Title: Resistance, tolerance and environmental transmission dynamics determine host extinction risk in a load-dependent amphibian disease

Running title: Resistance, tolerance and host extinction

Authors: Mark Q. Wilber[1,a], Roland A. Knapp[2,b], Mary Toothman[1,c], Cheryl J. Briggs[1,d]

Author affiliations
1. Ecology, Evolution and Marine Biology, University of California, Santa Barbara, Santa Barbara, CA, 93117
2. Sierra Nevada Aquatic Research Laboratory, University of California, Mammoth Lakes, CA 93546

Author emails
a. mark.wilber@lifesci.ucsb.edu
b. roland.knapp@lifesci.ucsb.edu
c. mary.toothman@lifesci.ucsb.edu
d. cherie.briggs@lifesci.ucsb.edu

Corresponding author
Mark Wilber
mark.wilber@lifesci.ucsb.edu, 805-893-2888
Ecology, Evolution and Marine Biology, University of California, Santa Barbara
Santa Barbara, CA, 93117

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Abstract

While disease-induced extinction is generally considered rare, a number of recently emerging infectious diseases with load-dependent pathology have led to extinction in wildlife populations. Transmission is a critical factor affecting disease-induced extinction, but the relative importance of transmission compared to load-dependent host resistance and tolerance is currently unknown. Using a combination of models and experiments on an amphibian species suffering extirpations from the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), we show that while transmission from an environmental Bd reservoir increased the ability of Bd to invade an amphibian population and the extinction risk of that population, Bd-induced extinction dynamics were far more sensitive to host resistance and tolerance than to Bd transmission. We demonstrate that this is a general result for load-dependent pathogens, where non-linear resistance and tolerance functions can interact such that small changes in these functions lead to drastic changes in extinction dynamics.

Introduction

Disease-induced extinction of a host population is generally considered rare (De Castro & Bolker 2005; Smith *et al.* 2006; McCallum 2012). This is because in many systems disease transmission is an increasing function of the density of infected hosts (i.e. density-dependent transmission) such that decreasing host density reduces disease transmission and prevents disease-induced extinctions (McCallum & Dobson 1995; Gerber *et al.* 2005). Theoretical models suggest that to drive a host population extinct, a disease needs to have alternative transmission dynamics such that declining host density does not prevent further disease transmission (De Castro & Bolker 2005; Smith *et al.* 2006; McCallum *et al.* 2009). For example, frequency-dependent transmission, in which transmission is proportional to the frequency of infected individuals (McCallum *et al.* 2001), or abiotic/biotic reservoirs for the disease-causing organism are two possible transmission scenarios that can lead to disease-induced host extinction. In both of these cases, decreasing host density does not necessarily lead to a decrease in disease transmission. Despite the general rarity of disease-induced host extinction (Smith *et al.* 2006), a number of wildlife diseases, such as chytridiomycosis in amphibians, white-nose syndrome in bats, and facial tumor disease in Tasmanian devils, have recently been identified as the causative agents of host declines and population extinctions (Skerratt *et al.* 2007; Blehert *et al.* 2008; McCallum *et al.* 2009).

While the characteristics of the transmission function in these emerging infectious diseases may ultimately determine whether a population experiences disease-induced extinction (McCallum 2012), extinction dynamics will also be influenced by how resistant (i.e. the ability of a host to reduce or

eliminate disease conditional on exposure; Boots *et al.* 2009; Medzhitov *et al.* 2012) and/or tolerant (i.e. the ability of a host to persist with a disease load that is typically lethal for non-tolerant individuals; Roy & Kirchner 2000; Medzhitov *et al.* 2012) a host is to the disease. For example, if a particular host phenotype is more tolerant to a disease than another it will experience a lower rate of disease-induced mortality and a slower decline to either extinction or a population size of conservation concern. Therefore, when managing disease-induced declines and extinctions, it may be important to manage not only for the transmission dynamics, but also the level of host tolerance and resistance in a population (Kilpatrick 2006; Langwig *et al.* 2015, 2017; Epstein *et al.* 2016). However, the conditions under which it might be more effective to manage for resistance and tolerance instead of transmission are currently unknown.

Understanding the relative importance of resistance and tolerance compared to transmission in driving extinction dynamics has important implications for managing the emerging amphibian disease chytridiomycosis. Chytridiomycosis is caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd) and has resulted in widespread amphibian declines and extinctions (Daszak *et al.* 2003; Skerratt *et al.* 2007). Bd is a cutaneous fungus that disrupts the osmoregulatory ability of amphibian skin, leading to the potentially fatal disease chytridiomycosis (Voyles *et al.* 2007, 2009). While a number of transmission-related factors, including an environmental pool of Bd zoospores and biotic reservoirs, are hypothesized to contribute to disease-induced extinction of amphibian populations (Rachowicz & Briggs 2007; Mitchell *et al.* 2008; Briggs *et al.* 2010; McCallum 2012; Doddington *et al.* 2013), few studies have attempted to quantify the transmission function itself (but see Rachowicz & Briggs (2007) and Bielby *et al.* (2015)). Quantifying the transmission function is important when considering disease-induced extinction because it determines the ability of a pathogen to invade a population as well as drive a host population extinct (Gerber *et al.* 2005).

In addition to the transmission function, extinction dynamics in amphibian-Bd systems also depend on the dynamics of Bd load on an amphibian host (Briggs *et al.* 2010). Varying Bd load dynamics among amphibian populations, potentially induced by varying resistance and tolerance mechanisms, can promote population-level persistence following epizootics (Briggs *et al.* 2010; Savage *et al.* 2015; Grogan *et al.* 2016; Savage & Zamudio 2016). This variation in resistance and tolerance could be due to a number of different mechanisms including innate and acquired immune responses (Ellison *et al.* 2015), differences in host susceptibility (Knapp *et al.* 2016), variation in the amphibian microbiome (Harris *et al.* 2009; Jani & Briggs 2014), temperature-dependent Bd growth (Knapp *et al.* 2011), and variable virulence in Bd strains (Rosenblum *et al.* 2013). Load dynamics are important in Bd systems because disease-induced mortality of amphibians is highly load-dependent, with survival probability sometimes decreasing rapidly at high Bd loads (Stockwell *et al.* 2010; Vredenburg *et al.* 2010). This attribute of

load-dependent survival leads to a simple, but largely untested hypothesis in host-pathogen systems: host populations that are either able to prevent large increases in pathogen load (via resistance mechanisms) or tolerate high pathogen loads (via tolerance mechanisms), will experience reduced disease-induced extinction risk, even when the transmission rate is high. This is a general hypothesis for load-dependent wildlife diseases and amphibian-Bd interactions provide an ideal system in which to test it.

The above hypothesis can be phrased as the following question: How important is transmission compared to host tolerance and resistance for mitigating disease-induced extinction? Answering this question requires robustly quantifying the transmission function, something rarely done in amphibian-Bd systems (Kilpatrick *et al.* 2010). Moreover, understanding the role of this transmission function in the ability of Bd to invade an amphibian population will be important for accurately understanding any subsequent Bd-induced extinctions (Gerber *et al.* 2005). Therefore, we also ask two additional questions: What is the nature of the transmission function in amphibian-Bd systems? How does this transmission function affect the ability of Bd to invade an amphibian population? We use a combination of experiments and dynamical models to show that empirical patterns of Bd transmission are best modeled using an environmental Bd pool and that this pool significantly increases the ability of Bd to invade a population and the population-level extinction risk. However, despite this large effect of the environmental pool, we show that host resistance and tolerance are far more influential on Bd-induced extinction dynamics than transmission. This is likely a general property of load-dependent diseases in which non-linear resistance and/or tolerance functions interact such that managing for resistance and tolerance can be more effective for mitigating disease-induced extinction than managing for transmission.

