Disentangling Disease Transmission as Exposure and Susceptibility

Bradley Lufkin

Spring 2013

Spencer Hall
Associate Professor
ABSTRACT

Epidemics of infectious disease can powerfully impact host populations, entire communities, and ecosystems. Given this strong potential, ecologists and managers must better explain, predict, and respond to disease outbreaks. Therefore, because it potently shapes disease dynamics at the population level, we must better understand disease transmission. Two processes contribute to disease transmission: exposure and susceptibility. Here we illustrate a new technique to simultaneously measure both. We use two experiments to demonstrate that these important traits vary across host genotypes and environmental contexts using a *Daphnia*-fungus system. The hosts (*Daphnia dentifera*) feed non-selectively and can become infected after inadvertently consuming free-living spores of the virulent fungal parasite (*Metschnikowia bicuspidata*). In both experiments, we exposed hosts to parasites and tracked their foraging rates and eventual infection. By combining foraging and infection data simultaneously using a model, we partitioned exposure rate and host susceptibility. In the first experiment, four host genotypes and another species, *Ceriodaphnia*, varied significantly in both traits. The susceptibility, however, best predicted overall disease transmission. In the second experiment, the contaminant copper significantly altered transmission primarily by increasing susceptibility (perhaps through immunotoxic effects), even though exposure decreased. Overall, our results show that separating susceptibility and exposure enables powerful connections between components of disease transmission and genetic and environmental factors. These results could have implications for host evolution, drivers of disease epidemics, and strategies used to control disease.
1. INTRODUCTION

Epidemics substantially impact many ecosystems, and a variety of factors can influence disease spread. Disease harms important species of conservation or economic concern. For instance, whirling disease induces irregular, corkscrew-like swimming in juvenile fish, particularly economically important species such as trout. These infections increase vulnerability to predation and impair foraging through neurological and skeletal damage (Vincent 1996). Pathogens that affect keystone species (such as Phytophthora remorum, the agent of Sudden Oak Death, SOD) can alter the physical structure and function of forest ecosystems (Kelly and Meentemeyer 2002; Cobb et al. 2012). Outbreaks of SOD increase forest heterogeneity and patchiness, altering the movement and habitat of many other species. Furthermore, treating diseases of managed species (e.g., livestock or timber) can be costly (Rizzo and Garbelotto 2003). Given the impacts of disease on conservation, communities, and economics, it remains crucial to predict and explain underlying mechanisms that promote disease spread (Bennett 2003). To uncover these mechanisms, the fundamental process of parasite transmission (the acquisition of infection among individuals in a population) must be understood and quantified.

Transmission is fundamental component of disease ecology because its mechanistic underpinnings drive population-level disease spread (Anderson and May 1992, McCallum et al. 2001, Civitello et al. 2013). However, understanding transmission remains challenging for several reasons. For all parasites, transmission itself depends on two core processes: exposure and susceptibility. Exposure is the rate at which hosts contact parasites, and susceptibility is the probability of becoming infected after contacting a specific number of parasites (Hall et al. 2010). These core components, exposure and susceptibility, could be driven by environmental or genetic variation. Thus, transmission may vary when one, the other, or both of these components...
is altered (Hall et al. 2007, 2010). For instance, fish kairomones (a semiochemical) depress exposure but boost susceptibility (Bertram et al. in press). By developing a method that separately measures the response of each side of the “transmission partition” to genetic or environmental factors, we can predict why and how transmission varies. Quantifying this variability could enhance understanding and prediction of disease dynamics.

In this study, we illustrate how to separately quantify exposure and susceptibility. Furthermore, in two experiments, we showed how each could depend on either host genotype or an environmental factor (the common pollutant copper) using a planktonic zooplankton-fungus system. The “transmission partition” methods involve simultaneously measuring exposure as foraging rate (also known as “water clearance rate” in the Daphnia literature) and ultimate infection via visual diagnosis. The foraging data directly estimates the exposure of hosts to parasites. Combining these data with observations of infections enables us to infer (per parasite) susceptibility of hosts. Furthermore, we employ a novel statistical model, based on copulas, that accounts for the potential correlation between exposure and infection within individuals. The partition provides insights into genetic and environmental components of transmission. Prior work using this system has already revealed variation in transmission with genotype (Duffy et al. 2008; Hall et al. 2010) and copper (Civitello et al. 2012). Now, with this transmission partition, we show how host genotypes vary in both exposure and susceptibility while copper imposes antagonistic effects between them.

