Check for updates

Received: 12 April 2021 | Accepted: 27 September 2021

DOI: 10.1111/1365-2656.13612

Journal of Animal Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Variability in environmental persistence but not per capita transmission rates of the amphibian chytrid fungus leads to differences in host infection prevalence

Samantha L. Rumschlag[1,2] | Sadie A. Roth[2,3] | Taegan A. McMahon[4,5] | Jason R. Rohr[1,2] | David J. Civitello[6]

[1]Department of Biological Sciences, Environmental Change Initiative, and Eck Institute for Global Health, University of Notre Dame, Notre Dame, IN, USA
[2]Department of Integrative Biology, University of South Florida, Tampa, FL, USA
[3]Department of Natural Resources Management, Texas Tech University, Lubbock, TX, USA; [4]Department of Biology, University of Tampa, Tampa, FL, USA
[5]Department of Biology, Connecticut College, New London, CT, USA
[6]Department of Biology, Emory University, Atlanta, GA, USA

Correspondence
Samantha L. Rumschlag
Email: srumschl@nd.edu

Funding information
National Institutes of Health, Grant/Award Number: 1R01GM135935-01, R01TW010286 and KK2022; U.S. Geological Survey; US Department of Agriculture, Grant/Award Number: 2021-38420-34065; National Science Foundation, Grant/Award Number: DEB-1518681, DEB-2017785, IOS-1754862, IOS-1754868 and IOS-1755002

Handling Editor: Andy Fenton

Abstract

1. Heterogeneities in infections among host populations may arise through differences in environmental conditions through two mechanisms. First, environmental conditions may alter host exposure to pathogens via effects on survival. Second, environmental conditions may alter host susceptibility, making infection more or less likely if contact between a host and pathogen occurs. Further, host susceptibility might be altered through acquired resistance, which hosts can develop, in some systems, through exposure to dead or decaying pathogens and their metabolites. Environmental conditions may alter the rates of pathogen decomposition, influencing the likelihood of hosts developing acquired resistance.
2. The present study primarily tests how environmental context influences the relative contributions of pathogen survival and per capita transmission on host infection prevalence using the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; Bd) as a model system. Secondarily, we evaluate how environmental context influences the decomposition of Bd because previous studies have shown that dead Bd and its metabolites can illicit acquired resistance in hosts. We conducted Bd survival and infection experiments and then fit models to discern how Bd mortality, decomposition and per capita transmission rates vary among water sources [e.g. artificial spring water (ASW) or water from three ponds].
3. We found that infection prevalence differed among water sources, which was driven by differences in mortality rates of Bd, rather than differences in per capita transmission rates. Bd mortality rates varied among pond water treatments and were lower in ASW compared to pond water.
4. These results suggest that variation in Bd infection dynamics could be a function of environmental factors in waterbodies that result in differences in exposure of hosts to live Bd. In contrast to the persistence of live Bd, we found that the rates of decomposition of dead Bd did not vary among water sources, which may suggest that exposure of hosts to dead Bd or its metabolites might not commonly

vary among nearby sites. Ultimately, a mechanistic understanding of the environmental dependence of free-living pathogens could lead to a deeper understanding of the patterns of outbreak heterogeneity, which could inform surveillance and management strategies.

KEYWORDS
amphibians, *Batrachochytrium dendrobatidis*, host-pathogen dynamics, *Osteopilus septentrionalis*, pathogen decomposition, pathogen mortality, transmission

1 | INTRODUCTION

Strategies for the surveillance and management of wildlife infectious diseases are informed by a mechanistic understanding of the distribution and persistence of pathogens in host populations and the environment (Barnett & Civitello, 2020; Cunningham et al., 2017). Yet, characterizing these mechanisms remains immensely challenging because of the complexity and natural variability of host-pathogen systems and the environments in which they exist (Gandon et al., 2016; Plowright et al., 2008; Tompkins et al., 2011). For instance, infection prevalence can vary widely among neighbouring host populations (Favier et al., 2005; Munster et al., 2007; Ostfeld et al., 2005; Wood et al., 2007). Some of this variability may be a function of the net effects of environmental conditions on host exposure to pathogens and host susceptibility to infection (Civitello et al., 2013; Civitello & Rohr, 2014; Rohr et al., 2008; Rumschlag et al., 2019). Variation in environmental conditions may influence disease dynamics through effects on mortality of infectious, free-living pathogens, leading to differences in host exposure. For instance, the survival of free-living stages of many marine trematode species are sensitive to temperature, salinity and pH which leads to spatial variability in their abundance in intertidal zones (Koprivnikar et al., 2010; Lei & Poulin, 2011). In addition, accounting for temperature-dependent fitness and mortality of free-living stages of nematodes has allowed for accurate development of predictive climate change models in caribou (Molnár et al., 2013).

Simultaneously, environmental variability could lead to differences in the transmission of pathogens through effects on host susceptibility to infections. For instance, herbicides can increase susceptibility of amphibian hosts to trematode infections by compromising host immunity, leading to variability in infection loads across space (Rohr, Schotthoefer, et al., 2008; Rumschlag et al., 2019). In addition, after exposure to petroleum hydrocarbons from the Exxon Valdez oil spill, the susceptibility of fish to gill ectoparasites increased (Khan, 1990). The net effects of environmental conditions on host exposure to pathogens and host susceptibility to infection may explain variability in the prevalence of pathogens (Estrada-Peña et al., 2014; Tompkins et al., 2011). For many wildlife disease systems, the influence of local environmental conditions on exposure and per capita transmission has not been adequately evaluated, which is likely leading to challenges in the development of effective strategies for disease surveillance and management.

Understanding how environmental variability impacts host-pathogen systems can be particularly important when associated with catastrophic host declines. *Batrachochytrium dendrobatidis* (Bd), for example, is a generalist pathogen that causes the disease chytridiomycosis in amphibians and has been implicated in population declines, extirpation and extinctions of hundreds of amphibian species world-wide (Kilpatrick et al., 2010; Venesky et al., 2014). Bd is an aquatic pathogen with a motile and infectious zoospore stage, which penetrates and colonizes host tissues containing keratin (Berger et al., 1998; McMahon, Brannelly, et al., 2013; McMahon & Rohr, 2015). After colonization, zoospores develop into zoosporangia, which produce and release new zoospores. New zoospores reinfect the same host or are released into the environment (Berger et al., 2005).

Host population persistence and response to Bd are heterogeneous (Brannelly et al., 2021); some amphibian host populations are suddenly extirpated, while others persist with endemic Bd transmission (Briggs et al., 2010; Longo & Burrowes, 2010; Pilliod et al., 2010). While many studies have investigated the sources of variability in Bd infection patterns at large spatial scales (regional to global), which include temperature regime (Cohen et al., 2017; James et al., 2015; Liu et al., 2013), precipitation (Becker & Zamudio, 2011; James et al., 2015), altitude (Lambertini et al., 2021; Muths et al., 2003; Rohr & Raffel, 2010), host community diversity (Cohen et al., 2016; Venesky et al., 2014) and land use practices (Padgett-Flohr & Hopkins, 2010; Rumschlag & Boone, 2020; Rumschlag & Rohr, 2018), less is known about the mechanisms that drive local transmission dynamics. For instance, currently, the relative importance of environmental variation in driving Bd exposure and per capita transmission remains uncertain, which could have important ramifications for explaining the patterns of Bd infection prevalence across host populations.

Neighbouring populations of amphibian hosts of the same species can vary tremendously in their Bd infection prevalence (Chestnut et al., 2014; Raffel et al., 2010; Rumschlag & Boone, 2020; Strauss & Smith, 2013; Venesky, Liu, et al., 2014). This local-scale variability in the patterns of infections could be caused by variation in zoospore mortality, leading to differences in Bd exposure among sites, or by variation in host susceptibility to Bd infection. The main

environmental sources of Bd mortality are likely consumption by predators and poor water quality. Protozoa, zooplankton and filter-feeding tadpoles can consume zoospores suspended in the water column, decreasing Bd exposure, and lowering infection rates and loads in amphibian hosts (Buck et al., 2011; Hamilton et al., 2012; Schmeller et al., 2014; Searle et al., 2013; Venesky, Liu, et al., 2014). In addition, water quality parameters including pH (Kärvemo et al., 2018; Piotrowski et al., 2004), salinity (Clulow et al., 2018; Heard et al., 2014; Stockwell et al., 2015), temperature (Raffel et al., 2015; Voyles et al., 2012) and the presence of contaminants (McMahon et al., 2013; Rohr et al., 2017; Rumschlag et al., 2014; Rumschlag & Rohr, 2018) can influence the survival of zoospores and ability to infect hosts. Simultaneously, local-scale variability in infection prevalence could be a result of differences in host susceptibility. In particular, the susceptibility of species may vary as a result of differences in water quality (Greenspan et al., 2019; Jani & Briggs, 2018) or intraspecific population-level differences in susceptibility (Bradley et al., 2015).

