# Introduction

Most parasites must navigate a complex environment in order to complete their life cycle and are constrained by the availability and suitability of hosts as well as environmental conditions. Generalist parasites contend with changes in the relative and absolute abundance of their various hosts, and parasites that are transmitted environmentally must rely on favorable conditions for persistence until they are taken up by a new host. Much attention has been paid to anthropogenic-induced changes in the biotic and abiotic conditions experienced by parasites, including introduction and extirpation of species, climate warming, pollution, and land conversion (Altizer et al. 2013; Patz et al. 2004; Young et al. 2017). Many of these changes feed into the dilution effect hypothesis, which posits that transmission (in general, or relative to a focal host species) is augmented as host species richness declines, often in response to anthropogenic influence (P. T. J. Johnson, Roode, and Fenton 2015; Keesing, Holt, and Ostfeld 2006). However, biotic and abiotic change also occurs naturally over time and space. Moreover, the joint effects of abiotic and biotic drivers of epizootics are rarely considered together (Bienentreu and Lesbarrères 2020; Dillon and Meentemeyer 2019; Hall et al. 2010; North et al. 2015; Scott-Baumann and Morgan 2015), and theoretical prediction for such interaction is underdeveloped. In part, this is due to the notion that transmission is influenced by abiotic factors at large spatial scales and by biotic factors at smaller scales (Halliday et al. 2019; Rohr et al. 2019). However, host mobility often means transmission is occurring at intermediate spatial scales, where communities of hosts are connected, and in these metacommunities, the joint influence of host community structure and environmental conditions on parasite transmission is not well understood. In response to this knowledge gap, we combine theoretical and empirical approaches to study how biotic and abiotic factors interact to promote transmission of ranavirus in amphibian communities. We demonstrate that focusing on the relative abundance of species, in concert with total community size and environmental conditions, provides a more detailed explanation for spatiotemporal variation in outbreak risk that cannot be solely explained by biotic or abiotic variables alone. ~~In addition, we build upon existing theory regarding the relationship between host biodiversity and parasite transmission by demonstrating that host species evenness, rather than richness, can be used to relate community composition to outbreak risk.~~

Generalist parasites, such as ranaviruses, are commonly found in nature and often use a range of alternative host species to reproduce (Park et al. 2018; Poulin et al. 2011). The transmission potential of a generalist parasite for a given host species may vary due to differences in host species abundances (Fenton et al. 2002; Hopkins et al. 2020), host competence (i.e. the ability for an individual host to become infected and pass infection on to others (Martin et al. 2019)), and the environmental conditions in which the interaction takes place (Altizer et al. 2013; Cable et al. 2017; Rohr et al. 2011). The combination of these factors makes it difficult to assess how multi-species host communities are differentially at risk of parasite invasion and onward transmission to other communities (P. T. J. Johnson, Roode, and Fenton 2015). One method to separately quantify the effects of host abundance and competence on parasite transmission is to use community competence: the weighted average of competence values of host species in the community (Pieter T. J. Johnson et al. 2013). In addition, community competence is advantageous because it considers the relative abundance of a species rather than the presence of a species alone. For example, a commonly occurring, low-competence host could be responsible for more transmission than a rare, high-competence host (Holt et al. 2003). In contrast to host species richness, which is the typical predictor variable in the dilution effect hypothesis literature, community competence and host abundance are more directly related to the parasite basic reproductive number, R0 (Dobson 2004; Rohr et al. 2019).

Ranaviruses (genus Ranavirus, family Iridoviridae) are well-suited for studying host-parasite-environment interactions at the community scale and are associated with worldwide amphibian declines, making them a parasite of global concern (Lesbarrères et al. 2012). The Ranavirus genus of double-stranded DNA viruses (Gray, Miller, and Hoverman 2009), affects most amphibian species (Jesse L. Brunner et al. 2015) and host species exhibit a wide range of competence for the parasite (Hoverman et al. 2011). At the Savannah River Site (South Carolina, USA), a spatially-distributed set of 20 wetlands has been extensively sampled in the context of ranavirus infection over a 6-month period. We focus on larval amphibian communities which are bound by their aquatic habitat but connected through the overland movement of their adult stages (Mihaljevic, Hoye, and Johnson 2018). Ranavirus is transmitted via intra- and interspecific direct transmission, which exhibits a positive, but ultimately saturating, relationship with host abundance (Jesse L. Brunner et al. 2017). Additionally, ranavirus can be transmitted environmentally via free-living infectious virions shed into water by infected live individuals or decaying carcasses (Brenes et al. 2014). Ranavirus persistence times in water have been found to diminish at high ambient temperatures (Nazir, Spengler, and Marschang 2012) suggesting that the relative importance of environmental transmission, versus contact-based transmission, should depend on environmental conditions. We hypothesize that community-scale prevalence of ranavirus will have a positive relationship with host abundance and community competence but a negative relationship with increasing water temperatures.

We begin with a theoretical model, where we use the community basic reproductive number, R0, to estimate the transmission potential of ranavirus in multi-host communities (Dobson 2004). R0 provides a valuable lens into transmission potential, clarifying conditions necessary for parasite invasion and persistence. Calculation of R0 at the community scale explicitly considers the role of community composition in the transmission of a multi-host parasite along with host abundance. In our case it also includes the relative force of infection from free-living infectious virions, versus infected hosts. Multiple transmission routes can increase overall transmission potential, as evidenced by contact and environmental transmission in models of avian influenza virus (Breban et al. 2009) and causal agents of diarrheal diseases (Tien and Earn 2010) as well as contact and sexual transmission in models of Ebola transmission (Abbate et al. 2016; Vinson et al. 2016). From our model, we analyzed the relative contribution of direct and environmental transmission in allowing the parasite to exceed the critical value of R0=1. These two routes are linked because environmental transmission relies on the shedding of viral material from infected individuals and higher environmental transmission rates will generate more opportunities for subsequent direct transmission. Specifically, we determine how transmission from each route facilitates parasite invasion under different scenarios of community composition, host abundance, and virus decay rate (half-life) in water, both as separate factors and acting in concert.