2. METHODS

We use two examples to illustrate this technique for the transmission partition. Both use a focal host-parasite system from the plankton. In this system, a crustacean zooplankton host
(Daphnia dentifera) inadvertently eats spores of a virulent yeast fungus (Metschnikowia bicuspidata; hereafter “fungus”) while it forages for algal food (Ebert et al. 2000; Ebert 2005; Fels 2005; Hall et al. 2007). Once infected, hosts release needle-like spores only upon death from infection or predation (Ebert 2005; Duffy 2009; Caceres et al. 2009). Those released spores, if distributed into the host’s habitat, can infect new hosts, potentially causing an epidemic. Our approach formalizes the connection between foraging and transmission. Importantly, Daphnia non-selectively filter feed. Thus, exposure is a direct function of the host’s foraging rate on algae. Daphnia’s foraging rate is influenced by both genetic and environmental factors (Sarnelle and Wilson 2008; Hall et al. 2010). Simultaneously, susceptibility may vary as well.

**Epidemiological model**

We built a model for parasite transmission that explicitly incorporates exposure to parasites, susceptibility to infection, and depletion of parasites from the environment. Typically, transmission rate is modeled simply as a constant rate, $\beta$. Here, we separate the rate as the product of exposure ($f$) and susceptibility ($u$) (so, $\beta = uf$). The model is:

\[
\frac{dS}{dt} = -ufSZ \quad \text{Equation 1a}
\]

\[
\frac{dI}{dt} = ufSZ \quad \text{Equation 1b}
\]

\[
\frac{dA}{dt} = -f(S + I)A \quad \text{Equation 1c}
\]

\[
\frac{dZ}{dt} = -f(S + I)Z \quad \text{Equation 1d}
\]

Susceptible hosts ($S$) become infected ($I$) as they contact spores ($Z$) at the exposure rate ($f$). Given exposure, hosts become infected with per parasite susceptibility probability, $u$ (equ. 1a). Once infected, susceptible hosts move into the infected class (equ. 1b). Exposure to parasites
occurs through foraging, and since *Daphnia* are non-selective feeders, foraging depletes both algal food, \( A \) (equ. 1c) and infectious spores, \( Z \) (equ. 1d) from the environment at an equal per *Daphnia* per prey (algae or spores) rate, \( f \). Algae (\( A \)) and spores (\( Z \)) are consumed by both classes (\( S + I \)) (equ. 1c, d).

**Empirical Methods**

We performed experiments that tracked infection and exposure to estimate parameters for this model. Specifically, we directly measured exposure (\( f \)) and infection. We measured \( f \) through comparison of pre- and post-foraging measurements of algae concentration. Using these infection and foraging data, we inferred susceptibility (\( u \)).

(i) *Culturing conditions*

We used two water treatments for the two experiments. For the genotype experiment (#1), we cultured and tested hosts in filtered lake water (A/E, 1.0 μm pore size). We reared hosts at 20 °C and fed chemostat-grown algal food daily (1.0 mg L\(^{-1}\) dry weight of *Ankistrodesmus falcatus* reared in WC media). In the pollution experiment (#2), hosts were maintained and tested in high hardness COMBO without EDTA, a synthetic media that successfully supports both algae and zooplankton growth, at 20 °C (Kilham 1998). We reared fungal spores *in vivo* in a single host clone of *D. dentifera* (Ebert 2005).

(ii) *General experimental procedures*

Both experiments followed the same general procedures. Each used a set or subset of clonal genotypes of focal host *D. dentifera* and another host *Ceriodaphnia sp*. We collected
neonate hosts over 24 hours, six days prior to each experiment, to ensure similarity in age and size. We placed six-day-old individuals into culture tubes containing 20 ml of media (filtered lake water or COMBO) at 20 °C and added algal food (1.0 mg dry mass L⁻¹), freshly harvested fungal spores, and copper (Exp. #2). We exposed hosts to parasites for 20 (Exp. #1) and 6 (Exp. #2) hours in darkness at 20 °C on a tube rotator (2 rpm) to continuously resuspend spores and algae. Following exposure, hosts were transferred to new water containing no spores and abundant algal food (1.0 mg dry mass L⁻¹ d⁻¹). Upon host removal, we used in vivo fluorimetry to calculate concentration of algae following the assay. We also measured the fluorescence of several Daphnia-free replicates in each experiment to estimate the initial concentration of algae. After maintaining individuals for 12 days, we visually diagnosed infections at 40X using a dissecting microscope (Ebert 2005).

