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ORIGINAL ARTICLE

The role of small-scale environmental gradients on trematode infection

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Abstract

1. Parasitism rates can vary dramatically across large and small spatial scales. Such heterogeneity in infection is driven by variation in environmental factors that influence the probability of host and parasite encountering each other (encounter filters) and of genetic and physiological factors that alter whether that encounter results in a successful infection (compatibility filters). Here, we investigated how infection in first-intermediate hosts (snails) by trematode parasites is influenced by small-scale variation in environmental variables (final host relative abundance, nutrients, and temperature) and population origin across multiple ponds within an artificial flow-through wetland system in Atlanta, GA, U.S.A.
2. We placed individually marked, lab-reared F1 generation snails of the same age from three different populations into field enclosures at nine ponds along a 1-km stretch of connected, flow-through ponds for 86 days and allowed them to accrue infections naturally. This highly controlled field experiment allowed us to disentangle the underlying factors influencing small-scale variation in infection risk, which can be masked when looking only at natural patterns of infection prevalence across space.
3. We found high heterogeneity in infection risk. The probability of infection was highest in ponds downstream and with high levels of bird activity; population origin was not important.
4. This work provides experimental evidence that infection risk varies across small spatial scales (tens of metres), driven by steep gradients in influential environmental drivers, emphasising the importance of controlled field manipulations to understand infection risk. Identifying small-scale drivers of infection risk can help mitigate infection in hosts of interest (e.g., humans, fish), and should be acknowledged in organismal field studies, where infection and infection risk may influence the ecology, physiology, or behaviour of animals.

KEYWORDS
birds, compatibility filter, encounter filter, population, wetland

1 | INTRODUCTION

Parasitism is often heterogeneous across the landscape, varying at spatial scales that are regional (across latitudes), local (across sites), and sub-local (within sites) (e.g., Altman & Byers, 2014; Galaktionov, 1996; Granovitch & Johannesson, 2000; Kuris & Lafferty, 1994; Robson & Williams, 1970; Smith, 2007; Sousa, 1990; Williams & Ellis, 1975). Elucidating the drivers of this heterogeneity is vital to understand how parasites may be transmitted to new areas or how disease dynamics will be altered by climate change and other anthropogenic changes (Díaz-Morales et al., 2022).

For parasites to successfully infect hosts, the parasites must be present in the environment (part of the regional species pool; Cornell & Harrison, 2014) and then successfully pass through two sets of filters: encounter and compatibility filters (Combes, 2001). Encounter filters dictate what parasites are available in an area, how long they survive, and whether they make contact with a host (pre-infection). Compatibility filters dictate what parasites can successfully infect, survive, and replicate within a given host (post-contact).

Encounter filters can be both abiotic and biotic. For parasites with complex life cycles (such as trematodes), the spatial heterogeneity found in infection of first intermediate hosts can often be tied to the behaviour and abundance of definitive (i.e., final) hosts (Altman & Byers, 2014; Byers et al., 2008, 2010; Fredensborg et al., 2006; Hechinger & Lafferty, 2005; Smith, 2001), which deposit parasite eggs in their faeces and which are significantly more mobile than first intermediate hosts. This variation in final host abundance can often be due to small microhabitat differences that cause final hosts to aggregate (Byers et al., 2015; Smith, 2001; Zimmermann et al., 2014, 2016). Byers et al. (2015) found that infection risk was greatest at higher tidal elevations where birds congregated, and subsequently defaecated, during low tide. Abiotic variables, such as temperature, salinity, and water quality, can also limit the production, viability, and duration of parasitic free-swimming stages (e.g., Johnson et al., 2007; Lawson & Wilson, 1980; Pechenik & Fried, 1995; Pietrock & Marcogliese, 2003; Studer & Poulin, 2012; Thieltges & Jennifer, 2006), thus altering infection prevalence and intensity in hosts. For instance, an increase in total dissolved nitrogen caused infected snails to produce and release nearly twice as many cercariae, increasing transmission to secondary and definitive hosts (Johnson et al., 2007). Abiotic variables can also limit infection by altering parasite dispersal to hosts (Cáceres et al., 2006; Zimmermann et al., 2016) or masking host cues. For example, Zimmermann et al. (2016) found that within a given lake, microhabitat differences in substrata influenced the prevalence and intensity of metacercarial trematode infection in the pulmonate snail, *Helisoma anceps*, potentially because leaf litter physically obstructed the cercariae from finding and reaching their host.