Using empirical data, we then apply metacommunity theory and dimensionality reduction to explain how biotic and abiotic factors contribute to community composition (M. A. Leibold et al. 2004), and then we evaluate these communities in their ability to differentially facilitate transmission. Community compositions change over space or time and can include dynamic, predictable patterns of assembly or disassembly (Pieter T. J. Johnson et al. 2019; Ostfeld and LoGiudice 2003). These changes in community composition can then influence transmission potential of a parasite and may map to dilution or amplification effects (Luis, Kuenzi, and Mills 2018; North et al. 2015). According to theory, a dilution effect may occur whenever hosts vary in competence and reductions in diversity are associated with increases in mean competence (P. T. J. Johnson, Roode, and Fenton 2015). In contrast, an amplification effect can occur when increased host diversity causes an increase in mean competence (Keesing, Holt, and Ostfeld 2006). ~~Considering these conflicting patterns, we relate species evenness, in addition to species richness, as a predictor for both community competence and infection prevalence.~~ In the ranavirus system, we measure competence as a species’ average viral load, which estimates the differential propensity for hosts to generate new infections. While the amphibian community comprises several species, their viral loads are bimodal and communities can be simplified into two groups (high and low competence), which is especially useful in model building and analysis.

Modeling results show that the effect on R0 by community composition, host abundance, and environmental persistence of virus are independently important such that weak contribution to R0 by a factor can be compensated by appropriately strong contributions from other factors. In addition, we find that each of these factors combined can have an appreciably greater than additive effect, meaning some host communities can be disproportionately at risk of epizootics. The empirical data confirm that outbreaks are frequently associated with ‘perfect storms’ of large communities dominated by competent species at relatively low temperatures. ~~Importantly, neither species richness nor species evenness were found to be powerful predictors for ranavirus prevalence. However, species evenness and community competence emerged as informative metrics for identifying mechanism in the diversity-disease relationship.~~ Our results improve our understanding of the location and timing of outbreaks of multi-host parasites and help to predict when host communities may be most vulnerable, supporting the study of zoonotic diseases by identifying potential disease hot spots that may form due to natural or anthropogenic causes. We contend that the study of abiotic factors directly influencing transmission and indirectly driving community composition holds great promise to advance diversity-disease research.

# Methods

## Transmission Model

According to known transmission, ranaviruses can infect a wide range of amphibian hosts and infectious periods can range from a few days up to weeks (Gray, Miller, and Hoverman 2009). Transmission can occur both directly and indirectly and exposure appears to induce an adaptive immune response in surviving hosts. Accordingly, we modeled a host community using an SIR framework for each species (Table 1). For model tractability, we limited the community to two host types that could vary in key traits including abundance and competence. As well as facilitating model analysis, this choice also reflects the empirical observation that viral loads of host species are bimodal (Supplementary Figure 1). In addition to intra- and inter-specific direct transmission, we included environmental transmission, whereby infectious host individuals shed virus into the environment where it persists for some finite time and can cause new infections without host-to-host contact (Gray, Miller, and Hoverman 2009). We included host demography via a constant birth rate and a constant per capita mortality rate, resulting in a disease-free equilibrium for each host species given by the ratio of the birth rate and mortality rate. For the model to reflect the viral load-based definition of host competence, infectivity, but not susceptibility, varied between the high and low competence host. This means that the rate of transmission from infectious individuals did not depend on whether transmission was to an intra- or inter-specific host, but rather on the high or low competence status of the infectious animal. For example, a high competence individual is likely to infect many individuals of either species when infectious and a low competence individual is expected to infect fewer individuals. In keeping with the assumption of no difference in host susceptibility between species, the environmental transmission rate was set to be the same for both species.

Using the next-generation matrix method (Odo Diekmann, Heesterbeek, and Metz 1990; O. Diekmann, Heesterbeek, and Roberts 2009), we calculated the community basic reproductive number (Dobson 2004), hereafter referred to as R0, for our community of hosts to determine the conditions necessary for parasite invasion (R0>1). Consequently, we determined how the boundary R0=1 operates as a function of parameters for communities with varying characteristics, specifically the effects of community composition, total host abundance, and viral half-life. To illustrate these effects, we created a reference community and 4 manipulated communities that were designed to make the communities more suitable for parasite invasion. The reference community had an equal number of both species, a total host abundance of 150 individuals, and a viral half-life of 1.35 days. The composition-manipulated community was altered to be dominated by the more competent species by a ratio of 2:1. The abundance-manipulated community was altered only in abundance, to a value of 175 individuals, and the half-life-manipulated community was altered by doubling the viral half-life to 2.7 days. The final manipulated community combined each of these single-factor manipulations. For each community, we calculated R0 over a range of values for environmental and contact transmission rates of the more competent species while holding the contact transmission rate for the less competent species constant. This allowed us to characterize the extent to which parasite invasion was more likely, i.e., occurring for an increased set of transmission parameters that included combinations previously associated with failure to invade (R0<1).