(iii) Experiment #1 (Genotype)

First, we assessed genetic variation for transmission, exposure, and susceptibility. We used four genotypes of the focal host (STD, Bristol-10, A4-5, and A4-3) and one co-occurring species (Ceriodaphnia sp.). Clonal isolates were collected from several lakes (Baker, Bristol, and Warner) in Barry County, Michigan, USA. Within each genotype, individuals were fed a constant concentration of algae (1.0 mg L⁻¹ dry weight) and exposed to one of two densities of spores (100 or 400 spores mL⁻¹). The number of replicates within each spore density was determined strategically, based on the genotypes’ known historical vulnerability. For more vulnerable genotypes, 15 replicates were tested at 100 spores mL⁻¹ and 5 replicates were tested at 400 spores mL⁻¹. For less vulnerable genotypes, we allocated more replicates to higher spore levels, i.e., 5 replicates at 100 spores mL⁻¹ and 15 replicates at 400 spores mL⁻¹. Additionally, we
used 16 control replicates (8 at each spore density) containing no hosts to measure algal concentrations in ungrazed conditions. All replicates were maintained for 20 hours prior to host removal and fluorimetry.

(iv) *Experiment #2 (Pollution)*

We then determined how a common environmental pollutant affected transmission, exposure, and susceptibility. A single focal host genotype, Bristol-6, was used to partition transmission under the influence of a pollutant, copper. Sixty-four replicates of hosts were fed a constant concentration of algal food (1.0 mg L\(^{-1}\) dry weight) and exposed to 150 spores/mL. We then added a gradient of copper as copper sulfate pentahydrate (CuSO\(_5\)H\(_2\)O), a common aquatic algicide and pesticide (Dorsey et al. 2004). Environmentally realistic concentrations of 5, 10, 15, and 20 μg Cu L\(^{-1}\) were used, each with 16 replicates. We also added 4 replicates of ungrazed controls for each copper density. All replicates were maintained for 6 hours prior to host removal and fluorimetry.

**Statistical analysis**

We parameterized the transmission model using maximum likelihood estimation. We used an integrated modeling approach based on copulas (Trivedi and Zimmer 2005; Song et al. 2009) to simultaneously fit the transmission model to data on infection status, \(I(t)\), and final algal density, \(A(t)\), for each experiment. We used likelihood ratio tests to evaluate significance of genotype (experiment 1) or copper (experiment 2) on the focal parameters (\(u\) and \(f\)). The model simultaneously predicts infection prevalence, \(\widehat{P}(t)\), as well as the density of algae, \(\widehat{A}(t)\) and spores, \(\widehat{Z}(t)\) remaining. These quantities depend on the initial densities of susceptible hosts, \(S_0\),
free-living spores, $Z_0$, algae, $A_0$, and exposure duration, $t$:

$$\overline{P}(t) = 1 - \exp\left(\frac{uZ_0}{S_0}\exp(-fS_0t - 1)\right)$$  \hspace{1cm} \text{Equation 2}

$$\overline{Z}(t) = Z_0 \exp(-fS_0t)$$  \hspace{1cm} \text{Equation 3}

$$\overline{A}(t) = A_0 \exp(-fS_0t)$$  \hspace{1cm} \text{Equation 4}

Combining datasets on infection and foraging (exposure) facilitates better parameter estimates, especially because infection data alone cannot be used to separately infer the exposure rate, $f$, and susceptibility, $u$. However, these bivariate outcomes (infection status and final algal density) are not independent. For example, an individual host that consumes more parasites could be more likely to become infected. Therefore, we must account for their potential correlation in order to accurately estimate their joint likelihood. We account for this correlation by using a Gaussian copula (Song et al. 2009). Copulas link univariate marginal distributions by specifying a correlation between their cumulative density functions. For bivariate cases, the Gaussian copula has a single parameter, $\rho$, which species the correlation between the cumulative density functions between the two univariate, marginal functions. Ignoring this correlation incorrectly overestimates the information content of each pair of observations and increases the chance of Type-I error (Song et al. 2009).

