A Model for the Interaction of Frog Population Dynamics with *Batrachochytrium dendrobatidis*, *Janthinobacterium lividum* and Temperature and its Implication for Chytridiomycosis Management

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Abstract: Chytridiomycosis is an emerging disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) that poses a serious threat to frog populations worldwide. Several studies have shown that inoculation of bacterial species *Janthinobacterium lividum* (*Jl*) can mitigate the impact of the disease. However, there are many questions regarding this interaction. A mathematical model of a frog population infected with chytridiomycosis is developed to investigate how the inoculation of *Jl*

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could reduce the impact of *Bd* disease on frogs. The model also illustrates the important role of temperature in disease dynamics. The model simulation results suggest possible control strategies for *Jl* to limit the impact of *Bd* in various scenarios. However, a better knowledge of *Jl* life cycle is needed to fully understand the interactions of *Jl*, *Bd*, temperature and frogs.

Keywords: *Batrachochytrium dendrobatidis*, Chytridiomycosis, Frog population, *Janthinobacterium lividum*, Mathematical modeling, Temperature.

1 Introduction

The disease chytridiomycosis poses a serious threat to many frog populations around the world [4, 19, 38, 39]. It has infected over 350 species of amphibians and been linked to devastating population declines and extinctions of over 200 species [13, 39, 44]. Chytridiomycosis has been detected in 52% of the threatened amphibian species [42]. Chytridiomycosis occurs when the skin of a frog is infected by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) which interferes with normal skin function. Eventually frogs die of cardiac arrest [27]. Despite much research dedicated toward understanding the natural history of the disease, chytridiomycosis continues to spread (e.g., see <http://www.bd-maps.net/>). Hence, it is necessary for conservation agencies to devote their research to determining mitigation strategies against chytridiomycosis.

Several studies [6, 9, 16] show that a microbial community on amphibian skin, *Janthinobacterium lividum* (*Jl*), can protect amphibians against *Bd*. The proposed mechanism is that the strains of *Jl* that protect frogs from chytridiomycosis produce violacein, a fungicide. However, not all amphibians have these symbiotic bacteria and are thus protected [24]. For example, while *Jl* is not present naturally on the skin of mountain yellow-legged frog (*Rana muscosa*) [16], it has been experimentally demonstrated that inoculating frogs of this species with *Jl* protected them from infection with *Bd* [16]. Thus, inoculation with *Jl* is a promising strategy to control chytridiomycosis [16, 20]. While inoculation has been successful with other wildlife diseases [26, 43], what might be an effective inoculation strategy for frogs is not well understood. For example, important questions related to inoculation strategies that remain to be answered include: What is the optimal timing of inoculation in order to best control the disease? How frequently should one inoculate? Mathematical models are important tools for testing hypotheses and checking what may happen in various scenarios. In this study, we develop a mathematical model to examine inoculation strategy as it relates to the interaction of *Bd*, *Jl*, frog life history and temperature.

Temperature plays an important role in the development of the disease on frogs, not just

because that frog activities such as breeding and hibernation are triggered by temperature, but also because that growth rate of *Bd* and its pathogenicity are temperature dependent [15, 19]. For example, *Bd* grows within a wide temperature range ($4^{\circ}C - 25^{\circ}C$), with optimal growth between $17^{\circ}C - 25^{\circ}C$ [5, 28]. *Bd* does not grow well in temperatures above $28^{\circ}C$ [28]; 50% of colonies of *Bd* are killed after 8 days at $30^{\circ}C$ [28], and 100% mortality occurred within 96 hours at $32^{\circ}C$ and within 4 hours at $37^{\circ}C$ [3]. Therefore, we adopt temperature as an important factor in our model.

The main objectives of this paper are: i) to develop mathematical models that can be used to investigate how a variety of factors including temperature and disease parameters influence the chytridiomycosis, ii) to use this model to determine an efficient control strategy of the disease under various scenarios, and iii) to provide insights into how to implement the effective inoculation strategy. We organize this paper as follows: in section 2 we present the general mathematical model, model parameters utilized in the numerical simulations, temperature profiles and numerical scheme. In section 3 we present the numerical simulation results. Discussion and conclusions are provided in section 4.

2 Materials and Methods

2.1 A mathematical model

We consider a frog population in a single pond and we divide individuals in this population into two stages: frogs and tadpoles. *Bd* fungus is able to infect frogs, as well as tadpoles. Since tadpoles live in water and frogs are amphibious, we assume in the model that the transmission between individuals is within stage only (i.e., frogs only directly infect frogs and tadpoles only directly infect tadpoles). Figure 1 illustrates the proposed transmission network. Several studies [10, 33] indicate that there are two routes for *Bd* fungus to spread in frog populations:

- (i) by exposure to motile zoospores in the environment such as water and moist or wet soil that were released from infected tadpoles and frogs, and
- (ii) by direct skin contact between infected and uninfected individuals of the same life history stage.

