia were fixed for preservation using 90% ethanol and the prey were dyed to aid visualisation, by adding 10 µl of diluted Rose Bengal solution (0.5 g in 100 ml of water) to each well and incubating at room temperature for two hours. Remaining prey were then counted under a compound microscope at 4x magnification (Olympus CX23).

2.3. Determining experimental duration

To determine the duration of the functional response experiments, cyclopoids taken from stock colonies after the 24-hour acclimatisation period were added to 2 cm³ wells of a 25 well plate containing 2 ml of sterile water along with 30, 40 or 50 miracidia ($n = 9$; 3 replicates each). Every ten minutes, for three hours, miracidia were counted under a GX stereo microscope. From this pilot study, we determined that 45 min was an adequate time to observe the consumption of miracidia, as the number of miracidia remaining plateaued thereafter (Fig. S2).

2.4. Functional response to *F. hepatica* miracidia and *P. caudatum*

Feeding rates were estimated for ostracods and cyclopoids on single prey species, *F. hepatica* miracidia and *P. caudatum*, at five different prey densities (5, 10, 20, 40, 60; $n = 6$ per treatment) without prey replacement. Ostracods and cyclopoids were taken from stock colonies after the 24-hour acclimatisation period and isolated in single well plates at 20 ± 2 °C for a 24-hour starvation period. The following day, miracidia were collected from stock colonies using a 200 µl pipette and counted under a GX stereomicroscope and then divided into the five prey densities in single wells of a 25 well plate with 2 ml of sterile, room temperature water after which, a single starved predator was added to each well. A control treatment was included with the same prey densities but with no predator. After 45 min, miracidia and *P. caudatum* were counted using the above protocol.

2.5. Functional response to mixed prey sources

For both ostracods and cyclopoids, experiments were conducted, in which *P. caudatum* was maintained at a density equal to the maximum ingestion rate as determined previously by the single prey experiments (see results 3.2), while miracidia were added at five different densities (5, 10, 20, 40, 60; $n = 6$ per density per predator). Functional responses to mixed prey treatments were then compared to single prey functional responses conducted at the same time, to ensure there was no effect of experiment day on consumption rates.

2.6. Data availability and statistical analysis

The data that supports the findings of this study are available on Mendeley Data (<https://doi.org/10.17632/tyhs5bt6y3.1>). All analyses were carried out in the programming software R, version 4.0.5 (R core team, 2021).

2.6.1. Consumption of *F. hepatica* miracidia

A beta regression model using the betareg package (Cribari-Neto and Zeileis, 2010) was conducted to determine differences in miracidia removal between the control, and ostracod and cyclopoid treatments.

2.6.2. Functional responses

To determine the form of the functional response for cyclopoids and ostracods, logistic regressions were fitted to data for each predator type consuming a single prey species using 'frair_test' in the friar package (Pritchard et al., 2017). Type I responses are linear and are characterised by a n attack rate. Type II responses are characterised by a significant negative first order term (decreasing consumption with increasing prey density), with the parameters attack rate and handling time, whereas type III responses have a significant positive first order term then a significant negative second order term resulting in a sigmoidal shape as a result of the scaling exponent (Pritchard et al., 2017). Functional response mod-

els were fit using 'frair_fit'. Where 'frair_test' did not indicate a particular model fit, estimated starting values for attack rate, handling time and the scaling exponent were fit using type I, Roger's type II and a Hassell's type III equation. Time was treated as minutes in an hour, i.e. $T = 0.75/1$ to represent 45 min. The different equations were applied to account for the potential problem of estimating functional responses in experiments in which there is prey depletion (Rosenbaum and Rall, 2018). To determine the best fitting model the AICcs of each were compared and the lowest value with a difference > 2 was chosen as the best fit (Pritchard et al., 2017). In cases where differences were not found, the lowest AICc was chosen for visualisation purposes. Best fit models were bootstrapped ($n = 999$) using the 'frair_boot' function to generate 95% confidence intervals (Dixon, 2001).