Next, we built the joint likelihood function for these bivariate observations. The key aspect of this likelihood function is that it accounts for the potential correlation between foraging data and infection status (see Song et al. 2009 for an explicit derivation of this likelihood function). First, we specified the two univariate distributions for observations of infection status, $I(t)$, and the final density of algae, $A(t)$. For our analyses, we assumed that infection status ($0 =$ uninfected, $1 =$ infected) followed a Bernoulli distribution. We also assumed that the density of algae remaining was log-normally distributed with a common standard deviation across all
treatments. Therefore, \( \log(A(t)) \), should be normally distributed with the common standard
deviation, \( \hat{\sigma} \). We log-transformed the algal density data, and updated Equation 4:
\[
\log(\overline{A}(t)) = \log(A_0) - fS_0 t
\]
Equation 5
Thus, the estimation procedure becomes a joint regression analysis for mixed normal and binary
outcomes (Song et al. 2009). The joint likelihood for the bivariate response vector for each
replicate, \( L(\log(A(t)), I(t) ) \), follows:
\[
L(\log(\overline{A}(t)), I(t)) = \begin{cases}
\varphi\left(\log(\overline{A}(t)), \log(\overline{A}(t)), \hat{\sigma}\right) \left(1 - C^*(P(t), Z_A|\rho)\right) & \text{if } I(t) = 0 \\
\varphi\left(\log(\overline{A}(t)), \log(\overline{A}(t)), \hat{\sigma}\right) \left(C^*(P(t), Z_A|\rho)\right) & \text{if } I(t) = 1 
\end{cases}
\]
Equation 6
Here, \( \varphi(a, b, c) \) represents the probability density of observation \( a \) based on the normal
distribution with mean \( b \) and standard deviation \( c \). Additionally,
\[
Z_A = \left[\log(A(t)) - \log(\overline{A}(t))\right] / \hat{\sigma},
\]
the standard deviation of the observed algal density from the predicted value. Lastly, \( C^*(x, y|z) = \Phi\left(\frac{\varphi^{-1}(x) - yz}{\sqrt{1-z^2}}\right) \), where \( \Phi \) represents the cumulative density
function for the standard normal distribution and \( \Phi^{-1} \) is the inverse of that function.

Using this likelihood function, we fit the transmission model to both datasets from each
experiment. We estimated the parameters of the transmission model by minimizing the negative
log-likelihood summed over all of the replicates, \( \mathcal{L} = -\Sigma \log(L) \), using the optim function in the R
Statistical Computing Language (R Development Core Team 2008). For experiment 1, we also
fit models in which the focal parameters, \( u \) and \( f \), varied among genotypes. In experiment 2, we
fit models in which these parameters became linear functions of the external copper
concentration, e.g., \( u(\text{[Cu]}) = u_0 + u_{\text{Cu}}\text{[Cu]} \). We tested for the significant effects of these factors
using likelihood ratio tests (Hilborn and Mangel 2013).
3. RESULTS

(a) Experiment 1 (Genotype)

(i) Exposure ($f$), susceptibility ($u$), and transmission ($\beta$)

Exposure rate ($f$) of hosts to parasites significantly varied among the five genotypes (likelihood ratio test: d.f. = 4, $P = 9 \times 10^{-12}$; figure 1a). A4-5, the genotype with the highest exposure rate (0.0171 L host$^{-1}$ day$^{-1}$), was exposed to approximately 3.5x as many parasites as Ceriodaphnia, the genotype with the lowest exposure rate (6.40x10$^{-3}$ L host$^{-1}$ day$^{-1}$).

Susceptibility ($u$) also varied significantly among the clones used (likelihood ratio test: d.f. = 4, $P = 1 \times 10^{-7}$; figure 1b). Combined, variation in both traits together produced very large variation among genotypes for overall transmission rate ($\beta$) (likelihood ratio test: d.f. = 4, $P = 3 \times 10^{-13}$; figure 1c). Transmission between the most vulnerable genotype (A4-5) and the least vulnerable (Ceriodaphnia sp.) ranged 56-fold.