In [16], the symbiont *Jl* was used to inoculate frogs against *Bd* infection. We therefore assume in the model that tadpoles and frogs that are inoculated with *Jl* gain protection from *Bd* infection. We assume that if an individual is infected with *Bd*, it either dies of chytridiomycosis (henceforth

chytrid) or recover from infection by acquiring Jl . Finally, we assume that Jl inoculation only lasts for a limited amount of time before the Jl is cleared from the skin [16], rendering the frog or tadpole susceptible to infection from Bd once again.

We divide both tadpole and frog stages into three classes: (i) susceptible individuals, (ii) individuals infected with Bd , and (iii) individuals inoculated with Jl and thus are protected from Bd infection. In [5], it was found that the frogs experienced upto 100% mortality rate when exposed to Bd unless inoculated with Jl . For this reason and the simplicity of the model, we did not include a recovered class. We assume that tadpoles dynamics are structured by age, while frogs dynamics are structured by size (e.g., body length) and represent the density of animals in each class depending on time t due to the seasonality of such populations (cf. the frog population model in [1] without Bd , Jl , or temperature). The frog stage is modeled by the following system of hyperbolic partial differential equations.

$$\begin{aligned} \frac{\partial}{\partial t} F_s + \frac{\partial}{\partial x} (g_s F_s) + m_s F_s &= -\lambda_F(x, t) F_s(x, t) - \omega_F(x, t) F_s(x, t) - \nu_{FBd}(x) Bd(t) F_s(x, t) \\ &\quad - \nu_{FJl}(x) Jl(t) F_s(x, t) + \eta_{FJl}(x, t) F_{Jl} \\ \frac{\partial}{\partial t} F_{Bd} + \frac{\partial}{\partial x} (g_{Bd} F_{Bd}) + m_{Bd} F_{Bd} &= \lambda_F(x, t) F_s(x, t) + \nu_{FBd}(x) Bd(t) F_s(x, t) \\ \frac{\partial}{\partial t} F_{Jl} + \frac{\partial}{\partial x} (g_{Jl} F_{Jl}) + m_{Jl} F_{Jl} &= \omega_F(x, t) F_s(x, t) + \nu_{FJl}(x) Jl(t) F_s(x, t) - \eta_{FJl}(x, t) F_{Jl} \end{aligned} \tag{2.1}$$

Here, $F_s(x, t)$, $F_{Bd}(x, t)$ and $F_{Jl}(x, t)$ denote the density of frogs of size x at time t that are susceptible, infected with Bd and inoculated with Jl , respectively. Suppose that size x varies from $x_{\min}$ to $x_{\max}$; for example, $x_{\min} = 1.5$ cm and $x_{\max} = 6$ cm for Green Tree Frog (GTF) (*Hyla cinerea*) based on a 6 year Capture-Mark-Recapture (CMR) field experiment [1]. Then the frog population at time t in each class could be calculated by integrating the densities. That is,

$$R_s(t) = \int_{x_{\min}}^{x_{\max}} F_s(x, t) dx, \quad R_{Bd}(t) = \int_{x_{\min}}^{x_{\max}} F_{Bd}(x, t) dx, \quad R_{Jl}(t) = \int_{x_{\min}}^{x_{\max}} F_{Jl}(x, t) dx.$$

The function g_s represents the individual growth rate of susceptible frogs. This growth rate may be nonlinear and depends on population of susceptible and/or infected frogs. The growth rate for frogs infected by Bd and Jl is denoted by g_{Bd} and g_{Jl} , respectively. The functions m_s, m_{Bd}

91 and m_{Jl} account for the individual mortality rate for frogs that are susceptible, infected by Bd
 92 and inoculated by Jl , respectively. The term $\nu_{FBd}(x)Bd(t)F_s$ represents Bd infection rate due
 93 to susceptible frogs getting exposed to environment containing motile Bd zoospores. The term
 94 $\lambda_F(x, t)F_s(x, t)$ denotes Bd infection rate due to skin contact of uninfected frogs with frogs infected
 95 by Bd , where $\lambda_F(x, t) = k_F(x) \int_{x_{min}}^{x_{max}} h_F(s)F_{Bd}(s, t)ds$ and $h_F(s)$ denotes size specific infectivity
 96 (i.e., frogs of size 5 cm might be more infectious than frogs of size 3 cm). For example, we take
 97 $h_F(s) = 0.001 s$ in the numerical simulations in section 3. Analogously, the term $\nu_{FJl}(x)Jl(t)F_s(x, t)$
 98 represents the rate at which susceptible frogs are infected by Jl through exposure to environment
 99 containing Jl zoospores. The rate at which frogs acquiring Jl through skin contact with frogs
 100 infected by Jl are described by the term $\omega_F(x, t)F_s(x, t)$, where $\omega_F(x, t) = \int_{x_{min}}^{x_{max}} c_F(s)F_{Jl}(s, t)ds$
 101 and $c_F(s)$ denotes size specific Jl acquiring ability.