Compatibility filters can be genetic, but also environmental in nature. For instance, host susceptibility or resistance to infection depends in part on genotype (e.g., Grosholz, 1994; Lively, 1989). Different parasite pressures over long time periods (due to encounter filters) can result in different susceptibility between host populations (e.g., Keogh et al., 2016, 2017; Weber et al., 2017, 2021). For hosts with limited dispersal, local adaptation may lead to differential susceptibility to infection (Weber et al., 2017, 2021). Additionally, environmental variables, such as temperature, may influence transmission success through changing how well the host immune system is able to suppress or fight off infection and how well the parasite is able to reproduce and transmit the infection to new hosts (Gehman et al., 2018; Mordecai et al., 2019). For instance, transmission success of miracidia into snail first intermediate hosts increases with temperature (and then crashes at very high temperatures; Morley & Lewis, 2015). Finally, environmental and genetic compatibility filters can interact via host genotype-by-environment interactions, resulting in differential infectivity of host genotypes under different environmental conditions (see Wolinska & King, 2009 for review). Together, these filters can create a patchwork of infection at regional, local, and small scales.

Importantly, movement of infected hosts can erase the signature of these filters. For instance, Byers et al. (2015) found through a controlled field experiment using sentinel snails (uninfected snails placed out to quantify infection risk) that infection risk was greatest at high tidal elevations where bird activity was highest. However, due to movement post-infection, prevalence of infection in the snails was highest at low tidal elevations. Thus, the infection risk was decoupled from the infection prevalence in this system, emphasising the need for controlled infection experiments in the field to truly understand the drivers of small-scale variation in infection risk.

To understand how small-scale encounter filters and compatibility filters influence the probability of trematode infection, we placed uninfected F1 sentinel snails across a wetland system constructed for wastewater treatment. Constructed wetlands make model field systems because they are fed by the same source of water flowing through replicated sets of connected ponds. By contrast, nutrients and other factors change across connected ponds, as excess nutrients should be taken up by the aquatic vegetation in each subsequent pond in a series. The compact nature of these wetland systems means that many environmental variables are identical, allowing us to focus on those variables that have sharp gradients over small spatial scales. In this study, we focused on nutrients, temperature, and bird activity, but also included population origin to investigate whether there were potential genetic or epigenetic differences between populations (i.e., ponds) that affected infection outcomes at this small scale. We predicted the following relationships:

1. Bird abundance should correlate positively with infection, since birds (particularly water and shore birds) are definitive hosts for many trematode species.
2. Nitrogen (nitrate, ammonium) and phosphorus (phosphate) should correlate positively with infection, as has been shown in other snail–trematode systems (Altman & Byers, 2014; Hartson et al., 2011; Johnson et al., 2007; Richgels et al., 2013). This relationship may result from bottom-up effects that increase cercarial

production and host density, ultimately increasing transmission through local hosts (Johnson et al., 2007). There is no clear mechanism for how other nutrients (dissolved inorganic carbon, calcium, etc.) might impact infection, and so we predict that these nutrients will have no effect.

3. Temperature should have a positive relationship with infection due to changes in the ability of miracidia to successfully infect snail hosts.
4. Population origin should not impact infection as high gene flow is expected at this small spatial scale.