(b) Experiment 2 (Pollution)

(i) Exposure ($f$), susceptibility ($u$), and transmission ($\beta$)

Copper significantly reduced the exposure rate ($f$) of Daphnia to parasites (likelihood ratio test: d.f. = 1, $P = 2 \times 10^{-8}$; figure 2a). The exposure rate in uncontaminated conditions was 0.024 L host$^{-1}$ day$^{-1}$, but in the most contaminated conditions (20 μg Cu L$^{-1}$), it was halved. However, copper significantly increased susceptibility ($u$) to infection (likelihood ratio test: d.f. = 1, $P = 0.0016$; figure 2b). Along the copper gradient, susceptibility experienced a 5-fold increase. This large increase in susceptibility overwhelmed the decrease in exposure. Thus, the overall transmission rate ($\beta$) significantly increased 2.5-fold across the gradient of copper
(likelihood ratio test: d.f. = 1, $P = 0.05$; figure 2c).

4. DISCUSSION

**Experiment #1 (Genotype)**

Host genotypes often vary in vulnerability and resistance to lethal parasites. Here, we saw that overall transmission ($\beta$) varied with clonal genotype. The focal host genotype Bristol-10 and the co-occurring host *Ceriodaphnia* had relatively low overall transmission rates compared to the three others (STD, A4-3, and A4-5). These two most resistant clones (lowest $\beta$) also had the lowest feeding rate ($f$). The transmission partition revealed an approximate 4-fold difference between the exposure rates of A4-5 and Ceriodaphnia. Susceptibility, however, had a larger influence on transmission; susceptibility, rather than exposure, more closely matched the overall pattern of transmission. Furthermore, there was approximately a 24-fold difference between the high and low values of susceptibility. However, it remains unknown if these clones represent *Daphnia* populations at large – in natural populations. Future work could determine whether natural genetic variation is greater for exposure or susceptibility.

Genetic variation in exposure and susceptibility matters for a rapid evolution of hosts during epidemics. For example, variation in exposure could drive key trade-offs among hosts, such as fecundity vs. resistance (Hall et al. 2010). Fast feeders have high exposure to parasites (thus, high transmission rate) but also can acquire food resources quickly, thus having high birth rates (Hall et al. 2007, 2009, 2010). This feeding rate-generated tradeoff means that hosts can evolve towards increased resistance during large epidemics, despite the fecundity-related costs stemming from slow feeding. Although, during small epidemics, hosts can evolve toward increased transmission rate due to the fecundity advantages of fast feeding (Boots and Haraguchi...
1999; Boots et al. 2009; Duffy et al. 2012). However, among the subset of clones here, variation in susceptibility, rather than exposure rate, played a larger role in shaping transmission. Such large variation in susceptibility could undermine a feeding-based fecundity tradeoff.

Nevertheless, susceptibility itself could correlate with fecundity of other key life history traits (thereby preserving tradeoffs among genotypes). Thus, the genetic (co-)variation among these key could greatly influence evolutionary response of hosts during epidemics.

Genetic variation in transmission-related traits also matters for the relationship between host diversity and disease risk. The dilution effect hypothesis proposes that resistant, non-host species that divert infectious propagules (or bites from disease vectors, i.e., “diluters”) could reduce disease spread in populations of susceptible hosts (Keesing et al. 2006; Hall et al. 2009; Thieltges et al. 2009). Thus, management strategies to control disease might exploit good diluters by enhancing their density. Our framework can identify great diluters – they should have high rates of exposure ($f$) and extremely low susceptibility ($u$). For instance, *Ceriodaphnia* consume a moderate number of parasites (moderate exposure), but rarely become infected (low susceptibility) by the fungus. Thus, *Ceriodaphnia* might serve as a good diluter. The Bristol-10 genotype of the focal host shared similar traits, so the presence of genotypes like Bristol-10 might also reduce the size of fungal epidemics within host populations. However, effects of other interactions among diluters and focal hosts must be assessed as well. For instance, although less susceptible hosts may sequester spores, they simultaneously compete with other species for resources such as algal food (Hall et al. 2009). Given negative consequences of competition, introduction of diluter-competitors may result in a net harm on densities of focal hosts, regardless of any disease control benefits that may (or may not) accompany diluters. Managers must weigh this risk when considering the addition of diluters, but the transmission partition can
help.