Methods

To answer the questions posed above, we focused on Bd-induced extinction dynamics in the Mountain yellow-legged frog complex (*Rana muscosa* and *Rana sierrae*, henceforth *R. muscosa*). *R. muscosa* are native to California's Sierra Nevada mountains and have experienced significant Bd-induced population declines and extinctions over the last four decades (Briggs *et al.* 2005; Vredenburg *et al.* 2010; Briggs *et al.* 2010; Knapp *et al.* 2016). Using this host-parasite system, the *Methods* section is organized as follows.

First, we used a laboratory experiment to quantify the well-known importance of temperature-dependent Bd growth dynamics on *R. muscosa* (Andre *et al.* 2008; Wilber *et al.* 2016). Second, we used a mesocosm experiment to quantify the nature of the transmission function in the *Rana muscosa*-Bd system, testing for both density-dependent transmission, frequency-dependent transmission, and transmission from an environmental zoospore pool. Third, we used the results from these experiments

to build a discrete-time, host-parasite Integral Projection Model (IPM) and derived R_0 with and without an environmental zoospore pool. We used this result to explore the effects of the zoospore pool on Bd invasion. Finally, to answer our primary question regarding the relative importance of transmission compared to resistance and tolerance on extinction risk, we extended our IPM model to consist of a within-year component in which Bd transmission and disease-induced amphibian mortality occurred and a between-year component in which *R. muscosa* demographic transitions occurred (Figure 1). Using this hybrid model, we explored the sensitivity of Bd-induced extinction to transmission, resistance, and tolerance.

Laboratory and mesocosm experiments

We used the laboratory and mesocosm experiments to quantify the temperature-dependent load dynamics as well as the transmission dynamics in the *R. muscosa*-Bd system. The laboratory experiment is fully described in Wilber *et al.* (2016) and consists of 15 adult frogs housed at 3 different temperatures (4 °C, 12 °C, 20 °C; 5 frogs per temperature). In Wilber *et al.* (2016) we used this experiment to parameterize four functions relating to Bd load dynamics: a load-dependent host survival function as well as a temperature-dependent Bd growth function, loss of infection function, and initial infection function (Appendix Fig. 1, see Table 1 for function descriptions).

To quantify the nature of the transmission function in this system, we performed an additional mesocosm experiment that consisted of four different density treatments: 1, 4, 8, and 16 uninfected adult frogs per mesocosm (volume = 1 m^3). Each treatment was replicated four times for a total of 16 mesocosms. In addition to the uninfected adults, each mesocosm started with five infected tadpoles, which could release Bd zoospores into the environment and subsequently infected adults. All of the adults in a mesocosm were uniquely identifiable by pit tags, but the five tadpoles were not. The experiment ran for 74 days and every 4-8 days all adults and tadpoles in a mesocosm were swabbed and the zoospore load on each was determined using quantitative PCR (Boyle *et al.* 2004). Frogs and tadpoles within a mesocosm were always swabbed on the same day.

To estimate the transmission function, we measured the load transitions on all adult frogs from time t to $t+\Delta t$ over the first 32 days of the experiment ($\Delta t = 4-8$ days depending on the time between swabs in the experiment). We only used the first 32 days of the experiment as after this time point all amphibians experienced an unexplained decline in zoospore loads (see Appendix Fig. 2). However, because the load trajectories over these first 32 days were consistent with other lab experiments (e.g. Wilber *et al.* 2016) and transitions from uninfected to infected tended to occur before day 32, we felt confident in estimating transmission dynamics from only the first 32 days. As transmission is the probability of an uninfected

individual gaining an infection in a time step, we only included transitions where Bd load was 0 at time t ($n = 333$). If the load at time $t + \Delta t$ was positive we assigned this data point a value of 1 (infected) and if the load was still zero we assigned it a value of 0 (uninfected).

Uninfected frogs can acquire Bd infection through contact with other infected frogs and through contact with zoospores in an environmental Bd pool (Courtois *et al.* 2016). To account for these different pathways, we fit a number of transmission functions to the data. The first set of models we fit assumed a constant level of infection from the zoospore pool as well as either density-dependent or frequency-dependent transmission from conspecifics (Table 2). The second set of models allowed transmission to be a function of how many zoospores were in the zoospore pool at time t in addition to either density-dependent or frequency-dependent transmission. In Appendix 1, we provide a full account of how we defined and fit our transmission models that accounted for the dynamic zoospore pool. In short, we used the transmission function $\phi(t) = 1 - \exp(-\Lambda(Z(t), I(t))\Delta t)$ and allowed for the zoospore pool $Z(t)$ at time t to be an unobserved, dynamic variable that lost zoospores due to environmental decay and gained zoospores due to production from infected adults and tadpoles at every time step. $I(t)$ is the number of infected adults at time t. Table 2 gives the transmission functions and the resulting fits to the data from the mesocosm experiment.

The host-parasite IPM and R_0

The host-parasite IPM

Using the aforementioned laboratory and mesocosm experiments, we parameterized a host-parasite Integral Projection Model (IPM) where Bd load on an individual frog was the continuous trait being modeled (Fig. 1B; Metcalf *et al.* 2015; Wilber *et al.* 2016). Bd load on a frog is estimated as the number of copies of Bd DNA detected on standardized skin swabs via quantitative PCR (Boyle *et al.* 2004) and provides a continuous measure of infection intensity between 0 (uninfected) and an arbitrarily large Bd infection. The IPM is a discrete time model and here a single time step is three days. This time step is on the same scale as the generation of time of Bd, which ranges between 4-10 days depending on temperature (Woodhams *et al.* 2008). We used the discrete-time IPM because it is easily parameterized from laboratory data which is collected at discrete time intervals.

This IPM tracks two discrete stages at time t. These stages are the density of susceptible adults $S_A(t)$ in the population and the density of zoospores in the environment $Z(t)$. This model also tracks a continuous, infected stage $I_A(x, t)$ where x is ln Bd load and $\int_{L_x}^{U_x} I_A(x, t)dx$ gives the density of adult frogs with a ln Bd load between a lower bound L_x and an upper bound U_x at time t. This continuous, infected stage tracks the distribution of Bd loads at any time t in the population.