2 | METHODS

2.1 | Natural history

Helisoma (Planorbella) trivolvis (ramshorn snails) are pulmonate freshwater snails native to North America that are common in ponds and wetlands. They feed on both periphyton and macrophyte tissue (Lombardo & Cooke, 2002; Smith, 1989). *Helisoma trivolvis* snails are infected by a diversity of trematode parasites (Fried & LaTerra, 2002; Richgels et al., 2013; Sapp & Loker, 2000). Snails can become infected by two different routes, depending on the trematode parasite. For one route, trematode eggs are released from definitive hosts (that can be fish, birds, mammals) and are then directly eaten by the snail. For the second route, trematode eggs develop into free-swimming stages (miracidia) that actively seek out snail hosts, using a variety of tactile and chemosensory methods (Haas, 2003). Once a snail is infected, a single egg or miracidium develops into an entire colony of reproductive individuals, generally in the gonadal tissue, resulting in castration of the host. These reproductive individuals produce free-swimming stages (cercariae) that leave the snail to encyst on or in a secondary intermediate host, which can be either invertebrate or vertebrate. This host is then generally eaten by the definitive host, where sexual reproduction occurs, completing the life cycle. Because trematodes have obligate multi-host life cycles, there is no horizontal transmission of miracidia between first intermediate hosts, meaning that snails act as independent replicates of their environment (Byers et al., 2008).

2.2 | Field collection and lab-rearing of F1 generation

To create a standardised set of uninfected, sentinel snails for our experiments that were the same age and devoid of any differential environmental conditioning, we raised an F1 generation in the laboratory. Six to eight adult Ramshorn snails were collected from three ponds (G1, G2, A2) at the Huie Constructed Wetlands in Clayton County, GA, U.S.A. on 1 November 2019 (Figure 1; 33.487274, −84.300912). The Huie Constructed Wetlands provide tertiary water treatment for the Clayton County Water Authority. Each wetland is composed of a series of connected ponds, where treated water flows through from the treatment plant, eventually leaving the last pond and entering a reservoir. Snails from each pond were brought back to the lab to reproduce and create a standardised set of snails for our experiments that had been exposed to similar environmental conditions. We held snails from each source pond in their own plastic aquarium (30×20×21 cm) with media, an air stone, and spinach for food. This media was tap water treated with TetraSafe Dechlorinated Water with 1 g/L of Seachem's Equilibrium to maintain water hardness to promote shell growth. Snails rapidly began to lay egg masses in all three tanks. We allowed them to lay eggs from 2 to 5 November. We then removed all adult snails and placed them in fresh aquaria. We allowed the eggs from that 3-day period to mature on their own. Once hatched, they were fed spinach ad libidum. We then used this F1 generation from our three populations (G1, G2, A2) for our field experiment, allowing us to isolate effects of population origin (a proxy for potential genetic/epigenetic compatibility filters) on infection risk.

2.3 | Field experiment

To determine the environmental variables that influence infection risk in our snail hosts, we placed our F1 generation snails out into the field across nine different ponds (Figure 1). We used the first three connected ponds (referred to by their upstream position: 1, 2, 3) that were nearest the inflow pipes in each of three wetlands. To ensure that snails were mature enough to be infectable and also