**Experiment #2 (Pollution)**

A gradient of an environmental pollutant, copper, also altered transmission of the focal host. More specifically, copper increased transmission rate. However, this increase reflected the net interplay between the opposing effects on exposure and susceptibility. As copper dosages increased, exposure decreased. Previous studies on filter-feeding invertebrates like *Daphnia* have found reduced feeding rate with pollution (Jones et al. 1991; Allen et al. 1995). In another experiment with a different focal genotype, however, copper increased exposure due to stimulated consumption of parasites (Civitello et al. 2012). Thus, it remains to be seen if feeding response of hosts depends strongly on host genotype (e.g., Agra et al. 2011). Here, however, the increase in susceptibility outweighed the decrease in exposure, i.e., exposure dropped 2-fold (Figure 2c) but susceptibility increased 5-fold (Figure 2b). This increase in susceptibility may reflect immunotoxicity as seen in another *Daphnia* systems (Little and Killick 2007) and more broadly among aquatic hosts (Parry and Pipe 2004; Yeh et al. 2004). Thus, while both traits determine the overall effect of a pollutant on transmission, susceptibility dominated here.

These results have two implications for hosts inhabiting polluted or otherwise stressful habitats. First, our results may help to explain why more contaminated environments have more disease (Rohr et al. 2008; Van Bressem et al. 2009). In the example shown here, the host’s response of susceptibility overpowered the response of exposure. Many contaminants suppress host immunity, and these immune effects may often outweigh decreased exposure to increased risk of infection (Galloway and Handy 2003). Yet, in other cases, we may find that exposure wins – and perhaps in these cases, disease may decrease with pollution. Second, the pull between
exposure and susceptibility may speak to a broader response of transmission components to environmental stressors. For instance, another example involving kairomones (infochemicals) also illustrates this tension between exposure and susceptibility. *Daphnia* hosts responded to fish kairomones by decreasing their body size (Stibor and Lüning 1994; Bertram et al. in press). Smaller hosts have lower exposure (Hall et al. 2007; Bertram et al. in press). However, hosts contacting fish kairomones had increased susceptibility after accounting for exposure. In that example, decreased exposure and increased susceptibility net canceled each other (Bertram et al. in press). The bottom line, though, is that the transmission partition can always help identify and quantify stressor-driven tension between exposure and susceptibility.

As disease increases in many ecosystems, environmental managers continue to focus on control strategies (Lafferty et al. 2004; Van Bressem et al. 2009). Our results and the quantitative technique involved could contribute towards these efforts by focusing on transmission rate, a key trait involved in spread of outbreaks. Overall, the model framework constructed here disentangles transmission into exposure and susceptibility components. With this kind of information, managers might harness species interactions to depress prevalence of parasites using a dilution effect. The transmission partition might help screen for species or host genotypes with ideal traits (high exposure, low susceptibility) to reduce disease through a dilution effect (Ostfeld and Keesing 2000; Keesing et al. 2006). Additionally, the method can quantify how and why components of transmission rate could respond to environmental contaminants but likely also other stressors. With this information, managers can better prepare for increasing disease in a changing world.

5. ACKNOWLEDGMENTS
We thank for A. Strauss for his help with laboratory experiments. The Indiana University BSES program and the NSF generously supported this research (DEB 1120316). The findings and conclusions expressed herein are those of the authors and do not necessarily reflect the views of the National Science Foundation.

6. LITERATURE CITED


Boots, M., A. Best, M. R. Miller, and A. White. 2009. The role of ecological feedbacks in the evolution of host defence: what does theory tell us? Philosophical Transactions of the


Ebert, D. 2005. Ecology, Epidemiology, and Evolution of Parasitism in Daphnia [Internet].


Figure 1. The “transmission partition” and genotypic variation among the four *Daphnia* host genotypes A4-5, STD, A4-3, and Bristol-10 and a co-occurring host *Ceriodaphnia* sp. (a) Host genotypes varied with per capita foraging rate \( (f) \). (b) These hosts, however, varied nearly 24-fold in per parasite susceptibility \( (u) \), which was well correlated with \( \beta \). (c) Overall, host genotypes varied in their vulnerability to infection, as indexed by the density-dependent transmission rate \( (\beta) \). It is clear that \( u \) was better correlated with overall transmission rate than \( f \). Dots are point estimates with 95% profiled confidence intervals.
Figure 2. Influence of a gradient of copper sulfate on exposure, susceptibility, and overall transmission. (a) Copper caused exposure rate (f) to spores to decrease. (b) This decrease, however, is paired with an increase in per parasite susceptibility (u). (c) Overall, copper contamination increased host transmission rate (β). Thus, copper’s positive effect on susceptibility overwhelmed the negative effect on exposure, leading to higher overall transmission. Dots are point estimates with 95% profiled confidence intervals.