2.7. Ethics

Fasciola hepatica eggs were sourced from experimental infection of sheep at the University of Liverpool, under Home Office Licence PPL 40/3621 and PE77BFD98, in accordance with Animal (Scientific Procedures) Act 1986.

3. Results

3.1. Microinvertebrates consume *F. hepatica* miracidia

Cyclopoids and ostracods consumed a large percentage of miracidia during the experiment (estimate = 0.92, $se = 0.19$, $z = 4.84$, $p < 0.001$). Cyclopoids removed 93% of miracidia ($SE = 4.98$) and ostracods removed 86% ($SE = 5.27$), however, the difference between the two predators was insignificant (estimate = 0.28, $se = 0.33$, $z = 0.83$, $p = 0.4$) (Fig. S3). For control treatments with no predators, 100% of miracidia were recovered (Fig. S3).

3.2. Microinvertebrates show different functional responses when consuming *F. hepatica* miracidia

Cyclopoids were observed to have a type II functional response to miracidia (1st order term = -0.018 , $p < 0.001$; Fig. 1a; Table 1), however, for ostracods, a type I functional response was the most appropriate model fit as the proportion of prey consumed was not affected by increasing prey density. When *P. caudatum* was the prey species, ostracods and cyclopoids were observed to have a type II response (1st order term = -0.024 , $p < 0.001$; 1st order term = -0.032 , $p < 0.001$; respectively; Fig. 1b; Table 1). Functional responses towards *P. caudatum* plateaued at the highest focal density (60) with the maximum ingestion rate being 15 for cyclopoids consuming *P. caudatum* and 7 for ostracods. The maximum ingestion rate at the maximum provided focal density (60) for cyclopoids consuming miracidia was 25 and 15 for ostracods. Here, we chose the highest ingestion rates by cyclopoids to be the maximum ingestion rate and for each of miracidia 25 and *P. caudatum* (15). Under control treatments with no predators, the number of prey remaining was highly similar to the number of prey which started (Fig. 1c).

3.3. Mixed prey sources do not affect microinvertebrate consumption of *F. hepatica* miracidia

In mixed treatments, cyclopoids and ostracods consumed both prey species available. A type II functional response was the best fitting model for ostracods consuming miracidia presented in mixed prey sources (1st order term = -0.0097 , $p < 0.05$), reducing miracidia consumption as prey density increases (Fig. 2a; Table 1). In contrast, a type I response was the most appropriate descriptor