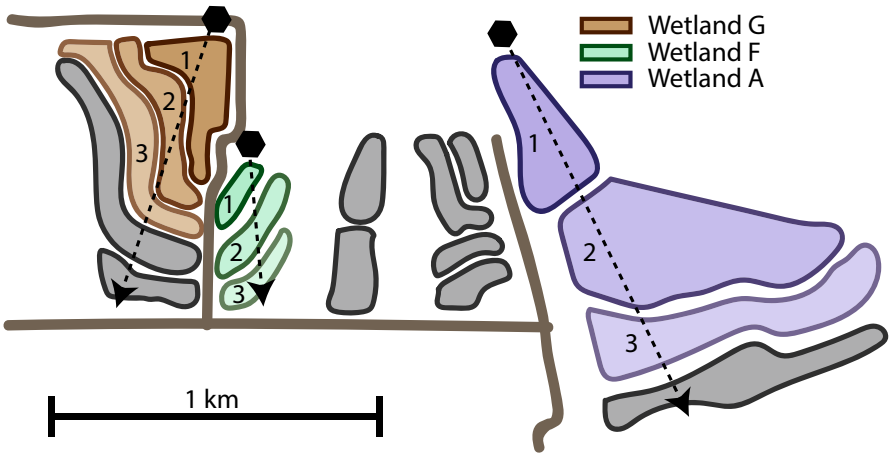

FIGURE 1 Experimental layout across Huie Constructed Wetlands. Snails were placed out across three wetlands (G, F, A) within the first three treatment ponds (upstream positions 1–3). Treated water from the plant (black hexagon) enters the first pond (upstream position 1), and then flows into the second pond (upstream position 2) and then the third pond (upstream position 3; Dashed line with arrow). Grey ponds were not used for this experiment.

large enough not to escape our field enclosures (maximum opening was 6 mm × 3.5 mm), we used lab-reared snails that were >6 mm (width from aperture lip). Enclosures were made of plastic minnow traps (Pentair Aquatic Eco-systems, Inc.) with window screen (1 mm × 1 mm mesh size) zip-tied to the opening at either end. Within each enclosure, five snails from each origin pond (G1, G2, A2, 15 total) were marked with nail polish to indicate individuals and pond origin. Enclosures were placed out across the first three ponds (upstream positions 1–3) at three wetlands (A, F, G; Figure 1). Wetlands A and G had three enclosures per pond ($n = 45$ snails), whereas F had two ($n = 30$ snails). Snails from the first two enclosures in each pond ($n = 30$) were measured (as above) before being placed in the field to investigate the relationship between host size and parasite infection.

All enclosures were zip-tied to a 2 m tall green T-post placed in the shallow (1–1.5 m depth) region of each pond. Emergent vegetation taken from each pond was placed in each of the enclosures to make sure snails had sufficient periphyton to eat. Enclosures were mostly submerged but kept at the surface to allow snails to emerge from the water if needed and so that the vegetation could receive enough light to survive. At each pond, an ONSET HOBO data logger (Water Temp Pro v2) was placed in one of the enclosures to log hourly temperature data at the microclimate level. Additionally, to capture potential final host activity, a motion-activated Bushnell Trophy Camera Model 119876 (Bushnell Outdoor Products) was placed on top of each T-post facing the water to estimate bird and mammal diversity and abundance. To investigate nutrient concentrations, 1-L water samples were collected monthly in acid-washed Nalgene containers from the surface of the water next to each T-post and frozen until analysis. The experiment ran for 86 days from 27 April 2020 to 22 July 2020. At the end of the experiment, snails (both dead and alive) from each enclosure were placed in pond water and brought back to the lab for further analysis.

2.4 | Nutrient analyses

Water samples were thawed and pooled across the 4 months to determine the average nutrient concentrations at each pond for the duration of the experiment. Pooled samples were filtered through a 0.45-μm filter and sent for nutrient analysis (ppm nitrate, phosphate, ammonium, dissolved organic carbon, dissolved inorganic carbon) to the Stable Isotope Ecology Laboratory at the Center for Applied Isotope Studies (CAIS) at University of Georgia. Calcium (μg/g) analyses were done at the CAIS Plasma Chem Laboratory.

2.5 | Camera trap data

Images from the motion-activated field cameras were visually assessed for animal activity. Animals (primarily birds) were identified to species where possible. We focus here on birds because we found few mammals. To prevent double counting of individuals, we used a combination of bird position and time stamps to determine whether multiple pictures were capturing a single individual. Certain cameras stopped recording before the end of the experiment, and so we controlled for this by dividing total bird abundance by duration that pictures were taken (Appendix S1). This yielded the number of birds per day as a standardised metric across all ponds.

