Notes

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Heterogeneities in the infection process drive ranavirus transmission

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Abstract. Transmission is central to our understanding and efforts to control the spread of infectious diseases. Because transmission generally requires close contact, host movements and behaviors can shape transmission dynamics: random and complete mixing leads to the classic density-dependent model, but if hosts primarily interact locally (e.g., aggregate) or within groups, transmission may saturate. Manipulating host behavior may thus change both the rate *and* functional form of transmission. We used the ranavirus–wood frog (*Lithobates sylvaticus*) tadpole system to test whether transmission rates reflect contacts, and whether the functional form of transmission can be influenced by the distribution of food in mesocosms (widely dispersed, promoting random movement and mixing vs. a central pile, promoting aggregations). Contact rates increased with density, as expected, but transmission rapidly saturated. Observed rates of transmission were not explained by observed contact rates or the density-dependent model, but instead transmission in both treatments followed models allowing for heterogeneities in the transmission process. We argue that contacts were not generally limiting, but instead that our results are better explained by heterogeneities in host susceptibility. Moreover, manipulating host behavior to manage the spread of infectious disease may prove difficult to implement.

Key words: amphibian; contact rates; density-independent; disease transmission; foraging behavior; mesocosm; susceptibility.

Introduction

Transmission is fundamental to the ecology of infectious disease. The magnitude and functional form of transmission determines whether a pathogen can invade, persist in, regulate, or cause the extinction of its host population, as well as the efficacy of management strategies (McCallum et al. 2001, de Castro and Bolker 2005). It is also one of the most difficult aspects of host–parasite interactions to study empirically. As a consequence, models of directly transmitted infections generally make one of two assumptions about transmission. The first is that hosts contact one another like molecules interacting in a chemical reaction, randomly and completely, so rates of contact and transmission increase with host density (McCallum et al. 2001, Hutchinson and Waser 2007). Moreover, below some threshold host density, contact rates are too low for sustained transmission and the infection fades from the population. These thresholds lay at the heart of many interventions to control or eradicate disease, although they may not be as distinct as we would like (Lloyd-Smith et al. 2005). Alternatively, if contact rates are constant then the rate of transmission scales with the proportion of those contacts that are with infected hosts. In this frequency-dependent model there is no threshold density for invasion or persistence and so transmission can (at least theoretically) continue even as the host declines to extinction (McCallum et al. 2001, de Castro and Bolker 2005).

Of course animals are not molecules and transmission in natural systems may not conform to these simple models (McCallum et al. 2001). By governing both the types and rates of intraspecific interactions, host behaviors such as mating, foraging, fighting, or flocking may be more important drivers of disease dynamics than density per se (Smith et al. 2009, VanderWaal and Ezenwa 2016). Indeed, empirical studies of disease transmission often find that neither the density- or frequency-dependent model adequately describes how observed transmission rates scale with densities of infected hosts (Knell et al. 1996, 1998, Roberts 1996, Barlow 2000, D'Amico et al. 2005, Greer et al. 2008, Smith et al. 2009) and so several alternate transmission terms have been proposed (reviewed in McCallum et al. 2001). Most of these terms lead to asymptotic rates of transmission, usually built on the assumption that hosts mix locally, so contacts and disease transmission occur more quickly at a small scale than they do in the population as a whole. While the logic that nonlinearities in transmission reflect underlying heterogeneities in contact

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rates has an intuitive appeal, indeed patterns of social interactions or contacts are often used as surrogates for disease transmission (VanderWaal and Ezenwa 2016), there are few empirical tests of this assertion, and alternative explanations exist. Dwyer et al. (1997), for instance, demonstrated that heterogeneities in susceptibility can lead to saturating rates of transmission. The idea is that very few infectious contacts are sufficient to infect the most susceptible hosts, leading to rapid increases in transmission at low densities of infected hosts, but the most resistant hosts remain uninfected even with a great deal of exposure.