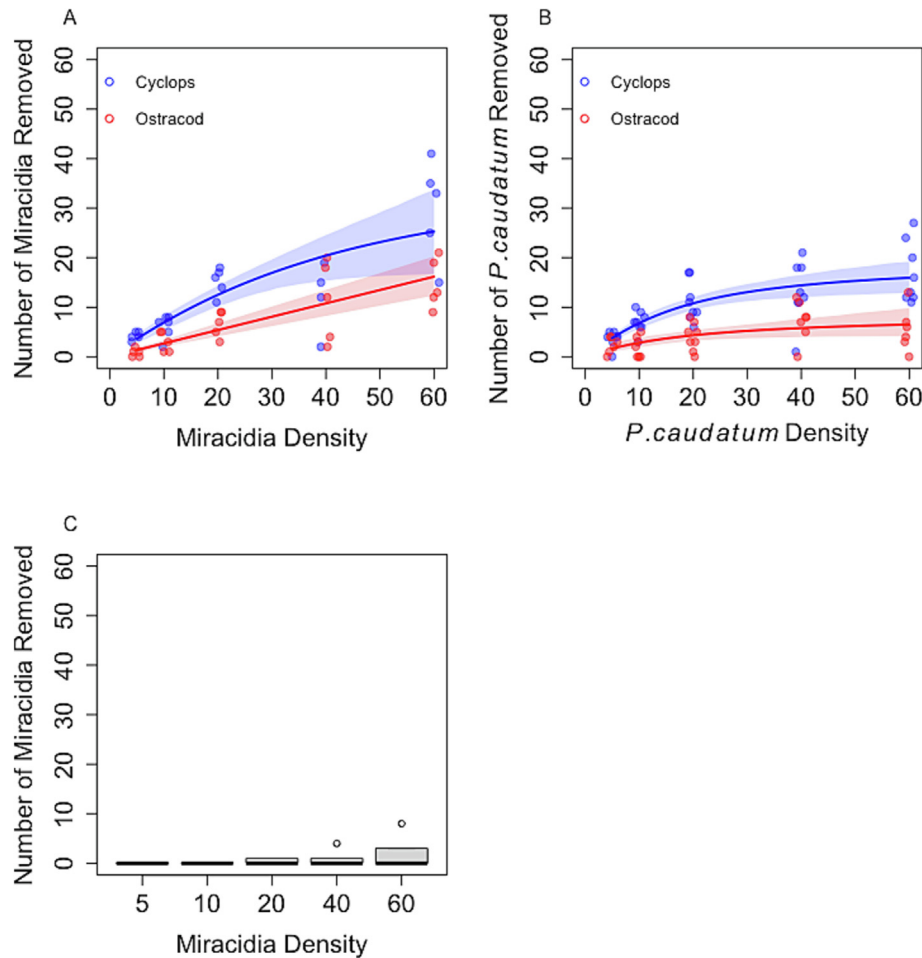


Fig. 1. Functional responses of cyclopoids (blue) and ostracods (red) for two different prey species, *F. hepatica* miracidia (A) and *P. caudatum* (B). Dots indicate the raw data, shaded areas represent 95% confidence intervals calculated from bootstraps ($n = 999$) and the block line the functional response. C) Boxplot showing the control treatments where 96–100% of miracidia were recovered. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Functional response type, results from the logistic regression and the attack rate and/or handling time for each experimental treatment.

Predator	Treatment	Prey	Functional response type	1st order term	<i>P</i> value	<i>a</i>	<i>h</i>
Ostracod	Single	Miracidia	I	–	–	0.3595	–
		<i>P. caudatum</i>	II	–0.0179899	<0.001	1.883	0.018
Cyclops		Miracidia	II	–0.0241877	<0.001	0.666	0.089
		<i>P. caudatum</i>	II	–0.0322043	<0.001	2.336	0.039
Ostracod	Mixed	Miracidia	II	–0.0097701	<0.05	1.543	0.009
Cyclops		Miracidia	I	–	–	0.758	–

for cyclopoids consuming miracidia as part of a mixed prey source (Fig. 2b; Table 1). Overlapping 95% confidence intervals, despite differences in functional responses between mixed and single prey treatments, indicate that the addition of alternate prey did not alter consumption rates.

4. Discussion

We have shown that ostracods and cyclopoids consume the miracidia of *F. hepatica*. In accordance with our hypothesis, we find that cyclopoids consume *F. hepatica* miracidia following a type II functional response, and that they consume more miracidia than filter feeding ostracods. In contrast, we did not observe differences in consumption between single and mixed prey treatments, and therefore, reject this hypothesis.

Previous studies have shown that *F. hepatica* miracidia are consumed by other aquatic microinvertebrates, *Daphnia pulex* and *Chaetogaster limnae limnae* (Christensen et al., 1977; Muñiz-Pareja and Iturbe-Espinoza, 2018). Our results show that two further aquatic microinvertebrates consume *F. hepatica* miracidia and consolidates the idea that *F. hepatica* may form an important component of the wider aquatic ecological community, acting not only as a parasite of *G. truncatula* and livestock, but also as an important food source for the microinvertebrate community. Our study is also the first to examine predation by fast swimming, motile predators and to estimate the functional feeding responses. Similar to studies focussing on the consumption of cercariae (Born-Torrijos et al., 2020; Mironova et al., 2020), we show that cyclopoids consumed the miracidia of *F. hepatica*, when the sole prey source, following a type II response. For ostracods, a type I response