At the same time, environmental variability in the decomposition of free-living pathogens may also contribute to differences in the patterns of infection. For instance, previous studies have documented that dead Bd zoospores and/or the metabolites they produced can induce acquired resistance in amphibian hosts (Ellison et al., 2014; McMahon et al., 2014; Savage et al., 2016). If environmental conditions cause zoospores to die rapidly once they are released from zoosporangia, hosts could be exposed regularly to dead Bd zoospores. However, if after death Bd decomposes quickly (either by chemical processes or by consumption), host exposure to dead Bd zoospores could be minimal. Conceivably, the decomposition of Bd could vary across environments according to water quality and communities of zoospore decomposers and scavengers. An important first step in evaluating the possibility of the importance of decomposition to infection dynamics is to characterize how decomposition of Bd zoospores could vary across different environments, which, to our knowledge, has not yet been attempted.

The primary objective of the current study is to test how environmental context influences the relative contributions of Bd mortality and per capita transmission to host infection prevalence. Our secondary objective is to evaluate how environmental context influences decomposition of Bd. To gain this mechanistic understanding of the potential for environmental variation in transmission dynamics of Bd, we conducted experiments with Cuban treefrog *Osteopilus septentrionalis* tadpole hosts, and fit transmission models, which evaluated how Bd mortality, decomposition and per capita transmission vary among water sources [artificial spring water (ASW) or water from three ponds]. We predicted that rates of Bd zoospore mortality and decomposition would be higher in pond water than in ASW. We speculated that mortality and decomposition may be lower in ASW because it lacks zoospore predators, competitors and scavengers and possibly toxic contaminants. High zoospore mortality, in turn, could lead to lower rates of transmission and infection prevalence in hosts in pond water compared to ASW.

2 | MATERIALS AND METHODS

2.1 | Animal collection and care

We collected multiple clutches of recently hatched Cuban treefrog *Osteopilus septentrionalis* tadpoles at Gosner stage 25 (Gosner, 1960) from the University of South Florida Botanical Gardens. We maintained the tadpoles in the laboratory for 9 days until the start of the second experiment (described below) at 21 ± 1°C with a 14:10-hr light:dark photoperiod in tanks containing 90% ASW (Cohen et al., 1980) and 10% rainwater. Tadpoles were fed ad libitum a mixture of fish flakes and spirulina suspended in agar, and water was changed daily. Tadpole survival was checked daily, and no mortality was observed prior to the start of the second experiment.

2.2 | Bd inoculate preparation

For both experiments, Bd (isolate SRS 810; isolated from *Lithobates catesbeianus* in South Carolina, USA) was grown on 1% tryptone agar plates at 23°C for 7–10 days. Immediately before use in each experiment, Bd plates were flooded with DI water for 30 min to suspend zoospores. Water containing zoospores were homogenized among plates. The zoospore concentration was determined using a haemocytometer and the solution was diluted to the given target concentration (described below).

2.3 | Experimental designs

To quantify the rates of Bd mortality, decomposition and per capita transmission in pond water and ASW, we conducted two experiments and used the results of the experiment to inform models estimating Bd mortality, decomposition and per capita transmission (Rumschlag et al., 2021). In the first experiment, we measured concentration of live and dead Bd zoospores in four water treatments (ASW or pond water from one of three locations: Flatwoods Park, Green Swamp Wilderness Preserve or Trout Creek Conservation Park) by destructively sampling experimental units across time (0, 6, 16, 24 or 48 hr). An experimental unit was 1 ml of zoospores at a concentration of 125,000 zoospores/ml plus 1 ml of a given water treatment, and each water treatment–time combination was replicated 10 times for a total of 200 experimental units. These three sources of pond water were selected because previous studies surveying amphibian populations for Bd infections at these three locations have not detected Bd (unpubl. data). All sites were within protected natural parks. Two closest sites (Trout Creek and Flatwoods) were 7 km apart, and the two furthest sites (Trout Creek and Green Swamp) were 48 km apart. Pond water was collected from the edge of the ponds and was not filtered because we wanted to maintain the natural zoospore predators, competitors and scavengers. ASW was used to control for the presence of any zoospore predators, competitors and scavengers or any potentially toxic contaminants.

The survival of zoospores was measured via counts of live and dead zoospores that were stained with Trypan blue (McMahon & Rohr, 2014). We added equal parts Trypan and zoospore-water treatment solution before counting live and dead zoospores on a haemocytometer. Cell counting of samples was randomly and evenly assigned to two people. Anecdotally, we observed other non-Bd microbes, likely protozoa, during these counts. Zooplankton were likely filtered out in the process of loading the haemocytometer slide. Dissolved oxygen and pH were taken from the stock sample for each of the water treatments used in the experiment: ASW (9.20 mg/L DO, 6.74 pH), Flatwoods (8.50 mg/L DO, 6.66 pH), Green Swamp (9.15 mg/L DO, 7.30 pH) and Trout Creek (9.40 mg/L DO, 6.38 pH). All water treatments were maintained 21°C during the experiment.

In a second experiment, we exposed Cuban treefrog tadpoles *Osteopilus septentrionalis* to Bd zoospores in four water treatments (ASW or pond water from one of three locations: Flatwoods Park, Green Swamp Wilderness Preserve or Trout Creek Conservation Park) at five time-lags between the introduction of Bd live zoospores (5×10^5 zoospores/ml) and the introduction of the tadpole (0, 3, 6, 12 and 24 hr) in 100 ml of the given water treatment. Each water treatment–time-lag combination was replicated 10 times with a single tadpole as the experimental unit for a total of 200 tadpoles. Tadpoles were exposed to Bd in their given water and time-lag treatments for 24 hr. Then, every tadpole was transferred to 450 ml ASW water for 2 weeks so that they could develop a detectable infection if present. The movement of all tadpoles to ASW allowed us to control for the effect of different water sources on the development of infection and disease. So, this experimental design specifically tested how per capita transmission and not disease or infection development varied across water sources. There was no tadpole mortality. Dissolved oxygen and pH were taken from the stock sample for each of the water treatments used the second experiment: ASW (7.0 mg/L DO, 7.52 pH), Flatwoods (7.3 mg/L DO, 6.76 pH), Green Swamp (7.9 mg/L DO, 7.84 pH) and Trout Creek (6.5 mg/L DO, 6.98 pH). The experiment was conducted at 21 ± 1°C with a 14:10 hr light:dark photoperiod. During the experiment, tadpole water was changed once per week and tadpoles were fed ad libitum a mixture of fish flakes and spirulina suspended in agar.

At the end of 2 weeks, tadpoles were euthanized, and their mouthparts were excised and analysed for Bd using quantitative PCR (qPCR). To determine the Bd quantity on each tadpole, we followed the qPCR protocol described by Hyatt et al. (2007). Briefly, we used PrepMan Ultra (Applied Biosystems) to extract DNA. To increase extraction efficiency, the mouthpart tissue was placed in a cell disruptor (Disruptor Genie, Scientific Industries) and agitated with 0.035 ± 0.005 g of zirconia/silica beads for a total of 2.25 min. We used TaqMan Exogenous Internal Positive Control Reagents (Applied Biosystems) to screen for inhibition in all samples and because we used this internal control in each well, we ran each sample in singlicate. We diluted and reran any samples with inhibition. We used the plasmid standards (Pisces Molecular LLC), which range from 2.1 to 2.1×10^6 molecules/μl.

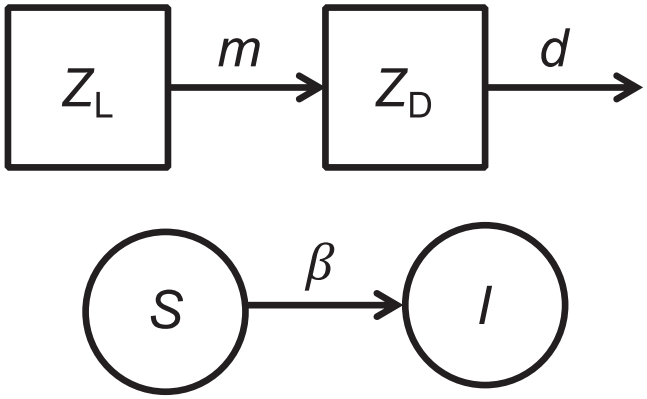

FIGURE 1 Conceptual diagram of the model which uses coupled ordinary differential equations to track the concentrations of live zoospores, Z_L, dead zoospores, Z_D, susceptible tadpoles, S, and infected tadpoles, I. The model assumes a constant background mortality of live zoospores, at per capita rate m, a constant decomposition rate of dead zoospores, at per capita rate d, and density-dependent transmission via zoospore-tadpole contact, at transmission rate β. Squares represent zoospores and circles represent tadpole hosts

2.4 | Mortality, decomposition and per capita transmission modelling and analysis