While heterogeneities in contacts and susceptibility both lead to saturating rates of transmission, and indeed they may be correlated (Hawley et al. 2011), their consequences for epidemiology and management options diverge. First, if saturating transmission reflects saturating rates of contacts, some individuals may escape infectious contacts in the short term, but given enough time every individual will eventually be contacted and infected. Alternatively, if there is great enough heterogeneity in susceptibility such that some individuals are very resistant to infection, then a fraction of the population can remain uninfected even over longer times or with a higher force of infection (Gomes et al. 2014). Second, if transmission is driven by contact rates then natural and anthropogenic factors affecting host behaviors can drive disease dynamics. For instance, the supplemental feeding grounds used to prevent elk from contacting cattle and transmitting *Brucella abortis* have had the unintended consequence of increasing contact and infections in the elk herds that feed on them (Cross et al. 2010, 2013). Alternatively, if heterogeneities in susceptibility shape the transmission process, then management strategies focused on physiological stressors, nutrition, or other factors that influence the distribution of host susceptibility may be critical (Madliger et al. 2016).

Here we describe an experiment designed to estimate the rate and functional form of the transmission of a *Ranavirus*, a genus of directly transmitted, often lethal viruses of ectothermic vertebrates, in populations of wood frog tadpoles (*Lithobates sylvaticus*) in small, homogeneous mesocosms. We also manipulated the distribution of food in order to alter the pattern of contacts within the mesocosms (haphazardly scattered food to reinforce random searching and contacts vs. a central pile of food to encourage aggregation) and, we expected, the way in which transmission scaled with the density of infected hosts.

Methods

Wood frog egg masses ($n = 12$) were reared to feeding stages (stage 25; Gosner 1960) in aged tap water, then fed algae discs (Hikari USA; Hayward, California, USA) ad libitum for roughly 10 d. They were then housed in 1,135-L black, cattle tanks (Newell Rubbermaid, Atlanta, Georgia, USA) filled with 10 cm (~350 L) of well water. Water was continuously pumped through ultraviolet sterilizers (Turbo Twister 2X; Coralife, Franklin, Wisconsin, USA) with 300 L/h. Eheim pumps (EHEIM GmbH, Deizisau, Germany) to clarify the water and inactivate pathogens. The tadpoles were haphazardly assigned to one of 18 pairs of mesocosms at one of 16 densities (11–320 tadpoles/tank; Appendix S1: Table S1) and acclimated for 4 d in the tanks and fed 0.03 g algae d^{-1} $tadpole^{-1}$. Each tank within a pair was assigned to one of two food treatments. In the central food treatment, all of the food was placed in a single pile in the center of the tank. In the dispersed food treatment, the same amount of food was ground into a powder and haphazardly scattered around the tank. The expectation was that tadpoles would be forced to aggregate while feeding on the central pile, but would forage more widely and contact each other less frequently and more randomly in the dispersed food treatment.

Ranavirus epidemics were started by introducing infected conspecifics ($n = 2$–48; Appendix S1: Table S1), which had been exposed via water bath to a high titer—10^5 plaque-forming units/mL of a Frog Virus 3-like ranavirus, AEC37 (Brunner et al. 2011), expected to infect and kill ~99% of the tadpoles (Warne et al. 2011)—5 d prior to ensure that they were infectious (Brunner et al. 2007). An equal number of susceptible tadpoles were removed to maintain a constant density. The infected tadpoles had been marked with a fluorescent visible elastomer (Northwest Marine Technology, Shaw Island, Washington, USA) injected along their dorsal tail fin under anesthesia (0.025 g/L MS-222) so initially infected and susceptible tadpoles could be distinguished. After 24 h, the susceptible tadpoles were removed with dip nets and housed in individual plastic containers with ~500 mL of well water for 5 d, at which point they were euthanized and frozen at −80°C.

Contact rates were estimated for each of four randomly selected infected tadpoles in 5 min of observations (or 10 min of observation when there were just two infecteds in a tank), and averaged across individuals to get an average contact rate for each tank. Contacts were defined as physical touch between the bodies or body and tail, but not tail-to-tail contacts, which tended to be fleeting and thus hard to detect, and not necessarily with unique individuals. We used the marked infected tadpoles as focal animals because they were easier to track than the unmarked susceptible tadpoles and ranavirus infection does not alter tadpoles' behavior soon after exposure (Reeve et al. 2013).

