International Journal for Parasitology xxx (xxxx) xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Host exposure history and priority effects impact the development and reproduction of a dominant parasite

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ARTICLE INFO

Article history:
Received 28 November 2020
Received in revised form 12 March 2021
Accepted 17 March 2021
Available online xxxx

Keywords:
Coinfection
Life history
Priority effects
Trematodes
Virulence

ABSTRACT

Within a single organism, numerous parasites often compete for space and resources. This competition, together with a parasite's ability to locate and successfully establish in a host, can contribute to the distribution and prevalence of parasites. Coinfection with trematodes in snail intermediate hosts is rarely observed in nature, partly due to varying competitive abilities among parasite taxa. Using a freshwater snail host (*Biomphalaria glabrata*), we studied the ability of a competitively dominant trematode, *Echinostoma caproni*, to establish and reproduce in a host previously infected with a less competitive trematode species, *Schistosoma mansoni*. Snails were exposed to *S. mansoni* and co-exposed to *E. caproni* either simultaneously or 1 week, 4 weeks, or 6 weeks post *S. mansoni* exposure. Over the course of infection, we monitored the competitive success of the dominant trematode through infection prevalence, parasite development time, and parasite reproductive output. Infection prevalence of *E. caproni* did not differ among co-exposed groups or between co-exposed and single exposed groups. However, *E. caproni* infections in co-exposed hosts took longer to reach maturity when the timing between co-exposures increased. All co-exposed groups had higher *E. caproni* reproductive output than single exposures. We show that although timing of co-exposure affects the development time of parasite transmission stages, it is not important for successful establishment. Additionally, co-exposure, but not priority effects, increases the reproductive output of the dominant parasite.

1. Introduction

Parasitism is a common consumer strategy across all forms of life, and hosts are frequently coinfected with numerous pathogens simultaneously. These pathogens interact as they compete for limited host resources and alter host immunity (Choisy and de Roode, 2010; Cusumano et al., 2012; Mideo, 2009; Zélé et al., 2018; Karvonen et al., 2019). Oftentimes, interactions are antagonistic, such as exclusion of caterpillar-infecting bacteria by other strains producing bacteriocins toxic to their competitors (Massey et al., 2004; Laidemitt et al., 2019). However, coinfection has also been shown to be beneficial or neutral for the parasites involved (de Roode et al., 2005; Telfer et al., 2010; Budischak et al., 2015). Thus, coinfection can have a variety of outcomes on the fitness of parasites involved.

Parasites may compete both directly and indirectly within the host. Direct interactions may occur between parasites in the same location in the host, including both competition for resources and intraguild predation (Polis et al., 1989; Cusumano et al., 2012; Mouritsen and Andersen, 2017). Alternatively, parasites indirectly compete through altered immune function or depletion of host resources (Ulrich and Schmid-Hempel, 2012; Rodrigues et al., 2016; Wuerthner et al., 2017). Both direct and indirect interactions are further influenced by the timing and sequence of arrival, known as priority effects (Fukami, 2015; Clay et al., 2019; Karvonen et al., 2019). Although there are many studies noting the effects of priority on prevalence or infection of intensity (Jackson et al., 2006; Johnson and Buller, 2011; Klemme et al., 2016; Wuerthner et al., 2017), only a few evaluate the importance of timing on the fitness of coinfecting parasites (de Roode et al., 2005; Ben-Ami et al., 2008).

Snails and trematodes have served as a model system for coinfection and within-host community structure for decades (Basch, 1970; Jourdane and Mounkassa, 1986; Sousa, 1993; Esch et al., 2001; Lagrue et al., 2018). Within each snail, space and resources are limited, resulting in strong competition among coinfecting species (Fernandez and Esch, 1991a, 1991b; Esch and Fernandez, 1994; Hendrickson and Curtis, 2002; Hechinger et al., 2008; Sulieman and Pengsakul, 2013). This competition has been inferred from lower than predicted coinfection rates in natural populations

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https://doi.org/10.1016/j.ijpara.2021.03.007
0020-7519/

(Fernandez and Esch, 1991a, 1991b; Sousa, 1992; Hendrickson and Curtis, 2002; Hechinger et al., 2008; Soldánová et al., 2012) and trials indicating intraguild predation between coinfecting trematodes (Heyneman et al., 1972; Lie, 1973; Basch and DiConza, 1975). However, studies investigating the timing of trematode coinfection (Heyneman et al., 1972; Soldánová et al., 2012) and the effects of coinfection on parasite fitness (Sandland et al., 2007) are less common.

