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Be afraid, be very afraid: how host cues determine attractiveness to parasite infectious stages and the resulting infection loads

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ABSTRACT

The likelihood of infection is influenced by both innate and environmental factors, including host defences and contacts with infectious stages. Although theory predicts that motile parasites ought to select susceptible host species, few studies have considered parasite preference among individuals of a single host species. By experimentally manipulating the presence, activity, and susceptibility of tadpoles, we tested the importance of host cues (chemical and mechanical) and host resistance on intraspecific host choice by free-swimming trematode (flatworm) cercariae. Cercariae could 'choose' among four chambers with these combinations in a first set of trials but could not contact (and infect) hosts. In a second set of trials with the same tadpoles, cercariae were allowed to select and infect hosts, allowing us to analyze the relationship between initial parasite choice and subsequent infection establishment. Cercariae showed a trend for greater attraction to anesthetized tadpoles over negative controls (empty chambers), suggesting the use of chemical cues to locate hosts, but were most attracted to active (non-anesthetized) tadpoles, indicating an important role for host movement. Cercariae showed no preference for tadpoles subjected to an immunosuppressive treatment, despite their greater susceptibility to infection. Importantly, the initial number of cercariae that chose each tadpole in the first round positively predicted parasite load in the second round of exposures. Highly active hosts, which initially attracted the most cercariae, ultimately supported the highest infections, either because parasites made 'good' host choices, or, alternatively, prior host exposure (without actual infection) increased susceptibility.

1. Introduction

Parasites are a ubiquitous threat for most organisms, with an estimated 6 million parasitic species accounting for almost 40% of all animal species worldwide (Dobson et al., 2008), and far more yet to be described (Carlson et al., 2020). However, parasite taxa vary enormously in their use of host species, from broad generalists to strict host-specialists (Woolhouse et al., 2001; Schmid-Hempel, 2011). This may result from selection to specialize on particular host species (Adamson and Caira, 1994; Poulin et al., 2011) or decisions made by parasites in ecological time, i.e., behavioural plasticity in host choice (Attwood, 2025). In both scenarios, host-parasite associations are the outcome of two "filters" that pertain to their likelihood of encounter and, given an encounter, their likelihood of compatibility (Euzet and Combes, 1980; Combes, 1991a, 2001). The former primarily reflects aspects of host and parasite ecology (e.g., habitat use, diurnal rhythms) while the latter typically involves physiology (e.g., immune response) and morphology.

For a parasite to successfully establish an infection, it must encounter a compatible host within a particular spatiotemporal window; thus, many parasites rely on motile stages and vectors to enhance their ability to find and contact suitable hosts (see reviews by Combes, 1991b; Hurd, 2003). The mass production of infectious stages can increase the odds of host encounter purely through numbers (Combes et al., 2002), without requiring any other tactics on the part of the parasites. However, motile parasites may additionally benefit from behavioural strategies to put themselves in the same space and time as potential hosts. Such strategies have been particularly well-documented for the free-swimming infectious stages of aquatic trematodes (flatworm parasites) known as cercariae, which are non-feeding larvae that emerge from their molluscan first intermediate hosts and often have <24 h to successfully infect the next host in their complex life cycle (Esch et al., 2002). For instance, cercariae can respond to both environmental (e.g., light and gravity) and host-generated (e.g., water current or chemicals) cues to place themselves in the same

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microhabitat as compatible hosts (reviewed by Combes et al., 1994; Haas, 1992, 1994).

Importantly, however, a parasite may come across different individuals that could serve as a host during this encounter phase. Parasites may thus be confronted with 'choosing' among different host species or among individuals of the same species, i.e., inter- and intraspecific choice, respectively (Combes, 1991b; Christe et al., 1998). While interspecific host preferences are important to understand given the implications for transmission and other key aspects of infectious disease dynamics (e.g., Simpson et al., 2012; Hopkins et al., 2022), the extent to which motile parasites show a preference for certain individuals (intraspecific choice), and why, is also highly consequential. For instance, the pathology induced by macroparasites is usually intensity-dependent (Anderson and May 1978; Wilber et al., 2016), such that individual hosts which are particularly attractive to motile parasites could bear heavier costs from the resulting increases in parasite burden. Most macroparasites exhibit highly aggregated (over dispersed) distributions among hosts (Shaw and Dobson, 1995; Shaw et al., 1998), which is classically thought to stem from variation in either host susceptibility or in parasite exposure (Poulin, 2013). For instance, male mammalian hosts often support heavier infections than females, potentially owing to differences in immune function or activity (Schalk and Forbes, 1997). However, few studies have considered the additional influence of parasite choice among host individuals of a given species in contributing to such aggregations. Consequently, understanding the occurrence of intraspecific choice by parasites, the driver(s) of such choices, and the cues potentially used to inform such decisions represent important knowledge gaps in the study of parasite ecology.