## Data Collection and Calculation of Community Competence

All empirical data were previously collected (Coleman 2018) at the Savannah River Site (South Carolina, USA). 20 wetlands were sampled monthly for 6 months from February to July in 2016 at the Savannah River Site. Of the 120 sampling events, 96 produced data, with the others being discounted due to wetlands being dry at the time of sampling. Each sampling event included an estimate of larval amphibian abundance ascertained from minnow traps and dip-net sweeps around the perimeter and center of the wetland. In addition to abundance, a single individual per species was collected per dip-net sweep or minnow trap. These individuals were tested for ranavirus load using qPCR in triplicate which were averaged to determine the viral load for an individual. At the species-level, all individuals that were analyzed for viral load were then averaged to provide a species level estimate of viral load, which was used as a proxy for competence. Using species-level competence, we then calculated community competence as the weighted average of each species’ competence, with weights given by the relative abundance of each species. Each site-month combination was treated as a distinct community in these calculations. Empirical data on community composition, viral loads, and infection prevalence enabled us to analyze host communities according to their community competence and relate these to diversity metrics, as detailed below.

## Host Community Analysis

First, using a metacommunity framework, we created a site-by-species matrix that included species presence or absence, and where ‘site’ referred to a unique wetland-month combination, referred to herein as a community. Within this matrix, communities were ordinated by minimizing the number of embedded absences and were then tested for key metacommunity patterns including nestedness and block replacement using the “metacom” package in R (Dallas 2014; Mathew A. Leibold and Mikkelson 2002). Following this, we used principal component analysis to order communities according to their similarity in community composition. We did this by ranking each community according to the score of their first principal component (PC1), which captured 71% of the variance. We then used this list of ranked communities to ascertain the congruence between host community composition and community competence, testing the assumption that similarly composed communities will be similarly competent. We used a generalized additive model with a geo-additive term to test the relationship between community competence and time (sampling month), space (wetland coordinates), and host abundance to establish the effects of these variables on community competence.

Following this, we treated community competence and infection prevalence as separate response variables and used species richness and species evenness as predictor variables. Community competence is used to measure an instantaneous property of the community in terms of its propensity to support a parasite, but infection prevalence is expected to manifest after sufficient transmission has occurred and so was lagged by a month (the period of time between measurements and a plausible estimate of the parasite generation time (J. L. Brunner and Collins 2009)). We estimated infection prevalence for the community by combining the observed host abundances and the raw infection prevalence of individual host organisms that were sampled for parasite presence using qPCR. The sampled hosts provided a percentage for each species that were found to be infected and this percentage was then weighted by the observed abundance of that species in that community. ~~Species richness was calculated as the sum of unique species present and species evenness was measured by Pielou’s J (Pielou 1984). Species richness was used to test if basic patterns of assembly and disassembly were able to predict transmission potential via dilution or amplification effects, whereas species evenness was used to detect if relative abundances of species were controlling transmission via community competence~~. In an effort to more closely examine the effects of species evenness on community competence, we ordered sites according to community competence and identified trends in the relative abundance of species and the phylogenetic relationships of our host species. We expected phylogenetically clustered or overdispersed species to co-occur less frequently, and we additionally determined the extent to which competence was clustered (versus dispersed) within the phylogeny so that we could evaluate the extent to which multiple competent species may co-occur and potentially dominate a community~~.~~ Finally, we tested for statistically significant relationships between infection prevalence and community competence, abundance, and mean water temperature using both multivariate (generalized linear model) and univariate tests (Spearman Rank correlation test with a correction for multiple comparisons). Mean water temperature was included to serve as a proxy for environmental persistence time of free-living infectious virions; as mean water temperature increases the viral half-life is expected to decrease (Nazir, Spengler, and Marschang 2012).

# Results

## Transmission Model

Model analysis considered the independent and joint effects of community composition, host abundance, and environmental persistence of the parasite and showed that each of these factors can contribute to the likelihood of an epizootic. In a system where transmission is driven by both contact and environmental transmission, these factors are all expected to enhance transmission of the parasite. Furthermore, the model shows that changes in community composition that increase overall community competence may enhance transmission via contact transmission while increases in viral half-life enhances transmission via environmental transmission (Figure 1A and 1C). Increases in overall abundance does not favor either transmission mode, and a combination of community composition, abundance, and viral half-life shows an effect that is greater than any single factor alone (Figure 1B and 1D). The existence of both contact and environmental transmission may allow for multiple factors to have significant contributions to the ability for a parasite to invade a host community successfully.

## Host Community Analysis

Using the empirical data, correlations between community competence, host abundance, and mean water temperature aligned with our predictions. Community competence and host abundance correlated positively with prevalence while mean water temperature correlated negatively with prevalence. These data did not provide sufficient statistical power for a multivariate model, but univariate analyses showed significant results for community competence (P < 0.0001; figure 5), abundance (P < 0.01; figure 5), and mean water temperature (P = 0.021; figure 5).