Trematodes of the genera *Echinostoma* and *Schistosoma* utilise a freshwater snail as an intermediate host and infect vertebrates as the definitive host (Moravec et al., 1974) (Fig. 1). *Echinostoma caproni,* a species found in Africa (Ataev et al., 1997; Langand and Morand, 1998), and *Schistosoma mansoni,* found throughout Africa, Asia and South America (Morgan et al., 2001), have both been established in laboratory-maintained lifecycles with the host *Biomphalaria glabrata* (Rollinson, 2011). Laboratory studies with *B. glabrata*, *E. caproni* and *S. mansoni* have been crucial in understanding snail-trematode interactions and these species are considered model species for understanding snail immunology, the biology of digenetic parasites, and intrahost trematode interactions (Coustau et al., 2009, 2015; Hanington et al., 2010). Studies have documented the ability of *E. caproni* to outcompete *S. mansoni* during simultaneous exposure to the parasites (Heyneman et al., 1972; Sandland et al., 2007). *Echinostoma caproni* larvae in the first intermediate host, known as rediae, possess a mouth and muscular pharynx that allow them to ingest smaller *S. mansoni* larvae, called sporocysts (Esch et al., 2001). Previous work suggests that simultaneous coinfection does not affect the ability of *E. caproni* to establish in the host but can result in life history changes such as altered reproductive output by the parasite (Sandland et al., 2007).

In this study, we varied the timing of coinfection of *E. caproni* with a subordinate competitor, *S. mansoni*, and quantified the impacts on the establishment and reproduction of the dominant competitor. We expected that the success of *E. caproni* would decrease with increased time after *S. mansoni* infection, as a more developed and mature *S. mansoni* infection would be more difficult for *E. caproni* to outcompete. As a result, we predicted a decrease in prevalence and reproductive output of the dominant competitor with increasing infection maturity of the subordinate competitor.

2. Materials and methods

2.1. Parasites

NMRI strain *Schistosoma mansoni* was used with M-line *Biomphalaria glabrata* as an intermediate host (Biomedical Research Institute, USA). The parasite life cycle was maintained in the laboratory using male BALB/c mice as the definitive host. In preparation for this experiment, mice were exposed to 150–200 *S. mansoni* cercariae (pooled between 4–6 snails) for 1 h via tail dip (Olivier and Stirewalt, 1952). Infections matured for 7–8 weeks, at which point mice were euthanized using CO_2 and cervical dislocation (Purdue Animal Care and Use Committee Protocol #1111000225, Purdue University, USA). For each miracidial exposure, 1–2 mouse livers were removed and blended in an 0.8% NaCl solution. This solution was run through a series of vacuum filtrations to separate the eggs, which were then placed into fresh Millipore water to stimulate miracidial hatching. Miracidia were used within 1–2 h of hatching (Tucker et al., 2013).

Echinostoma caproni was acquired from Dr. Thomas Platt at St. Mary's College, USA. *Biomphalaria glabrata* was used for both first and second intermediate hosts and laboratory bred white leghorn chickens, *Gallus gallus domesticus*, from Purdue University Poultry Farm, USA, served as the definitive host. Chicks were fed 50–100 *E. caproni* metacercaria (pooled between 10–20 snails) the day they hatched, before consuming any other food (Detwiler et al., 2010). After 14–16 days of maturation, the chickens were euthanized using CO_2 and cervical dislocation (Purdue Animal Care and Use Committee Protocol #1111000225 Purdue University, USA). The intestines of 10–12 chickens were removed and searched for *E. caproni* worms. Adult *E. caproni* worms were then dissected to remove eggs from the uterus. Eggs were stored in the dark for 15–17 days (Detwiler et al., 2010), at which point they were placed under lights to stimulate miracidial hatching. Miracidia were used within 1–2 h of hatching.

2.2. Treatment groups

All treatment groups were comprised of 50 *B. glabrata* snails, 10–12 mm in diameter, representing snails that were reproductively mature (Loker et al., 1987; Gérard et al., 1993; Gower and Webster, 2005). Three control groups were established. Snails in the first group were not exposed to either parasite. Two single exposure control groups were established in which snails were exposed to either eight *E. caproni* miracidia or eight *S. mansoni* miracidia.

Four experimental groups were exposed to both eight *E. caproni* miracidia and eight *S. mansoni* miracidia for a total of 16 miracidia. Previous work demonstrated that in mature snails, the total miracidial dose of coinfecting parasites does not impact host mortality (Sandland et al., 2007), infection prevalence (Sandland et al., 2007; Theron et al., 2008), or infection intensity (Gérard et al., 1993; Blair