Infection status of the initially susceptible tadpoles was determined with a quantitative real time polymerase chain reaction assay specific to the major capsid gene of ranaviruses (see Appendix S1 for details). Samples were run in duplicate and scored as positive if both wells showed clear signs of amplification. Samples lacking signs of amplification were re-run, scored as positive if there was clear amplification after multiple reactions, and scored as negative without repeatable amplification.

The number of tadpoles was fixed in these experimental epidemics (i.e., no birth or death occurred) and tadpoles

did not recover, so susceptible animals (S) were only lost to infection at rate $dS/dt = \phi S$, where ϕ is the force of infection (Rachowicz and Briggs 2007). The probability that a susceptible tadpole remained uninfected after the 24-h exposure is thus $\exp(-\phi\tau)$, where $\tau = 1$ d is the duration of the epidemic (Rachowicz and Briggs 2007), which was short enough that secondary transmission and the loss of infecteds could be discounted (Brunner et al. 2007).

We considered several transmission terms described in McCallum et al. (2001) including density- and frequency-dependent transmission, the power relationship, $\beta S^p I^q$, where $p < 1$ and $q < 1$ indicate transmission saturates with the density of susceptible and infected hosts, respectively, and the negative binomial term, $kS \ln(1 + \beta I/k)$, where small k corresponds to a highly aggregated transmission process. We also fit the generalized model of Smith et al. (2009), $\beta S K^q I/N^q$, where K^q is a rescaling constant and q controls how contact rates change with host density: $q = 0$ leads to density-dependent transmission and $q = 1$ is frequency-dependent transmission. When $0 < q < 1$ contact rates saturate with increasing density. To avoid estimating extra parameters, we fit this term with just two parameters: the product $\beta' = \beta K^q$ and q.

Last, reasoning that transmission is a function of the contact rate, which we estimated directly, and the fraction of those contacts that were with infected hosts (i.e., the initial ratio of I to N), we fit a model in which the force of infection is $\phi_{\text{contact}} = \beta \times c_i(I/N)$, where c_i is the number of contacts a tadpole experiences in 24 h in mesocosm i and β now represents the proportion of contacts with infected hosts that result in transmission. In this way, we could determine whether contact rates alone were sufficient to explain observed transmission. In order to avoid extrapolations from extreme values (e.g., no contacts were observed in some mesocosms during the 5 min of observations, but some contacts certainly occurred in 24 h), values of c_i were derived from contact rates predicted from a linear regression of the average contact rate against the density in the mesocosm.

We fit these transmission terms to the number of tadpoles remaining uninfected (out of the total number tested in each mesocosm) in each treatment assuming binomially distributed outcomes, as in Rachowicz and Briggs (2007) and Greer et al. (2008). Models were fit by minimizing the negative log-likelihood using the mle2 function in the bbmle package (version 1.0.17; Bolker and R Development Core Team 2014) in R (version 3.2.1; R Core Team 2015) and compared with Akaike's Information Criterion adjusted for sample size (AIC_c) and AIC_c weights (w_i; Burnham and Anderson 2002). Note that, while there was support for letting the parameter β vary between experimental blocks (see Appendix S1; the second block had higher rates of transmission), the results did not change qualitatively and so for simplicity we do not include this effect in the results we report here.

Results

Of the 447 initially susceptible tadpoles that were tested for ranavirus infection, 187 (41.8%) were infected, but infection prevalence varied considerably among mesocosms and treatments (average = 35.8% in the central food treatment and 47.2% in the dispersed food treatment). The power relationship fit best to the data from both treatments, although several other models were reasonably well supported. In particular, the power relationships, negative binomial, and general model, all of which led to transmission saturating with density, collectively received 91.2% of the evidentiary weight in the central food treatment and 74.9% in the dispersed food treatment (Fig. 1, Table 1).