For parasites capable of choosing among individual hosts (inter- or intraspecific host choice), one possibility is that parasites select hosts in accordance with their competence to support infection. That is, parasites should be under selective pressure to choose hosts that are particularly suitable to supporting and subsequently transmitting a given infection (Martin et al., 2016; Manzoli et al., 2018). The role of competence as a driver of interspecific host preference has been explored through theoretical approaches (e.g. Best et al., 2014; Forbes et al., 2017), but there is limited empirical support for it to date (Manzoli et al., 2021); even among similar host-parasite systems, study findings are often highly variable. As an example, geographic isolates of a parasitoid wasp (*Leptopilina boulardi*) preferred to oviposit in the insect host species in which they showed the greatest ability to counteract host immune defences (Dubuffet et al., 2006). Yet in direct contrast, endemic parasitoids frequently attack introduced host species of low competence, despite poor outcomes for their offspring (e.g., Konopka et al., 2018). Similar variability is evident for trematode cercariae. Sears et al. (2012) found that the cercariae of an amphibian-infecting trematode were attracted to the larvae of four different host species in an order similar to their susceptibility, with similar findings reported for *Echinostoma trivolvis* cercariae in infecting alternative snail species that varied in suitability (Wojdak et al. 2013). In contrast, Johnson et al. (2019) found that, while *Ribeiroia ondatrae* cercariae showed a consistent preference among larval amphibians of four different species, this pattern was not reflective of host competence. In such cases, the lack of selection in favor of highly competent hosts may be due to an inability to discriminate among host species (Konopka et al., 2018) or to the potential costs of parasite choosiness (e.g., time to discriminate among hosts), which may outweigh any fitness benefits of selecting particular hosts, especially for parasites with a short transmission window (Attwood, 2025).

Importantly, host competency is also relevant to intraspecific host preference. Thus, any preference exhibited by parasites for particular hosts within a species may be tied to individual traits, including those related to susceptibility and competence. Among studies of ectoparasites and juvenile birds, the "tasty chick" hypothesis posits that colonizing parasites prefer the smallest chicks within a brood because they have the lowest resistance relative to their larger siblings (Christe et al., 1998), thereby leading to higher loads on these smaller and more vulnerable individuals. However, other studies with similar ectoparasite systems have found that parasites preferred to feed on hosts with sufficient resources (blood meal size), rather than those of high susceptibility (Heylen and Matthysen, 2011; see review by Attwood, 2025). For instance, Bize et al. (2008) reported that louse flies avoided feeding on Alpine swift nestlings with a weak cutaneous immune response, likely because these individuals also had poor body condition. Similarly, the mite *Macrocheles subbadius* preferentially infected cactus flies (*Drosophila nigrospiracula*) with high respiration rates as this likely reflects high host resource availability via metabolic state (Horn et al., 2018). Alternatively, intraspecific choice could also simply reflect parasite responses to stimuli uncorrelated with subsequent infection success or persistence. As an example, while beer consumption increases individual attractiveness to human-feeding mosquitoes in experimental trials, there is no obvious advantage to this for the mosquito (Lefèvre et al., 2010).

Given that most investigations of intraspecific host preference to date have involved ectoparasites in a range of host taxa from birds (e.g., Christe et al., 1998) to lizards (e.g., Pollock et al., 2012) to mammals (e.g., Christe et al., 2003, 2007; Dallas and Foré, 2013), it is important to consider other host-parasite systems to determine the extent to which intraspecific choice occurs, as well as the potential basis for parasite attraction. Additionally, while many of these studies have found that parasites do exhibit intraspecific host choice (or preferences), the majority are correlative or do not manipulate host susceptibility or host cues directly, often making it difficult to explicitly test the underlying basis for parasite preference (Attwood, 2025). There is thus a need for investigations that experimentally decouple these, as well as those that consider the possible cues used by parasites in making intraspecific choices.

Here, we investigated intraspecific preference of trematode cercariae for individual hosts. Previous studies have reported on the interspecific preferences of cercariae from two trematode species for tadpoles of different amphibian species (Sears et al., 2012; Johnson et al., 2019). However, Sears et al. (2012) also found unexplained yet repeatable differences in the attractiveness of individual tadpoles used in recurrent interspecific choice tests, regardless of their species identity, indicating some degree of inherent appeal. Given the high degree of aggregation by larval trematodes within amphibian hosts in nature (e.g., Koprivnikar and Redfern, 2012; Johnson and Hoverman, 2014), we set out to explore whether specific traits of individual hosts contributed deterministically to variation in both host attractiveness to infective stages and the resulting parasite loads. By experimentally manipulating the presence, activity, and immunity of tadpole hosts, we compared the importance of host-generated physical (i.e., movement) and chemical (i.e., effluent) cues in attracting cercariae (see reviews by Haas, 1994, 2003), alongside the potential contribution of variation in host susceptibility (i.e., innate resistance) in determining cercariae choice. We complemented the cercariae choice component of our study with subsequent infection trials to examine the extent to which individual tadpole susceptibility, as well as attractiveness in the previous choice trials, jointly predicted their resulting infection load after permitting contact by cercariae. Taken together, these experiments aimed to evaluate the factors that influence parasite choice and the degree to which infection can be explained by individual host traits and their attractiveness.

2. Materials and methods

2.1. Experimental design

Our study was comprised of two steps: 1) examining cercariae attraction to individual tadpoles of the same species based on their movement and susceptibility to infection without the possibility of contact ('choice trials'); and 2) considering the effect of cercariae attraction in step one on resulting tadpole infection load after allowing contact to new cercariae ('infection trials'). For step one, we provided cercariae the opportunity to choose among four options in an experimental arena: A) chamber with an empty mesh cup ('no host'), B) chamber with mesh cup containing an anesthetized tadpole ('immobile host'), C) chamber with mesh cup containing a CORT-exposed tadpole ('increased susceptibility host'), and D) chamber with mesh cup containing a control tadpole with sham exposure to exogenous corticosterone (CORT) treatment ('control host'). The CORT treatment was intended to reduce tadpole resistance by raising endogenous levels of CORT (see Belden et al., 2005; Belden et al., 2010; Fraker et al., 2021), thereby resulting in greater trematode infection, as demonstrated in previous studies (e.g., Belden and Kiesecker, 2005; LaFonte and Johnson, 2013).