102 Similarly, the tadpole stage is modeled by the following system of hyperbolic partial differential
 103 equations.

$$\begin{aligned}
 \frac{\partial}{\partial t}T_s + \frac{\partial}{\partial a}T_s + \mu_s T_s &= -\lambda_T(a, t)T_s(a, t) - \nu_{TBd}(a)Bd(t)T_s(a, t) - \nu_{TJl}(a)Jl(t)T_s(a, t) \\
 &\quad - \omega_T(a, t)T_s(a, t) \\
 \frac{\partial}{\partial t}T_{Bd} + \frac{\partial}{\partial a}T_{Bd} + \mu_{Bd}T_{Bd} &= \lambda_T(a, t)T_s(a, t) + \nu_{TBd}(a)Bd(t)T_s(a, t) \\
 \frac{\partial}{\partial t}T_{Jl} + \frac{\partial}{\partial a}T_{Jl} + \mu_{Jl}T_{Jl} &= \omega_T(a, t)T_s(a, t) + \nu_{TJl}(a)Jl(t)T_s(a, t)
 \end{aligned} \tag{2.2}$$

105 Here, $T_s(a, t), T_{Bd}(a, t), T_{Jl}(a, t)$ denote the density of tadpoles that are susceptible, infected with
 106 Bd , inoculated with Jl , respectively. Tadpoles are characterized by age which varies between 0 and
 107 a_{max} (metamorphosis age). The tadpole population at time t in each class could be obtained by
 108 integrating the densities. That is,

$$109 \quad Q_s(t) = \int_0^{a_{max}} T_s(a, t)da, \quad Q_{Bd}(t) = \int_0^{a_{max}} T_{Bd}(a, t)da, \quad Q_{Jl}(t) = \int_0^{a_{max}} T_{Jl}(a, t)da.$$

110 The terms $\nu_{TBd}(a)Bd(t)T_s$ and $\lambda_T(a, t)T_s$ denote Bd infection rate of susceptible tadpoles due to
 111 exposure to environment containing motile Bd zoospores and contact with tadpoles infected by
 112 Bd , respectively. Here, $\lambda_T(a, t) = k_T(a) \int_0^{a_{max}} h_T(\alpha)T_{Bd}(\alpha, t)d\alpha$ calculates the infection rate with
 113 $h_T(\alpha)$ being age specific infectivity and $k_T(a)$ age specific susceptibility. For example, we take
 114 $h_T(\alpha) = 0.1 \alpha$ in the numerical simulations in section 3. Analogously, the rate at which susceptible
 115 tadpoles are infected by Jl through exposure to the environment containing Jl zoospores and contact

with tadpoles infected by Jl are described by the terms $\nu_{TJl}(a)Jl(t)T_s(a, t)$ and $\omega_T(a, t)T_s(a, t)$, respectively. Here, $\omega_T(a, t) = \int_0^{a_{max}} c_T(\alpha)T_{Jl}(\alpha, t)d\alpha$, where $c_T(\alpha)$ denotes age specific Jl acquiring ability.

Rapid changes occur in the immune system during metamorphosis [32]. For this reason, we assume that the age/size specific infectivity $k_T(a)$ and $k_F(x)$ are highest during metamorphosis (i.e. the oldest tadpoles and the smallest frogs are most susceptible). We account for this variability in our model by choosing $k_T(a)$ as an increasing function of age a and $k_F(x)$ as a decreasing function of size x . For example, we take $k_T(a) = 10.4a$ and $k_F(x) = \frac{6-x}{4.5}$ in the numerical simulations in section 3. Frogs behavior is affected by temperature and long periods of seasonal inactivity or hibernation, our model accounts for this behavioral variability by making explicitly time dependent vital rates such as breeding; hence directly linking behavior and temperature. Previous studies have been conducted to understand how long Jl can stay on the skin of frogs [16]. Experiments show that once Jl was acquired through inoculation, it stayed on the skin of frogs for at least 20 weeks, and perhaps longer [16]. To model the rate at which Jl gets cleared and thus move a frog from inoculated class into the susceptible class we adopt the terms $\eta_{FJl}(x, t)F_{Jl}$. We do not have any data on how fast Jl is cleared in tadpoles but in frogs it exceeds 20 weeks, which is longer than tadpoles remain as tadpoles. We therefore assume tadpoles with Jl are protected through metamorphosis. After metamorphosis tadpoles with Jl become frogs with Jl and hence protected from Bd . It is assumed that there is no direct transmission from frogs to tadpoles of either Jl or Bd , that is, all of the newly hatched tadpoles are susceptible. Therefore,