To test if and how amphibian communities change across wetlands, we used the sampling unit of community (a unique wetland-month combination). First, we established that community patterns were non-random (coherence: P < 0.00001), and were then able to characterize the pattern further as block-replacement, meaning groups of species tended to appear and disappear together from community to community (turnover: P < 0.00001; boundary clumping: P < 0.00001; Supplementary Figure 2). To reduce the complex, high-dimensional characterization of communities for visualization and analysis, we ordinated the community-by-species matrix (maximizing between-community and between-species similarity) and obtained the community score along the first principal component axis (which captured 71% of the variation), with more similar scores representing communities of similar composition. The rank order of these scores confirms that community similarity well explains the emerging property of community competence (Figure 2), and that communities of different compositions may have similar values of community competence (e.g., Figure 2, communities ranked ~5 and ~30), essentially because several combinations of weighted competence scores can evaluate to the same overall level of community competence. Beyond community composition, which is linked to competence mechanistically (cite Johnson), statistically, both season and community size, but not geographical location, also contribute to community competence (generalized additive model - sampling month, P < 0.00001; community size, P < 0.0001; wetland coordinates, P = 0.34). Overall, communities of high competence occurred in February through May (versus June and July, Supplementary Figure 3a). The importance of sampling month likely represents the direct effect of temperature influencing environmental transmission via the viral half-life and not the indirect effect of temperature-mediated competence via community composition because composition was not strongly dictated by temperature (Figure 2, color) whereas air and water temperature at sites align with the second principal component (which explained 26% of the variance, Supplementary Figure 4). Both small and large communities could result in high competence (Figure 2, shape size), but the trend was generally positive (Supplementary Figure 3b).

~~Species evenness, which captures the extent to which species in a community occur at similar abundances, versus are dominated by only one or a few species, reveals that high evenness communities tend to have intermediate competence, whereas low evenness communities diverge to either low or high competence communities, depending on the species that dominate (Figure 3). Furthermore, we found that communities of low evenness and high community competence were generally of higher abundance and had higher future infection prevalence (Figure 3, circle sizes and colors, respectively). There were some notable exceptions to this pattern where communities with low or moderate competence still had high future infection prevalence. In some of these cases, community size appears to be driving transmission (Figure 3; i and ii), whereas low sample size may explain disconnections between measured competence in month~~ *~~t~~* ~~and prevalence in month~~ *~~t~~*~~+1 in other cases (Figure 3).~~ Species richness was not predictive of infection prevalence meaning we did not detect a classic dilution effect pattern (Supplementary Figure 5; Spearman’s rho = 0.15, P = 0.14). We found that the most competent communities were dominated by either one or a few highly competent species (Figure 4), and in the host phylogeny, we see that these highly competent species are moderately dispersed, suggesting that limiting similarity may not be hindering coexistence and the ability for multiple competent species to achieve high abundance in the same community (Figure 4, bottom panel; Supplementary Figure 6).

# Discussion

Using a blend of theoretical model predictions and empirical data, we have shown that the effects of host abundance, community composition, and the environment can simultaneously affect the transmission potential of a generalist parasite in communities of hosts. In our mathematical model, we demonstrated that these independent factors can all enhance transmission and that when combined, can have a more than additive effect. Using empirical data, we confirmed that both biotic and abiotic factors can have detectable effects on parasite transmission at an intermediate spatial scale. Importantly, we also found that species evenness was a more informative predictor for parasite transmission potential compared to species richness. Moreover, our results inform the risk of amphibian communities to ranavirus epizootics and extend theory regarding the relationship between biodiversity and the transmission of generalist parasites by including environmental transmission and the factors that promote it.

From our theoretical model, we learned that the inclusion of both contact-based and environmental transmission allowed for each transmission mode to compensate for the other; a low strength of one transmission mode could be supplemented by the other transmission mode, resulting in a broader range of favorable conditions for the parasite (Vinson et al. 2016). In other studies, it has been noted that the incorporation of environmental transmission can provide dramatically different estimates of R0 and allow for persistence of parasites at population sizes that would not be supported by contact transmission alone (Breban et al. 2009; Breban, Drake, and Rohani 2010; Tien and Earn 2010). We build upon these findings by demonstrating how the relative strength of transmission modes can emphasize the importance of different factors relevant to parasite transmission. In a community where the strength of contact-based transmission is high and environmental transmission strength is low we expect community composition to have a significant impact on transmission. Conversely, when environmental transmission is high and contact-based transmission is low, we expect community composition to be less important for overall transmission. This suggests that in natural systems where both modes of transmission are available, the influence of both may be present but obscured by the other. When we enhanced absolute host abundance, we anticipated that contact-based transmission would be favored, but our analysis showed that the increase in available hosts can augment both transmission modes equally. We understood this to be because as the number of hosts increases, the quantity of free-living infectious virions also increases proportionally due to a greater number of infected hosts shedding viral material as well as simply increasing host density. This results in an increase in the force of infection for both contact-based transmission and environmental transmission.

~~From the empirical data, we found that community composition and evenness were far more informative in predicting transmission potential compared to species richness, in spite of the fact that the diversity-disease literature includes many correlative studies that investigate the relationship between species richness and infection prevalence. Indeed, recent research has emphasized the importance of a more mechanistic understanding of diversity-disease relationships, and host species evenness may aid in this effort (Merrill and Johnson 2020; Rohr et al. 2019). Evenness, rather than richness, can more directly relate the composition of a community to its transmission potential. At low evenness, the identity of the dominant species drives community competence, and we found that communities with the highest absolute abundance were usually of lower evenness and dominated by a few highly competent species. This pattern is supported in theory connecting life history traits to host competence, where hosts with a faster pace-of-life invest heavily into rapid growth and reproduction and less into immunity, resulting in highly abundant, highly competent hosts (Joseph et al. 2013). Together, host abundance, evenness, and community competence have the potential to offer a more mechanistic lens into community compositions and how they will affect infection prevalence.~~