For the experimental arenas, we used choice chambers (Carolina™ Large Choice Chamber Kit, item #143051) with two extra chambers to create a 4-chamber apparatus – the same setup as used by Johnson et al. (2019) to examine cercariae choice among larvae of different species of amphibians. This apparatus includes gates that can be added and removed to prevent movement in and out of the central portion. As per Johnson et al. (2019), the cups in each chamber consisted of reusable coffee filters (https://www.walmart.ca/en/ip/re-fil-ter-reuseable-coffee-filters-5-pack/6000195373620) that were 6.10 cm in diameter and made of 14 μm mesh. Pilot trials verified that cercariae (*Alaria* sp.) with a smaller size than those used here could not pass through the mesh. Consequently, step one allowed cercariae to experience cues produced by hosts but not to physically contact and infect tadpoles. In step two, we individually exposed the control and increased susceptibility tadpoles to new cercariae, but this time we allowed for contact, and thus infection.

2.2. Host and parasite sourcing and maintenance

We obtained northern leopard frog (*Rana,* a.k.a. *Lithobates, pipiens*) egg masses from a commercial supplier (Boreal Science) and kept them at room temperature (~22°C) with a 14:10 h light:dark cycle until tadpoles had hatched. Tadpoles were housed in ten, 5-L tanks containing ~100 individuals in each for ~3 weeks until enough had reached Gosner developmental stage 25 (Gosner, 1960), which better tolerate exposure to trematodes and exogenous CORT (e.g., Belden and Kiesecker, 2005; LaFonte and Johnson, 2013; O'Dwyer et al., 2024). During this maintenance period, we changed the water in the tanks every second day using dechlorinated water, i.e., DW (tap water containing Nutrafin Aquaplus, A7928 Hagen®, and Nutrafin Cycle, A7906 Hagen®) and fed the tadpoles *ad libitum* with store-bought organic spinach softened with boiled DW as well as rabbit food pellets (Hagen®). Animals were used in accordance with the Canadian Council on Animal Care guidelines, and with institutional approval from the local animal care committee (AUP 699).

We chose to use trematodes of the genus *Echinostoma* for this study as many infect tadpoles as their second intermediate host, with cercariae forming cysts (metacercariae) in the nephric system that can compromise renal function and decrease survival at high infection loads (e.g., Schotthoefer et al., 2003, Szuroczki and Richardson, 2009). Cercariae of trematodes in this genus are known to use chemical cues (small molecular peptides, amino acids, ammonia and urea) for locating aquatic snails that can also serve as second intermediate hosts (e.g., Haas et al., 1995). However, they are also attracted to samples of water conditioned by fish, tadpole, or leeches diluted to the same total amino acid concentrations, suggesting a general response to organic molecules (Körner and Haas, 1998). This indicates the potential for *Echinostoma* sp. chemoattraction to actual tadpoles, similar to that for fish and larval amphibians shown by the cercariae of *Diplostomum spathaceum*, which will encyst in both (Haas et al., 2002).

To obtain cercariae for this study, we collected aquatic snails (*Helisoma* sp.) from two local ponds and screened them in the lab for trematode infection using established methods (see Szuroczki and Richardson, 2009). This involved stimulating cercariae emergence by exposing snails to heat and light from a lamp. We identified emerged cercariae using a standard key (Schell, 1985); any snails infected with *Echinostoma* sp. cercariae (based on the presence of collar spines) were moved into separate containers with DW and fed raw spinach *ad libitum*. For use in the cercariae choice aspect of this study (step one), *Echinostoma* sp. cercariae were collected as described above, pooled among snails, and then divided among 1.5 mL microcentrifuge tubes such that each contained 50 cercariae in DW.

2.3. Tadpole corticosterone treatment

As we required a total of 54 tadpoles for the immobile, increased susceptibility, and control host treatments in the choice trials, we haphazardly removed five or six tadpoles that had reached at least Gosner (1960) stage 25 (based on visual inspection) from each of the 10 group housing containers. We then exposed 18 tadpoles to one of the following solution treatments: i) DW only for the immobile hosts (these would later be temporarily anesthetized during the choice trials); ii) DW containing corticosterone (Sigma-Aldrich® ≥98.5%) at 0.1 μM for the increased susceptibility hosts, made by dissolving 0.0003464 g of corticosterone in 0.2 ml of 80% ethanol before adding it to 1 L of DW; or, iii) DW containing the solvent ethanol for the control/sham CORT treatment (0.2 ml of 80% ethanol per 1 L). For the increased susceptibility and control host treatments, we followed protocols similar to previous studies for exogenous CORT-induced immunosuppression of tadpoles (Belden and Kiesecker, 2005; LaFonte and Johnson, 2013; O'Dwyer et al., 2024). During the 14-day exposure period, tadpoles were individually housed in randomly arranged 1-L plastic containers (diameter ~10 cm) holding 500 mL of the respective treatment solution and fed boiled organic spinach *ad libitum*. We renewed the treatments every second day by replacing the appropriate solution. However, because the choice trials required two days (see below), we staggered the 14-day exposure periods so that enough tadpoles from each treatment would be available on each day, i.e., ensuring a consistent 14 days of treatment exposure beforehand.