$$T_s(0, t) = \int_{x_{min}}^{x_{max}} \beta_s(y, t)F_s(y, t)dy + \int_{x_{min}}^{x_{max}} \beta_{Jl}(y, t)F_{Jl}(y, t)dy + \int_{x_{min}}^{x_{max}} \beta_{Bd}(y, t)F_{Bd}(y, t)dy, \quad (2.3)$$

where $\beta_s(y, t)$, $\beta_{Jl}(y, t)$, and $\beta_{Bd}(y, t)$ denote the individual birth rate of susceptible frogs, frogs with Jl , and frogs with Bd , respectively, and time t is linked to a regional temperature profile. We assume that a frog begins breeding once it attains a critical size (e.g., 3cm for GTF [1]) and temperature on that particular day along with the average temperature of the past three weeks is above a critical temperature.

It is assumed that when tadpoles reach their maximum age, a_{max} , they metamorphose into frogs with minimum size, x_{min} . Evidence shows that there is a transition of Bd infection from mouthparts to hind limbs during metamorphosis [25]. Therefore, we assume that tadpoles with Bd

metamorphose to frogs with *Bd*. We also assume that susceptible tadpoles and tadpoles with *Jl* metamorphose to susceptible frogs and frogs with *Jl*, respectively. This process is modeled through the following condition

$$g_{\Theta}(x_{min}, R_{\Theta}(t)) F_{\Theta}(x_{min}, t) = T_{\Theta}(a_{max}, t) \quad \text{for} \quad \Theta = s, Bd, Jl. \quad (2.4)$$

Temperature plays an important role in the spread of *Bd* through frog populations where transmission occurs between 4° and 27°C. Between 27°C and 29°C transmission declines, and *Bd* dies at or above 30° C. *Bd* is able to reproduce through sporogenesis [29]. The reproduction rate of *Bd* zoospores is modeled by $\gamma^{Bd} \exp(-\xi^{Bd} Bd(t))$, where $\exp(-\xi^{Bd} Bd(t))$ describes the *Bd* population density impact on this rate. Infected frogs and tadpoles release zoospores at a constant rate given by ρ^{FBd} and ρ^{TBd} , respectively. Since mortality of *Bd* zoospores differ widely above 28°C [18], we let the mortality rate ψ_{Bd} depend on temperature. The dynamics of the free living stage of *Bd* are then modeled by the following ordinary differential equation:

$$\begin{aligned} \frac{d}{dt}(Bd) = & \gamma^{Bd} Bd(t) \exp(-\xi^{Bd} Bd(t)) - \psi_{Bd}(t) Bd(t) + \rho^{TBd} \int_0^{a_{max}} T_{Bd}(a, t) da \\ & + \rho^{FBd} \int_{x_{min}}^{x_{max}} F_{Bd}(x, t) dx - Bd(t) \int_0^{a_{max}} \nu_{TBd}(a) T_s(a, t) da \\ & - Bd(t) \int_{x_{min}}^{x_{max}} \nu_{FBd}(x) F_s(x, t) dx. \end{aligned} \quad (2.5)$$

Here, the last two terms represent the rate at which *Bd*/*Jl* free living zoospores spread from the environment to the skin of susceptible frogs.

Since the transmission mechanism of *Jl* is rarely understood, we model modes of transmission of *Jl* the same way as *Bd*. Thus, the dynamics of the free living stage of *Jl* are described by

$$\begin{aligned} \frac{d}{dt}(Jl) = & \gamma^{Jl} Jl(t) \exp(-\xi^{Jl} Jl(t)) - \psi_{Jl}(t) Jl(t) + \rho^{TJl} \int_0^{a_{max}} T_{Jl}(a, t) da + \rho^{FJl} \int_{x_{min}}^{x_{max}} F_{Jl}(x, t) dx \\ & - Jl(t) \int_0^{a_{max}} \nu_{TJl}(a) T_s(a, t) da - Jl(t) \int_{x_{min}}^{x_{max}} \nu_{FJl}(x) F_s(x, t) dx \end{aligned} \quad (2.6)$$