~~Detection of diversity-disease relationships is facilitated by predictable community structures (Faust et al. 2017).~~ Classic dilution-effect systems typically operate by nested structures influencing parasite transmission through biodiversity loss (LoGiudice et al. 2003). In these examples, species losses follow a pattern where less competent hosts are lost before more competent hosts (Pieter T. J. Johnson et al. 2013). In contrast, our study revealed block-replacement of sets of host species, where transmission potential was not changing due to species richness, but rather by species evenness and identity; often, ranavirus attained high prevalence due to correlated competence and abundance of certain key species. However, the generality of this depends on how competence is typically distributed among a community of host species and how relationships among those species affect their probability of co-occurring and abundance. The amphibian species in our study showed evidence of phylogenetic repulsion and environmental filtering, as has been detected, for example, in avian communities (Weinstein, Graham, and Parra 2017). When co-occurring, species that were closely related or distantly related to their neighbors had low relative abundance (Supplementary Figure 6). In contrast, species that were intermediately related to others on average attained high relative abundance. Combined with the fact that high competence was a relatively well-dispersed trait in the studied amphibian clade, this created the conditions for competent species to co-occur and attain high relative abundance. By corollary, if competent species were clustered phylogenetically, then high community competence would be enhanced by the presence of an environmental filter and absence of phylogenetic repulsion. Future work may benefit from considering host communities simultaneously in terms of the rules that govern species co-occurrence and abundance and the distribution of competence levels among those species.

The study of individual host competence and how it scales to a community can provide us with valuable insight into how well host communities can support generalist parasites (Becker, Seifer, and Carlson 2020). However, host competence can be a challenging trait to measure because it is multifactorial, relying on components such as exposure, susceptibility, suitability, and transmissibility (Downs et al. 2019). In our study, we use mean viral load as a proxy for host competence which is useful because it informs shedding rate, infectivity, and host suitability. However, viral load is not a holistic measure of host competence because it does not address, for example, the infectious period or susceptibility. Further research on host competence should incorporate more robust measures of competence such as those derived from controlled infection assays (Merrill et al. 2019). Factors within host species such as age and sex will also likely change the competence of a host and while there may be the ability to measure competence with genetic methods, there is likely to be phenotypic plasticity within the trait (Gervasi et al. 2015).

Ranaviruses in amphibians are a useful and important system for studying diversity-disease relationships because of their highly generalist nature and role in global amphibian biodiversity declines (Lesbarrères et al. 2012; North et al. 2015). Environmental transmission of ranavirus and the connection of wetlands by amphibian adults made this system especially useful for our study of the effects of host composition, host abundance, and environmental factors. However, these features are not limited to this study system and other multi-host generalist parasite systems can benefit from our findings. Furthermore, our research focused on natural variations in biodiversity, but it is important to recognize how anthropogenic influence will alter these patterns as well (Altizer et al. 2013; Rohr et al. 2011). Amphibian species, in particular, have been extremely sensitive to anthropogenic disturbance and have been considered proxies for overall environmental health (Scheele et al. 2019). Land use and climate change are also factors that affect amphibian communities globally and it is important to understand the implications of these changes (Wilkinson et al. 2018). Altogether, the ecology of ranavirus in amphibians and the global relevance of amphibian biodiversity make this system a valuable case study for the joint influence of biotic and abiotic factors on parasite transmission under natural and artificial settings.

By focusing on parasites with multiple transmission modes, we have highlighted how biotic or abiotic factors may differentially exacerbate the impact of parasites introduced into a host community. In addition, we have expanded theory regarding diversity-disease relationships to advocate the use of species evenness and community competence as informative predictors for the impact of generalist parasites in multi-host communities. These metrics can facilitate analysis that is more focused on the mechanics of dilution or amplification rather than correlative association between disease presence and underlying assembly rules that may be inconsistent across systems, space, and time. Furthermore, we advocate for deeper analysis of host community phylogeny in the context of diversity-disease relationships which can provide insight into patterns of host species co-occurrence and host traits that may correlate with competence. Together, these developments combined with the contextual understanding of specific host-parasite ecology should allow for mechanistic understanding of many generalist parasite systems.

# References

Abbate, Jessica L., Carmen Lia Murall, Heinz Richner, and Christian L. Althaus. 2016. “Potential Impact of Sexual Transmission on Ebola Virus Epidemiology: Sierra Leone as a Case Study.” *PLoS Neglected Tropical Diseases* 10 (5): e0004676. <https://doi.org/10.1371/journal.pntd.0004676>.

Aickin, Mikel, and Helen Gensler. 1996. “Adjusting for Multiple Testing When Reporting Research Results: The Bonferroni Vs Holm Methods.” *American Journal of Public Health*. <https://doi.org/10.2105/ajph.86.5.726>.

Altizer, S., R. S. Ostfeld, P. T. J. Johnson, S. Kutz, and C. D. Harvell. 2013. “Climate Change and Infectious Diseases: From Evidence to a Predictive Framework.” *Science (American Association for the Advancement of Science)* 341 (6145): 514–19. <https://doi.org/10.1126/science.1239401>.

Becker, Daniel J., Stephanie N. Seifer, and Colin J. Carlson. 2020. “Beyond Infection: Integrating Competence into Reservoir Host Prediction.” *Trends in Ecology and Evolution* 35 (12): 1061–62. <https://doi.org/10.1016/j.tree.2020.08.014>.

Bienentreu, Joe-Felix, and David Lesbarrères. 2020. “Amphibian Disease Ecology: Are We Just Scratching the Surface?” *Herpetologica* 76 (2): 153. <https://doi.org/10.1655/0018-0831-76.2.153>.