We estimated the rates of mortality, decomposition and per capita transmission of Bd zoospores by fitting a model to the results of the survival and infection experiments. The model used coupled ordinary differential equations to track the concentrations of live zoospores, Z_L, dead zoospores, Z_D, susceptible tadpoles, S, and infected tadpoles, I (Equations 1–4, Figure 1). It assumes a constant background mortality of live zoospores, at per capita rate m, a constant decomposition rate of dead zoospores, at per capita rate d and density-dependent transmission via zoospore–tadpole contact, at transmission rate β. For tractability, it assumes no host mortality and that depletion of zoospores via infection (sensu Civitello & Rohr, 2014) is negligible relative to zoospore mortality:

$$\frac{dZ_L}{dt} = -mZ_L, \tag{1}$$

$$\frac{dZ_D}{dt} = mZ_L - dZ_D, \tag{2}$$

$$\frac{dS}{dt} = -\beta S Z_L, \tag{3}$$

$$\frac{dI}{dt} = \beta S Z_L. \tag{4}$$

Integrating this model yields analytical solutions for the key quantities observed in the experiments: $Z_L(t)$, $Z_D(t)$ and $S(t)$. In our infection experiments, we added zoospores to containers at time t_0, and then added a single susceptible host at time t_1, such that $S(t_1) = 1$. We then

removed the host individual after an exposure period, at final time t_2 (in our experiments $t_2 - t_1 = 1$ day for all treatments). Therefore, we focus on these time steps in the following equations, noting that $t_2 > t_1 > t_0$. The density of live zoospores at the final time, $Z_L(t_2)$, follows an exponential decay function, depending on the mortality rate, m, the initial density of live zoospores, $Z_L(t_1)$, and the time difference, $t_2 - t_0$:

$$Z_L(t_2) = Z_L(t_0)e^{-m(t_2-t_0)}. \quad (5)$$

The model also yields an analytical solution for $Z_D(t_2)$, the density of dead zoospores at the final time, assuming no dead zoospores initially:

$$Z_D(t_2) = Z_L(t_0)\frac{m}{d-m}(e^{-d(t_2-t_0)} - e^{-m(t_2-t_0)}). \quad (6)$$

We fit these functions to paired observations of these quantities from the zoospore survival experiment. We assumed independent log-normal errors to obtain likelihoods:

$$\log(Z_i(t_2)_{\text{Observed}} + 0.1) \sim \text{Normal}(\log(Z_i(t_2)_{\text{Predicted}} + 0.1), SD_i), \quad (7)$$

where $i = L, D$ and SD_i are the standard deviations of predictive errors. We added a small quantity, 0.1, to each observation and prediction to avoid taking log(0).

Tadpole hosts are predicted to be exposed upon introduction to the concentration of zoospores remaining alive at time t_1, $Z_L(t_1)$, and the entire trial ends at the total time t_2. The model also yields an analytical solution for $S(t_2)$, which provides a well-established method for estimating the per capita transmission rate, β, via a likelihood function derived from the binomial distribution (Allen, 2010; Rachowicz & Briggs, 2007):

$$S(t_2) = S(t_1)e^{\frac{\beta Z_L(t_1)}{m}(e^{-m(t_2-t_1)}-1).} \quad (8)$$

We then calculated $Z_L(t_1)$ from $Z_L(0)$, which reflects the initial concentration of zoospores that we added at the start of these experiment, using Equation 5, substituted this result into Equation 8:

$$S(t_2) = S(t_1)e^{\frac{\beta Z_L(0)e^{-mt_1}}{m}(e^{-m(t_2-t_1)}-1)}. \quad (9)$$

In infection trials such as these, which have been simplified to short time-scales to exclude host births and deaths, the total density of tadpoles never changes, thus $S(t_1)$ and $S(t_2)$ fully specify prevalence at time t_2, $p(t_2)$:

$$p(t_2) = 1 - \frac{S(t_2)}{S(t_1)} = 1 - e^{\frac{\beta Z_L(0)e^{-mt_1}}{m}(e^{-m(t_2-t_1)}-1)}. \quad (10)$$

Although this model generates a deterministic prediction for $S(t_2)$ and therefore prevalence at time t_2, $p(t_2)$, the exposure outcomes for individual frog hosts are more appropriately viewed as stochastic outcomes. There is only a single 'transition' that is possible for an initially uninfected host: it may become infected. Thus, an equivalent discrete-individual stochastic representation of these dynamics can be represented as a Markov chain 'simple death' process, in which the probability distribution for the number of infected individuals is the binomial distribution with probability = $p(t_2)$ and size = $S(t_1)$ (Allen, 2010). This probability distribution provides likelihood for observations of infection status for all hosts. We combined both likelihood functions (Equations 7 and 10) to generate an overall likelihood for all observations (live zoospores, dead zoospores and infected hosts) across the two experiments.

With the theoretical and statistical models in place, we estimated parameters (zoospore mortality, zoospore decomposition and per capita transmission) for a series of 15 transmission models (see Table 1) using the mle2() function in the BBLME package in R (Bolker & R Development Core Team, 2020). Using model selection approaches, we tested models that assumed these three parameters were either constant across all water sources, could vary across all four sources or could vary between ASW versus the pond waters (as a group). To evaluate the relative performance of these 15 models, the AICc, ΔAICc and Akaike weights for each candidate model were calculated. In addition, Akaike weights for each parameter were summed across models to determine relative importance scores (Burnham & Anderson, 2002). An importance score of 1 would indicate that all of the model weight was accounted for by models containing the given parameter, while an importance score of 0 would indicate that all of the model weight was accounted for by models that did not contain the parameter.

The best-fit transmission model was selected from among models with ΔAICc < 2. Given that uninformative parameters within models with low AIC scores can lack enough explanatory power to justify their inclusion in the best-fit model (Arnold, 2010), we selected the simplest model from among models with ΔAICc < 2 and used a log-likelihood ratio test to evaluate if additional model complexity was warranted. In addition, we compared mean estimates of model parameters with 95% confidence intervals to evaluate if there was meaningful variation in estimates across water treatments. Based on the estimated mortality and decomposition rates from the best-fit transmission model, we calculated the expected life span and the expected persistence time of a single zoospore by dividing 24 hr by the mortality or decomposition rates respectively. We evaluated relationships among Bd zoospore mortality rates and pH and DO measurements using correlation coefficients.

3 | RESULTS

The comparison of the 15 transmission models via model selection (Table 1) indicated that the model with mortality rate, m, varying among water sources and a common estimate for decomposition, d, and per capita transmission, β, rates was the best-fit transmission model. Three models had ΔAICc scores less than 2, all of which contained varying parameter estimates for m according to the four water sources. These models that included varying estimates of d and β

TABLE 1 Results of the model selection analysis for 15 transmission models fit to the Bd zoospore mortality, decomposition and infection data. Models differed based on whether mortality (*m*), decomposition (*d*) and transmission (β) parameters were fit with a single, common estimate among the four water sources (Parameters with Single Estimates), with two estimates that corresponded to whether the water source was artificial spring water (ASW) or pond water (Parameters with Two Estimates) or with four estimates that corresponded to each water source (ASW or pond water from Flatwoods Park, Green Swamp Wilderness Preserve or Trout Creek Conservation Park). Model competition results suggest that mortality, *m*, varied among water sources, but the other two parameters do not

Parameters with single estimates	Parameters with two estimates	Parameters with four estimates	*df*	AICc	ΔAICc	Weight
β	–	*m*, *d*	12	1,377.9	0	0.40
–	–	*m*, *d*, β	15	1,379	1.1	0.24
d, β	–	*m*	9	1,379.2	1.2	0.22
d	–	*m*, β	12	1,380.2	2.2	0.13
β	*m*, *d*	–	8	1,385	7.1	0.01
d, β	*m*	–	7	1,389.1	11.2	0.002
–	*m*, *d*, β	–	9	1,389.7	11.8	0.001
m, β	*d*	–	7	1,391.8	13.9	<0.001
m	*d*, β	–	8	1,392	14.1	<0.001
d	*m*, β	–	8	1,393.9	15.9	<0.001
m, β	–	*d*	9	1,395	17.1	<0.001
m, *d*, β	–	–	6	1,396.5	18.6	<0.001
m, *d*	β	–	7	1,396.7	18.8	<0.001
m	–	*d*, β	12	1,397.2	19.3	<0.001
m, *d*	–	β	9	1,398.7	20.8	<0.001