The exponents in the best-fit power relationship models ($p_{\text{Central}} = 0.72$, 95% CI 0.457–0.976 and $q_{\text{Central}} = 0.447$, 0.161–0.734, and $p_{\text{Dispersed}} = 0.513$, 0.266–0.753 and $q_{\text{Dispersed}} = 0.547$, 0.307–0.793) indicate that transmission rates increase less than linearly with host densities (Fig. 1c). Estimates of similar parameters in the negative binomial (overdispersion parameter, $k_{\text{Central}} = 0.141$, 0.035–0.366, and $k_{\text{Dispersed}} = 0.156$, 0.051–0.333; Fig. 1a), and the general model (scaling parameter $q_{\text{Central}} = 0.698$, 0.445–0.966, and $q_{\text{Dispersed}} = 0.887$, 0.695–1.086; Fig. 1d) strongly suggest transmission saturates, often quite quickly, with host densities. Because transmission saturated so quickly with density in the dispersed food treatment, transmission rates overall were largely independent of host densities and so the frequency-dependent model received substantial support in this treatment ($w_i = 0.251$). Still, there was little evidence that the functional form of the transmission term varied between the treatments. In addition to the power relationship being the best-supported model when fit to both treatments separately, it had 98.9% of the evidentiary weight when the models were fit to both data sets together and allowing β to vary between treatments; there was no support for letting the parameters p and q vary by treatment (not shown). The parameter estimates and the fit of this model, as measured by the negative log-likelihood (LL), were essentially equivalent when fit to each treatment individually ($-\text{LL} = 49 + 47.3 = 96.3$) and together ($-\text{LL} = 97$), suggesting a consistently saturating relationship with only the rate, β, varying by treatment.

The density-dependent model was a very poor fit to the data in both treatments ($\Delta AIC_c = 31.7$ and 84.2 in the central and dispersed food treatments, respectively). The only model that performed worse was the model in which transmission scaled with the contact rates estimated from our observations (Table 1). Contact rates between the focal infected tadpoles and susceptible tadpoles in their mecosoms varied from a low of 0 to a high of 7.6 contacts min^{-1} tadpole^{-1} and generally increased with density (Fig. 2; $t = 4.168$, $P < 0.001$). While contact rates were higher immediately after adding food (by about 1.3 contacts min^{-1} tadpole^{-1}), they was no clear effect of the treatment on contact rates ($t = -0.133$, $P = 0.895$),