Considering these discrete and continuous stages, the amphibian-Bd IPM can be written as follows (Fig. 1B)

$$S_A(t+1) = S_A(t)s_0(1-\phi) + \int_{L_x}^{U_x} I_A(x,t)s_A(x)l_A(x)dx \quad (1)$$

$$I_A(x',t+1) = \int_{L_x}^{U_x} I_A(x,t)s_A(x)(1-l(x))G(x',x)dx + S_A(t)s_0\phi G_0(x') \quad (2)$$

$$Z(t+1) = Z(t)\nu + \mu_A \int_{L_x}^{U_x} \exp(x)I_A(x,t)dx - \psi(S_A(t), Z(t)) \quad (3)$$

$S_A(t+1)$ describes the density of uninfected (susceptible) adults at time $t+1$ and is determined by the number of adults that survive and do not become infected in a time step (first term in equation 1) and the number of infected adults that survive and lose their infection in a time step (second term in equation 1). $I_A(x', t+1)$ describes the density of infected adults with a ln Bd load x' at time $t+1$ and is determined by infected adults who survive with load x, do not lose their load x, and experience a change in load from x to x' in a time step (first term in equation 2) and from uninfected adults who survive, become infected, and gain an initial Bd load of x' in a time step (second term in equation 2). The vital rate functions contained in equations 1-3 are described in Fig. 1B and Table 1.

The equation $Z(t+1)$ gives the density of zoospores in the zoospore pool at time $t+1$. $Z(t+1)$ depends on three distinct terms: the survival probability of the zoospores in the environment from time t to $t+1$ (ν), contribution of zoospores from infected adults where μ_A is the proportion of total zoospores on adults contributed to the zoospore pool over a time step, and removal of zoospores from the zoospore pool by frogs transitioning from uninfected to infected. This final removal term $\psi(S_A(t), Z(t))$ had very little effect on the dynamics of the system and we do not consider it further.

Based on the temperature-dependent laboratory experiment described above, we also allowed various vital rate functions in the IPM to be temperature-dependent (Table 1, Appendix Fig. 1).

R_0 with an environmental reservoir

Using the IPM described in equations 1-3, we calculated R_0 in order to quantify how temperature and the transmission dynamics affected the ability of Bd to invade a *R. muscosa* population – a necessary condition for Bd-induced population declines and extinction. R_0 describes the average number of secondary infections produced over the lifetime of an average infected agent (Diekmann *et al.* 1990; Dietz 1993). When $R_0 \leq 1$, a pathogen cannot invade a fully susceptible host population. When $R_0 > 1$, a pathogen can invade a fully susceptible host population with probability $1-(1/R_0)$ (Gerber *et al.* 2005; Allen 2015).

R_0 for continuous-time disease models with an environmental reservoir has been shown to be an additive result of transmission from host contact and infection from the environmental reservoir (Rohani *et al.* 2009). In Appendix 2, we extend this result to discrete-time IPMs with an environmental reservoir and analogously show that R_0 is combination of transmission from host contact and the environment. We use this result in combination with our fully parameterized host-parasite IPM to calculate the temperature-dependent R_0 for *R. muscosa*-Bd systems with and without infection from the environmental zoospore pool.

The hybrid model

The model described by equations 1-3 may be sufficient to describe the dynamics of an initial epizootic, but in order to examine Bd-induced extinction dynamics in *R. muscosa* populations a number of additions need to be made. We briefly describe the hybrid model that accounts for the within-year Bd dynamics in *R. muscosa* as well as the between year demography of *R. muscosa*. Fig. 1 gives a visual representation of the hybrid model and Appendix 3 gives a full description of the model.

The within-year component (Fig. 1B.), is identical to the IPM given in equations 1-3 with the addition of three tadpole stages. The tadpole stage of *R. muscosa* has been shown to play an important role in generating enzootic dynamics in *R. muscosa* populations (Briggs *et al.* 2005, 2010). We assumed all tadpoles were immediately infected with Bd and had a constant contribution to the zoospore pool. This is justified by the observation that most tadpoles in *R. muscosa* populations carry high fungal loads, even in enzootic populations (Briggs *et al.* 2010). *R. muscosa* tadpole survival is not affected by Bd infection. Therefore, the within-season dynamics of the tadpole stages were simply given by the probability of a tadpole surviving from time t to $t+1$. Infected tadpoles also contributed to the zoospore pool at each time step (Fig. 1B).

R. muscosa populations also experience seasonal temperature fluctuations in which winter lake temperatures drop to approximately 4 °C in the winter (in the unfrozen portion of a lake where the frogs overwinter) and reach approximately 20 °C in the summer (Knapp *et al.* 2011). We accounted for this seasonal variability by imposing a deterministically fluctuating environment on the *R. muscosa*-Bd IPM (Fig. 1A). At each discrete time point within a season, a new temperature was calculated based on the sinusoidal curve and the temperature-dependent vital rate functions were updated accordingly.

The between-year component of the hybrid model accounted for yearly maturation and metamorphosis of the tadpole stages as well as reproduction of adults (Fig. 1C). We assumed that the recruitment of metamorphed tadpoles into the adult stage was density-dependent and that all tadpoles entered the adult stage as uninfected (i.e. all individuals are infected as tadpoles, but lose their infection at meta-

morphosis). Because we have no empirical evidence for Bd-induced fertility reduction in *R. muscosa*, we assumed that reproduction in uninfected adults was the same as reproduction in infected adults.

Simulating the hybrid model

After parameterizing the hybrid model using the above experiments, we used the model to make predictions about the probability of disease-induced host extinctions. Because demographic stochasticity is particularly important when predicting extinction for small populations (Lande *et al.* 2003), we included it into the hybrid model (Caswell 2001; Schreiber & Ross 2016). To do this, we first discretized the within-season IPM using the mid-point rule and 30 mesh points (Easterling *et al.* 2000) and then determined the transition of an individual frog or zoospore to another state (including death) as a draw from a multinomial distribution with probabilities given by the discretized hybrid model at that time step (Appendix 4). In addition, we assumed that both the production of tadpoles that occurs once a year in the spring and the number of zoospores shed into the zoospore pool at each time step followed a Poisson distribution (Appendix 4).

To answer our question regarding the importance of transmission, resistance, and tolerance on Bd-induced extinction, we performed two analyses. First, we focused on transmission and examined how different transmission functions parameterized from our mesocosm experiment affected extinction risk. Using the three transmission functions with a dynamic zoospore pool described in Table 2 and the parameter values given in Table 1, we performed 500 stochastic simulations of the hybrid model to generate time-dependent extinction curves over a 25 year period. All simulations were started with 10 uninfected adult frogs, 85 T_1 tadpoles, 12 T_2 tadpoles, and 3 T_3 tadpoles. The relative proportions of adult frogs and tadpoles were assigned based on the stable stage distribution in the Bd-free model. While the initial conditions necessarily affect the absolute time to extinction, they do not affect the shapes of the extinction curves for different transmission functions relative to each other. All simulations started in the winter at 4 °C, with reproduction occurring in the first spring at 12 °C (Fig. 1A). Given our analysis of R_0 in the previous section, we assumed that Bd could invade with a probability of one, such that tadpoles were immediately infected with Bd and began contributing to the zoospore pool $Z(t)$. For each simulation, we calculated time-dependent extinction curves as the mean probability of going extinct in a given time step over all 500 simulations.

Second, we performed a sensitivity analysis on the hybrid model to assess the relative importance of transmission compared to resistance and tolerance. Transmission was determined by the parameters in the transmission function ϕ, resistance was determined by the parameters in the growth function $G(x', x)$, the loss of infection function $l(x)$, and the initial infection burden function $G_0(x')$, and tolerance was

determined by the parameters in the survival function $s(x)$. To perform the sensitivity analysis, we ran 1000 simulations using the parameter values given in Table 1 and the initial conditions described in the previous section. For each simulation we recorded whether or not a *R. muscosa* population went extinct in ≤ 8 years, as this was where extinction probability was approximately 50% with the default parameter values.