2.2 Model parameters and temperature data

(a) Vital rates estimates

Vital rates for susceptible animals are based on [1]. Frogs or tadpoles inoculated with *Jl* are assumed

to possess the same vital rates as those of susceptible frogs or tadpoles, respectively. It is assumed that the mortality rate depends linearly on density (logistic type density-dependent mortality) and exponentially on the size of the frog. Because of the seasonal dynamics of such animals and the fact that they hibernate during winter, the mortality rate was assumed to depend on time explicitly. Due to the relative short duration for the tadpole stage, it's assumed for simplicity and for lack of other information that the mortality rate of susceptible tadpoles is a constant. To incorporate the disease impact on the mortality rate we assume that mortality rate of frogs infected with *Bd* is 20% higher compared to that of susceptible and *Jl* frogs. However, the mortality rate might vary with a range from 0% to 100%, depending on various factors including species susceptibility [37], innate immune defenses [41] and environmental temperature [40]. We also assume *Bd* frogs will reproduce fewer tadpoles compared to susceptible or *Jl* frogs. We choose the growth rate of a susceptible frog to be $g(x, Q) = 0.489(6 - x)$ as in [1]. We assumed a 10% reduction in growth rate of *Bd* frogs to account for the *Bd*'s impact. Since there are no field data estimating the per capita contact rate ν_{FBd} between *Bd* zoospores and susceptible frogs, we adopted the range of transmission rates explored in [14]. The rate at which *Bd* zoospores released by tadpoles infected with *Bd* has been estimated by [21] through parameter estimation between $1 \times 10^4 \text{ yr}^{-1}$ and $6.6 \times 10^5 \text{ yr}^{-1}$ and we used the same *Bd* zoospore release rate for the infected frogs. The infection rate is the rate at which an uninfected frog becomes infected with either *Bd* or *Jl* via zoospores. The rate per zoospore per year is the same for *Jl* and *Bd*, and set to 6×10^{-9} for tadpoles [21]. The frog infection rate is set to 4×10^{-8} . This infection rate that causes 100% of the susceptible frogs to become infected is chosen in order to investigate the potential of *Jl* inoculation. The frog infection (ν_{FBd}) rate chosen is the minimum rate needed for *Bd* persistence in the model. We refer Table 1 for model parameters and their corresponding descriptions with units; Table 2 contains all the parameter values used in our simulations.

(b) Initial population estimates

Our simulations begin with $t = 0$ representing January 1, 2004 and no tadpoles are present in the pond. Hence, we assume that the initial density of tadpoles in all of the three tadpole classes is zero (i.e., $T_s(a, 0) = T_{Jl}(a, 0) = T_{Bd}(a, 0) = 0$). Based on the work in [1], we choose the initial density for frogs at time $t = 0$ as $F_s(x, 0) = 615.96 \exp(-0.75x)$ which integrates to $R_s(0) = 257.2$ frogs in the pond. We assume all frogs at the initial time are susceptible by letting $F_{Jl}(x, 0) = F_{Bd}(x, 0) = 0$.

(c) Temperature profiles

Temperature plays an important role in both frogs and *Bd* dynamics. To capture the effect of day-to-day and seasonal temperature variability we performed simulations with three natural temperature profiles based on weather data from Lafayette, LA, Los Angeles, CA, and Phoenix, AZ for a six-year period from 2004 to 2009. The three locations were chosen because they represent seasonal temperature patterns that are representative of the potential thermal ranges that *Bd* and frog populations might experience. We downloaded average daily temperatures for each location (<http://www.wunderground.com>) as shown in Figure 2. We then ran the simulations in six-year cycles for thirty years.

2.3 Numerical scheme

A finite difference scheme is developed to approximate solutions of model (2.1) -(2.6) in a similar manner to those used for other structured models [2]. As described in section 2.1, there is an upper bound $a_{\max}$ for the age of tadpoles. The sizes (that is, body length) of frogs vary between $x_{\min}$ and $x_{\max}$. Based on the six-year CMR experiment data for GTF in [1], we take $a_{\max} = 5/52$ (5 weeks), $x_{\min} = 1.5$ cm, $x_{\max} = 6$ cm. We consider the model dynamics over a time period $[0, T]$, where $T = 30$ years. Uniform mesh sizes are used with $\Delta a = \frac{5}{52 \times 160}$, $\Delta x = \frac{4.5}{320}$ and $\Delta t = \frac{30}{3200}$. Therefore, mesh points are given by: $a_i = i\Delta a, i = 0, 1, \dots, m$, $x_j = x_{\min} + j\Delta x, j = 0, 1, \dots, n$, $t_k = k\Delta t, k = 0, 1, \dots, l$. For $\Theta = s, Bd$ or Jl , we denote by $T_{\Theta,i}^k, F_{\Theta,j}^k, Q_{\Theta,i}^k$, and $R_{\Theta,j}^k$ the finite difference approximation of $T_{\Theta}(a_i, t_k)$, $F_{\Theta}(x_j, t_k)$, $Q_{\Theta}(a_i, t_k)$, and $R_{\Theta}(x_j, t_k)$, respectively. We also let

$$g_{\Theta,j}^k = g_{\Theta}(x_j, t_k, R_{\Theta,j}^k), \quad \mu_{\Theta,i}^k = \mu_{\Theta}(a_i, t_k, Q_{\Theta,i}^k), \quad m_{\Theta,j}^k = m_{\Theta}(x_j, t_k, R_{\Theta,j}^k), \quad \beta_{\Theta,j}^k = \beta_{\Theta}(x_j, t_k, R_{\Theta,j}^k),$$

where $\Theta = s, Bd$ or Jl .