2.6 | Infection status

In the laboratory, snails were investigated for nail polish to determine their identity and population origin. Snails that had no nail polish on them were classified as unmarked and not included in subsequent analyses, since they could either represent snails that infiltrated the enclosures when small (<6 mm), were born in the enclosures from our sentinel snails, or whose nail polish had come off. The majority of recovered snails did have nail polish markings and could be matched to a specific pre-recorded individual. All recovered snails were measured and all surviving recovered snails were housed in 162-ml plastic cups with freshwater media and agar food cubes under fluorescent lights for 9 weeks with water changes twice a week. This duration allowed all immature infections to mature. Each cup was checked twice a week for cercariae (free-swimming parasite stage) or egg masses. Both egg masses and cercariae could be seen with the naked eye. However, we also looked at each cup under the dissecting microscope once a week to ensure that small cercariae were not overlooked. The fluorescent lights created a bright, warm environment that prompted trematodes to release cercariae from infected snails (a process called shedding). Snails that shed cercariae were marked as infected and those that did not shed and laid eggs (indicating intact gonads) were marked as uninfected. This method was used rather than simply dissecting the snails because infected and uninfected snails were used for a subsequent experiment (Resetarits et al., 2023). Cercariae were identified to morphotype under a light microscope using relevant literature (Schell, 1985).

2.7 | Statistical analyses

Since we only measured a subset of our snails before the experiment, we first investigated whether initial snail size influenced probability of infection to determine if we could exclude snail size in our models and use all recovered snails for analyses. Because our experimental snails came from the same lab-reared cohorts, their initial sizes were all somewhat similar. However, some variation in size did exist (mean = 8.58, min = 6.00, max = 14.15). To analyse the effect of size on the subset of snails for which we had measured original size ($n = 102$), we used a binomial generalised linear mixed model with infection status (0/1) as the dependent variable, original size as the independent variable, and pond number (1–9) as the random effect. We found that original size did not significantly predict infection status ($\chi^2_1 = 0.097$, $p = 0.756$). Therefore, we used all recaptured, marked snails ($n = 137$) regardless of whether original size was measured (i.e., including all three

TABLE 1 The total number of alive, recovered snails ($n = 136$), and infected snails ($n = 32$) used to estimate infection prevalence across ponds. Abiotic and biotic variables measured at the pond level ($n = 8$) used for model selection: birds/day, nitrate (ppm), ammonium (ppm), phosphate (ppm), inorganic carbon (ppm), organic carbon (ppm), calcium (μg/g), and mean water temperature (°F). Water samples were collected monthly ($n = 4$) during the field experiment and pooled before nutrient analyses. Water temperature was taken hourly during the field experiment and averaged over the entire duration.

Wetland	Upstream position	Snail n	Infected snails	Infection prevalence	Birds/day	Nitrate (ppm)	Ammonium (ppm)	Phosphate (ppm)	Inorganic carbon (ppm)	Organic carbon (ppm)	Calcium (μg/g)	Temp (°F)
A	1	20	0	0.00	1.03	4.68	0.24	0.12	9.57	11.15	8.81	76.66
A	2	22	2	0.09	1.05	2.56	0.20	0.14	8.14	13.32	8.56	77.63
F	1	5	0	0.00	1.40	4.54	0.31	0.27	14.94	10.65	8.53	75.18
F	2	8	0	0.00	0.88	0.13	0.25	0.37	12.21	16.13	9.41	72.83
F	3	18	10	0.56	3.05	1.95	0.22	0.24	13.56	11.55	8.61	75.59
G	1	11	0	0.00	0.80	0.23	0.24	0.35	14.54	14.20	7.81	72.45
G	2	28	9	0.32	1.58	0.10	0.09	0.29	14.39	15.40	9.15	77.05
G	3	24	11	0.46	1.74	0.12	0.15	0.38	16.43	14.42	9.081	75.09

enclosures for A and G wetlands) for the following analyses to maximise our sample size. Because there were no infections in any ponds closest to inflow (i.e., within upstream position 1 for A, F, or G wetlands), we coded one live uninfected snail (Wetland A, Cell 1, snail origin A2) as infected to provide a non-zero variability that helped the model run properly. Additionally, we removed pond A3 from our analyses because only one snail survived, probably because the enclosures became submerged under a floating mat of vegetation. Thus, our final sample size for snail parasite analyses was $n = 136$.