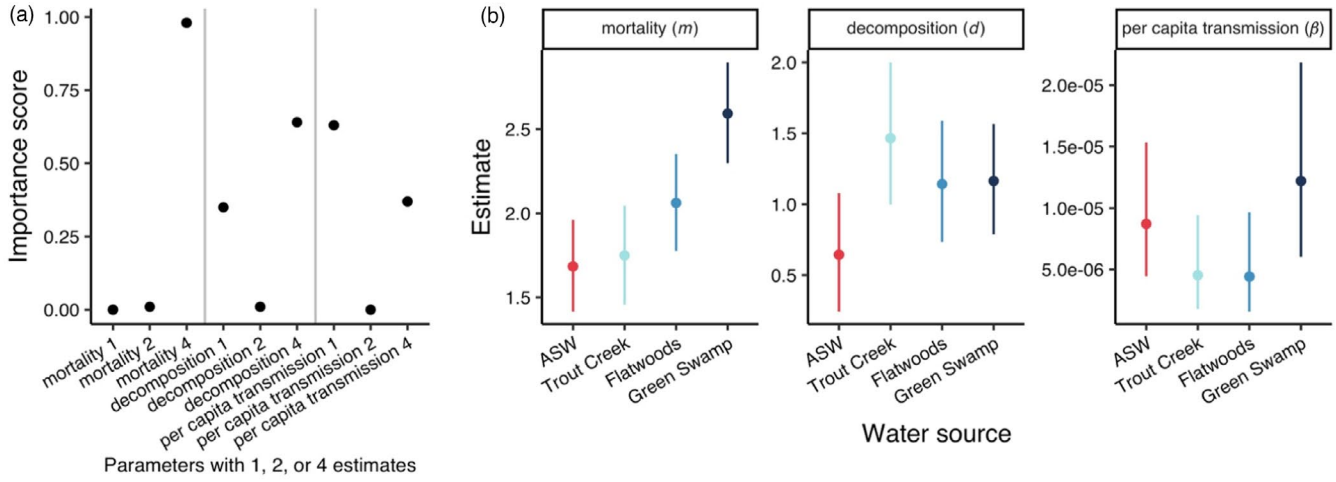

FIGURE 2 (a) Importance scores of mortality, *m*, decomposition, *d*, and per capita transmission, β, parameters fit with single estimates, two estimates [artificial spring water (ASW) or pond water] or four estimates (ASW or pond water from Flatwoods Park, Green Swamp Wilderness Preserve or Trout Creek Conservation Park). Importance scores were calculated as the sum of the Akaike weights of models including the given parameter (Table 1). Importance scores were greatest for mortality and decomposition rates fit with four estimates according to water sources, while importance score was greatest for per capita transmission fit with a single estimate. (b) Parameter estimates for mortality, *m*, decomposition, *d*, and per capita transmission,β, from a model including four separate estimates according to water source for each parameter. Error estimates are 95% confidence intervals. Mortality varied significantly among water treatments with the lowest rates of mortality in artificial spring water (ASW). Decomposition did not show as great of differences among water treatments compared to mortality, though the largest differences in decomposition were between ASW and pond water from Trout Creek. Per capita transmission did not vary among the four water sources

were not substantially different from the model that varied *m* alone (i.e. more than two AICc units different). In corroboration, mortality rate fit with four estimates received an importance score of almost 1, and per capita transmission rate fit with a single estimate received a greater importance score than per capita transmission rates fit with two or four estimates (Figure 2a). While the importance score for

decomposition rate was highest when it was fit with four estimates (Figure 2a), this result likely reflected a small difference between decomposition in ASW and pond water from Trout Creek (Figure 2b). A log-likelihood ratio test supported that the lowest AICc model with the additional complexity of m and d fit with four parameters was not a significant improvement above the model with a single parameter estimate for d and β and four parameter estimates according to water treatments for m ($\chi^2 = 7.581$, $df = 3$, $p = 0.056$). So, with all the lines of evidence from model selection results, we have strong evidence that m varied among water sources and weak evidence that d and β varied among water sources, suggesting varied estimates of d and β are uninformative and do no explain enough variation to justify inclusion or be interpreted as having an ecological effect.

Predictions from the best-fit transmission model supported lower rates of zoospore mortality in ASW compared to pond water (Figure 2b). The expected life span of a zoospore (±95% CI) was 14.4 ± 2.3 hr in ASW, 13.3 ± 2.2 hr in pond water from Trout Creek, 11.4 ± 1.6 hr in pond water from Flatwoods and 9.4 ± 1.1 hr in pond water from Green Swamp. In evaluation of correlations between mortality rates and DO and pH measurements, we did not find evidence of strong relationships. These differences in mortality rates led to differences in the concentrations of live and dead zoospores across time (Figure 3). The model predicted that the concentration of live zoospores in ASW was consistently greater across time than the concentration of live zoospores in water from any of the three ponds (Figure 3a).

While the zoospore mortality rates varied across water treatments, the best-fit transmission model suggested that decomposition rates of zoospores did not. Overall, the expected persistence time of a zoospore (±95% CI) was 21.6 ± 5.2 hr. Persistence time in the model was defined as the average time from the death of a zoospore to complete decomposition, which would include both abiotic (e.g. chemical processes) and biotic (e.g. consumption) pathways. The concentration of dead Bd zoospores over time was non-monotonic, with an initial increase as zoospores died and then a decrease after 16 hr, caused by decomposition of dead zoospores (Figure 3b). While the rate of decomposition of zoospores did not vary across water treatments, the mean concentration of dead zoospores experimentally measured was substantially greater in ASW at the 48-hr experimental time point (Figure 3b). This difference could suggest that decomposition rates increase more rapidly across time in pond water compared to ASW, but our experimental design did not allow us to test for this possibility.

The best-fit transmission model suggested that the inferred per capita rate of transmission, β, was $7.1 \times 10^{-6} \pm 2.6 \times 10^{-6}$ (95% CI), and that this rate did not vary across water treatments. Predicted infection prevalence, which depends on both per capita rate of transmission, β, and zoospore mortality rate, m, decreased across time as zoospores died (Figure 4a). Differences in zoospore mortality rate, but not per capita transmission rate, led to differences in infection prevalence across time; infection prevalence was greater in ASW compared to pond water sources across time (Figure 4a). The best-fit transmission model also predicted a positive association between host infection prevalence and concentration of live zoospores with the highest rates of infection prevalence and the greatest concentration of live zoospores occurring in ASW (Figure 4b).

4 | DISCUSSION

To gain a mechanistic understanding of the potential for local variation in transmission dynamics, we conducted survival and infection experiments and then fit models to evaluate how pathogen mortality, decomposition and per capita transmission rates varied among water sources. Results showed that infection prevalence differed among water sources, which was driven indirectly by differences in mortality

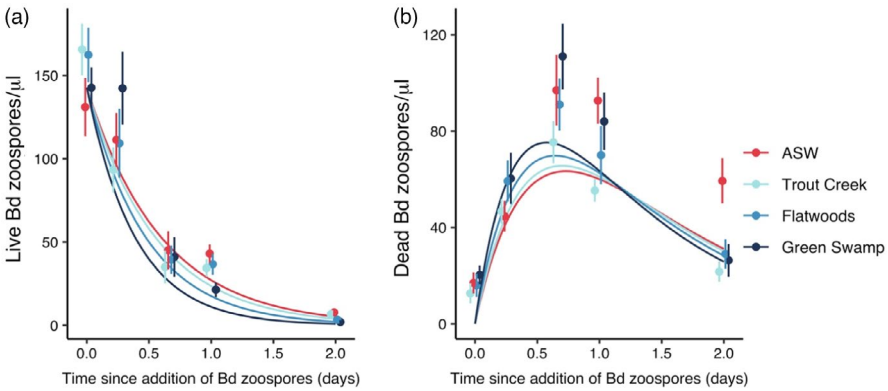

FIGURE 3 Predictions from the best-fit transmission model of concentrations of (A) live and (B) dead zoospores across time. (a) The concentration of live zoospores decreased across time, driven by differences in mortality rates across the four water treatments [artificial spring water (ASW) or pond water from Flatwoods Park, Green Swamp Wilderness Preserve or Trout Creek Conservation Park]. The highest concentrations of live zoospores were in ASW, as predicted. Measured means and standard errors of concentrations of live zoospores across time are provided. (b) The concentration of dead Bd zoospores over time was non-monotonic, with an initial increase as zoospores died, driven by differences in mortality rates across water sources, and then a decrease after 16 hr, caused by decomposition or scavenging of dead zoospores. The model predicted the concentration of dead zoospores did not differ across water treatments; although at 48 hr, the measured concentration of dead zoospores was significantly greater in ASW, suggesting lower rates of decomposition or scavenging in accordance with predictions. Measured means and standard errors of concentrations of dead zoospores across time are provided