A first order nonstandard finite difference scheme is adopted to discretize model (2.1)

$$\begin{aligned} \frac{F_{s,j}^{k+1} - F_{s,j}^k}{\Delta t} + \frac{g_{s,j}^k F_{s,j}^{k+1} - g_{s,j-1}^k F_{s,j-1}^{k+1}}{\Delta x} + m_{s,j}^k F_{s,j}^{k+1} &= -\lambda_F^k F_{s,j}^{k+1} - \omega_F^k F_{s,j}^{k+1} - \nu_{FBd,j} Bd^k F_{s,j}^{k+1} \\ &\quad - \nu_{FJl,j} Jl^k F_{s,j}^{k+1} + \eta_{FJl}^{k+1} F_{Jl,j}^k \\ \frac{F_{Bd,j}^{k+1} - F_{Bd,j}^k}{\Delta t} + \frac{g_{Bd,j}^k F_{Bd,j}^{k+1} - g_{Bd,j-1}^k F_{Bd,j-1}^{k+1}}{\Delta x} + m_{Bd,j}^k F_{Bd,j}^{k+1} &= \lambda_F^k F_{s,j}^{k+1} + \nu_{FBd,j} Bd^k F_{s,j}^{k+1} \\ \frac{F_{Jl,j}^{k+1} - F_{Jl,j}^k}{\Delta t} + \frac{g_{Jl,j}^k F_{Jl,j}^{k+1} - g_{Jl,j-1}^k F_{Jl,j-1}^{k+1}}{\Delta x} + m_{Jl,j}^k F_{Jl,j}^{k+1} &= \omega_F^k F_{s,j}^{k+1} + \nu_{FJl,j} Jl^k F_{s,j}^{k+1} - \eta_{FJl}^{k+1} F_{Jl,j}^k \end{aligned} \quad (2.7)$$

220 We discretize model (2.2) in a similar manner.

$$\begin{aligned}
& \frac{T_{s,i}^{k+1} - T_{s,i}^k}{\Delta t} + \frac{T_{s,i}^{k+1} - T_{s,i-1}^{k+1}}{\Delta a} + \mu_{s,i}^k T_{s,i}^{k+1} = -\lambda_T^k T_{s,i}^{k+1} - \nu_{\text{TBd},i} B d^k T_{s,i}^{k+1} - \nu_{\text{TJl},i} J l^k T_{s,i}^{k+1} - \omega_T^k T_{s,i}^{k+1} \\
& \frac{T_{Bd,i}^{k+1} - T_{Bd,i}^k}{\Delta t} + \frac{T_{Bd,i}^{k+1} - T_{Bd,i-1}^{k+1}}{\Delta a} + \mu_{Bd,i}^k T_{Bd,i}^{k+1} = \lambda_T^k T_{s,i}^{k+1} + \nu_{\text{TBd},i} B d^k T_{s,i}^{k+1} \\
& \frac{T_{Jl,i}^{k+1} - T_{Jl,i}^k}{\Delta t} + \frac{T_{Jl,i}^{k+1} - T_{Jl,i-1}^{k+1}}{\Delta a} + \mu_{Jl,i}^k T_{Jl,i}^{k+1} = \omega_T^k T_{s,i}^{k+1} + \nu_{\text{TJl},i} J l^k T_{s,i}^{k+1}.
\end{aligned}
\tag{2.8}$$

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222 Model (2.5) and (2.6) are approximated by the following finite difference method