Breban, Romulus, John M. Drake, and Pejman Rohani. 2010. “A General Multi-Strain Model with Environmental Transmission: Invasion Conditions for the Disease-Free and Endemic States.” *Journal of Theoretical Biology* 264 (3): 729–36. <https://doi.org/10.1016/j.jtbi.2010.03.005>.

Breban, Romulus, John M. Drake, David E. Stallknecht, and Pejman Rohani. 2009. “The Role of Environmental Transmission in Recurrent Avian Influenza Epidemics.” *PLoS Computational Biology* 5 (4): e1000346. <https://doi.org/10.1371/journal.pcbi.1000346>.

Brenes, Roberto, Matthew J. Gray, Thomas B. Waltzek, Rebecca P. Wilkes, and Debra L. Miller. 2014. “Transmission of Ranavirus Between Ectothermic Vertebrate Hosts.” *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0092476>.

Brunner, J. L., and J. P. Collins. 2009. “Testing Assumptions of the Trade-Off Theory of the Evolution of Parasite Virulence.” *Evolutionary Ecology Research* 11 (8): 1169–88. <https://search.proquest.com/docview/21258486>.

Brunner, Jesse L., Lynne Beaty, Alexandra Guitard, and Deanna Russell. 2017. “Heterogeneities in the Infection Process Drive Ranavirus Transmission.” *Ecology* 98 (2): 576–82. <https://doi.org/10.1002/ecy.1644>.

Brunner, Jesse L., Andrew Storfer, Matthew J. Gray, and Jason T. Hoverman. 2015. *Ranavirus Ecology and Evolution: From Epidemiology to Extinction*. Springer International Publishing. <https://doi.org/10.1007/978-3-319-13755-1_4>.

Cable, Joanne, Iain Barber, Brian Boag, Amy R. Ellison, Eric R. Morgan, Kris Murray, Emily L. Pascoe, Steven M. Sait, Anthony J. Wilson, and Mark Booth. 2017. “Global Change, Parasite Transmission and Disease Control: Lessons from Ecology.” *Philosophical Transactions. Biological Sciences* 372 (1719): 20160088. <https://doi.org/10.1098/rstb.2016.0088>.

Coleman, Austin Lee. 2018. “Incorporating Environmental Factors into Discussions of Diversity-Disease Relationships.” PhD thesis, University of Georgia.

Dallas, Tad. 2014. “Metacom: An R Package for the Analysis of Metacommunity Structure.” *Ecography* 37 (4): 402–5. <https://doi.org/10.1111/j.1600-0587.2013.00695.x>.

Diekmann, Odo, Hans Heesterbeek, and J. A. J. Metz. 1990. “On the Definition and the Computation of the Basic Reproduction Ratio R0 in Models for Infectious Diseases in Heterogeneous Populations.” *Journal of Mathematical Biology* 28 (4): 365–82. <https://www.narcis.nl/publication/RecordID/oai:cwi.nl:2026>.

Diekmann, O., J. A. P. Heesterbeek, and M. G. Roberts. 2009. “The Construction of Next-Generation Matrices for Compartmental Epidemic Models.” *Journal of the Royal Society Interface* 7 (47): 873–85. <https://doi.org/10.1098/rsif.2009.0386>.

Dillon, Whalen W., and Ross K. Meentemeyer. 2019. “Direct and Indirect Effects of Forest Microclimate on Pathogen Spillover.” *Ecology (Durham)* 100 (5): e02686–n/a. <https://doi.org/10.1002/ecy.2686>.

Dobson, Andrew. 2004. “Population Dynamics of Pathogens with Multiple Host Species.” *The American Naturalist* 164 (S5): S64–78. <https://doi.org/10.1086/424681>.

Downs, Cynthia J., Laura A. Schoenle, Barbara A. Han, Jon F. Harrison, and Lynn B. Martin. 2019. “Scaling of Host Competence.” *Trends in Parasitology* 35 (3): 182–92. <https://doi.org/10.1016/j.pt.2018.12.002>.

Faust, Christina L., Andrew P. Dobson, Nicole Gottdenker, Laura S. P. Bloomfield, Hamish I. McCallum, Thomas R. Gillespie, Maria Diuk-Wasser, and Raina K. Plowright. 2017. “Null Expectations for Disease Dynamics in Shrinking Habitat: Dilution or Amplification?” *Philosophical Transactions of the Royal Society B: Biological Sciences* 372 (1722). <https://doi.org/10.1098/rstb.2016.0173>.

Fenton, A., J. P. Fairbairn, R. Norman, and P. J. Hudson. 2002. “Parasite Transmission: Reconciling Theory and Reality.” *Journal of Animal Ecology* 71 (5): 893–905. <https://doi.org/10.1046/j.1365-2656.2002.00656.x>.

Gervasi, Stephanie S., David J. Civitello, Holly J. Kilvitis, and Lynn B. Martin. 2015. “The Context of Host Competence: A Role for Plasticity in Host–Parasite Dynamics.” *Trends in Parasitology* 31 (9): 419–25. <https://doi.org/10.1016/j.pt.2015.05.002>.

Gray, Matthew J., Debra L. Miller, and Jason T. Hoverman. 2009. “Ecology and Pathology of Amphibian Ranaviruses.” *Diseases of Aquatic Organisms* 87 (3): 243–66. <https://doi.org/10.3354/dao02138>.