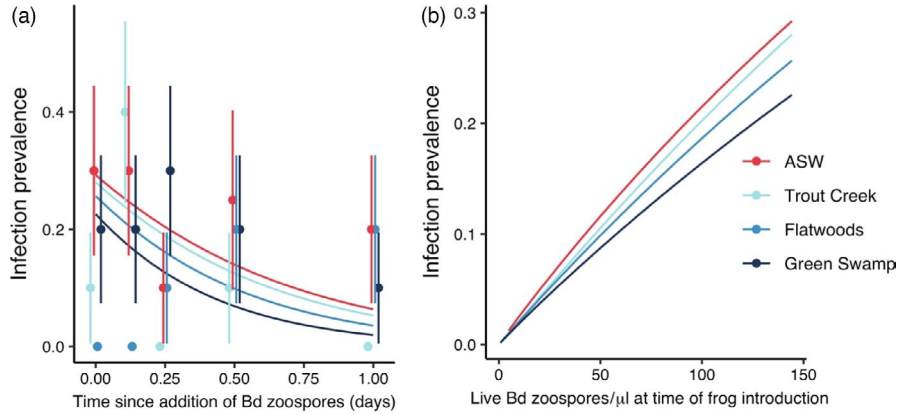

FIGURE 4 (a) Predictions from the best-fit transmission model of prevalence of Bd infections across time from the transmission model. Infection prevalence, which was informed by both zoospore mortality and transmission, decreased across time as zoospores died. Differences across water sources in zoospore mortality, but not per capita transmission, led to differences in infection prevalence with the highest infection prevalence across time in artificial spring water (ASW). Measured means and binomial standard errors of infection prevalence are provided. (b) Predictions from the best-fit transmission model of prevalence of Bd infections against concentration of live Bd zoospores. The model predicts a positive association between host infection prevalence and concentration of live zoospores with the highest rates of infection prevalence and the greatest concentration of live zoospores occurring in ASW

rates of Bd zoospores, the free-living infectious stage, and not by differences in per capita transmission rates. More specifically, zoospore mortality rates varied among pond water sources and were lower in ASW compared to pond water. These differences in mortality suggest that local variation in infection dynamics could be a function of differences in pathogen exposure. This result underscores that environmental dependence of free-living pathogens is critical to the accurate predictions of disease dynamics. In contrast to the differences in mortality rates of live zoospores among water sources, we found that the rates of decomposition of dead zoospores did not vary. These results could suggest that exposure of amphibian hosts to dead Bd and its metabolites, which have been shown to induce acquired resistance (Barnett et al., 2021; Ellison et al., 2014; McMahon et al., 2014; Savage et al., 2016), might not commonly vary among nearby sites.

Consistent with our predictions, we found that Bd mortality rates varied among water treatments with the lowest rates of mortality in ASW compared to pond water from two of the three sites (Flatwoods and Green Swamp). Differences in Bd mortality across water treatments resulted in differences in infection prevalence with the greatest infection prevalence in ASW compared to all pond water sources. This result underscores the broader conclusion that accurate characterization of the environmental dependence of free-living pathogens and parasites is crucial for the prediction of spatial and temporal infection dynamics. The environmental dependence of free-living pathogens and parasites has been well-documented in many disease systems. The environment may influence free-living pathogen and parasite survival through biotic or abiotic mechanisms. For instance, parasites are commonly consumed by predators (Fincher, 1973; Johnson et al., 2010) or endure direct mortality from environmental conditions (Civitello et al., 2012; Koprivnikar et al., 2010; Lei & Poulin, 2011; McMahon, Romansic, et al., 2013). In particular, the environmental dependence of free-living pathogens and parasites can alter infection dynamics through variation in the per-propagule probability of infection; in other words, the environment can affect the probability of an individual pathogen or parasite finding and infecting a host before its death (Cortez & Duffy, 2021; Rohr et al., 2015).

We hypothesized that differences between ASW and pond water were driven either by differences in the predators and/or competitors of live Bd zoospores or by differences in water quality. Protozoa and zooplankton can consume live zoospores in the water column, decreasing live zoospore persistence and thus infection rates and loads in amphibian hosts (Buck et al., 2011; Hamilton et al., 2012; Schmeller et al., 2014; Searle et al., 2013). Among ponds, mortality rates differed (e.g. Trout Creek versus Green Swamp, Figure 2b), which conceivably could have been driven by differences in the densities and/or compositions of predators and consumers. While trends in mortality rates across water treatments did not align with pH and dissolved oxygen measurements, given the relatively low sample size and the lack of additional water quality metrics, the direct effects of water quality on Bd mortality cannot be ruled out as an alternative or additional mechanism driving observed differences. Other studies have documented direct negative effects of poor water quality on Bd. Factors shown to influence the survival of zoospores and their ability to infect hosts include pH (Kärvemo et al., 2018; Piotrowski et al., 2004), salinity (Clulow et al., 2018; Heard et al., 2014; Stockwell et al., 2015) and the presence of contaminants (McMahon, Romansic, et al., 2013; Rohr et al., 2017; Rumschlag et al., 2014; Rumschlag & Rohr, 2018).

Across treatments, infection prevalence was positively predicted by the concentration of live Bd zoospores. We suggest that consumption of live Bd zoospores by protozoa and zooplankton or differences in water quality could have led to decreases in the rates of Bd prevalence in pond water treatments; however, future field and laboratory experiments, which examine variability among Bd population dynamics, host infections, zooplankton and

protozoa communities, and water quality through time or space should test this hypothesis explicitly and parse how much variation in exposure and infections can be attributed to these two hypothesized mechanisms.

While the model with mortality varying by water treatment simultaneously fit the combined data on zoospore mortality, zoospore decomposition and host infection better than the other models, variation in infection still exists that is left to be explained. Part of this unexplained variation reflects a statistical compromise of maximizing fit across these three types of observations. Biologically, however, it also reflects that there are likely factors not included in these mechanistic models that could also influence infection outcomes. For example, slight differences in developmental stage of hosts, activity levels of hosts or other traits could drive variation in exposure rates and/or susceptibility. In addition, stochastic variation among the two experiments and among experimental units within each experiment might lead to residual variability in host infection.

We found no evidence that differences in per capita Bd transmission rates, β, across water treatments directly influenced infection prevalence. Given that the per capita transmission rate is a composite parameter that is simultaneously a function of the contact rate of hosts and infectious agents and the probability that a contact results in a new infection ('infectivity', from a parasite-centric perspective, or 'susceptibility' from a host-centric perspective) (McCallum et al., 2017), no support for varying β across water treatments suggests that the contact rate of hosts, infectivity and susceptibility either do not vary across water treatments or they vary across water treatments in such a way that their net effect would be cancelled out, which seems unlikely. So, we suggest that there is a lack evidence that the contact rate of hosts, infectivity and susceptibility vary across water treatments.

In addition, the lower rates of mortality in ASW compared to pond water sources suggest laboratory studies of Bd dynamics are likely to consistently overestimate infection rates relative to field or mesocosm studies. More often than not, laboratory studies use a medium, like ASW, distilled water or sterilized pond water, to expose hosts to Bd, and these media lack natural Bd predators and competitors, likely leading to low Bd mortality and higher infection rates in hosts compared to field or mesocosm studies, which include Bd predators and competitors (Sauer et al., 2020). Experimental designs for studies that seek to predict Bd transmission should consider including a natural water source, given the influence of pond water sources on Bd zoospore mortality rates found in the present study.

Finally, we found that because of similarities in the decomposition rates of Bd zoospores across water sources, the concentrations of dead Bd through time did not vary among water sources. We know of no other study that has directly estimated the decomposition rates of any life stage of Bd, which could be an important consideration in understanding spatial and temporal variability in infection dynamics in natural host populations. For instance, one promising avenue that scientists are currently considering is how to aid amphibian hosts in developing acquired resistance against Bd as a management strategy to limit the spread of chytridiomycosis through repeated host exposures to dead Bd and its metabolites (Barnett et al., 2021; Ellison et al., 2014; McMahon et al., 2014; Savage et al., 2016). Our results may suggest that exposure of amphibian hosts to dead Bd and its metabolites might not commonly vary among local sites, assuming a comparable degree of similarity among water sources in nature compared to those in the current study. While we did not directly examine the rates of decomposition of Bd metabolites, we hypothesize that the rates of decomposition of Bd zoospores and metabolites are similar, although future studies should test this hypothesis explicitly.

While considerable efforts have been undertaken by researchers over the last 20 years to uncover the environmental drivers of the spread of Bd (Fisher & Garner, 2020; James et al., 2015; Kilpatrick et al., 2010), additional insights into variability in Bd zoospore mortality, decomposition and per capita transmission could aid in understanding outbreak heterogeneity among host populations. Our results show that differences in infection prevalence can be mechanistically driven by differences in Bd zoospore mortality, but we show no evidence that per capita transmission rates drive differences in infection prevalence. These results indicate that differences in mortality of Bd zoospores among neighbouring sites have the potential to drive local-scale patterns of disease dynamics, underscoring the broader take-home message concerning the importance of understanding the environmental dependence of free-living pathogens and parasites in disease dynamics. Further, we show that Bd decomposition is similar across water sources from neighbouring sites; these results suggest that exposure of hosts to dead Bd could be similar at small spatial scales. Increased attention towards the effects of local-scale variability in pathogen mortality, decomposition and per capita transmission may increase the efficacy of efforts to surveil and manage wildlife infectious diseases (Barnett & Civitello, 2020; Cunningham et al., 2017).