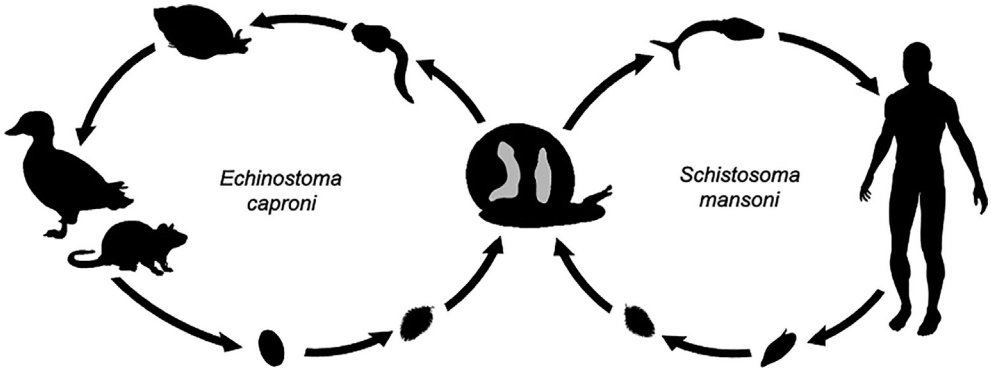

Fig. 1. Life cycles of *Schistosoma mansoni* and *Echinostoma caproni*. Adult worms infect the definitive hosts (most often humans for *S. mansoni* and waterfowl or rodents for *E. caproni*) and release eggs. Once released into the environment, eggs hatch into miracidia which search for a molluscan intermediate host. Within the snail, *S. mansoni* sporocysts and *E. caproni* rediae compete directly for limited host resources and produce free-living larval stages called cercariae. *Schistosoma mansoni* cercariae will infect the definitive host, while *E. caproni* cercariae encyst in a second molluscan intermediate host which is consumed by the definitive host to complete the life cycle. (Images adapted from Centers for Disease and Control, USA (https://www.cdc.gov/dpdx/schistosomiasis/modules/Schistomes_LifeCycle_lg.jpg; https://www.cdc.gov/dpdx/echinostomiasis/modules/Echinostoma_LifeCycle_lg.jpg)).

and Webster, 2007) within the range of miracidial doses used in this study. The first experimental group was simultaneously exposed to miracidia of both parasites during a single exposure event. The remaining groups were first exposed to *S. mansoni* and later exposed to *E. caproni* at 1 week, 4 weeks, and 6 weeks post schistosome exposure (Supplementary Fig. S1), representing various intervals of *S. mansoni* development. At 1 week post exposure, *S. mansoni* miracidia have transformed into mother sporocysts and remain within the tissue of the head-foot, where they will remain for 2–3 weeks (Hanington et al., 2010; Humphries, 2011). By 4 weeks post exposure, daughter sporocysts have migrated to the digestive gland-gonad complex of the snail, but very few, if any, sporocysts are reproductively mature and releasing cercariae (Hanington et al., 2010; Humphries, 2011, Supplementary Fig. S3). By 6 weeks post schistosome exposure the *S. mansoni* infection will have reached maturity and be actively releasing cercariae. While the nature of the delayed co-exposures resulted in *E. caproni* exposures to hosts that had been maintained under experimental conditions longer, natural host mortality does not differ significantly over the time frame examined within this study (Supplementary Table S1). Additionally, there is evidence that host resistance to infection does not significantly increase after snails have reached maturity (Loker et al., 1987), nor does parasite cercarial output (Niemann and Lewis, 1990). No treatment groups were established with *S. mansoni* exposure following *E. caproni* exposure as previous studies indicate superinfection with a sporocyst producing parasite in a host previously infected with a redial producing parasite is rare (Lie et al., 1968; Heyneman et al., 1972; Jourdane and Mounkassa, 1986; Fernandez and Esch, 1991a, 1991b; Sousa, 1992; Soldánová et al., 2016) and not recorded in this laboratory system (Sandland et al., 2007). Additionally, a recent study evinces that schistosome miracidia avoid snails infected with echinostome parasites (Vannatta et al., 2020).

All snails within a single treatment group were exposed on the same day using the same batch of miracidia. Due to life cycle constraints and available host populations, unexposed, *E. caproni* single exposure, and *S. mansoni* single exposure groups were run simultaneously, but not concurrently with any co-exposure groups. Co-exposure groups were staggered in exposure to *S. mansoni* but were exposed to the same batch of *E. caproni* miracidia. However, considering low genetic diversity recorded in laboratory strains (Sloss et al., 1995; Stohler et al., 2004), the low number of generations required to achieve high inbreeding (Gower and Webster, 2005; Theron et al., 2008), and steps taken during life cycle maintenance to ensure mixing of parasite populations (see above methods), significant genetic heterogeneity among miracidial batches is unlikely.

Snails were individually exposed to miracidia in 12 mL well plates. After miracidial exposure, snails were left in wells overnight (>12 h) before being returned to individual 120 mL glass jars. Unexposed controls were sham exposed by placing the snails into well plates for the same duration of time, but without adding parasites.

2.3. Snail maintenance

Snails were housed individually in jars for the entirety of the experiment, beginning 10 days prior to first parasite exposure. Well water was changed weekly and snails were fed romaine lettuce ad libitum. Snails were maintained in a 12:12 h light:dark cycle at approximately 25 °C. Egg masses laid in the jars were removed weekly. Snail mortality was monitored and recorded twice weekly.

Beginning at 4 weeks post first miracidia exposure and continuing for 12 weeks post *E. caproni* exposure, snails were monitored weekly for infection status and reproductive output of the parasites. Snails were placed in individual wells containing 10 mL of well water and set under fluorescent light for 90 min to stimulate cercarial release (Lewis et al., 1986; Sandland et al., 2007; Thiele and Minchella, 2013). As both parasites are diurnal shedders and laboratory maintenance of parasite lineages selects for daytime shedding, the 90 min under fluorescent lighting never started prior to 12:00 h or ended after 14:00 h to maximise the chance of observing peak cercarial shedding for the day and minimise variation from circadian patterns in cercarial shedding (Théron, 2015; Hannon et al., 2018). Cercarial counts were continued for the lifespan of the host to capture long-term weekly variations in cercarial output. Distinct morphology and swimming patterns allowed for cercariae of each species to be easily distinguished. Wells were examined for the presence of *S. mansoni* and *E. caproni.* If positive for *S. mansoni*, water was homogenised, and *S. mansoni* cercariae were counted in a 1 mL sample from the well (Gleichsner et al., 2018). For snails releasing *E. caproni*, all cercariae in the 10 mL were counted (including cercariae that had lost their tails) to determine reproductive output.