$$\begin{aligned}
& \frac{B d^{k+1} - B d^k}{\Delta t} = \gamma^{\text{Bd}} B d^k e^{-\xi^{\text{Bd}} B d^k} - \psi_{\text{Bd}}^k B d^{k+1} + \rho^{\text{TBd}} \sum_{i=1}^m T_{Bd,i}^{k+1} \Delta a + \rho^{\text{FBd}} \sum_{j=1}^n F_{Bd,j}^{k+1} \Delta x \\
& \quad - B d^{k+1} \sum_{i=1}^m \nu_{\text{TBd},i} T_{s,i}^{k+1} \Delta a - B d^{k+1} \sum_{j=1}^n \nu_{\text{FBd},j} F_{s,j}^{k+1} \Delta x, \\
& \frac{J l^{k+1} - J l^k}{\Delta t} = \gamma^{\text{Jl}} J l^k e^{-\xi^{\text{Jl}} J l^k} - \psi_{\text{Jl}}^k J l^{k+1} + \rho^{\text{TJl}} \sum_{i=1}^m T_{Jl,i}^{k+1} \Delta a + \rho^{\text{FJl}} \sum_{j=1}^n F_{Jl,j}^{k+1} \Delta x \\
& \quad - J l^{k+1} \sum_{i=1}^m \nu_{\text{TJl},i} T_{s,i}^{k+1} \Delta a - J l^{k+1} \sum_{j=1}^n \nu_{\text{FJl},j} F_{s,j}^{k+1} \Delta x.
\end{aligned}
\tag{2.9}$$

223

224 The boundary and initial conditions are approximated by

$$T_{s,0}^{k+1} = \sum_{j=1}^n \beta_{s,j}^{k+1} F_{s,j}^{k+1} \Delta x + \sum_{j=1}^n \beta_{Jl,j}^{k+1} F_{Jl,j}^{k+1} \Delta x + \sum_{j=1}^n \beta_{Bd,j}^{k+1} F_{Bd,j}^{k+1} \Delta x,$$

$$g_{\Theta,0}^{k+1} F_{\Theta,0}^{k+1} = T_{\Theta,m}^{k+1},$$

225

$$T_{\Theta,i}^0 = T_{\Theta}^0(a_i), \quad i = 1, 2, \dots, m,$$

$$F_{\Theta,j}^0 = F_{\Theta}^0(x_j), \quad j = 1, 2, \dots, n,$$

226 for $\Theta = s, Bd$ or Jl .

227 An important characteristic of the above chosen nonstandard finite difference approximations is

228 that this method preserves nonnegativity of numerical solutions which is biologically relevant. It is

229 also efficient computationally.

3 Results

To demonstrate appropriate patterns in frog dynamics, the two possible outcomes of disease emergence are as follows: (i) with only *Bd* and (ii) with both *Bd* and *Jl*. We thus simulated frog population dynamics with three different scenarios: (a) Control scenario, where neither *Bd* nor *Jl* is present (b) Disease scenario, where only *Bd* is present and (c) Inoculation scenario, where both *Bd* and *Jl* are present.

(a) Control scenarios

We first simulated the frog (control) population dynamics over 30 years without *Bd* and *Jl* in the model (see Figure 3) under all three different temperature profiles. From observation of the CMR experiment, GTFs breed (as indicated by calling activity and tadpole metamorphosis to frogs) when daily temperatures for the previous three weeks averaged 27°C (Carter *et al.*, unpublished data and analyses). However, this critical breeding temperature may vary from year to year, in different locations and amongst different species. For example, in Louisiana there are winter breeding species in the treefrog family, Hylidae, (e.g., *Hyla crucifer* and various *Pseudacris* species) that breed at temperatures as low as 5°C [12]. In our simulations, when we used the critical breeding temperature of 27°C , the frog population persisted in the Lafayette and Phoenix temperature profiles. However, it did not persist in the Los Angeles temperature profile because it was too cool to permit breeding (Figure 3(a)). When we used the lower critical breeding temperature of 18°C , GTFs was able to persist in all three temperature profiles (Figure 3(b)). As we are interested in understanding the impact of *Bd* on the dynamics of frogs in different temperature profiles, we will fix the critical breeding temperature to 18°C (a level where the frog population persists in all temperature profiles considered here) for the remainder of the discussion.

(b) Disease scenarios

To illustrate the dependency of *Bd* and frog dynamics on temperature, in Figure 4 we simulated the system dynamics for all the three temperature profiles given in Figure 2. Because chytridiomycosis does not greatly affect the survivorship of tadpoles [4, 7] our analyses focused on frogs.

Figure 4 supports the result that GTF is not susceptible to chytridiomycosis in Lafayette, LA where the temperature is not suitable for *Bd* growth [8]. Chytridiomycosis died out in the

Lafayette and Phoenix simulations because their temperature profiles were too warm to allow *Bd* to survive [8]. Thus our model is in agreement with field observations, *Bd* should not be naturally occurring in GTF populations in those regions where average temperature during breeding season is above 25°C [33]. Our model is consistent with the hypothesis that the chance of a frog being infected with *Bd* decreases rapidly with the increase of the time-window where temperatures remain above the *Bd*'s upper optimal temperature [11, 34]. Figure 4 shows *Bd* persisted in Los Angeles, CA temperature profile since *Bd*'s growth is more active during optimal temperature window there ($17^{\circ}\text{C} - 25^{\circ}\text{C}$). In order to explore mitigation strategies, for the rest of our analyses we will focus on the Los Angeles, CA temperature profile.