First, we investigated whether the probability of infection differed based on population origin (A2, G1, G2; prediction 4), upstream position (1, 2, 3), or wetland (A, F, G). We used a binomial generalised linear model with infection status (0, 1) of each snail as our dependent variable. We used the package emmeans() in R to calculate estimated marginal means for each variable (while controlling for the two other predictive variables in the model) and determined significant differences between levels of each variable using the Tukey method. We visualised these results using the visreg() package in R (Breheny & Burchett, 2017). To plot this, visreg applies an inverse logistic transformation to the regression line and confidence bands.

Because of issues of singularity and model overfitting that might stem from over-parameterised models, we then used a series of univariate models to investigate how each pond-level environmental factor of interest (bird activity, nutrients, water temperature) explained variation in the probability of infection across ponds (Table 1; predictions 1–3). Because we had many correlated nutrient variables, we also ran a principal component analysis (PCA) on nutrient variables to see if a single axis of variation of collective nutrient effects could better explain individual infection status. For probability of infection, we used a binomial generalised linear mixed model with infection status (0,1) as the dependent variable, one environmental explanatory variable as the independent variable (i.e., bird activity, temperature, each individual nutrient, nutrient PC1, nutrient PC2), and pond number (1–8, since A3 was excluded) as a random effect. Because of our use of multiple tests, we used a Bonferroni p-value of 0.005 to correct for our multiple (10) tests. We used the package visreg() in R to visualise effects of our significant models.

3 | RESULTS

We found that the probability of getting infected was predicted by upstream position ($\chi^2_2 = 17.559$, $p < 0.0001$, Figure 2a), but not by wetland ($\chi^2_2 = 1.451$, $p = 0.484$) or snail origin ($\chi^2_2 = 1.074$, $p = 0.585$). Snails placed in ponds with upstream position 3 (furthest downstream) were significantly more likely to be infected than snails placed in ponds with upstream position 1 (furthest upstream; z-ratio = −2.907, $p < 0.05$) or upstream position 2 (z-ratio = −2.347, $p < 0.05$). We recovered 136 snails alive (43%, not including the single snail in A3), of which 32 were infected. Of those infected, 29 were infected with a brevifurcate–apharyngeate cercarial morphotype (families either Spirorchiidae or Schistosomatidae) and three

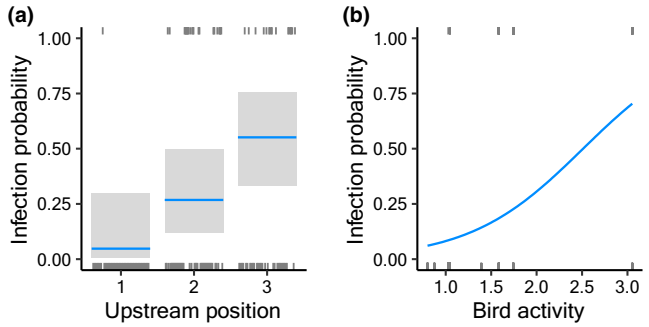

FIGURE 2 (a) The probability of infection was dependent on upstream position, with no infections (minus the one added for statistical analysis) occurring at upstream position 1 (closest to the treatment plant inflow) and >50% probability of infection occurring for snails at upstream position 3 (farthest downstream). (b) Predicted relationship between bird activity (birds/day) and infection probability from binomial generalised linear mixed model. Bird activity significantly and positively correlated with infection prevalence. Blue lines represent predicted values and grey shading represents confidence bands. Rug marks are on the top and bottom of each figure, representing the distribution of the data along the *x* axis.

were infected with a magnacauda cercarial morphotype (families either Echinostomatidae or Psilostomidae).