On each run of the simulation we perturbed sixteen lower-level transmission, resistance and tolerance parameters by, for each parameter, drawing a random number from a log normal distribution with median 1 and dispersion parameter $\sigma_{\text{sensitivity}} = 0.3$ and multiplying the given parameter by this random number (Sobie 2009). Our results were robust to our choice of σ and our method of perturbation (Appendix Fig. 3). For each simulation, we saved the perturbed parameter values and stored them in a 1000 by 16 parameter matrix. Upon completion of the simulation, we used both regularized logistic regression (using 5 fold cross validation to identify the optimal L2 regularization parameter) and a Random Forest classifier ($n = 1000$ bootstrapped trees with 4 randomly selected predictors) in which our response variable was whether or not a given simulation went extinct and our predictor variables were the scaled (i.e. z-transformed) matrix of perturbed predictors (Harper *et al.* 2011; Pedregosa *et al.* 2011; Lee *et al.* 2013). Using this approach, we could then identify the relative importance of each parameter in the vital rate functions in predicting whether or not extinction occurred (Sobie 2009). Moreover, we also built a pruned regression tree to visualize the interactive effects of transmission, resistance, and tolerance parameters on host extinction risk (Harper *et al.* 2011). All the code and data necessary for replicating the analysis are provided at X.

Results

Question 1: What is the structure of the transmission function?

Accounting for the dynamics of the environmental zoospore pool resulted in significantly better transmission models compared to those that did not. The transmission model with density-dependent host to host transmission as well as transmission from a dynamic zoospore pool was generally a better model than all other transmission models considered (Table 2, Appendix Fig. 4). While the frequency-dependent model was a worse model in terms of WAIC, it was able to capture the marginal pattern of increasing transmission with increasing infection prevalence (Appendix Fig. 5).

Question 2: How does the transmission function affect the ability of Bd to invade?

Using the temperature-dependent vital functions described in Table 1 (Appendix Fig. 1) and the best-fitting density-dependent transmission function with an environmental zoospore pool (Table 2), we examined how host density and temperature affected the ability of Bd to invade a *R. muscosa* population. When transmission was density-dependent, but did not depend on the environmental zoospore pool, Bd was able to invade *R. muscosa* populations over a large range of densities and temperatures, though there was a slight protective effect of low temperatures and low densities (Fig. 2A). Including transmission from the environmental zoospore pool substantially increased the region in which Bd could invade and invasion was highly probable for most temperatures and host density combinations (Fig. 2B, C).

Question 3: How sensitive is disease-induced extinction to transmission, resistance, and tolerance?

Using the hybrid model in combination with the three transmission functions that included a dynamic zoospore pool (Table 2), we found that the time-dependent probability of extinction was similar between these transmission functions (Fig. 3A). The similarity between these curves was due to the overwhelming influence of the infection probability from the zoospore pool, which swamped out the well-known differences between frequency-dependent and density-dependent transmission functions. Drastically decreasing zoospore survival probability below what has been observed in laboratory experiments (Woodhams *et al.* 2008), led to an expected reduction in extinction risk as the transmission probability then declined with decreasing host density (given density-dependent transmission, Fig. 3A).

A sensitivity analysis of Bd-induced host extinction to transmission, host resistance, and host tolerance showed that, regardless of the transmission function used, *R. muscosa* extinction was more sensitive to the parameters relating to host resistance and tolerance than to parameters relating to transmission (Fig. 4). In particular, the most important parameter across all transmission functions was the slope of the growth function $b_{1,1}$, which is a parameter affecting host resistance. For a given temperature, the logistic regression analysis showed that decreasing this parameter, which roughly corresponds to decreasing the mean Bd load on a host for a given temperature, decreased the probability of disease-induced extinction for all transmission functions (Fig. 4A-C). Bd-induced *R. muscosa* extinction was also sensitive to the parameters of the survival function, particularly the intercept of the survival function $b_{0,0}$. This parameter can be thought of as the threshold as which Bd-induced mortality begins to occur given a fixed slope in the survival function. The logistic regression analysis showed that increasing this param-

eter, which corresponds to increasing the threshold at which *R. muscosa* begins to suffer load-dependent Bd mortality, decreased the probability of extinction (Fig. 4A-C).

Resistance and tolerance parameters also showed significant interactions when affecting host extinction risk. Random forests and pruned regression trees showed the importance of the slope of the growth function (Fig. 4A-C) as well the importance of the interaction between this parameter and the intercept of the survival function (a tolerance parameter) and the temperature-dependency in the growth function (a resistance parameter) (Appendix Fig. 6A-C).

Discussion

Wildlife conservation in the face of disease emphasizes the importance of the transmission function in extinction risk (McCallum 2012). This is a reasonable emphasis as the transmission function is ultimately the most important aspect of disease-induced extinction: if a host does not get infected with a disease it will not suffer disease-induced mortality. In amphibian-Bd systems it has been hypothesized that both amphibian density and an environmental pool of zoospores can affect transmission (Rachowicz & Briggs 2007; Briggs *et al.* 2010; Courtois *et al.* 2016), but the nature of this transmission has rarely been quantified. We experimentally quantified the transmission function in the *R. muscosa*-Bd system and used these results, in combination with a dynamic model, to predict how the environmental zoospore reservoir affected the ability of Bd to invade an amphibian population. Consistent with previous theory (Godfray *et al.* 1999; Rohani *et al.* 2009), we found that including an environmental zoospore pool substantially increased R_0 for *R. muscosa*-Bd systems, such that Bd was able to invade a *R. muscosa* population for most realistic temperatures and host densities. To our knowledge, this is the first estimation of R_0 in an amphibian-Bd system, and the large value of R_0 across all temperatures and densities is consistent with field observations that temperature and density have little protective effect in the *R. muscosa/sierrae* system (Knapp *et al.* 2011, R. A. Knapp et al., unpublished). These results suggest that attempting to prevent Bd invasion into a system may be largely futile and management should be focused on mitigating post-invasion Bd impacts (Langwig *et al.* 2015).

Conditional on Bd invasion, we used our parameterized model to explore the importance of the transmission function on Bd-induced amphibian extinction and found that the extinction dynamics were similar between all transmission models with a dynamic zoospore pool. This was due to the large number of zoospores shed by infected amphibians combined with the laboratory-estimated decay rate of zoospores outside the host, leading to a zoospore pool that remained large even for rapidly declining host populations (Fig. 3B). Only considering this result, we would then expect *R. muscosa* populations to be at substantial risk of disease-induced extinction given that the persisting zoospore pool prevents a

decrease in transmission rate with declining host density (Anderson & May 1981; Godfray *et al.* 1999; De Castro & Bolker 2005). This finding is consistent with a number of other studies that have found that the dynamics of the Bd zoospore pool are critical for determining Bd-induced amphibian extinctions (Mitchell *et al.* 2008; Briggs *et al.* 2010; Doddington *et al.* 2013). If abiotic or biotic factors such as temperature, stream flow, water chemistry, and/or zoospore consumption by aquatic organisms are able to substantially increase zoospore death rate beyond the values seen in the lab (Tunstall 2012; Venesky *et al.* 2013) [Water chemistry citation?], then we might expect a reduction, though not an elimination, of amphibian extinction risk.