Snails were determined to be not infected if fewer than five cercariae were observed in the well for multiple consecutive weeks. This filter impacted very few observations (n = 9 of 1,321) and was included to account for accidental cross-contamination of wells that could occur if the well plate was bumped during shedding events. Prevalence was calculated using the last known infection status of snails before mortality or the end of the experiment. In some co-exposed snails, the snails first released *S. mansoni* cercariae until *S. mansoni* was replaced with *E. caproni* cercarial release. In some individuals, simultaneous release of both species did occur for 1–3 weeks until *S. mansoni* ceased and only *E. caproni* was released. Because *S. mansoni* cercarial output ended prematurely (infections are naturally chronic; Hanington et al., 2010) those hosts were recorded for prevalence calculations as only *E. caproni*-infected and not *S. mansoni*-infected so that no individual was counted twice in prevalence calculations. Snails that died prior to parasite exposure were not included in prevalence calculation, while snails that were exposed but died prior to shedding cercariae were recorded as uninfected.

2.4. Statistical analysis

Mortality of uninfected and single exposure group snails was compared using a Cox proportional hazard ratio. For experimental groups, mortality was compared among co-exposure groups and between co-exposure groups and single exposure controls two ways: (i) using days post *S. mansoni* exposure and (ii) using days post *E. caproni* exposure. In the first survival analysis, individuals that died during the 10 day acclimation period were not included and day 0 indicates the day of exposure to *S. mansoni*. In the second survival analysis, day 0 indicates the day of exposure to *E. caproni* and any individuals that died prior to this point (i.e. during the 10 day acclimation period or between *S. mansoni* and *E. caproni* exposures) were not included.

Infection prevalence of both *E. caproni* and *S. mansoni* was evaluated with separate binomial generalised linear mixed models for each species. The model used co-exposure group as a categorical variable and the simultaneous co-exposure as the reference group. The same method was used to compare prevalence of co-exposure groups to the single exposure groups, with the single exposure group as a reference. Weeks until cercarial release for *E. caproni* and *S. mansoni* were compared between all co-exposure groups and single exposures using Cox proportional hazard analysis for each trematode species. Cercarial output among co-exposed groups was compared using a negative binomial generalised linear mixed model (GLMM) with exposure group as a categorical fixed

factor. Snail ID was included as a random factor to control for repeated measure of individuals.

All statistical analyses were performed in R version 3.6.2 (R Core Team, 2019). We completed Cox proportional hazard models using the package "survival" (Therneau and Grambsch, 2000) and pairwise comparisons using the package "survminer" (Kassambara et al., 2019). We used the package "MASS" (Venables and Ripley, 2002) to perform negative binomial GLMMs and the package "emmeans" (Lenth, 2019) for pairwise comparisons between treatment groups. We used the package "ggplot2" (Wickham, 2016) to visualise data. Multiple comparisons were corrected using a Holm adjustment (Aickin and Gensler, 1996; Holm, 1979). *P* values below 0.05 were considered significant.

2.5. Data accessibility

Data is available through Github (https://github.com/saracarpenter/echino-schisto-coexposure).

3. Results

Host survival decreased relative to the unexposed control group in both *E. caproni* single exposure ($z = 5.757$, $P < 0.0001$) and *S. mansoni* single exposure groups ($z = 3.434$, $P = 0.0004$), with significantly higher mortality in *E. caproni*-exposed snails than *S. mansoni*-exposed snails ($z = 4.436$, $P = 0.0001$; Supplementary Fig. S2). Survival of the simultaneous co-exposure group post *S. mansoni* exposure did not differ from any other co-exposure group (Fig. 2A, Table 1). Simultaneous and 1 week co-exposure had higher mortality than the *S. mansoni* single exposure group, but 4 week and 6 week co-exposures did not differ from the single exposure group (Supplementary Table S2). When compared using days post *E. caproni* exposure (Fig. 2B), there was no difference between the simultaneous co-exposure group and any other co-exposure group (Table 1). In addition, mortality post *E. caproni* exposure did not differ between the *E. caproni* single exposure and any co-exposure group (Supplementary Table S2).