2.4. Cercariae choice trials

On each morning or early afternoon of the cercariae choice trials, we stimulated emergence from infected snails as described above, timing this so that they would be 3–5 h old when tested to avoid reductions in activity as reported for echinostomatid cercariae (McCarthy, 1999). Emerged cercariae from multiple snails were first transferred via pipet into a Petri dish with DW and then counted into aliquots of 50 into individual 1.5 mL microcentrifuge tubes. We used three choice arenas, each set up under a tripod holding a digital camera (2 JVC HD 203 Everio digital and 1 Canon

VIXIA HF R700). This allowed us to record the behaviour of each tadpole in its filter cup. We placed empty cups in the centre of each choice chamber and then added 1.5 L of DW to fill the arena before adding gates to close these off from the central acclimation chamber (Fig. 1). The assignment of the options for the cercariae (tadpoles from each of the treatments or an empty cup) was randomized among the chambers for each arena, as was the assignment of individual tadpoles among the nine trials for their recording day (three trials/camera).

Prior to starting the cercariae choice trials, the tadpoles from the DW-only exposure were placed for 2 min into a container with 1-L of a buffered 0.1% solution of the anesthetic MS-222 (Sigma Aldrich), a concentration effective for short-term immobilization (e.g., Koprivnikar et al., 2006), followed by a transfer to another container of DW for rinsing and then placement into the cup of their assigned chambers. We then immediately added the CORT- and sham-CORT tadpoles (increased susceptibility and control treatments, respectively) to the cups of their assigned chambers, followed by gently adding the entire contents of a cercariae-containing microcentrifuge tube into the central compartment of each arena. After 10 min to let the cercariae disperse within the latter, as well as allow any tadpole cues to permeate within each chamber, we removed the gates and began recording for 30 min, after which the gates were replaced. We then removed the filter cup from each chamber and returned the tadpoles to their individual 1-L housing containers.

After completing the first part of the study (cercariae choice trials), only the CORT and sham-CORT tadpoles were kept for the second part of the study (infection trials) while anesthetized tadpoles from the immobile group were euthanized by further exposure to the MS222 solution and then frozen for later parasite examination to verify that cercariae could not pass through the mesh and encyst during the choice trials. As the infection trials occurred over a 48-h period, it would not be possible to safely keep the immobile group of tadpoles anesthetized for that length of time. In addition, we opted to only expose the CORT and sham-CORT tadpoles to cercariae during the infection trials as including those that had been anesthetized could have introduced another variable potentially affecting the resulting infection load, i.e., any resulting from prior MS222 exposure. This also allowed us to determine how the CORT treatment affected tadpole susceptibility to infection by comparing these only to the sham-CORT individuals.

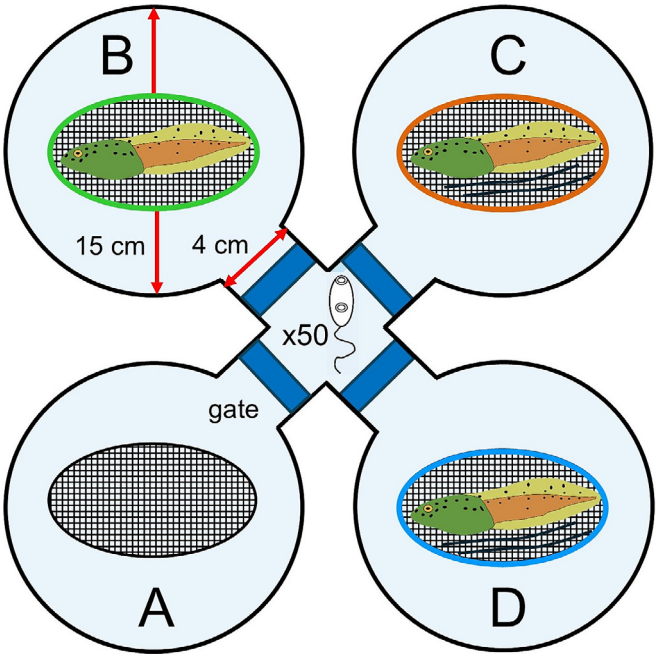

Fig. 1. Experimental set-up of choice trials by 50 cercariae of *Echinostoma* sp. presented with four different options among chambers: (A) empty mesh cup, (B) anesthetized tadpole (temporarily immobile), (C) a corticosterone (CORT) exposed tadpole (capable of movement), and (D) control tadpole (sham CORT-exposed and capable of movement). The cups were made of 14 μm mesh that allowed host chemical and physical cues into water but no direct parasite contact.

To conduct final cercariae counts in the choice trials, we slid each arena under a boom-arm dissecting microscope (Nikon SMZ 745-T) and counted the number of cercariae in each chamber (including the central compartment) with a clicker by removing them individually via pipet, repeating this for each arena. After multiple re-checks to ensure all cercariae were removed, the arenas and filter cups were thoroughly rinsed with hot water and left to dry before re-use. To assess tadpole movement, we reviewed the individual recordings and noted whether hosts were moving every 30 sec during the 30-min recording period to determine the proportion of time that they were active for these 61 time points, an approach consistent with previous studies of tadpole behaviour (e.g., Koprivnikar et al., 2014; O'Dwyer et al., 2024).