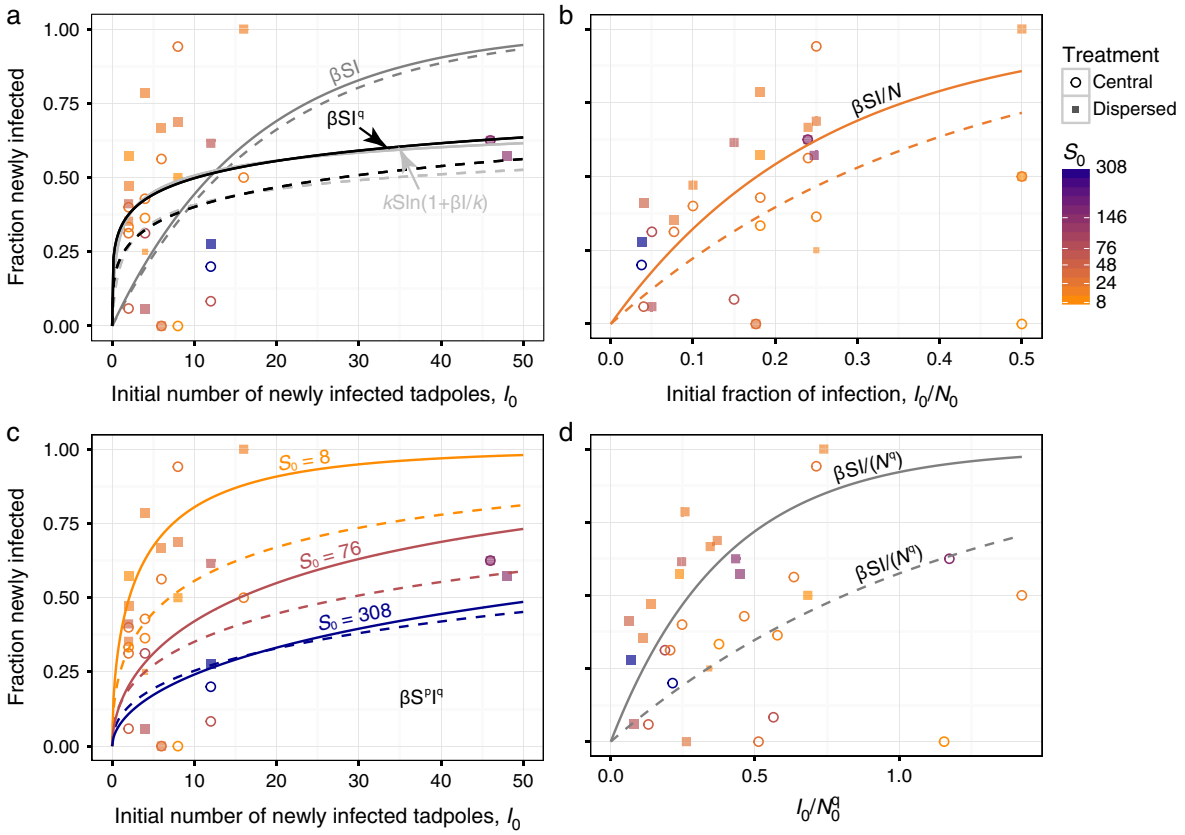

FIG. 1. The fraction of initially susceptible tadpoles that became infected with ranavirus in a 24-h period as a function of (a) and (c) the initial density of infected tadpoles (I_0), (b) the initial frequency of infection (I_0/N_0, where N_0 is the initial density of all tadpoles), and (d) a modified frequency from the general transmission model of Smith et al. (2009). The color represents the number of initially susceptible tadpoles (S_0) in in each replicate mesocosm. The lines illustrate the probability of infection predicted by (a) density-dependent (βSI), negative binomial ($kS \ln[1+\beta I/k]$), and power of I (βSI^q), (b) frequency-dependent ($\beta SI/N$), (c) power of I and S ($\beta S^p I^q$), and (d) the general transmission ($\beta SI/[N^q]$) models fit to the data from the central food (dashed line) and dispersed food (solid line) treatments. [Color figure can be viewed at wileyonlinelibrary.com]

nor was there an effect of experimental block ($t = -1.314$, $P = 0.196$).

DISCUSSION

In disease ecology, determining the form of the transmission function is critical to predicting the dynamics and outcome of infectious disease (McCallum et al. 2001, de Castro and Bolker 2005). The classic, density-dependent transmission term remains the most common in models of wildlife disease, both because of its analytic tractability and the intuitive notion that contact rates, and thus transmission, tend to increase with host density (McCallum et al. 2001). Our results, however, add to a growing list of empirical studies suggesting that this density-dependent model is far from adequate (Knell et al. 1996, 1998, Roberts 1996, Dwyer et al. 1997, Begon et al. 1998, Barlow 2000, D'Amico et al. 2005, Greer et al. 2008, Smith et al. 2009).

This is rather surprising, because our experimental conditions in many ways mirrored the conditions assumed by the density-dependent model. Our mesocosms were relatively small (2.56 m^2), homogeneous environments, so that the tadpoles could mix thoroughly and homogeneously. The tadpoles were kept under high-food conditions, which we expected to minimize social structuring (Peacor and Pfister 2006), although there was variability in developmental stages, which can influence tolerance to ranavirus infections (Warne et al. 2011), if not the likelihood of infections (J. L. Brunner, *unpublished data*). And the densities we used ($N \leq 320$ tadpoles/mesocosm) were never so high that we would expect contact rates to saturate. However, while contact rates did increase with density (Fig. 2), the density-dependent transmission term and the ad hoc term using empirical estimates of contact rates both failed to describe the actual amount of transmission we observed (Fig. 1a; Table 1). Rather, as with many previous studies, the large majority of evidentiary weight fell behind the models that allowed transmission to saturate with density.