Bird activity per day significantly predicted the probability of infection across our eight ponds ($\chi^2_1 = 10.26$, $p < 0.005$; Table 2; Figure 2b). Bird activity varied from 0.80 to 3.05 birds/day, with great blue herons (*Ardea herodias*), great egrets (*Ardea alba*), red-winged blackbirds (*Agelauis phoeniceus*), and Canada geese (*Branta canadensis*) being the most common. Nutrients (mean ammonium, nitrate, phosphate, inorganic carbon, organic carbon, calcium) and temperature varied little between ponds and did not significantly explain infection prevalence across the ponds (Table 1; Table 2). PC1 and PC2 explained 54% and 23% of the variation in nutrients across our pond, respectively, but did not significantly predict probability of infection (Figure S1; Table 2).

4 | DISCUSSION

Using similar-aged F1 sentinel snails placed across eight ponds, we investigated potential encounter and compatibility filters (nutrients, host activity, temperature, population origin) impacting infection risk. We found that even across the small spatial scale examined here, the probability of infection increased downstream, and that bird activity was predictive of infection, supporting prediction 1. Nutrients and temperature were not significant (predictions 2 and 3). Finally, there was no effect of population origin on infection risk (prediction 4), suggesting no genetic or epigenetic basis for susceptibility/resistance at this small scale. This is perhaps not surprising, considering that the ponds were within 1 km of each other and in some cases contiguous (G1 flows into G2). Thus, snails (and therefore genes) could potentially mix over such a scale. However, bird activity was also measured at this same small spatial scale and did differ considerably and influence infection risk.

TABLE 2 Significance testing for univariate models. All models are binomial generalised mixed models with infection status (0/1) as the dependent variable, and Pond Number (1–8) as a random effect. Df is the degrees of freedom. Significance level after Bonferroni correction for $p < 0.005$ is denoted by *.

Variable	χ^2	Df	*p*-Value
Bird activity	10.26	1	0.0014*
Ammonium	3.22	1	0.073
Nitrate	1.08	1	0.299
Phosphate	0.11	1	0.738
Calcium	0.97	1	0.325
Inorganic carbon	2.16	1	0.142
Organic carbon	0.01	1	0.933
Nutrient PC1	1.3	1	0.254
Nutrient PC2	0.24	1	0.623
Temperature	1.15	1	0.284

Infection prevalence in our experiment varied at the pond level from 0% to 56%. This is similar to natural infection levels seen in *Helisoma trivolvis* in other areas (Richgels et al., 2013). Two different cercarial morphotypes infected our sentinel snails: brevifurcate–apharyngeate and magnacauda. Brevifurcate–apharyngeate cercarial morphotypes, which were far more common, were either in the family Spirorchiidae or Schistosomatidae (Schell, 1985). Spirorchiidae use turtles as final hosts and Schistosomatidae use wild birds and mammals (Schell, 1985). The magnacauda cercarial morphotype was either in the family Echinostomatidae or Psilostomidae. Echinostomatidae use reptiles, birds, and mammals as final hosts, and Psilostomidae use birds and mammals (Schell, 1985).

The probability of infection was strongly related to upstream position, with no infections occurring in ponds closest to water inflow (upstream position 1), and almost half of the snails being infected in the most downstream ponds (upstream position 3), regardless of wetland. There is evidence that areas with unidirectional flow may be able to accumulate parasites, resulting in high parasitism downstream (Blasco-Costa et al., 2013; Theron et al., 1978). For example, Theron et al. (1978) sampled along a small canal in Guadeloupe and found that infection prevalence with *Schistosoma mansoni* in *Biomphalaria glabrata* snails increased downstream. However, other studies have found the opposite trend, with higher parasitism upstream in headwater sites (Zemmer et al., 2020), although the mechanism for this is unclear. Flow may explain some of the variation in infection prevalence in our system since flow rate was unidirectional (albeit seemingly slow), and may have caused eggs or miracidia (that infect snails) to accumulate downstream. However, we do not believe that flow rate alone can explain the large effect of upstream position on the probability of infection.