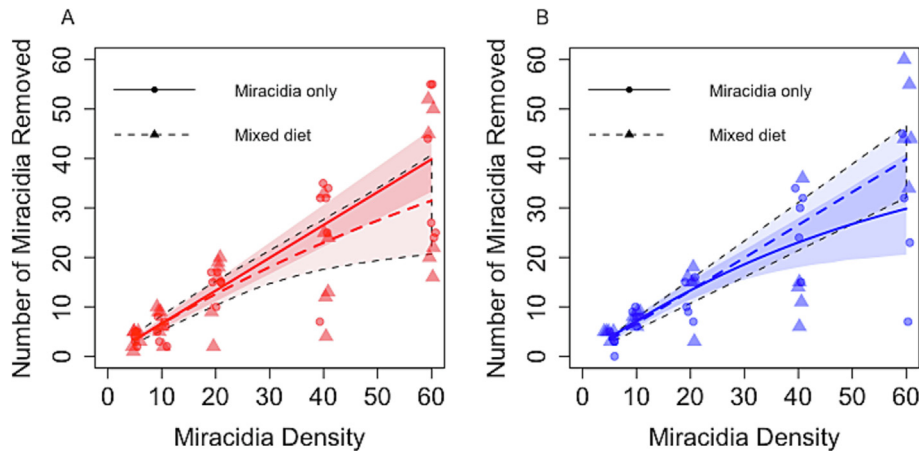


Fig. 2. The consumption of *F. hepatica* miracidia by ostracods (A) and cyclopoids (B) when *P. caudatum* at a constant initial density. Colours denote the different predators with cyclopoids (blue) and ostracods (red). The block line represents the functional response to a single prey source and the dashed line to a mixed prey source. Shapes denote a single prey source (circles) and mixed prey (triangles). Shaded areas represent 95% confidence intervals of the predicted mean calculated from bootstraps ($n = 999$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was the best fit when consuming miracidia, suggesting that the handling time is minimal and that ostracods were not fully satiated (Jeschke et al., 2004).

The consumption of miracidia by cyclopoids and ostracods, albeit non-significant due to overlapping error bars (Fig.S4) was slightly greater than the consumption of *P. caudatum*, and this pattern remains true at all prey densities for cyclopoids. Miracidia are quicker than *P. caudatum* with a speed of 1.25 mm/s compared to 0.9 mm/s at 25 °C (Cerini et al., 2023; Villa-Mancera et al., 2015), which could lead to higher encounter rates between predators and miracidia (McCoy and Bolker, 2008) in a closed system. Slower swimming ciliates may also be better at avoiding detection by active predatory microinvertebrates (Rabette et al., 1998), suggesting quicker ciliates, such as miracidia, may be an easily detected prey source. Further, miracidia were 1.13 times larger than *P. caudatum* in this study and may be more visible, which could further explain a greater consumption of miracidia than *P. caudatum*. The lower consumption of prey by ostracods can be explained by their feeding behaviour. Ostracods gather multiple prey items which they transfer to the mouth, grinding up prey (Smith et al., 2015). Cyclopoids on the other hand are active, raptorial predators and can consume prey items whole (Thackeray and Beisner, 2024).

It is important to explore the effects of alternate food on predator–prey interactions as any disruption in the feeding patterns could limit the disruptive effect of *F. hepatica* infection dynamics. Crabs (*Hemigrapsus takanoi*) and oysters (*Crassostrea gigas*), consuming the cercariae of *Himasthla elongata*, for example, were not affected by the presence of alternate food while barnacles (*Semibalanus balanoides*) and shrimp (*Crangon crangon*) showed reduced consumption of cercariae when presented with additional alternate prey sources (Welsh et al., 2017). In our mixed food treatments, the consumption rates of miracidia were shown to be robust to the presence of alternate prey, suggesting that alternate prey may not affect the removal of *F. hepatica* miracidia. Consumption rates of miracidia may be altered by seasonal population dynamics of their predators. Ostracod and cyclopoid densities peak in late spring and early summer (Hansen and Jeppesen, 1992; Shah et al., 2013), whereas *F. hepatica* miracidia densities peak in March and miracidia continue to emerge from eggs through to November (Beltrame et al., 2018). Consumption rates may, therefore, be greater during early spring when there are high prey numbers and lower predator numbers compared to later in the year when there are low densities of both miracidia and ostracods/cyclopoids.