However, considering only the transmission function ignores the fact that, conditional on infection, increasing resistance or tolerance to a disease can also reduce disease-induced mortality and thus provide alternative mechanisms by which to manage disease-induced extinction risk (Kilpatrick 2006; Vander Wal *et al.* 2014; Langwig *et al.* 2015). Using our model, we found that Bd-induced extinction risk was far more sensitive to host resistance and tolerance than to the transmission dynamics of Bd. In particular, extinction risk of *R. muscosa* populations was most sensitive to the vital rate functions dictating the growth rate of Bd on a host, the load-dependent survival probability, and the interaction between these two functions.

This result highlights the importance of accounting for the load-dependent nature of vital rate functions when considering extinction risk in load-dependent diseases such as chytridiomycosis. In this study, consistent with results observed in the field (Vredenburg *et al.* 2010), the survival function of Bd was strongly non-linear such that above ≈ 9 ln zoospores $= 8103$ zoospores the survival probability of *R. muscosa* rapidly declined (Appendix Fig. 1B). When the survival function is load-dependent and highly non-linear, as observed in some amphibian-Bd systems (Stockwell *et al.* 2010; Vredenburg *et al.* 2010, but see Clare *et al.*), small changes in host resistance or tolerance can lead to abrupt changes in survival probability. In particular, non-linearities in the survival function (i.e. tolerance) need to be considered in the context of the shape of the growth function (i.e. resistance) of a parasite on its host.

To illustrate the generality of this result, consider the following graphical argument. Take a pathogen growth function ($G(x', x)$) that predicts a static mean pathogen load near the threshold at which the survival function predicts a drastic decrease in survival probability (Appendix Fig. 7). Slightly shifting the slope (or the intercept) of the growth function (i.e. changing resistance) up or down will increase or decrease the static mean pathogen load and move a host into the region of the survival curve where either mortality or survival is almost certain (Appendix Fig. 7). Similarly, holding the growth function constant and altering the survival function (i.e. changing tolerance) will change how close the static mean pathogen load is to the survival function threshold (Appendix Fig. 7). This suggests that identifying

how the growth function of a pathogen changes in resistant host populations, whether by decreasing the slope, decreasing the intercept or by transitioning from a linear to a non-linear function (Langwig *et al.* 2017), is important for understanding the sensitivity of extinction risk to both the growth function (resistance) and the survival function (tolerance). Identifying whether or not a disease system shows this strong interaction between resistance and tolerance can help determine whether disease mitigation should focus on reducing parasite loads via strategies such as inducing acquired immunity, microbial treatments, or selecting for resistance (Harris *et al.* 2009; Langwig *et al.* 2015) or decreasing parasite transmission via strategies such as culling and/or treating the disease reservoir (Cleaveland *et al.* 2001).

The importance of host resistance and tolerance for our model's predictions of disease-induced extinction indicates that these host strategies could promote population persistence in *R. muscosa*-Bd populations, as they have in other species of amphibians suffering from chytridiomycosis, bats suffering from white-nose syndrome, and Tasmanian devils suffering from facial tumor disease (Hoyt *et al.* 2016; Savage & Zamudio 2016; Epstein *et al.* 2016; Langwig *et al.* 2017). In fact, a recent study showed that many populations of *R. sierrae* that experienced Bd-induced population declines over the last four decades are recovering in the presence of Bd, but with reduced Bd loads relative to naive populations (Knapp *et al.* 2016). This result is generally consistent with resistance mechanisms reducing Bd load and thus increasing host survival probability. While changes in resistance mechanisms, and not tolerance mechanisms, are putatively responsible for persistence in a number of load dependent diseases (Savage & Zamudio 2011; Epstein *et al.* 2016; Langwig *et al.* 2017), our results show that the shape of the tolerance function is critical for understanding the effects of resistance on host persistence (Appendix Fig. 7).

While these results provide compelling empirical evidence for the importance of resistance mechanisms in preventing disease-induced extinction, it is still unclear how resistance mechanisms might emerge to rescue populations from Bd-induced extinction. One common hypothesis is that standing variation in resistance and/or tolerance traits upon Bd invasion could act to prevent Bd-induced host extinction (Orr & Unckless 2014; Savage *et al.* 2015; Savage & Zamudio 2016). Testing this hypothesis will require identifying the source of standing variation in resistance (Tunstall 2012) as well as its genetic basis and inheritance structure (Mazé-Guilmo *et al.* 2014; Ellison *et al.* 2015). Moreover, it will be important to identify any trade-offs between host resistance/tolerance mechanisms and host fitness, which can have enormous effects on the evolutionary dynamics of resistance and tolerance (Boots *et al.* 2009; Duffy & Forde 2009).

In conclusion, the emergence of a number of diseases of conservation concern, where the pathogen load critically influences host-parasite dynamics, have highlighted the importance of considering disease-induced extinction in the context of load-dependent parasite dynamics. We show that while the dynamics

of the environmental zoospore pool substantially increased the Bd invasion probability and host extinction risk, extinction dynamics were most sensitive to mechanisms affecting resistance and tolerance. We highlight the generality of this result across host-parasite systems by illustrating that non-linearities in load-dependent host-parasite vital rate functions can lead to mechanisms affecting host resistance and tolerance having larger effects on extinction risk than transmission mechanisms. Our results show that simultaneously considering transmission, resistance, and tolerance, in conjunction with load-dependent pathogen dynamics, provides important insight regarding how to best manage emerging infectious diseases.

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Table 1: Parameters used in the *Rana muscosa*-Bd hybrid model. All b and c parameters had a Normal prior with mean 0 and standard deviation 5. All ν parameters had a half-Cauchy prior from 0 to ∞ with scale parameter 1. For all statistical models, convergence was assessed using traceplots and ensuring that the Gelman-Rubin statistic was < 1.05 (Gelman *et al.* 2014). *logit* specifies a logistic link, x is ln zoospore load, and T is temperature. All probabilities are over a three day time step.