We found that, across these eight ponds, bird activity significantly predicted the probability of a snail becoming infected during the experiment, supporting prediction 1. This makes sense because

birds are probably one of the dominant definitive hosts for these trematode parasites. While mammals may also be definitive hosts for these trematodes, our camera data captured very little mammal activity and so we were unable to investigate mammals in this study. Previous studies investigating the relationship between definitive host abundance and infection prevalence at small spatial scales have shown mixed results. Fredensborg et al. (2006) found a positive relationship between trematode prevalence and bird abundance on large spatial scales (between sites) but not small spatial scales (within sites), whereas other small scale-studies have found significant relationships between bird abundance and trematode parasite prevalence in first intermediate snail hosts (Byers et al., 2015; Smith, 2001). This discrepancy may be partly due to the fact that infection prevalence is not always an accurate estimator of infection risk (Byers et al., 2015), emphasising the importance of controlled infection studies such as this. It is unclear why bird activity might increase farther downstream from the water source, but could be due to a change in the plant/aquatic community, which could also affect other pond species, for instance, fish. Bird activity, therefore, may increase downstream due to an increase in prey abundance.

We found no effect of nutrients or temperature on infection. Previous studies have found a positive relationship between trematode infection prevalence and both nitrogen (Altman & Byers, 2014; Johnson et al., 2007; Richgels et al., 2013) and phosphorus (Hartson et al., 2011). In this study, contrary to prediction 2, we found no effect of nitrogen (nitrate, ammonium) or phosphorus (phosphate). As predicted, other nutrients (calcium, phosphate, inorganic carbon, organic carbon) did not predict infection prevalence. Temperature also did not predict infection prevalence, refuting prediction 3. However, nutrients and temperature did not vary substantially between the ponds, and so there may not have been enough variation in these parameters to see an effect on infection, even if these variables might be influential in other contexts.

Despite the highly controlled nature of this field experiment, there are a few shortcomings of this study. One issue was that some enclosures became submerged below a thick mat of aquatic vegetation (i.e., A3), decreasing sunlight availability for vegetation within the enclosures, potentially explaining some of the discrepancies we see in mortality rate. These mortality rates may result in skewed estimations of infection risk, if infected snails are more likely to die in the field. Evidence from the lab suggests that infected snails are more likely to die, so this may also hold true in the field (Resetarits et al., 2023). This mortality resulted in small numbers of surviving snails in some of the ponds, which probably increased stochasticity and limited some of our analyses. Second, because we did not identify cercarial morphotypes to species, we were unable to pinpoint final host identity. Finally, our data suggested that population origin did not matter at this spatial scale (Prediction 4). This is probably caused by limited genetic differences between ponds due to their close proximity and thus presumed high gene flow. However, we did not test for genetic differences between source ponds, which limited our ability to definitively determine if high gene flow was the mechanism for the lack of population origin effect at this spatial scale.

The strength of our experimental approach lies in quantifying infection risk in highly standardised individual hosts, namely F1 generation snails of the same age from three different populations that were reared in the lab and subsequently individually tracked in our field experiment. This level of control allowed us to investigate what encounter and compatibility filters (nutrients, final host activity, temperature, population origin) influenced infection risk at this small spatial scale (<1 km). This work provides additional support that biotic drivers of infection, such as final host presence can vary substantially at small (<1 km) spatial scales, resulting in meaningful differences in infection risk. This work underscores the importance of controlled experiments (using F1 sentinel snails) to isolate the drivers of infection risk in the field.

AUTHOR CONTRIBUTIONS

Conceptualisation, data interpretation, writing: EJR, JEB. Conducting the research, data analysis, preparation of figures and tables: EJR.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available on Dryad (doi:10.5061/dryad.wwpzgmsq9).

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SUPPORTING INFORMATION

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