Individual predation rates, for the same miracidia density, varied substantially (e.g. 5–55 miracidia consumed at the highest miracidia density) despite our experimental design controlling for factors that can affect consumption rates, such as temperature, experimental volume and starvation period (Born-Torrijos et al., 2020; Koprivnikar et al., 2023). The number of miracidia used for prey in this study is similar to that used by Christensen et al (1977) (between 15 and 75 miracidia used per replicate), however, there is no information on miracidial densities in aquatic habitats. Future experiments should, therefore, include higher densities of *F. hepatica* miracidia to find the saturation point. Further, gender may influence consumption rates of ostracods used in this study as Sex-specific consumption by has not been studied for this predator, however in cyclopoids, gender was not found to alter maximum consumption rates when body size was controlled (van Someren Gréve et al., 2017).

Uncovering the predator–prey relationship between *F. hepatica* miracidia and cyclopoids and ostracods highlights their potential for use as biocontrol agents. There is precedent for this sort of biocontrol to have impacts on infection dynamics, with the consumption of cercariae by damselflies altering transmission dynamics of *Ribeiroia ondatrae* to their amphibian hosts (Orlofske et al., 2012). By assessing functional responses to ascertain predation efficacy, we would assume a type II is most beneficial, as this will remove more prey at low densities than predators showing a type III response (Rall et al., 2008). Type II functional responses could, therefore, lead to the localised spatial and temporal eradication of *F. hepatica* miracidia compared to predators showing type III responses where small numbers of miracidia would remain, and, therefore, infection of the intermediate host snail (Alexander et al., 2012). Predation may be more effective depending on the behaviour and distribution of trematode free-living stages. Certain species of trematode cercariae can form large aggregations upon emergence from a group of snail hosts, accumulating in areas where definitive hosts feed (see review by Morley, 2012), and such accumulations may make it easier for predators to capture cercariae in large numbers. Miracidia may also aggregate when attempting to infect susceptible snail hosts. Studies on the symbiont *Chaetogaster limnaei limnaei*, that live on intermediate snail hosts of *F. hepatica*, have demonstrated that this close association with the host snail enables the predator to feed effectively on *F. hepatica* miracidia and cercariae (Muñiz-Pareja and Iturbide-Espinoza, 2018). Our system offers further advantages, however,

due to the motile capacity of our predators and because setting up cultures of these microinvertebrates is likely to be simple and offer scalability to industrial settings (Marten et al., 1997).

Our work provides the first evidence of ostracods and cyclopoids as predators of *F. hepatica* miracidia. Cyclopoids removed more miracidia than ostracods in both single prey and mixed prey treatments suggesting that cyclopoids are a more competent predator. Interestingly, additional food sources had a limited impact on the ability of either predator to reduce the number of miracidia. Nevertheless, the effect of a broader range of alternative prey species on miracidial consumption should be explored. Ostracods and cyclopoids, therefore, have the potential to disrupt liver fluke infection dynamics. Important next steps will set out to determine the effect of this miracidial predation on infection dynamics of the intermediate host snail and ultimately on the dynamics of the adult in the definitive host.

CRediT authorship contribution statement

Daniel McDowell: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sarah E. Perkins:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Frank Van Veen:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Joanne Lello:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Authors contributions

All authors contributed to the study conception and design. Material preparation, data collection, analysis, visualisation and validation were performed by Daniel McDowell. The first draft of the manuscript was written by Daniel McDowell and all authors reviewed and edited previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of AI usage

Authors did not use AI during the preparation of this manuscript.

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Data availability

The data that supports the findings of this study are available on Mendeley Data at: McDowell, D; Van Veen, F; Perkins, S, E; Lello, J (2025), “Microinvertebrate consumption rates of *Fasciola hepatica* miracidia are not affected by alternate food.”, Mendeley Data, V1, <https://doi.org/10.17632/tyhs5bt6y3.1>.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2025.05.004>.

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