2.5. Tadpole infection trials

Forty-eight hours after the cercariae choice trials (step 1), hosts from the CORT and control/sham-CORT exposure treatments were placed individually into 1-L containers with 20 *Echinostoma* sp. cercariae (collected as previously described) that allowed for contact between host and parasite as well as infection. In these trials, cercariae interacted with only a single tadpole in 500 mL of water (i.e., these trials measured only infection success relative to the number of added cercariae). After another 48 h (sufficient time for cysts to establish), we euthanized tadpoles by immersion in a MS222 solution and froze them for later necropsies to determine infection load and developmental stage. After thawing all frozen tadpoles, we examined them under a dissecting microscope to determine their Gosner developmental stage followed by dissections to examine the nephric system and count the number of *Echinostoma* sp. cysts. Because of this latter necessity, we could not assess whole-body CORT levels in the tissues of individual tadpoles (see also Belden and Kiesecker, 2005; LaFonte and Johnson, 2013; O'Dwyer et al., 2024). While assays that consider water-borne CORT as a proxy for endogenous CORT levels in larval amphibians can be useful for non-invasive measurement (Narayan et al., 2019), the relationship between the two measures is not consistent among species and development stages (McClelland and Woodley, 2021). Importantly, such water-borne assays may not be a reliable way to assess endogenous CORT for pre-metamorphic (Gosner stage 25–35) northern leopard frog tadpoles (McClelland and Woodley, 2021).

2.6. Data analysis

Statistically, we analyzed the effect of tadpole treatment on the number of cercariae per chamber using a generalized linear mixed effects model (GLMM) with a Poisson distribution and log-link function (using the glmmTMB package in R (Brooks et al., 2017)). Because not all cercariae made a 'choice' (i.e., some fraction remained in the central compartment), we used an offset term to scale by the number of cercariae detected across the four choice chambers, consistent with Johnson et al. (2019). As fixed effects, we included treatment as a categorical effect with four levels (CORT, anesthetized, control, and empty chamber) along with a categorical fixed effect for the day on which a trial was conducted. We incorporated a random intercept term for trial to account for the non-independence of chambers within the same apparatus and run period. To evaluate how tadpole activity and developmen-

tal stage influenced parasite choice within the same experiment, we repeated the analysis above after subsetting to treatments with hosts (i.e., omitting the empty chamber condition). Tadpole developmental stage (Gosner) and tadpole activity (proportion of trial observations in which the animal was active) were included as fixed effects following standardization (i.e., using the 'scale' function to subtract the mean and divide by the standard deviation), and the total number of cercariae in the offset was adjusted to omit the empty chamber condition.

Finally, we assessed how parasite choice (in the 'choice trials') and host treatment determined subsequent parasite load in the same tadpoles when cercariae were allowed to contact and infect hosts (in the 'infection trials'). This analysis focused specifically on the CORT and sham-CORT (control) tadpole treatments given that the anesthetized tadpoles were excluded from the infection trials. Once again, the response (number of *Echinostoma* sp. metacercariae per tadpole) was modeled using a Poisson distribution, which yielded a lower AIC value relative to alternative distributions such as negative binomial, Poisson with observation-level random effect, or Gaussian.

Across all analyses, we evaluated the effect of individual fixed effects using a likelihood ratio test comparing models with and without the term. For the categorical effect of treatment, detection of a significant effect of treatment was followed by use of Tukey-Kramer tests to isolate which conditions differed while correcting for multiple tests. Model diagnostics were assessed using 'performance' and 'DHARMa' in R (Hartig, 2024) to provide information on model fit, collinearity, over dispersion, and zero inflation.

2.7. Data availability

The data that support the findings of this study are available through the Toronto Metropolitan University online data repository, Rshare, (https://library.torontomu.ca/rshare/) – a publicly-accessible resource.

3. Results

3.1. Cercariae choice

On average, 97.2% ± 2.8% (S.D.) of cercariae administered in each trial were recovered successfully; of these, 63.7% were detected in the treatment chambers relative to 36.3% that remained in the central acclimation compartment (i.e., failed to make a choice). Cercarial choice among chambers was strongly influenced by treatment (likelihood ratio test [LRT] against null model without treatment; chi-square = 60.64, $P < 0.0001$), such that nearly twice as many cercariae were recovered from chambers with tadpoles from the CORT and control conditions, relative to anesthetized tadpoles and empty chambers (Fig. 2a; Tukey-Kramer contrasts, $P < 0.001$). There was no difference between cercariae counts in the CORT and control treatments ($P = 0.692$), but a trend for more cercariae to choose chambers holding anesthetized tadpoles over empty chambers ($P = 0.0819$). The conditional R^2 of the model was 0.22 with no evidence of significant overdispersion, collinearity, or zero inflation. Among conditions containing tadpoles (i.e., removing the empty chamber condition and adjusting the offset accordingly), tadpole movement had a strong, positive effect on cercarial choice (Fig. 2b; LRT chi-square = 28.76, $P < 0.0001$), whereas there was no significant effect of developmental stage (LRT chi-square = 1.47, $P = 0.22$) or trial day (LRT chi-square = 0.05, $P = 0.81$). This effect persisted even if the anesthetized tadpole treatment was removed, suggesting that the influence of movement on parasite choice was robust.