Description	**Functional Form**	**Parameters**	**Details of Parameterization**
Infected survival function, $s(x)$: Probability of host survival from t to $t+1$ with load x	$\text{logit}[s(x)] = b_{0,0} + b_{1,0}x$	$b_{0,0} = 5.295$ $b_{1,0} = -2.595$	*Likelihood* Bernoulli($s(x)$), x was z-transformed Appendix Fig. 1B.
Growth function, $G(x', x)$: Probability density of host transitioning from load x to load x' at time $t+1$	$\mu(x,T) = b_{0,1} + b_{1,1}x + b_{2,1}T$ $\sigma^2(x) = \nu_{0,1}\exp(2c_{0,1}x)$	$b_{0,1} = 0.012$ $b_{1,1} = 0.799$ $b_{2,1} = 0.092$ $\nu_{0,1} = 5.92$ $c_{0,1} = -0.049$	*Likelihood* Normal($\mu(x,T), \sigma^2(x)$) Appendix Fig. 1A.
Loss of infection function, $l(x)$: The probability of host having infection of load x at t and losing it by $t+1$	$\text{logit}[l(x,T)] = b_{0,2} + b_{1,2}x + b_{2,2}T$	$b_{0,2} = 1.213$ $b_{1,2} = -0.472$ $b_{2,2} = -0.151$	*Likelihood* Bernoulli($l(x,T)$) Appendix Fig. 1C.
Initial infection burden function, $G_0(x')$: Probability density of being uninfected at t and gaining an infection of x' at $t+1$	$\mu(T) = b_{0,3} + b_{1,3}T$ $\sigma^2(T) = \nu_{0,3}\exp(2c_{0,3}T)$	$b_{0,3} = 0.642$ $b_{1,3} = 0.137$ $\nu_{0,3} = 0.59$ $c_{0,3} = 0.063$	*Likelihood* Normal($\mu(T), \sigma^2(T)$) Appendix Fig. 1D.
Transmission function, ϕ: The probability of an uninfected host gaining an infection at time $t+1$	Functional forms vary	See Table 2	See Table 2
Uninfected adult survival probability over three day time step, s_0	Constant	$s_0 = 0.999$	Yearly survival from Briggs *et al.* 2005 converted to a three day time scale
Temperature-dependent zoospore survival probability, ν	$\nu = f(T)$	Non-parametric	Cubic smoothing spline based on data from Woodhams *et al.* 2008 Appendix Fig. 1E.
Proportion of zoospores contributed to Z by infected adult	Constant	$\mu_A = 1$	Likely a conservative estimate of how infected adults contribute to the zoospore pool
Mean tadpole zoospore load, μ_{T_i}	Constant	$\mu_{T_i} = 1487.036$	Mean zoospore load of tadpoles in mesocosm experiment described in text
Probability of tadpole i surviving a three day time step	Constant	$s_{T_1} = 0.987$; $s_{T_2} = 0.997$; $s_{T_3} = 0.997$	Yearly survival from Briggs *et al.* 2005 converted to a three day time scale
Probability of tadpole i not metamorphosing, p_{T_i}	Constant	$p_{T_1} = 1$; $p_{T_2} = 0.5$; $p_{T_3} = 0$	Briggs *et al.* 2005
Probability of tadpole i surviving metamorphosis, m_{T_i}	Constant	$m_{T_1} = 0.9$; $m_{T_2} = 0.9$; $m_{T_3} = 1.0$	Briggs *et al.* 2005
Probability of adult reproducing, p_A	Constant	$p_A = 0.25$	Briggs *et al.* 2005
Mean adult reproductive output, λ	Constant	$\lambda(x) = \lambda_0 = \lambda = 50$	Leads to realistic population-level growth rate of $\lambda_{\text{growth rate}} \approx 1.46$ in the disease-free, density-independent model (Briggs *et al.* 2005)
Carrying capacity parameter, K	Constant	$K = 5$	Leads to equilibrium adult densities between 10-15 adults per m^3 in disease-free model

Table 2: The results of fitting transmission functions of the form $\phi = 1 - \exp(-\Lambda)$ to the *R. muscosa*-Bd mesocosm experiment. I is the total number of infected adults in a mesocosm at the beginning of a time interval, A is the total number of adults in a tank, Z is the number of zoospores in the mesocosm at the beginning of the time interval as estimated from a latent zoospore pool model (Appendix 1), and Δt is the time between swabbing events in the experiment (between 4-8 days). All β parameters had a half-Cauchy prior from 0 to ∞ with scale parameter equal to 1. Models with lower WAICs and higher weights are better models.

Name	**Function**	**Parameters**	**WAIC (weight)**
Constant zoospore pool	$\Lambda = (\beta_0)\Delta t$	$\beta_0 = 8.07\text{x}10^{-2}$ day^{-1}	401.1 (0)
Density-dependent w/ constant zoospore pool	$\Lambda = (\beta_0 + \beta_1 I)\Delta t$	$\beta_0 = 4.18\text{x}10^{-2}$, $\beta_1 = 5.25\text{x}10^{-2}$	336.7 (0)
Frequency-dependent w/ constant zoospore pool	$\Lambda = (\beta_0 + \beta_1 \frac{I}{A})\Delta t$	$\beta_0 = 4.28\text{x}10^{-2}$, $\beta_1 = 0.551$	336.6 (0)
Dynamic zoospore pool	$\Lambda = (\beta_0 \ln(Z+1))\Delta t$	$\beta_0 = 1.09\text{x}10^{-2}$	345.32 (0)
Density-dependent w/ dynamic zoospore pool	$\Lambda = (\beta_0 \ln(Z+1) + \beta_1 I)\Delta t$	$\beta_0 = 5.29\text{x}10^{-3}$, $\beta_1 = 7.52\text{x}10^{-2}$	**301.18** (0.99)
Frequency-dependent w/ dynamic zoospore pool	$\Lambda = (\beta_0 \ln(Z+1) + \beta_1 \frac{I}{A})\Delta t$	$\beta_0 = 5.77\text{x}10^{-3}$, $\beta_1 = 0.627$	311.06 (0.01)