In co-exposure groups, 18 individuals (five in 6 week co-exposure, seven in 4 week co-exposure, five in 1 week co-exposure, and one in simultaneous co-exposure) were observed to first release *S. mansoni*, which was later replaced by release of *E. caproni* cercariae. Prevalence of *E. caproni* infection in the simultaneous co-exposures (prevalence = 36%, $n = 50$) did not differ from 1 week co-exposure (33%, $n = 48$, $z = -0.277$, Holm adjusted $P = 1.00$), 4 week co-exposure (21%, $n = 47$, $z = -1.585$, $P = 1.00$), or 6 week co-exposure (35%, $n = 43$, $z = -0.112$, $P = 1.00$; Fig. 3). Relative to *E. caproni* single exposure (45%, $n = 49$), *E. caproni* prevalence was not significantly different from the co-exposure group (simultaneous: $z = -0.901$, $P = 1.00$; 1 week: $z = -1.163$, $P = 1.00$; 4 week: $z = -2.411$, $P = 0.1591$; 6 week: $z = -0.975$, $P = 1.00$). *Schistosoma mansoni* prevalence in simultaneous co-exposure (28%, $n = 50$) was lower than 4 week co-exposure (56%, $n = 48$, $z = 2.789$, $P = 0.0318$), but did not differ from 1 week (24%, $n = 50$, $z = -0.456$, $P = 1.00$) or 6 week co-exposures (34%, $n = 47$, $z = 0.643$, $P = 1.00$). Prevalence of *S. mansoni* was lower in all co-exposure groups, but only marginally lower in the 4 week co-exposure group (simultaneous: $z = -4.922$, $P < 0.0001$; 1 week: $z = -5.241$, $P < 0.0001$; 4 week: $z = -2.479$, $P = 0.0659$; 6 week: $z = -4.368$, $P = 0.0001$) than in the the *S. mansoni* single exposure group (80%, $n = 50$).

Snails that were simultaneously co-exposed began releasing *E. caproni* cercariae sooner than 4 week co-exposed snails but did not differ from any other co-exposure group (Fig. 4, Supplementary Table S3). Snails in the 1 week co-exposure group also began releasing *E. caproni* earlier than 4 week and 6 week co-exposure groups, although this trend was significant for 4 week co-exposures only (Supplementary Table S3). The *E. caproni* single exposure snails began releasing cercariae sooner than simultaneous, 4 week, and 6 week co-exposure groups but did not significantly differ from 1 week co-exposures. Release of *S. mansoni* was delayed in simultaneous co-exposures relative to 4 week and 6 week co-exposures and *S. mansoni* single exposures (Supplementary Fig. S3, Table S4). Both simultaneous and 4 week co-exposures were slower to begin *S. mansoni* release than single exposure hosts (Supplementary Table S4).

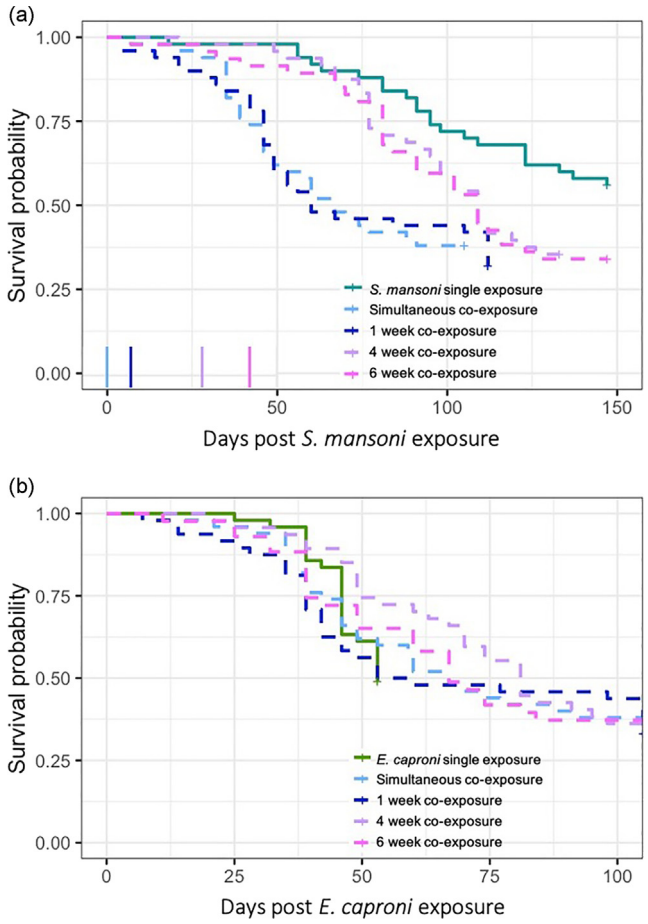

Fig. 2. Host survival following parasite exposure. (A) Host survival measured post *Schistosoma mansoni* exposure. Bars along the X axis represent timings of co-exposure to *Echinostoma caproni* for simultaneous, 1 week, 4 week, and 6 week co-exposures. (B) Host survival measured post *E. caproni* exposure. Both graphs show mortality for all individuals in an exposure group regardless of infection status.

Average weekly *E. caproni* reproductive output did not differ between simultaneously exposed snails (mean ± S.E.: 122.03 ± 12.56) and 1 week (122.16 ± 17.80, $P = 1.00$), 4 week (136.66 ± 18.70, $P = 1.00$ and 6 week co-exposures (165.83 ± 18.39, $P = 0.9752$; Table 2; Fig. 5). *Echinostoma caproni* reproductive output was significantly higher in all co-exposed groups than in *E. caproni* single exposed snails (57.58 ± 14.28; Table 2). Average weekly *S. mansoni* cercarial output was higher in 6 week co-exposed snails than single exposed or simultaneous co-exposures, but no other significant differences existed between treatment groups (Supplementary Table S5).