As noted above, saturating contact rates and heterogeneities in susceptibility both lead to a pattern of saturating

TABLE 1. Evidentiary support (ΔAIC_c and AIC_c weights, w_i) for each transmission term fit to the data from the central food or dispersed food treatments.

Model	Function	Central food				Dispersed food			
		ΔAIC_c	w_i	β	Additional parameters	ΔAIC_c	w_i	β	Additional parameters
Power of I and S	$\beta S^p I^q$	0.0	0.421	0.519	$p = 0.72$; $q = 0.447$	0.0	0.622	1.277	$p = 0.513$; $q = 0.547$
Power of I	βSI^q	1.4	0.205	0.258	$q = 0.297$	13.2	<0.001	0.399	$q = 0.237$
Negative binomial	$kS\ln\left(\frac{1+-\beta I}{k}\right)$	1.7	0.179	0.568	$k = 0.141$	13.5	<0.001	1.415	$k = 0.156$
General	$\beta' SI/N^q$	2.7	0.107	0.855	$q = 0.698$	3.2	0.126	2.540	$q = 0.887$
Frequency dependent	$\beta SI/N$	4.9	0.037	2.516	–	1.8	0.251	3.896	–
Density dependent	βSI	31.7	<0.001	0.054	–	84.2	<0.001	0.059	–
Constant	β	4.2	0.051	0.444	–	17.3	<0.001	0.639	–
Observed contacts	$\beta \times c_i(I/N)$	276.4	<0.001	0.002	–	308.3	<0.001	0.004	–

Notes: The maximum likelihood estimates of β and the other parameters are also provided. Note that the units of β and other parameters depend on the transmission function.

rates of transmission, so our transmission data alone cannot distinguish between the mechanism at work; indeed both may play a role. However, there are several lines of evidence suggesting that the more important heterogeneity is in susceptibility. First, in such small, homogeneous mesocosms, every tadpole would be "local" and easily contacted, and we did not observe any sign of contact rates leveling off (Fig. 2). Second, observed contact rates were quite high, even at low densities (~53.4 contacts tadpole^{-1} d^{-1}), and thus likely not (generally) the rate limiting step in the transmission process. Some individuals may have had substantially fewer contacts than predicted and we are not certain what kind of contact is necessary for transmission (but see Brunner et al. 2007). Still, it seems that, except at very low densities, susceptibles would have had more than enough contacts with infected tadpoles to acquire the infection. Similarly, Reeve et al. (2013) tracked the movements of tadpoles during ranavirus epidemics in essentially identical mesocosms and found a fivefold or greater difference in the velocity of tadpoles in mesocosms with and without predator cues, which should correlate with contact rates (Hutchinson and Waser 2007), but they did not see differences in the timing or outcome of the epidemics (Reeve et al. 2013). Lastly, the ad hoc model in which transmission was assumed to scale with observed contact rates was the worst-performing model by far (Table 1). A similar model using observed rather than predicted contacts, which could account for individual