Hall, Spencer R., Robyn Smyth, Claes R. Becker, Meghan A. Duffy, Christine J. Knight, Sally MacIntyre, Alan J. Tessier, and Carla E. Cáceres. 2010. “Why Are Daphnia in Some Lakes Sicker? Disease Ecology, Habitat Structure, and the Plankton.” *BioScience* 60 (5): 363–75. <https://doi.org/10.1525/bio.2010.60.5.6>.

Halliday, Fletcher W., Robert W. Heckman, Peter A. Wilfahrt, and Charles E. Mitchell. 2019. “Past Is Prologue: Host Community Assembly and the Risk of Infectious Disease over Time.” *Ecology Letters* 22 (1): 138–48. <https://doi.org/10.1111/ele.13176>.

Holm, Sture. 1979. “A Simple Sequentially Rejective Multiple Test Procedure.”

Holt, Robert D., Andrew P. Dobson, Michael Begon, Roger G. Bowers, and Eric M. Schauber. 2003. “Parasite Establishment in Host Communities.” *Ecology Letters* 6 (9): 837–42. <https://doi.org/10.1046/j.1461-0248.2003.00501.x>.

Hopkins, Skylar R., Arietta E. Fleming‐Davies, Lisa K. Belden, Jeremy M. Wojdak, and Nick Golding. 2020. “Systematic Review of Modelling Assumptions and Empirical Evidence: Does Parasite Transmission Increase Nonlinearly with Host Density?” *Methods in Ecology and Evolution* 11 (4): 476–86. <https://doi.org/10.1111/2041-210X.13361>.

Hoverman, Jason T., Matthew J. Gray, Nathan A. Haislip, and Debra L. Miller. 2011. “Phylogeny, Life History, and Ecology Contribute to Differences in Amphibian Susceptibility to Ranaviruses.” *EcoHealth* 8 (3): 301–19. <https://doi.org/10.1007/s10393-011-0717-7>.

Johnson, P. T. J., J. C. de Roode, and A. Fenton. 2015. “Why Infectious Disease Research Needs Community Ecology.” *Science (American Association for the Advancement of Science)* 349 (6252): 1259504. <https://doi.org/10.1126/science.1259504>.

Johnson, Pieter T. J., Dana M. Calhoun, Tawni Riepe, Travis McDevitt-Galles, and Janet Koprivnikar. 2019. “Community Disassembly and Disease: Realistic-but Not Randomized-Biodiversity Losses Enhance Parasite Transmission.” *Proceedings of the Royal Society B: Biological Sciences*. <https://doi.org/10.1098/rspb.2019.0260>.

Johnson, Pieter T. J., Daniel L. Preston, Jason T. Hoverman, and Katherine L. D. Richgels. 2013. “Biodiversity Decreases Disease Through Predictable Changes in Host Community Competence.” *Nature*. <https://doi.org/10.1038/nature11883>.

Joseph, Maxwell B., Joseph R. Mihaljevic, Sarah A. Orlofske, Sara H. Paull, and Richard Ostfeld. 2013. “Does Life History Mediate Changing Disease Risk When Communities Disassemble?” *Ecology Letters* 16 (11): 1405–12. <https://doi.org/10.1111/ele.12180>.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. “Effects of Species Diversity on Disease Risk.” *Ecology Letters* 9 (4): 485–98. <https://doi.org/10.1111/j.1461-0248.2006.00885.x>.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, et al. 2004. “The Metacommunity Concept: A Framework for Multi‐scale Community Ecology.” *Ecology Letters* 7 (7): 601–13. <https://doi.org/10.1111/j.1461-0248.2004.00608.x>.

Leibold, Mathew A., and Gregory M. Mikkelson. 2002. “Coherence, Species Turnover, and Boundary Clumping: Elements of Meta-Community Structure.” *Oikos* 97 (2): 237–50. <https://doi.org/10.1034/j.1600-0706.2002.970210.x>.

Lesbarrères, D., A. Balseiro, J. Brunner, V. G. Chinchar, A. Duffus, J. Kerby, D. L. Miller, et al. 2012. “Ranavirus: Past, Present and Future.” *Biology Letters* 8 (4): 481–83. <https://doi.org/10.1098/rsbl.2011.0951>.

LoGiudice, Kathleen, Richard S. Ostfeld, Kenneth A. Schmidt, and Felicia Keesing. 2003. “The Ecology of Infectious Disease: Effects of Host Diversity and Community Composition on Lyme Disease Risk.” *Proceedings of the National Academy of Sciences - PNAS* 100 (2): 567–71. <https://doi.org/10.1073/pnas.0233733100>.

Luis, Angela D., Amy J. Kuenzi, and James N. Mills. 2018. “Species Diversity Concurrently Dilutes and Amplifies Transmission in a Zoonotic Host–Pathogen System Through Competing Mechanisms.” *Proceedings of the National Academy of Sciences - PNAS* 115 (31): 7979–84. <https://doi.org/10.1073/pnas.1807106115>.

Martin, Alynn M., Hayley Ricardo, Adrianna Tompros, Tamieka A. Fraser, Adam Polkinghorne, and Scott Carver. 2019. “Burrows with Resources Have Greater Visitation and May Enhance Mange Transmission Among Wombats.” *Australian Mammalogy*. <https://doi.org/10.1071/AM18013>.

Merrill, Tara E. Stewart, Spencer R. Hall, Loren Merrill, and Carla E. Cáceres. 2019. “Variation in Immune Defense Shapes Disease Outcomes in Laboratory and Wild Daphnia.” *Integrative and Comparative Biology* 59 (5): 1203–19. <https://doi.org/10.1093/icb/icz079>.