4. Discussion

This experiment evaluated the effects of co-exposure timing on the establishment, development, and reproductive output of a

Table 1
Cox proportional hazard model coefficients, S.E., *z*, and *P* values for survival analyses. Both analyses run with simultaneous co-exposure group as the reference. The first model compares survival of simultaneous co-exposures to delayed co-exposures using days post *Schistosoma mansoni* exposure as the predictor variable. The second model compares survival of simultaneous co-exposures to delayed co-exposures using days post *Echinostoma caproni* exposure as the response variable. Holm adjusted *P* values are shown.

Response Variable	Factor	Coefficient	S.E.	*Z*	*P*
Days post *S. mansoni* exposure					
	1 week co-exposure	−0.1467	0.2544	−0.577	1.000
	4 week co-exposure	−0.6468	0.2627	−2.462	0.0736
	6 week co-exposure	−0.5990	0.2630	−2.278	0.1208
Days post *E. caproni* exposure					
	1 week co-exposure	0.1084	0.2521	0.430	1.000
	4 week co-exposure	−0.1216	0.2563	−0.492	1.000
	6 week co-exposure	−0.0079	0.2633	−0.030	1.000

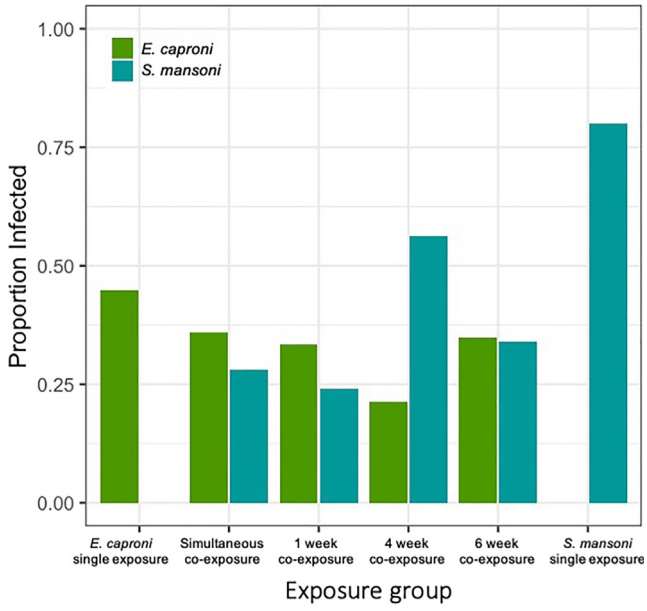

Fig. 3. Final prevalence of patent *Echinostoma caproni* and *Schistosoma mansoni* infections in all treatment groups. Prevalence was recorded as the last known infection status of the snail before mortality or when the experiment ended. Snails that died prior to parasite exposure are not included in the prevalence calculation. Snails that were exposed but died prior to shedding cercariae were recorded as uninfected.

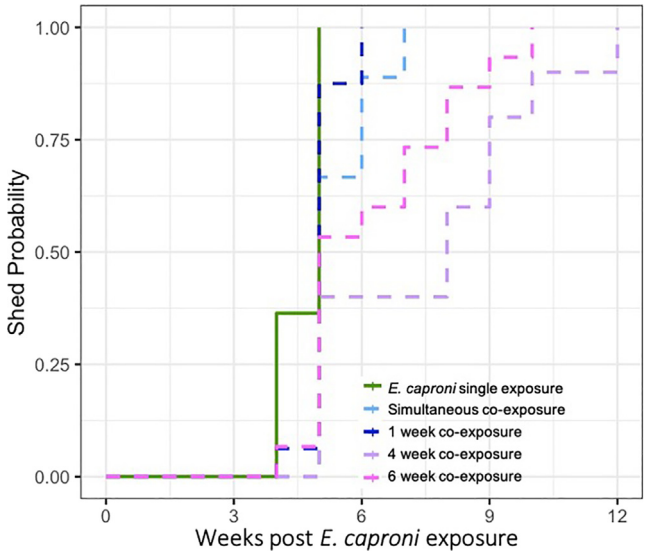

Fig. 4. Time to patency of *Echinostoma caproni* infections as measured by the first week that an infected individual was observed releasing cercariae. Time is measured as weeks post *E. caproni* exposure.

highly competitive trematode within its molluscan host. Echinostomes are able to directly consume schistosomes, and are therefore able to exclude schistosomes from snail hosts (Basch and DiConza, 1975). Through a laboratory experiment, we demonstrated that *E. caproni* development time, but not host mortality or *E. caproni* establishment success, is affected by varying time after *S. mansoni* exposure. Additionally, co-exposure to *S. mansoni*, but not the timing of co-exposure, significantly increased *E. caproni* reproductive output. This indicates that while timing of co-exposures can slow time to reproductive maturity, priority effects may have little impact on disease transmission.