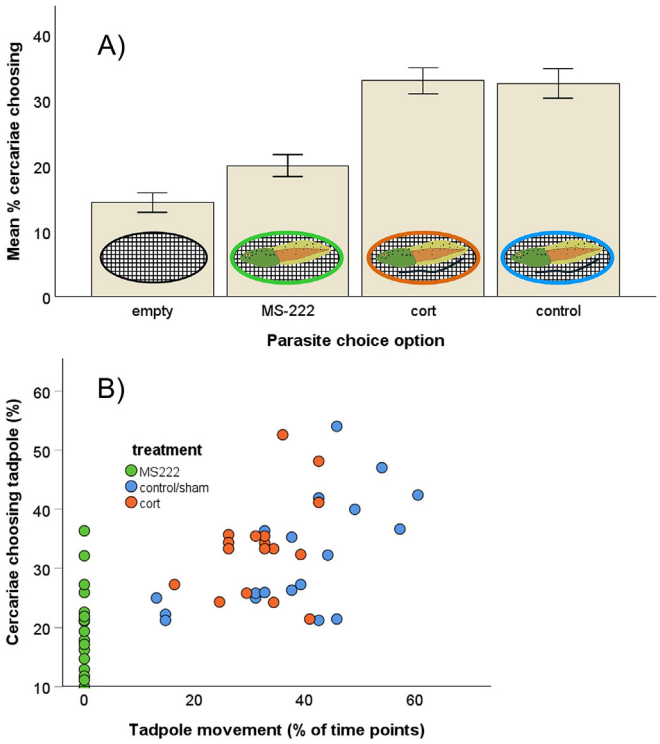

Fig. 2. Effects of experimental treatment and activity level on tadpole attractiveness to cercariae during choice trials. A) The mean (±1 S.E.) proportion of *Echinostoma* sp. cercariae choosing among the four different options presented to them in choice chambers: an empty mesh cup, a tadpole anesthetized with MS-222 (immobile host), a corticosterone (CORT) exposed tadpole (capable of movement), and a control/sham CORT-exposed tadpole (capable of movement). This proportion excludes any cercariae that failed to make a choice and remained in the central acclimation chamber. B) The influence of individual tadpole activity level during 30 min choice trials on cercariae selection of host chambers.

3.2. Tadpole infection loads

For the infection trials, tadpoles treated with CORT exhibited 21% greater infection loads, on average, relative to those in the control (sham-CORT) (Fig. 3a; LRT chi-square = 8.97, $P = 0.002$). CORT-exposed tadpoles had a mean (± S.E.) cyst load of 8.98 (± 0.95) while that of the control tadpoles was 6.49 (± 0.92). Parasite choice – the number of cercariae that selected a specific tadpole during the prior choice trials – was also a significant, positive predictor of the observed number of *Echinostoma* sp. metacercariae in the infection trials (Fig. 3b; LRT chi-square = 4.75, $P = 0.029$), with no significant interaction between CORT and parasite choice (LRT chi-square = 1.47, $P = 0.22$). Day of the trial had a negative influence on parasite load (LRT chi-square = 11.28, $P = 0.0008$) while there was no significant effect of tadpole development stage (LRT chi-square = 2.80, $P = 0.09$). The model conditional R^2 was 0.60.

4. Discussion

Here, we manipulated the presence of amphibian hosts, their susceptibility to infection, and their capacity for movement to investigate intraspecific host choice by free-swimming trematode stages (cercariae). Results of the experiment indicated that parasites were most attracted to chambers containing tadpoles (over empty chambers) and especially active hosts. Remarkably, the frequency of choice by *Echinostoma* sp. cercariae for chambers holding an immobile tadpole (~20%) was not significantly different from cercariae choice for empty chambers (~14%), emphasizing the importance of host movement. Overall, these results highlight

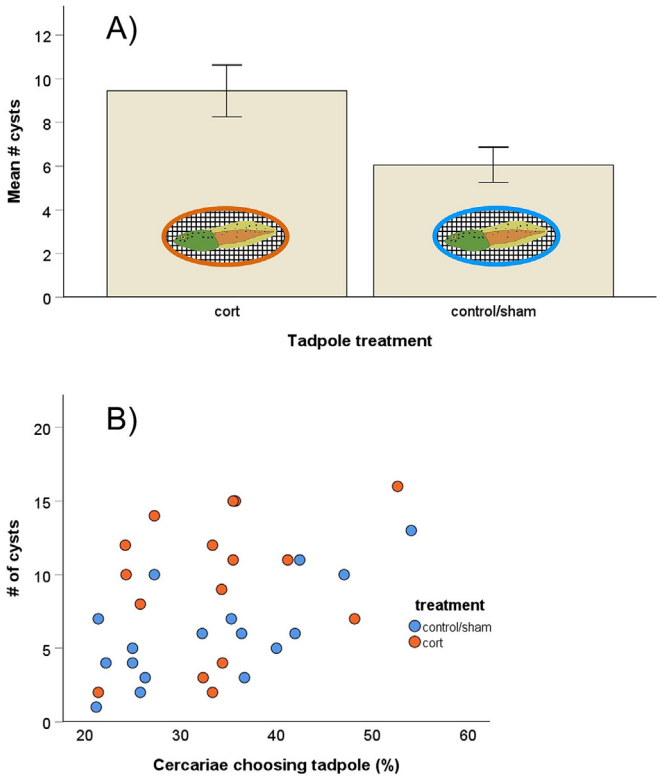

Fig. 3. Effects of tadpole host treatment through CORT- or sham-CORT exposure (A) and individual tadpole attractiveness during the choice trials (B) on each tadpole's subsequent infection load (metacercariae per host) after the infection trials (with individual tadpoles in 500 mL of water with 20 cercariae).

parasites' likely use of both chemical and physical cues to locate suitable hosts; however, cercariae did not show a preference for tadpoles subjected to an immunosuppressive treatment (CORT), with virtually identical proportions (32%) choosing the CORT- and sham-CORT (control) tadpoles, respectively. Instead, the more active an individual tadpole, regardless of treatment, the more cercariae chose to spend time in proximity to it, despite the lack of contact opportunity.