ACKNOWLEDGEMENTS

This research was supported by grants from the National Science Foundation (J.R.R.: DEB-2017785, DEB-1518681, IOS-1754868, T.A.M.: IOS-1754862, D.J.C: IOS-1755002), National Institutes of Health (J.R.R.: R01TW010286; T.A.M.: 1R01GM135935-01, subaward: KK2022), US Department of Agriculture (2021-38420-34065) to J.R.R., and the John Wesley Powell Center for Analysis and Synthesis, funded by the US Geological Survey, as a part of the Analyses of Contaminant Effects in Freshwater Systems Working Group to S.L.R. and J.R.R. The authors acknowledge Joyce Longcore for providing the Bd isolate.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

S.L.R., T.A.M., D.J.C. and J.R.R. designed the experiment; S.A.R. and S.L.R. conducted the experiments; T.A.M. completed the qPCR analyses; S.L.R. and D.J.C. analysed the data; S.L.R. wrote

the manuscript; T.A.M., D.J.C. and J.R.R. provided funding, and all authors contributed to editing the manuscript.

DATA AVAILABILITY STATEMENT

Data are available via figshare https://doi.org/10.6084/m9.figshare.16814701.v1 (Rumschlag et al., 2021).

ORCID

Samantha L. Rumschlag https://orcid.org/0000-0003-3125-8402
Taegan A. McMahon https://orcid.org/0000-0002-0995-7445
Jason R. Rohr https://orcid.org/0000-0001-8285-4912
David J. Civitello https://orcid.org/0000-0001-8394-6288

REFERENCES

Allen, L. J. S. (2010). *An introduction to stochastic processes with applications to biology*. Chapman and Hall/CRC.

Arnold, T. W. (2010). Uninformative parameters and model selection using Akaike's Information Criterion. *The Journal of Wildlife Management*, *74*, 1175–1178. https://doi.org/10.1111/j.1937-2817.2010.tb01236.x

Barnett, K. M., & Civitello, D. J. (2020). Ecological and evolutionary challenges for wildlife vaccination. *Trends in Parasitology*, *36*, 970–978. https://doi.org/10.1016/j.pt.2020.08.006

Barnett, K. M., Detmering, S. E., McMahon, T. A., & Civitello, D. J. (2021). Asymmetric cross-strain protection for amphibians exposed to a fungal-metabolite prophylactic treatment. *Biology Letters*, *17*, 20210207. https://doi.org/10.1098/rsbl.2021.0207

Becker, C. G., & Zamudio, K. R. (2011). Tropical amphibian populations experience higher disease risk in natural habitats. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 9893–9898. https://doi.org/10.1073/pnas.1014497108

Berger, L., Hyatt, A. D., Speare, R., & Longcore, J. E. (2005). Life cycle stages of the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms*, *68*, 51–63. https://doi.org/10.3354/dao068051

Berger, L., Speare, R., Daszak, P., Green, D. E., Cunningham, A. A., Goggin, C. L., Slocombe, R., Ragan, M. A., Hyatt, A. D., McDonald, K. R., Hines, H. B., Lips, K. R., Marantelli, G., & Parkes, H. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 9031–9036. https://doi.org/10.1073/pnas.95.15.9031

Bolker, B., & R Development Core Team. (2020). *bbmle: Tools for general maximum likelihood estimation*. R package version 1.0.23.1. Retrieved from https://CRAN.R-project.org/package=bbmle

Bradley, P. W., Gervasi, S. S., Hua, J., Cothran, R. D., Relyea, R. A., Olson, D. H., & Blaustein, A. R. (2015). Differences in sensitivity to the fungal pathogen *Batrachochytrium dendrobatidis* among amphibian populations. *Conservation Biology*, *29*, 1347–1356.

Brannelly, L. A., McCallum, H. I., Grogan, L. F., Briggs, C. J., Ribas, M. P., Hollanders, M., Sasso, T., López, M. F., Newell, D. A., & Kilpatrick, A. M. (2021). Mechanisms underlying host persistence following amphibian disease emergence determine appropriate management strategies. *Ecology Letters*, *24*, 130–148. https://doi.org/10.1111/ele.13621

Briggs, C. J., Knapp, R. A., & Vredenburg, V. T. (2010). Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 9695–9700. https://doi.org/10.1073/pnas.0912886107

Buck, J. C., Truong, L., & Blaustein, A. R. (2011). Predation by zooplankton on *Batrachochytrium dendrobatidis*: Biological control of the deadly amphibian chytrid fungus? *Biodiversity and Conservation*, *20*, 3549–3553. https://doi.org/10.1007/s10531-011-0147-4

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (2nd ed.). Springer.

Chestnut, T., Anderson, C., Popa, R., Blaustein, A. R., Voytek, M., Olson, D. H., & Kirshtein, J. (2014). Heterogeneous occupancy and density estimates of the pathogenic fungus *Batrachochytrium dendrobatidis* in waters of North America. *PLoS ONE*, *9*, e106790. https://doi.org/10.1371/journal.pone.0106790

Civitello, D. J., Forys, P., Johnson, A. P., & Hall, S. R. (2012). Chronic contamination decreases disease spread: A Daphnia–fungus–copper case study. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 3146–3153.

Civitello, D. J., Pearsall, S., Duffy, M. A., & Hall, S. R. (2013). Parasite consumption and host interference can inhibit disease spread in dense populations. *Ecology Letters*, *16*, 626–634. https://doi.org/10.1111/ele.12089

Civitello, D. J., & Rohr, J. R. (2014). Disentangling the effects of exposure and susceptibility on transmission of the zoonotic parasite *Schistosoma mansoni*. *Journal of Animal Ecology*, *83*, 1379–1386.

Clulow, S., Gould, J., James, H., Stockwell, M., Clulow, J., & Mahony, M. (2018). Elevated salinity blocks pathogen transmission and improves host survival from the global amphibian chytrid pandemic: Implications for translocations. *Journal of Applied Ecology*, *55*, 830–840. https://doi.org/10.1111/1365-2664.13030

Cohen, J. M., Civitello, D. J., Brace, A. J., Feichtinger, E. M., Ortega, C. N., Richardson, J. C., Sauer, E. L., Liu, X., & Rohr, J. R. (2016). Spatial scale modulates the strength of ecological processes driving disease distributions. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, E3359–E3364. https://doi.org/10.1073/pnas.1521657113

Cohen, J. M., Venesky, M. D., Sauer, E. L., Civitello, D. J., McMahon, T. A., Roznik, E. A., & Rohr, J. R. (2017). The thermal mismatch hypothesis explains host susceptibility to an emerging infectious disease. *Ecology Letters*, *20*, 184–193. https://doi.org/10.1111/ele.12720

Cohen, L. M., Neimark, H., & Eveland, L. K. (1980). *Schistosoma mansoni*: Response of cercariae to a thermal gradient. *The Journal of Parasitology*, *66*, 362–364. https://doi.org/10.2307/3280843

Cortez, M. H., & Duffy, M. A. (2021). The context-dependent effects of host competence, competition, and pathogen transmission mode on disease prevalence. *The American Naturalist*, *198*, 179–194. https://doi.org/10.1086/715110

Cunningham, A. A., Daszak, P., & Wood, J. L. N. (2017). One Health, emerging infectious diseases and wildlife: Two decades of progress? *Philosophical Transactions of the Royal Society B*, *372*, 20160167.

Ellison, A. R., Savage, A. E., DiRenzo, G. V., Langhammer, P., Lips, K. R., & Zamudio, K. R. (2014). Fighting a losing battle: vigorous immune response countered by pathogen suppression of host defenses in the chytridiomycosis-susceptible frog *Atelopus zeteki*. *G3*, *4*, 1275–1289.

Estrada-Peña, A., Ostfeld, R. S., Peterson, A. T., Poulin, R., & de la Fuente, J. (2014). Effects of environmental change on zoonotic disease risk: An ecological primer. *Trends in Parasitology*, *30*, 205–214. https://doi.org/10.1016/j.pt.2014.02.003

Favier, C., Schmit, D., Müller-Graf, C. D. M., Cazelles, B., Degallier, N., Mondet, B., & Dubois, M. A. (2005). Influence of spatial heterogeneity on an emerging infectious disease: The case of dengue epidemics. *Proceedings of the Royal Society B: Biological Sciences*, *272*, 1171–1177. https://doi.org/10.1098/rspb.2004.3020

Fincher, G. T. (1973). Dung beetles as biological control agents for gastrointestinal parasites of livestock. *The Journal of Parasitology*, *59*, 396–399. https://doi.org/10.2307/3278842

Fisher, M. C., & Garner, T. W. J. (2020). Chytrid fungi and global amphibian declines. *Nature Reviews Microbiology*, *18*, 332–343. https://doi.org/10.1038/s41579-020-0335-x

Gandon, S., Day, T., Metcalf, C. J. E., & Grenfell, B. T. (2016). Forecasting epidemiological and evolutionary dynamics of infectious diseases. *Trends in Ecology & Evolution*, *31*, 776–788. https://doi.org/10.1016/j.tree.2016.07.010

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, *16*, 183–190.