Studies of coinfecting species or coinfecting genotypes have recorded disparate patterns of host mortality, with mortality: (i) increasing as a result of increasing exploitation of host resources due to multiple parasites (Ben-Ami et al., 2011; Louhi et al., 2015; Gleichsner et al., 2018), (ii) decreasing as a result of strong competition between parasites limiting growth of both parasites (Balmer et al., 2009; Wuerthner et al., 2017), or (iii) matching the mortality observed when the host is infected by the more virulent parasite alone (Massey et al., 2004; Ben-Ami et al., 2008, 2011). In our study, co-exposure of hosts to both *S. mansoni* and *E. caproni* at any time interval increased mortality relative to snails exposed to *S. mansoni* only. However, when examining host mortality after *E. caproni* exposure, there was no observed difference between hosts co-exposed to both parasites and those exposed to *E. caproni* only. These results are consistent with previous studies, indicating that mortality is driven by the more virulent parasite, and neither competition nor timing of coinfection have a significant impact on host mortality relative to the more virulent parasite (Sandland et al., 2007; Garcia et al., 2010).

The final prevalence of *S. mansoni* was lower in all co-exposure groups than in the *S. mansoni* single exposure control, a consequence of competitive exclusion by the dominant parasite, *E. caproni* (Fig. 3). Among co-exposure groups, *S. mansoni* prevalence differed between simultaneous and 4 week co-exposures, following predictions that older *S. mansoni* infections may be more resilient to dominance by *E. caproni*. However, this pattern is not seen with the 6 week co-exposure group. Co-exposure did not affect prevalence of the dominant parasite, *E. caproni*, regardless of timing. These results follow patterns from simultaneous coinfection studies that record no difference in prevalence of *E. caproni* (Sandland et al., 2007), indicating that timing of infection does not significantly affect the ability of a dominant competitor to establish and exclude the subordinate parasite. Interestingly, these results directly contradict the findings by Garcia et al. (2010) in which co-exposure of *B. glabrata* to *S. mansoni* and *E. paraensei* increased host susceptibility to *S. mansoni*. The contrast in results between these two studies may be due to the difference in immune response of the *B. glabrata* strain used for the study (Garcia et al., 2010). The *B. glabrata* snails in the Garcia et al. (2010) study

Table 2
Results of negative binomial general linearized mixed model evaluating weekly *Echinostoma caproni* cercarial counts between simultaneous co-exposure and *E. caproni* single exposure and delayed co-exposure groups. Results are given on a log scale. Holm adjusted *P* values are shown; bold indicates significant *P*-values.

Contrast	Estimate	S.E.	*z*	*P*
Simultaneous vs. 1 week co-exposure	−0.00106	0.216	−0.005	1.0000
Simultaneous vs. 4 week co-exposure	−0.11322	0.222	−0.5101	1.0000
Simultaneous vs. 6 week co-exposure	−0.3067	0.220	−1.397	0.9752
1 week vs. 4 week co-exposure	−0.1122	0.229	−0.489	1.0000
1 week vs. 6 week co-exposure	−0.3057	0.227	−1.345	0.9752
4 weeks vs. 6 week co-exposure	−0.1935	0.233	−0.831	1.0000
Single exposure vs. simultaneous co-exposure	−0.7511	0.194	−3.862	**0.0009**
Single exposure vs. 1 week co-exposure	−0.7521	0.203	−3.705	**0.0015**
Single exposure vs. 4 week co-exposure	−0.8643	0.209	−4.132	**0.0003**
Single exposure vs. 6 week co-exposure	−1.0578	0.207	−5.113	**<0.0001**

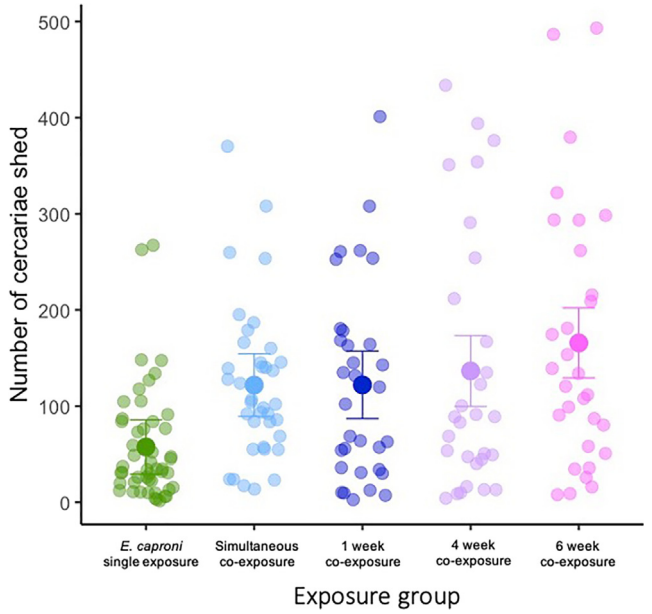

Fig. 5. Counts from weekly observation of *Echinostoma caproni* cercarial release. Each point represents cercarial output from a single individual in a single week; bars represent mean ± S.E.

evinced higher natural resistance to *S. mansoni* infection (20.5% infection prevalence) than the hosts in our study (80% prevalence, Fig. 3). Thus, differences in results between these studies may be due to the effect of relative strength of host immunity on parasite dynamics in which a weak host immune response in our system leads to greater importance of direct parasite-parasite interactions (Choisy and de Roode, 2010; Karvonen et al., 2019).