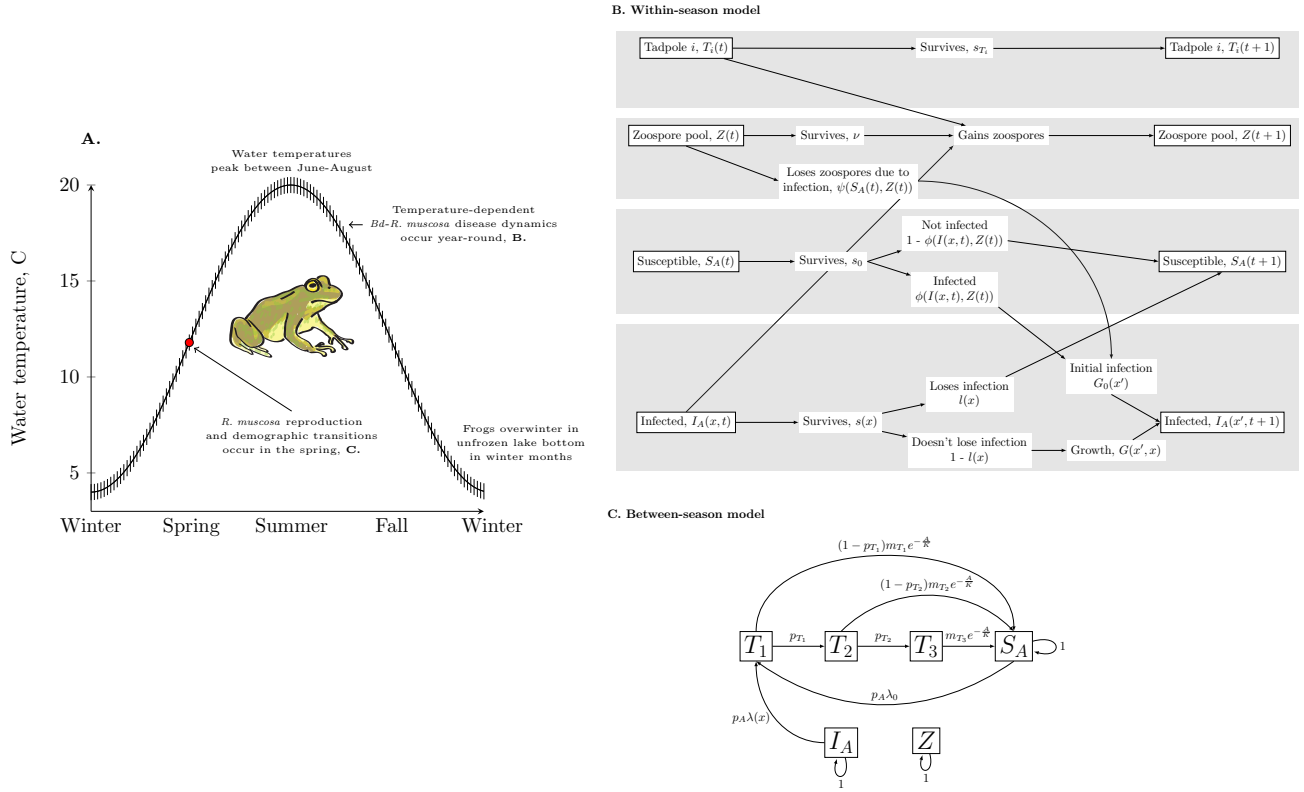

Figure 1: **A.** Diagram showing the temporal dynamics of the hybrid model of *R. muscosa*-Bd. Reproduction and demographic transitions occur once a year in the spring (red dot and C.). Disease dynamics are temperature-dependent and are updated every 3 days (vertical dashes and B.) over the course of the entire year. **B.** The within-season dynamics of *R. muscosa* and Bd based on equations 1 - 3. **C.** The between season demography of *R. muscosa* based on equations 6 - 11. Note that survival probability of susceptible adults (S_A), infected adults (I_A) and Bd zoospores in the environmental pool (Z) is one because their survival probabilities are already accounted for in the within-season model.

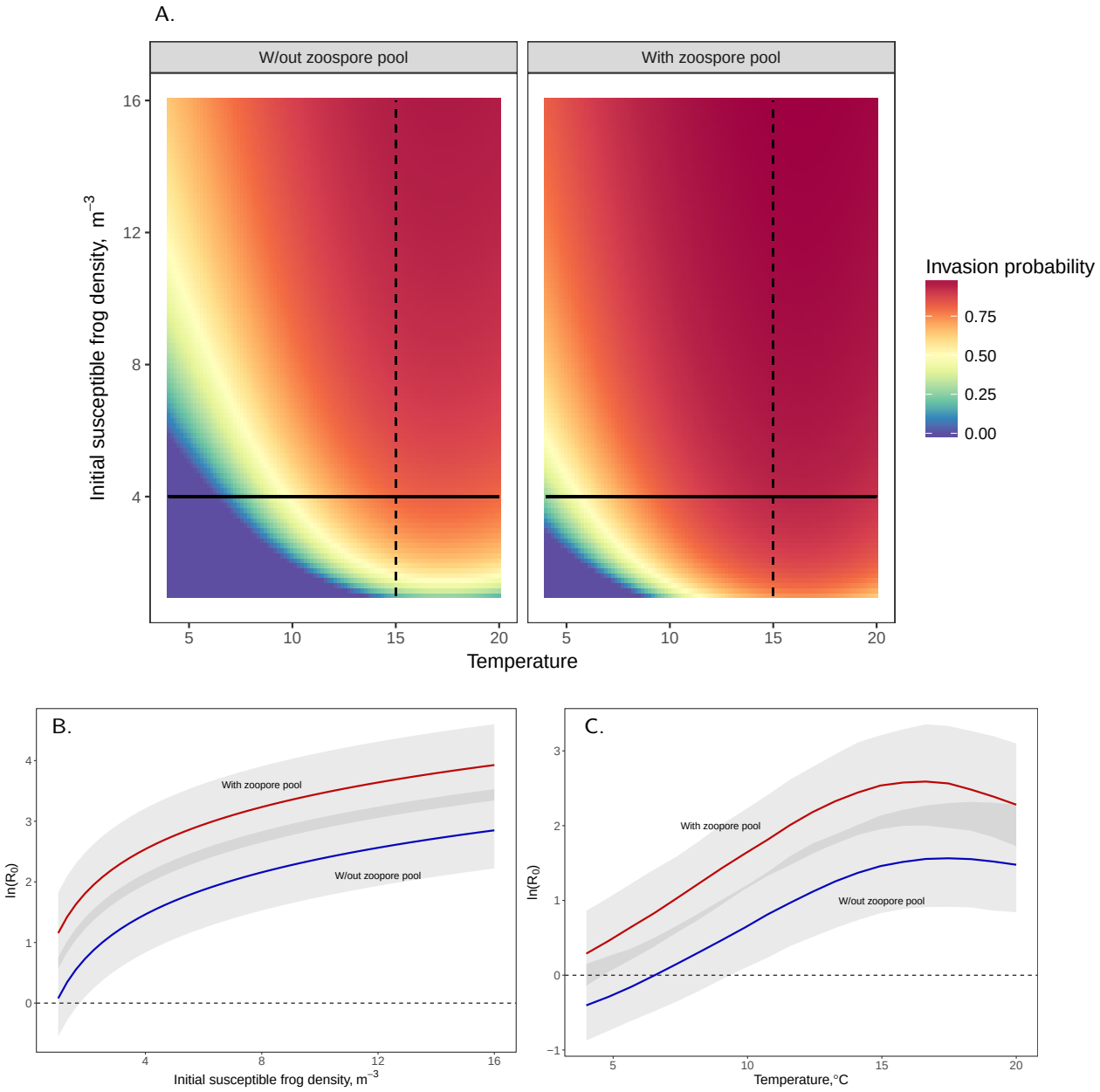

Figure 2: R_0 and the invasion probability of Bd $(1 - \frac{1}{R_0})$ for different temperatures and host densities with and without an environmental zoospore pool. This calculation of R_0 uses the parameters given in Table 1 and "Density dependent w/ dynamic zoospore pool" transmission function in Table 2. While R_0 will inevitably decrease without transmission from a zoospore pool (see Appendix 2), the magnitude of that decrease depends on the estimated transmission coefficient from the zoospore pool. **A.** Gives the invasion probability. The dashed, vertical lines in A. correspond to the curves shown in **B.**, where $\ln(R_0)$ is plotted against initial adult density when temperature is 15 °C. The solid, horizontal lines in A. correspond to the curves shown in **C.** where $\ln(R_0)$ is plotted against temperature when initial adult density is four adults per m^3. The gray regions give the 95% credible intervals. The dashed lines in B. and C. correspond to $R_0 = 1$ $(\ln(R_0) = 0)$, below which Bd cannot invade.