Greenspan, S. E., Lyra, M. L., Migliorini, G. H., Kersch-Becker, M. F., Bletz, M. C., Lisboa, C. S., Pontes, M. R., Ribeiro, L. P., Neely, W. J., Rezende, F., Romero, G. Q., Woodhams, D. C., Haddad, C. F. B., Toledo, L. F., & Becker, C. G. (2019). Arthropod–bacteria interactions influence assembly of aquatic host microbiome and pathogen defense. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1905), 20190924. https://doi.org/10.1098/rspb.2019.0924

Hamilton, P. T., Richardson, J. M. L., & Anholt, B. R. (2012). Daphnia in tadpole mesocosms: Trophic links and interactions with *Batrachochytrium dendrobatidis*. *Freshwater Biology*, *57*, 676–683. https://doi.org/10.1111/j.1365-2427.2011.02731.x

Heard, G. W., Scroggie, M. P., Clemann, N., & Ramsey, D. S. L. (2014). Wetland characteristics influence disease risk for a threatened amphibian. *Ecological Applications*, *24*, 650–662. https://doi.org/10.1890/13-0389.1

Hyatt, A. D., Boyle, D. G., Olsen, V., Boyle, D. B., Berger, L., Obendorf, D., Dalton, A., Kriger, K., Hero, M., Hines, H., Phillott, R., Campbell, R., Marantelli, G., Gleason, F., & Colling, A. (2007). Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms*, *73*, 175–192. https://doi.org/10.3354/dao073175

James, T. Y., Toledo, L. F., Rödder, D., Leite, D. D. S., Belasen, A. M., Betancourt-Román, C. M., Jenkinson, T. S., Soto-Azat, C., Lambertini, C., Longo, A. V., Ruggeri, J., Collins, J. P., Burrowes, P. A., Lips, K. R., Zamudio, K. R., & Longcore, J. E. (2015). Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: Lessons from the first 15 years of amphibian chytridiomycosis research. *Ecology and Evolution*, *5*, 4079–4097.

Jani, A. J., & Briggs, C. J. (2018). Host and aquatic environment shape the amphibian skin microbiome but effects on downstream resistance to the pathogen *Batrachochytrium dendrobatidis* are variable. *Frontiers in Microbiology*, *9*, 487. https://doi.org/10.3389/fmicb.2018.00487

Johnson, P. T. J., Dobson, A., Lafferty, K. D., Marcogliese, D. J., Memmott, J., Orlofske, S. A., Poulin, R., & Thieltges, D. W. (2010). When parasites become prey: Ecological and epidemiological significance of eating parasites. *Trends in Ecology & Evolution*, *25*, 362–371. https://doi.org/10.1016/j.tree.2010.01.005

Kärvemo, S., Meurling, S., Berger, D., Höglund, J., & Laurila, A. (2018). Effects of host species and environmental factors on the prevalence of *Batrachochytrium dendrobatidis* in northern Europe. *PLoS ONE*, *13*, e0199852. https://doi.org/10.1371/journal.pone.0199852

Khan, R. A. (1990). Parasitism in marine fish after chronic exposure to petroleum hydrocarbons in the laboratory and to the Exxon Valdez oil spill. *Bulletin of Environmental Contamination and Toxicology*, *44*, 759–763. https://doi.org/10.1007/BF01701799

Kilpatrick, A. M., Briggs, C. J., & Daszak, P. (2010). The ecology and impact of chytridiomycosis: An emerging disease of amphibians. *Trends in Ecology & Evolution*, *25*, 109–118. https://doi.org/10.1016/j.tree.2009.07.011

Koprivnikar, J., Lim, D., Fu, C., & Brack, S. H. M. (2010). Effects of temperature, salinity, and pH on the survival and activity of marine cercariae. *Parasitology Research*, *106*, 1167–1177. https://doi.org/10.1007/s00436-010-1779-0

Lambertini, C., Becker, C. G., Belasen, A. M., Valencia-Aguilar, A., Nunes-de-Almeida, C. H. L., Betancourt-Román, C. M., Rodriguez, D., da Silva Leite, D., Oliveira, I. S., Gasparini, J. L., Ruggeri, J., Mott, T., Jenkinson, T. S., James, T. Y., Zamudio, K. R., & Toledo, L. F. (2021). Biotic and abiotic determinants of *Batrachochytrium dendrobatidis* infections in amphibians of the Brazilian Atlantic Forest. *Fungal Ecology*, *49*, 100995. https://doi.org/10.1016/j.funeco.2020.100995

Lei, F., & Poulin, R. (2011). Effects of salinity on multiplication and transmission of an intertidal trematode parasite. *Marine Biology*, *158*, 995–1003. https://doi.org/10.1007/s00227-011-1625-7

Liu, X., Rohr, J. R., & Li, Y. (2013). Climate, vegetation, introduced hosts and trade shape a global wildlife pandemic. *Proceedings of the Royal Society B: Biological Sciences*, *280*, 20122506. https://doi.org/10.1098/rspb.2012.2506

Longo, A. V., & Burrowes, P. A. (2010). Persistence with chytridiomycosis does not assure survival of direct-developing frogs. *EcoHealth*, *7*, 185–195. https://doi.org/10.1007/s10393-010-0327-9

McCallum, H., Fenton, A., Hudson, P. J., Lee, B., Levick, B., Norman, R., Perkins, S. E., Viney, M., Wilson, A. J., & Lello, J. (2017). Breaking beta: Deconstructing the parasite transmission function. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*, 20160084. https://doi.org/10.1098/rstb.2016.0084

McMahon, T. A., Brannelly, L. A., Chatfield, M. W. H., Johnson, P. T. J., Joseph, M. B., McKenzie, V. J., Richards-Zawacki, C. L., Venesky, M. D., & Rohr, J. R. (2013). Chytrid fungus *Batrachochytrium dendrobatidis* has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 210–215. https://doi.org/10.1073/pnas.1200592110

McMahon, T. A., & Rohr, J. R. (2014). Trypan blue dye is an effective and inexpensive way to determine the viability of *Batrachochytrium dendrobatidis* zoospores. *EcoHealth*, *11*, 164–167. https://doi.org/10.1007/s10393-014-0908-0

McMahon, T. A., & Rohr, J. R. (2015). Transition of chytrid fungus infection from mouthparts to hind limbs during amphibian metamorphosis. *EcoHealth*, *12*, 188–193. https://doi.org/10.1007/s10393-014-0989-9

McMahon, T. A., Romansic, J. M., & Rohr, J. R. (2013). Nonmonotonic and monotonic effects of pesticides on the pathogenic fungus *Batrachochytrium dendrobatidis* in culture and on tadpoles. *Environmental Science & Technology*, *47*, 7958–7964.

McMahon, T. A., Sears, B. F., Venesky, M. D., Bessler, S. M., Brown, J. M., Deutsch, K., Halstead, N. T., Lentz, G., Tenouri, N., Young, S., Civitello, D. J., Ortega, N., Fites, J. S., Reinert, L. K., Rollins-Smith, L. A., Raffel, T. R., & Rohr, J. R. (2014). Amphibians acquire resistance to live and dead fungus overcoming fungal immunosuppression. *Nature*, *511*, 224–227. https://doi.org/10.1038/nature13491

Molnár, P. K., Kutz, S. J., Hoar, B. M., & Dobson, A. P. (2013). Metabolic approaches to understanding climate change impacts on seasonal host-macroparasite dynamics. *Ecology Letters*, *16*, 9–21. https://doi.org/10.1111/ele.12022

Munster, V. J., Baas, C., Lexmond, P., Waldenström, J., Wallensten, A., Fransson, T., Rimmelzwaan, G. F., Beyer, W. E. P., Schutten, M., Olsen, B., Osterhaus, A. D. M. E., & Fouchier, R. A. M. (2007). Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. *PLoS Pathogens*, *3*, e61.