While timing of co-exposure to *S. mansoni* did not affect host mortality of *E. caproni*-exposed snails nor the prevalence of *E. caproni,* it did impact the time to *E. caproni* reproductive maturity (Fig. 4), suggestive of some level of niche modification or preemption by *S. mansoni* (Fukami, 2015). Although not investigated in this study, the delay in *E. caproni* reproductive maturity may indicate differences in parthenitae dynamics within the host, which may also affect long-term patterns of peaks in shedding as well as *E. caproni* community structure (Theron, 1981a, 1981b). These effects have the potential to influence infection and disease in downstream hosts such as amphibians where small differences in host age or infection patterns can affect disease outcome (Johnson and Buller, 2011). For example, a delay in exposure of only 1 week can significantly decrease infection success of echinostomes and echinostome-like species encysting in larval amphibians (Hoverman et al., 2013). In addition to decreasing the parasite's ability to establish an infection in an older host, delaying parasite reproduction can disrupt priority effects in subsequent hosts, impacting disease outcomes. Early echinostome exposure of amphibians lessens infection prevalence of coinfecting trematodes (Hoverman et al., 2013; Billet et al., 2020) and decreases disease severity of microparasites (Wuerthner et al., 2017), potentially mediating disease in vulnerable host communities. Thus, trematode coinfections of snail hosts have the potential to influence infection and disease dynamics through small alterations in the timing of parasite reproduction.

However, regardless of the timing of co-exposure, *E. caproni* infections had a higher weekly reproductive output in co-exposed groups than in the *E. caproni* single exposure, corroborating increased reproduction seen by Sandland et al. (2007) in simultaneous co-exposures. Increased reproductive output of parasites in coinfections is often hypothesised to result from decreased host immune function (Budischak et al., 2015), an increased rate of host exploitation (Mouritsen and Andersen, 2017) or, in the case of direct predation between parasites, resources that are of higher quality or easier to attain (Lie, 1973; Mouritsen and Andersen, 2017). While it is possible that *S. mansoni* infection altered host immunity and allowed *E. caproni* to be more successful, previous studies have shown that echinostomes strongly suppress immune function (Loker et al., 1986; Hanington et al., 2010) and suggest that *S. mansoni* does not have as strong an effect (Loker and Adema, 1995; Guillou et al., 2007). Additionally, an increased rate of host exploitation is associated with increased morbidity and mortality (Gower and Webster, 2005; Sandland et al., 2007), while our study found no difference in mortality among snails exposed to *E. caproni* only or co-exposed to both parasites (Fig. 2B). Although there is debate about the hypothesis that competitors can increase resource quality (Sousa, 1992; Hendrickson and Curtis, 2002), evidence supporting it includes experiments demonstrating in vivo competitive abilities of rediae (Heyneman et al., 1972; Lie, 1973; Sandland et al., 2007) and in vitro predation of sporocysts (Basch and DiConza, 1975; Leung and Poulin, 2011; Mouritsen and Halvorsen, 2015). Alternatively, degenerating sporocysts have also been demonstrated as a suitable food source for rediae (Gorbushin and Shaposhnikova, 2002; Galaktionov et al., 2015), in which the cessation of *S. mansoni* cercarial production may be caused by decreased sporocyst production caused by competition for host resources. Rediae consuming coinfecting sporocysts have also been observed as larger than their counterparts in single infections (Lie et al., 1968), suggesting that this alternative food source enhances growth (Esch et al., 2001). Additionally, *S. mansoni* may cause reallocation of host resources, stimulating host growth (Minchella, 1985) and increasing the mobilisation of carbohydrates and degradation of amino acids (Bonfim et al., 2014). These alterations to host biology induced by *S. mansoni* may create a more favourable environment for *E. caproni* reproduction, perhaps explaining host selection by *E. caproni* for schistosome-infected snails over uninfected snails (Vannatta et al., 2020).

Our study demonstrates that the outcome of interactions between parasites can differ as the maturity of the early arriving parasites alters the life history of subsequent parasites. In view of our observations of higher *E. caproni* cercarial output in coinfected hosts, combined with no difference in prevalence and mortality, we predict that there will be higher total *E. caproni* cercarial release from a snail population when schistosome co-exposure is common. For some dominant parasites establishing in coinfected hosts, the interplay between their delayed development and their increased reproductive output will lead to an array of disease outcomes in the community. Moving forward, studies must consider not only how frequently coinfection occurs, but also how the exposure history of hosts affects developmental time and reproductive output of parasites, which ultimately may impact transmission dynamics. Host-parasite systems provide a valuable system in which the role of priority effects can be extensively observed. Physiological changes in hosts can be tracked to observe niche preemption and modification, infection rates may serve as proxies for immigration rates, and host density (i.e. patch density) is easily manipulated. The field of coinfection ecology provides a highly tractable system which is sure to influence our understanding of community assembly in years to come.

Acknowledgements

We would like to thank Tom Platt for providing *Echinostoma caproni* for this study and the members of the Minchella laboratory group for additional assistance. *Biomphalaria glabrata* snails provided by the National Institute of Allergy and Infectious Disease (NIAID) Schistosomiasis Resource Center of the Biomedical Research Institute (Rockville, MD, USA) through National Institute of Health-NIAID Contract HHSN272201700014I for distribution through BEI Resources, USA. S.C. was supported by a Cable-Silkman Fellowship for undergraduates in Parasitology, Purdue University, USA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2021.03.007.

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