When cercariae were allowed to contact and infect the same tadpoles during a second round of exposure ('infection trials'), prior exposure to exogenous CORT was associated with a 72% increase in observed parasite loads relative to control (sham-CORT) tadpoles. This finding is consistent with prior work involving CORT and trematodes and likely reflects the immunosuppressive effects of the hormone on innate resistance to cercariae invasion and establishments (e.g., Belden and Kiesecker, 2005; LaFonte and Johnson, 2013). Importantly, however, individual tadpole attractiveness from the first, non-contact round ('choice trials') was also a significant and positive predictor of parasite load, even after accounting for the influence of CORT. This indicates that the choices made by cercariae for particular hosts during the choice trials correlated positively with the susceptibility or competence of those same hosts when contact were allowed. Thus, cercariae exhibited a preference for more suitable host individuals (i.e., intraspecific choice) of a given species, a result similar to that reported by Sears et al. (2012) for larval amphibians and for a range of ectoparasite and host species (e.g., Dallas and Foré, 2013; Campbell and Luong, 2016). However, this choice did not perfectly reflect host susceptibility to infection; indeed, despite sharp increases in susceptibility among CORT-treated tadpoles, these hosts were not preferentially selected by cercariae in the choice trials. This suggests that parasite choice (either inter- or intraspecific) may involve different host attributes and cues, some of which could reflect host vulnerability (susceptibility) or quality/vigor (Attwood, 2025).

When it comes to potential drivers of cercariae choice, our findings also confirm and extend previous work on the cues cercariae may use to locate aquatic hosts. The *Echinostoma* sp. cercariae here used both physical and chemical cues to locate suitable hosts, exhibiting a much lower preference for empty cups as well as immobilized tadpoles. Echinostomatid cercariae employ photo- and geo-orientation to move into microhabitats most frequented by their potential hosts (e.g., Loy et al., 2001), but we are unaware of any reports that cercariae in this family respond to water currents or vibrations potentially signalling host proximity. Use of such physical cues from host movement is known from cercariae of various fish-, bird-, and mammal-infecting trematode species (see reviews by Haas, 1994, 2003; Combes et al., 2002). The cercariae of many trematode species also use chemical cues for host location (see reviews by Haas, 1994, 2003; Combes et al., 2002). Echinostomatid cercariae respond to hydrophilic organic molecules in a concentration-dependent manner to locate and orient towards aquatic snails that can also be used as second intermediate hosts (Haas et al., 1995). However, Körner and Haas (1998) reported that cercariae responses were similar to samples of water conditioned with different snail species, fish, tadpoles, and leeches when these were diluted to the same total amino acid concentrations, suggesting that particular mixtures of the latter were not necessary. In addition, Johnson et al. (2014) found that *E. trivolvis* cercariae were attracted to tadpole cues in the form of solutions containing homogenized nephric tissue.

Despite the clear effect of the exogenous corticosterone (CORT) treatment that increased tadpole susceptibility to infection, host hormone exposure did not significantly affect cercariae preference. As described earlier, the "tasty chick hypothesis" suggests that ectoparasites should prefer the least-developed nestlings in a brood if these are the most susceptible hosts (Christe et al., 1998), corresponding to the CORT-exposed tadpoles here. Our results also contrast with ectoparasite studies that considered host hormones. For instance, nymphal ticks preferred host blood without added glucocorticoids, indicating the potential importance of host physiological state in host selection (Vanroy et al., 2024). In another example, Christe et al. (2007) reported a clear choice by *Spinturnix* mites for adult females in the bat host species tested and suggested that steroid hormone levels could act as a host selection cue, especially if hormone variation also translated into detectable differences in body temperature or other biochemicals.

The preference of cercariae for active tadpoles in the choice trials regardless of host CORT treatment could thus simply reflect an intensity-dependent response to host-generated physical cues, such as water currents or vibrations within the confines of a chamber, especially given the small volume of water involved here. However, an alternative explanation is that tadpole movement may serve an indicator of host quality/vigor to cercariae in terms of potential resources available post-infection. Because most trematode species have life cycles involving trophic transmission such that a suitable vertebrate final host must ingest an infected second intermediate host (tadpoles here) harboring cysts (Esch et al., 2002), relatively motile tadpoles may represent individuals of higher quality which are more likely to tolerate infection and eventually encounter the final host. Notably, longer lifespans of intermediate hosts should promote the chances of successful transmission (Poulin, 1994). This may be particularly relevant for *Echinostoma* sp. if larval amphibians are less likely than metamorphosed or adult amphibians to encounter the mammals and birds used as final hosts by this trematode species, such as herons and

foxes (Toledo et al., 2009); thus, tadpole survival to metamorphosis and greater use of terrestrial habitats could be critical to its transmission.