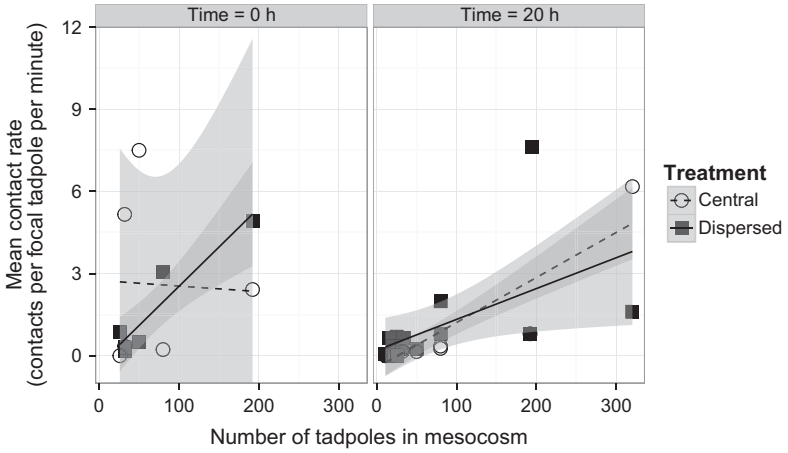

FIG. 2. The average rate of contacts made between marked focal infected tadpoles ($n = 2$ or 4 infected tadpoles/mesocosm) with others in the mesocosm immediately after food was added or 20 h later. Food was either added in a pile in the center or dispersed throughout the bottom of each mesocosm. Regression lines fit to each time and treatment are shown with their 95% confidence envelopes (shaded regions).

heterogeneity in contacts, faired no better (not shown). Transmission was not even coarsely related to contact rates. For instance, contact rates were somewhat, if not significantly higher in the central food treatment, whereas the estimates of β were higher in dispersed food treatment (Fig. 1, Table 1).

It is also worth noting that our results suggest that manipulating host behavior to alter transmission dynamics may prove difficult. Our manipulation of the distribution of food had only a transient effect on behavior and no clear effect on contact rates overall (Fig. 2). Behavioral responses and thus changes in contact rates may also vary with age, stage, condition, and physical setting. More importantly, while host-to-host contacts are necessary for transmission, they may not limit transmission and so even successful manipulations of host behavior may have little impact on transmission. Rather transmission dynamics may be shaped by other factors such as host susceptibility.

While we cannot be certain that the saturating rates of transmission we observed were, in fact, due to heterogeneities in susceptibility, such heterogeneities are commonly observed in ranavirus–amphibian systems, albeit indirectly. A shallow slope in a dose–response experiment (as opposed to sharp thresholds) implies a large amount of heterogeneity in the amount of pathogen required to infect or kill individuals in a population (Dwyer et al. 1997), so the fact that numerous dose–response challenges with amphibian ranaviruses have yielded rather shallow relationships between the dose of inoculum and the outcome suggests at least moderate heterogeneities in susceptibility or tolerance to infections (Pearman et al. 2004, Brunner et al. 2005, Warne et al. 2011). Further research will be needed to understand both the source(s) and consequences of heterogeneity in the infection process.

Last, it is worth considering the epidemiological implications of our results for ranavirus epidemics. With such high rates of transmission, epidemics should be rapid. Consider Reeve et al.'s (2013) data on ranavirus epidemics in essentially identical mesocosms (with $I_0 = 12$ and $S_0 = 60$ or 300). They found that ~95% of the tadpoles were infected when they died or metamorphosed 5–25 d later. The power relationship model predicts 95% of the tadpoles would be infected within 4.4 d (with $S_0 = 60$) or 9.7 d (with $S_0 = 300$), which fits quite well, even ignoring secondary transmission (which would occur on this time frame; Brunner et al. 2007). Transmission in the wild may be slower in larger, more structurally complex ponds that prevent homogeneous mixing (Greer and Collins 2008) or higher, because ranavirus can be transmitted from carcasses (Pearman et al. 2004, Brunner et al. 2007) or via water given sufficient doses (Brunner et al. 2005, 2007, but see Johnson and Brunner 2014), both of which we minimized in our experiment. But rough calculations with even conservative values for β (e.g., 1/10th of our estimates) suggest that ranavirus epidemics in wood frogs progress rapidly from an introduction (J. L. Brunner, *unpublished data*). This contrasts with the consistent observation of ranavirus-related die-offs occurring in the late summer, as ponds dry and tadpoles begin to metamorphose (Brunner et al. 2015). We suspect that this reflects a separation between the epidemic rise in ranavirus infection and the later mortality event. Perhaps tadpoles only succumb to infection when environmental conditions deteriorate (e.g., high temperatures; Brand et al. 2016), or during a critical window of vulnerability during development (Warne et al. 2011). A clearer understanding of the physiological basis of both susceptibility and tolerance will thus help us understand both the dynamics *and* outcome of ranaviruses and other pathogens.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.1644/suppinfo