Merrill, Tara E. Stewart, and Pieter T. J. Johnson. 2020. “Towards a Mechanistic Understanding of Competence: A Missing Link in Diversity-Disease Research.” *Review*.

Mihaljevic, Joseph R., Bethany J. Hoye, and Pieter T. J. Johnson. 2018. “Parasite Metacommunities: Evaluating the Roles of Host Community Composition and Environmental Gradients in Structuring Symbiont Communities Within Amphibians.” *The Journal of Animal Ecology* 87 (2): 354–68. <https://doi.org/10.1111/1365-2656.12735>.

Nazir, J., M. Spengler, and R. E. Marschang. 2012. “Environmental Persistence of Amphibian and Reptilian Ranaviruses.” *Diseases of Aquatic Organisms* 98 (3): 177–84. <https://doi.org/10.3354/dao02443>.

North, Alexandra C., David J. Hodgson, Stephen J. Price, and Amber G. F. Griffiths. 2015. “Anthropogenic and Ecological Drivers of Amphibian Disease (Ranavirosis).” *PloS One* 10 (6): e0127037. <https://doi.org/10.1371/journal.pone.0127037>.

Ostfeld, Richard S., and Kathleen LoGiudice. 2003. “Community Disassembly, Biodiversity Loss, and the Erosion of an Ecosystem Service.” *Ecology* 84 (6): 1421–27. <https://doi.org/10.1890/02-3125>.

Park, A. W., M. J. Farrell, J. P. Schmidt, S. Huang, T. A. Dallas, P. Pappalardo, J. M. Drake, et al. 2018. “Characterizing the Phylogenetic Specialism–Generalism Spectrum of Mammal Parasites.” *Proceedings of the Royal Society. B, Biological Sciences* 285 (1874): 20172613. <https://doi.org/10.1098/rspb.2017.2613>.

Patz, Jonathan A., Peter Daszak, Gary M. Tabor, A. Alfonso Aguirre, Mary Pearl, Jon Epstein, Nathan D. Wolfe, et al. 2004. “Unhealthy Landscapes: Policy Recommendations on Land Use Change and Infectious Disease Emergence,” April. <https://doi.org/10.1289/ehp.6877>.

Pielou, E. C. 1984. *The Interpretation of Ecological Data: A Primer on Classification and Ordination*. Wiley.

Poulin, R., B. R. Krasnov, D. Mouillot, and D. W. Thieltges. 2011. “The Comparative Ecology and Biogeography of Parasites.” <https://www.narcis.nl/publication/RecordID/oai:imis.nioz.nl:231270>.

Rohr, Jason R., David J. Civitello, Fletcher W. Halliday, Peter J. Hudson, Kevin D. Lafferty, Chelsea L. Wood, and Erin A. Mordecai. 2019. “Towards Common Ground in the Biodiversity–Disease Debate.” *Nature Ecology & Evolution* 4 (1): 24–33. <https://doi.org/10.1038/s41559-019-1060-6>.

Rohr, Jason R., Andrew P. Dobson, Pieter T. J. Johnson, A. Marm Kilpatrick, Sara H. Paull, Thomas R. Raffel, Diego Ruiz-Moreno, and Matthew B. Thomas. 2011. “Frontiers in Climate Change–Disease Research.” *Trends in Ecology & Evolution* 26 (6): 270–77.

Scheele, Ben C., Frank Pasmans, Lee F. Skerratt, Lee Berger, An Martel, Wouter Beukema, Aldemar A. Acevedo, et al. 2019. “Amphibian Fungal Panzootic Causes Catastrophic and Ongoing Loss of Biodiversity.” *Science* 363 (6434): 1459–63. <https://doi.org/10.1126/science.aav0379>.

Scott-Baumann, James F., and Eric R. Morgan. 2015. “A Review of the Nest Protection Hypothesis: Does Inclusion of Fresh Green Plant Material in Birds’ Nests Reduce Parasite Infestation?” *Parasitology* 142 (8): 1016–23. <https://doi.org/10.1017/S0031182015000189>.

Tien, Joseph, and David Earn. 2010. “Multiple Transmission Pathways and Disease Dynamics in a Waterborne Pathogen Model.” *Bulletin of Mathematical Biology* 72 (6): 1506–33. <https://doi.org/10.1007/s11538-010-9507-6>.

Vinson, John E., John M. Drake, Pejman Rohani, and Andrew W. Park. 2016. “The Potential for Sexual Transmission to Compromise Control of Ebola Virus Outbreaks.” *Biology Letters* 12 (6): 20151079. <https://doi.org/10.1098/rsbl.2015.1079>.

Weinstein, Ben G., Catherine H. Graham, and Juan Luis Parra. 2017. “The Role of Environment, Dispersal and Competition in Explaining Reduced Co-Occurrence Among Related Species.” *PloS One* 12 (11): e0185493. <https://doi.org/10.1371/journal.pone.0185493>.

Wilkinson, David A., Jonathan C. Marshall, Nigel P. French, and David T. S. Hayman. 2018. “Habitat Fragmentation, Biodiversity Loss and the Risk of Novel Infectious Disease Emergence.” *Journal of the Royal Society Interface* 15 (149): 20180403. <https://doi.org/10.1098/rsif.2018.0403>.

Young, Hillary S., Ingrid M. Parker, Gregory S. Gilbert, Ana Sofia Guerra, and Charles L. Nunn. 2017. “Introduced Species, Disease Ecology, and Biodiversity–Disease Relationships.” *Trends in Ecology & Evolution* 32 (1): 41–54.