The influence of host attractiveness in the choice trials on cyst load after the infection trials may have at least two explanations. The first is that cercariae showed the greatest attraction to tadpoles most inherently susceptible to infection, which would correspond to host vulnerability as the driving factor behind intraspecific choice. However, it is not obvious which cue(s) would be used by cercariae an indicator of tadpole resistance. It cannot be a lack of motility indicating individuals in poor condition (i.e., most likely to have low resistance) as cercariae were instead most attracted to relatively active tadpoles. Alternatively, tadpoles previously exposed to cercariae during the choice trials, even without direct contact and infection, may have become more susceptible to subsequent infection in an intensity-dependent manner, i.e., the more cercariae that came into close proximity to an individual tadpole in the choice trials, the heavier that tadpole's cyst load after the infection trials. This may reflect one of the "costs of parasite exposure" borne by potential hosts (Rohr et al., 2010), and more specifically, the "ecology of fear." This term has historically pertained to the non-consumptive effects (NCEs) of predators on their potential prey because the latter can exhibit a range of costly physiological and behavioral changes in response to predation risk without actual attack/consumption (see reviews by Peacor et al., 2013; Sheriff and Thaler, 2014; Sheriff et al., 2020). Given the many similarities between predators and parasites as natural enemies (Raffel et al., 2008), the ecology of fear and NCE paradigms have been extended to parasites (Buck et al., 2018; Weinstein et al., 2018; Daversa et al., 2021). Tadpoles perceiving a greater risk of parasitism during our choice trials may thus have undergone trait changes that subsequently increased their susceptibility during the infection trials.

Most studies examining host trait changes in response to parasite threat have focused on behavioural alterations, especially avoidance of high-risk situations. For example, tadpoles will forego foraging where cercariae are present (Koprivnikar and Penalva, 2015). However, indirect parasite exposure can also cause physiological changes in hosts such as increased metabolic rates (Luong et al., 2017). Non-contact exposure to cercariae here could thus have caused various trait changes in tadpoles that later increased their susceptibility to infection. Notably, a number of environmental stressors, including predator presence, can cause changes in hormones such as glucocorticoids (e.g., Sheriff et al., 2009; Dantzer et al., 2014). Whether there are similar hormone alterations because of parasite threat represents a significant gap in our knowledge (see review by Lopes, 2023). The possibility for indirect cercariae exposure to cause physiological changes in larval amphibians that affect their susceptibility warrants exploration given such effects have been reported in other host-parasite systems (e.g., Garvey et al., 2022), and especially because elevated levels of CORT are known to affect tadpole leukocyte profiles and cause greater trematode infections (e.g., Belden and Kiesecker, 2005; LaFonte and Johnson, 2013). Alternatively, 48 h post-exposure may not be enough time for tadpoles to undergo physiological changes that could affect immune defences, suggesting the importance of plasticity in other traits affecting tadpole resistance to trematode infection, such as anti-parasite behaviours.

Tadpoles from a range of amphibian species (including the *R. pipiens* used here) respond to the close proximity of live cercariae in small volumes of water by increasing their overall activity level, exhibiting sharp twists and turns to evade and remove cercariae (e.g., Taylor et al., 2004; Szuroczki and Richardson, 2012; Sears et al., 2013). More active tadpoles thus have lower infection loads (e.g. Koprivnikar et al., 2014; O'Dwyer et al., 2024) and suppressing their movement causes higher cyst burdens (e.g., Koprivnikar et al., 2006; Daly and Johnson, 2011). Here, non-contact exposure to cercariae during the choice trials could have made the most attractive tadpoles more susceptible during the infection trials if they showed altered behavioral responses. Whether prior non-contact exposure to cercariae later affects larval amphibian behaviours in an intensity-dependent manner is not known but this is certainly the case for predation risk (e.g., Ferrari et al., 2010; Ferrari and Chivers, 2010). Given that tadpoles exposed to cues signalling predation risk were less active in the presence of cercariae a week later compared to sham predator-exposed tadpoles (Koprivnikar and Urichuk, 2017), it is possible that prior indirect exposure to cercariae could have similar effects. However, this may depend on whether activity increases or decreases are more effective in this context. In our study, tadpoles that attracted a large number of cercariae during the choice trials may have showed a general reduction in activity during the infection trials so as to avoid parasite detection given that our results clearly indicate a strong preference by cercariae for relatively motile individuals, and they may have exhibited a diminished behavioural response even when contacted by cercariae.

Future studies of intraspecific preference by parasites should thus consider how non-contact exposure to infectious stages might consequently alter anti-parasite behaviours and other aspects of host defence that determine infection. The few studies that have examined such indirect effects of parasite exposure indicate that it can have persistent influences on host behaviour (e.g., Selbach et al., 2022; Liang and Luong, 2024), but more work is needed to understand whether such effects are widespread. The possibility for cercariae to choose inherently susceptible individuals, rather than causing fear-induced trait changes, could also be further explored by identifying whether there are reliable cues that allow parasites to identify more susceptible hosts (Attwood, 2025). A more complete understanding of the extent to which intraspecific choice by parasites occurs, as well as the cue(s) used, will also help provide insights into parasite distributions and potential pathology among hosts. While parasite aggregation among hosts (within a species) is frequently postulated to emerge from heterogeneity in both exposure and susceptibility (see Poulin, 2013; Johnson and Wilber, 2017), our results emphasize the additional role of parasite choice among hosts in affecting non-random patterns of parasite exposure and, ultimately, infection aggregation. A strong preference for particular host individuals could help drive aggregated macroparasite distributions, as reported for ticks and mice (Dallas and Foré, 2013), with consequences for intensity-dependent pathology. Intraspecific variation in host attractiveness may also play a role in evolutionary dynamics by promoting a Red Queen-style "arms race" between heritable host-generated cues and the ability of parasites to detect such cues (Sears et al., 2012). Further investigations of parasite choice and the non-consumptive effects of parasites are therefore essential given their relevance for infectious disease dynamics.

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CRediT authorship contribution statement

J. Koprivnikar: Writing – original draft, Visualization, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization. **L.M. Santos:** Writing – review & editing, Methodology,

Investigation. **P.T.J. Johnson:** Writing – original draft, Funding acquisition, Formal analysis, Conceptualization.

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