Muths, E., Stephen Corn, P., Pessier, A. P., & Earl Green, D. (2003). Evidence for disease-related amphibian decline in Colorado. *Biological Conservation*, *110*, 357–365. https://doi.org/10.1016/S0006-3207(02)00239-2

Ostfeld, R. S., Glass, G. E., & Keesing, F. (2005). Spatial epidemiology: An emerging (or re-emerging) discipline. *Trends in Ecology & Evolution*, *20*, 328–336. https://doi.org/10.1016/j.tree.2005.03.009

Padgett-Flohr, G. E., & Hopkins, R. L. (2010). Landscape epidemiology of *Batrachochytrium dendrobatidis* in central California. *Ecography*, *33*, 688–697. https://doi.org/10.1111/j.1600-0587.2009.05994.x

Pilliod, D. S., Muths, E., Scherer, R. D., Bartelt, P. E., Corn, P. S., Hossack, B. R., Lambert, B. A., Mccaffery, R., & Gaughan, C. (2010). Effects of amphibian chytrid fungus on individual survival probability in wild boreal toads: Toad survival of chytridiomycosis. *Conservation Biology*, *24*, 1259–1267. https://doi.org/10.1111/j.1523-1739.2010.01506.x

Piotrowski, J. S., Annis, S. L., & Longcore, J. E. (2004). Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia*, *96*, 9–15. https://doi.org/10.2307/3761981

Plowright, R. K., Sokolow, S. H., Gorman, M. E., Daszak, P., & Foley, J. E. (2008). Causal inference in disease ecology: Investigating ecological drivers of disease emergence. *Frontiers in Ecology and the Environment*, *6*, 420–429. https://doi.org/10.1890/070086

Rachowicz, L. J., & Briggs, C. J. (2007). Quantifying the disease transmission function: Effects of density on *Batrachochytrium dendrobatidis* transmission in the mountain yellow-legged frog *Rana muscosa*. *Journal of Animal Ecology*, *76*, 711–721. https://doi.org/10.1111/j.1365-2656.2007.01256.x

Raffel, T. R., Halstead, N. T., McMahon, T. A., Davis, A. K., & Rohr, J. R. (2015). Temperature variability and moisture synergistically interact to exacerbate an epizootic disease. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1801), 20142039. https://doi.org/10.1098/rspb.2014.2039

Raffel, T. R., Michel, P. J., Sites, E. W., & Rohr, J. R. (2010). What drives chytrid infections in newt populations? Associations with substrate, temperature, and shade. *EcoHealth*, *7*, 526–536. https://doi.org/10.1007/s10393-010-0358-2

Rohr, J. R., Brown, J., Battaglin, W. A., McMahon, T. A., & Relyea, R. A. (2017). A pesticide paradox: Fungicides indirectly increase fungal infections. *Ecological Applications*, *27*, 2290–2302. https://doi.org/10.1002/eap.1607

Rohr, J. R., Civitello, D. J., Crumrine, P. W., Halstead, N. T., Miller, A. D., Schotthoefer, A. M., Stenoien, C., Johnson, L. B., & Beasley, V. R. (2015). Predator diversity, intraguild predation, and indirect effects drive parasite transmission. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 3008–3013. https://doi.org/10.1073/pnas.1415971112

Rohr, J. R., & Raffel, T. R. (2010). Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 8269–8274. https://doi.org/10.1073/pnas.0912883107

Rohr, J. R., Raffel, T. R., Sessions, S. K., & Hudson, P. J. (2008). Understanding the net effects of pesticides on amphibian trematode infections. *Ecological Applications*, *18*, 1743–1753. https://doi.org/10.1890/07-1429.1

Rohr, J. R., Schotthoefer, A. M., Raffel, T. R., Carrick, H. J., Halstead, N., Hoverman, J. T., Johnson, C. M., Johnson, L. B., Lieske, C., Piwoni, M. D., Schoff, P. K., & Beasley, V. R. (2008). Agrochemicals increase trematode infections in a declining amphibian species. *Nature*, *455*, 1235–1239. https://doi.org/10.1038/nature07281

Rumschlag, S. L., & Boone, M. D. (2020). Amphibian infection risk changes with host life stage and across a landscape gradient. *Journal of Herpetology*, *54*, 347–354. https://doi.org/10.1670/19-107

Rumschlag, S. L., Boone, M. D., & Fellers, G. (2014). The effects of the amphibian chytrid fungus, insecticide exposure, and temperature on larval anuran development and survival. *Environmental Toxicology and Chemistry*, *33*, 2545–2550. https://doi.org/10.1002/etc.2707

Rumschlag, S. L., Halstead, N. T., Hoverman, J. T., Raffel, T. R., Carrick, H. J., Hudson, P. J., & Rohr, J. R. (2019). Effects of pesticides on exposure and susceptibility to parasites can be generalised to pesticide class and type in aquatic communities. *Ecology Letters*, *22*, 962–972. https://doi.org/10.1111/ele.13253

Rumschlag, S. L., & Rohr, J. R. (2018). The influence of pesticide use on amphibian chytrid fungal infections varies with host life stage across broad spatial scales. *Global Ecology and Biogeography*, *27*, 1277–1287. https://doi.org/10.1111/geb.12784

Rumschlag, S. L., Roth, S. A., McMahon, T. A., Rohr, J. R., & Civitello, D. J. (2021). Variability in environmental persistence but not per capita transmission rates of the amphibian chytrid fungus leads to differences in host infection prevalence. *Figshare*. https://doi.org/10.6084/m9.figshare.16814701.v1

Sauer, E. L., Cohen, J. M., Lajeunesse, M. J., McMahon, T. A., Civitello, D. J., Knutie, S. A., Nguyen, K., Roznik, E. A., Sears, B. F., Bessler, S., Delius, B. K., Halstead, N., Ortega, N., Venesky, M. D., Young, S., & Rohr, J. R. (2020). A meta-analysis reveals temperature, dose, life stage, and taxonomy influence host susceptibility to a fungal parasite. *Ecology*, *101*, e02979.

Savage, A. E., Terrell, K. A., Gratwicke, B., Mattheus, N. M., Augustine, L., & Fleischer, R. C. (2016). Reduced immune function predicts disease susceptibility in frogs infected with a deadly fungal pathogen. *Conservation Physiology*, *4*, cow011. https://doi.org/10.1093/conphys/cow011

Schmeller, D. S., Blooi, M., Martel, A., Garner, T. W. J., Fisher, M. C., Azemar, F., Clare, F. C., Leclerc, C., Jäger, L., Guevara-Nieto, M., Loyau, A., & Pasmans, F. (2014). Microscopic aquatic predators strongly affect infection dynamics of a globally emerged pathogen. *Current Biology*, *24*, 176–180. https://doi.org/10.1016/j.cub.2013.11.032

Searle, C. L., Mendelson, J. R., Green, L. E., & Duffy, M. A. (2013). Daphnia predation on the amphibian chytrid fungus and its impacts on disease risk in tadpoles. *Ecology and Evolution*, *3*, 4129–4138.

Stockwell, M. P., Clulow, J., & Mahony, M. J. (2015). Evidence of a salt refuge: Chytrid infection loads are suppressed in hosts exposed to salt. *Oecologia*, *177*, 901–910. https://doi.org/10.1007/s00442-014-3157-6

Strauss, A., & Smith, K. G. (2013). Why does amphibian chytrid (*Batrachochytrium dendrobatidis*) not occur everywhere? An exploratory study in Missouri ponds. *PLoS ONE*, *8*, e76035. https://doi.org/10.1371/journal.pone.0076035

Tompkins, D. M., Dunn, A. M., Smith, M. J., & Telfer, S. (2011). Wildlife diseases: From individuals to ecosystems. *Journal of Animal Ecology*, *80*, 19–38. https://doi.org/10.1111/j.1365-2656.2010.01742.x

Venesky, M. D., Liu, X., Sauer, E. L., & Rohr, J. R. (2014). Linking manipulative experiments to field data to test the dilution effect. *Journal of Animal Ecology*, *83*, 557–565. https://doi.org/10.1111/1365-2656.12159

Venesky, M. D., Raffel, T. R., McMahon, T. A., & Rohr, J. R. (2014). Confronting inconsistencies in the amphibian-chytridiomycosis system: Implications for disease management. *Biological Reviews*, *89*, 477–483. https://doi.org/10.1111/brv.12064

Voyles, J., Johnson, L. R., Briggs, C. J., Cashins, S. D., Alford, R. A., Berger, L., Skerratt, L. F., Speare, R., & Rosenblum, E. B. (2012). Temperature alters reproductive life history patterns in *Batrachochytrium dendrobatidis*, a lethal pathogen associated with the global loss of amphibians. *Ecology and Evolution*, *2*, 2241–2249. https://doi.org/10.1002/ece3.334

Wood, M. J., Cosgrove, C. L., Wilkin, T. A., Knowles, S. C. L., Day, K. P., & Sheldon, B. C. (2007). Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistes caeruleus*. *Molecular Ecology*, *16*, 3263–3273.

How to cite this article: Rumschlag, S. L., Roth, S. A., McMahon, T. A., Rohr, J. R., & Civitello, D. J. (2022). Variability in environmental persistence but not per capita transmission rates of the amphibian chytrid fungus leads to differences in host infection prevalence. *Journal of Animal Ecology*, 91, 170–181. https://doi.org/10.1111/1365-2656.13612