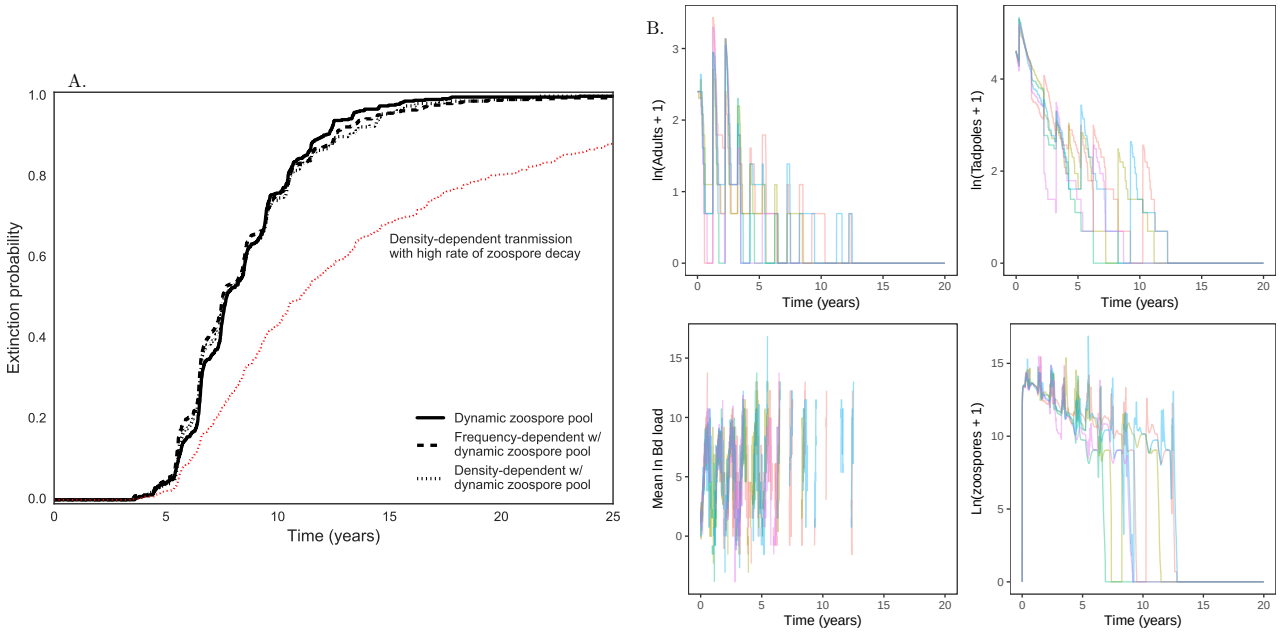

Figure 3: **A.** Time-dependent extinction curves of the hybrid model with three different transmission functions described in Table 1: dynamic zoospore pool, frequency-dependent transmission with a dynamic zoospore pool, density-dependent transmission with a dynamic zoospore pool. The curves were generated from 500 stochastic simulations of each model parameterized with the parameters given in Table 1 and 2. The red line gives the extinction curve when the zoospore survival probability was set to a constant 0.05 per time step, compared to the laboratory-estimated temperature-dependent survival probability of 0.8 at 15 °C. (Woodhams *et al.* 2008) **B.** Representative trajectories of adult, tadpole and zoospore pool population sizes from the hybrid model with a density-dependent transmission function and infection from a dynamic zoospore pool. Five stochastic trajectories are shown from the hybrid model (colored lines). The mean log Bd load, conditional on infection, is also shown. Gaps in trajectories for the mean Bd load indicate that no infected individuals were in the population at those time points.

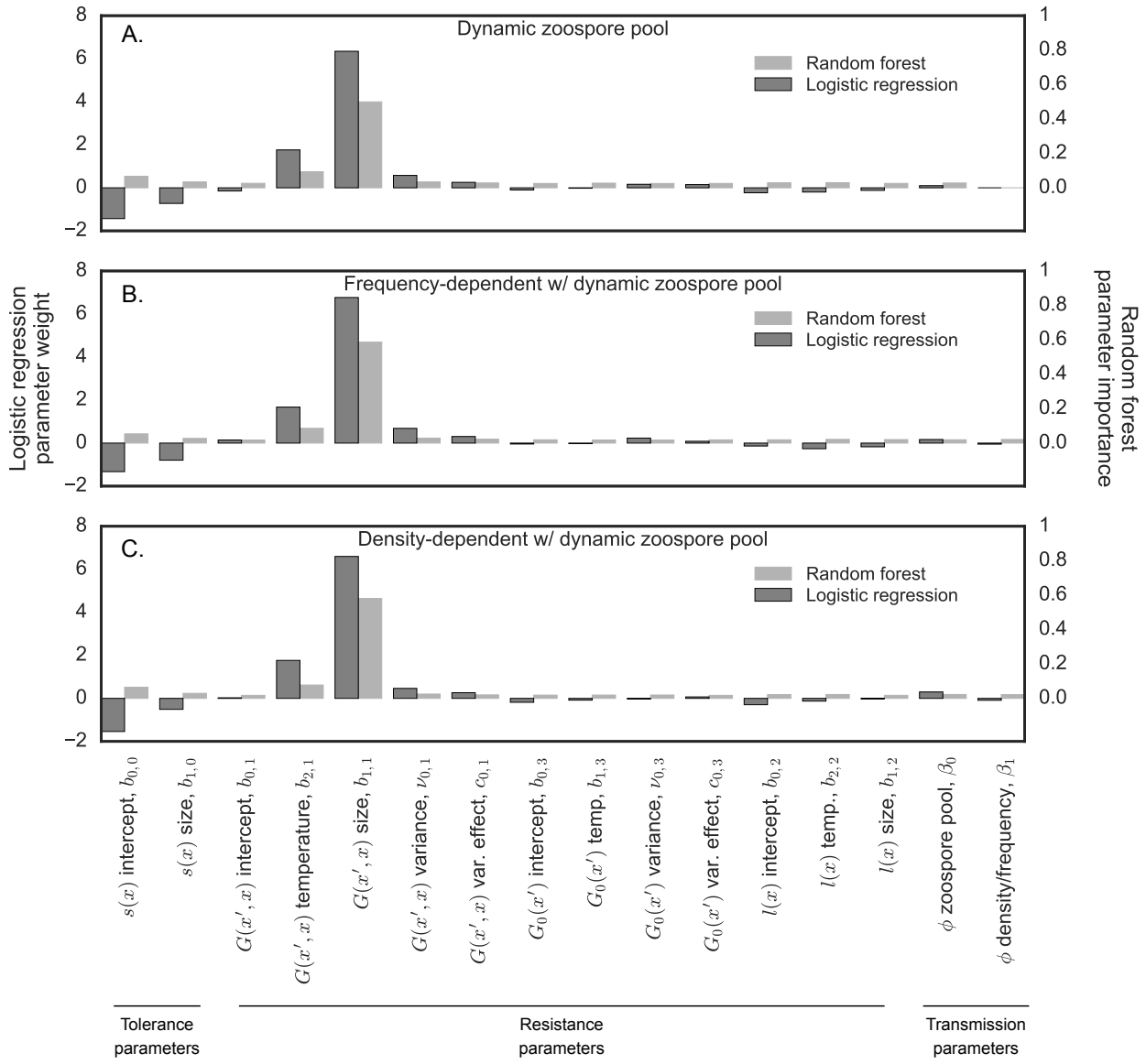

Figure 4: **A. - C.** The sensitivity of *R. muscosa* extinction probability to parameters dictating transmission, resistance, and tolerance of Bd for the three transmission functions used in the hybrid model. The dark gray bars give the weights of the various parameters when logistic regression is used to classify whether or not a simulation trajectory experienced extinction. The absolute height of the bar shows the relative importance of that parameter and the direction specifies what happens to the extinction probability when that parameter is increased. For example, increasing the $G(x', x)$ parameter $b_{1,1}$ increases the probability of extinction. The light gray bars give the relative importances of each parameter in predicting the extinction of a simulation when a Random Forest classifier was used to account the interactions between parameters on extinction probability. These values are all between zero and one and the height of a bar indicates the relative importance of a transmission, resistance, or tolerance parameter.