Next, we investigate the disease impact on different vital rates of frogs. Information on how *Bd* affects the vital rates is not available in the literature. Therefore, we simulate minor to severe impact of *Bd* on each vital rate in order to understand the effect of such impact on the overall dynamics of the system.

(i) *Impact of Bd on growth rate of frogs:* *Bd* infection can reduce the feeding rate in frogs [37], and in turn can affect their growth rate. We reduced the growth rate of frogs infected with *Bd* from 5% to 30%. The corresponding frog dynamics is shown in Figure 5. At 30% *Bd*'s impact on the frog growth rate reduced the proportion of breeding frogs in the frog population to where there were not enough breeders to maintain the frog population.

(ii) *Impact of Bd on mortality rate of frogs:* *Bd* mortality rate (the rate at which frogs infected with *Bd* die) was increased from 5% to 40% of the susceptible frog mortality rate. At 40% the frog population has a dramatic drop of 98.5% and reached a level at which it can hardly persist (Figure 6).

(iii) *Impact of Bd on frogs birth rate:* The reduction of young produced by infected frogs was between 5%-55%. The frog population had a dramatic decline of 97.2% at a 55% reduction (Figure 7).

(iv) *Impact of Bd on tadpole mortality rate:* The mortality rate for tadpoles infected with *Bd* was varied between 5% and 40% of the susceptible tadpole. The frog population was not affected significantly (Figure 8).

(c) Inoculation scenarios

Our model assumed that *Jl*, when *Bd* is absent, did not have an impact on the life history parameters of frogs and tadpoles. We used similar mechanisms as *Bd* to model *Jl* infection transmission, but with different parameters and with no effect of temperature on *Jl* life history parameters. In this model because of the assumption that frogs (tadpoles) with *Jl* are protected from *Bd*, and *Bd* infected frogs (tadpoles) do not acquire *Jl*, our focus was on how *Jl*'s life history parameters relative to those of *Bd* affect frog survival. Although we simulated inoculation scenarios for a particular set of parameters, additional simulations show that protectiveness of the inoculation holds under reasonable changes in the parameter values, that is, inoculation effect is robust with respect to small changes in the parameters.

(i) *Infection rate*: We varied the infectivity of *Jl* relative to *Bd*. Our simulation results are presented in Figure 9. The minimum infectivity ratio needed to mitigate *Bd* was approximately 1:1. If *Jl* had an infection rate lower than *Bd* then it had no effect on controlling *Bd*. Ratios higher than 1:1 had increasing impact on mitigating *Bd*. A 5:1 infectivity ratio almost eliminated *Bd*. Our results suggest that to be effective any proposed *Jl* bioaugmentation species should be as infective as *Bd*, or sufficient number of *Jl* zoospores must be added to overcome any infectivity advantage *Bd* might have.

(ii) *Growth rate of free living stage*: The initial growth (reproduction) rates for *Jl* and *Bd* as free-living organisms were 20 zoospores per year per infectious organism. Figure 10 suggest that the minimum growth rate ratio (*Jl* growth rate/*Bd* growth rate) at which *Jl* had no impact on frog population dynamics was approximately 0.75:1. To efficiently control *Bd*'s impact on frog population, our results indicated that the *Jl* growth rate has to be more than 5 times of the growth rate of *Bd*.

(iii) *Jl loss rate*: In [16] it's demonstrated that *Jl* infection lasted for 20 weeks however they did not demonstrate that infection was permanent. We simulated the effect of frogs losing their *Jl* infection anywhere between 20 to 40 weeks. This did not have a significant impact on the dynamics, as shown in Figure 11. The question wasn't relevant for the tadpole stage since tadpoles metamorphosed in under 20 weeks.

(iv) *Dose and timing of inoculation:* When it comes to chytridiomycosis control and management, one natural question to ask is when it would be a best time to inoculate with *Jl*. Our model simulation indicated that inoculating during the breeding season was more effective than during hibernation (Figure 12(a)). We also investigated whether one needs to inoculate annually to control the disease. As shown in Figure 12(b), inoculating for a few years mitigates the *Bd* impact temporarily. We evaluated the effectiveness of the number of *Jl* zoospores inoculated in controlling *Bd* infection by increasing the number of *Jl* zoospores inoculated from 10^{12} to 10^{16} . Our simulations indicated that it is necessary to inoculate every year in perpetuity.

4 Discussion and Conclusions

The goal of this study was to investigate how to integrate what is known about *Bd*, *Jl*, temperature and frog population dynamics in order to propose effective inoculation strategies to control the spread of chytridiomycosis. Our model simulations produced three main results. First, we demonstrated the importance of temperature in *Bd* dynamics and the potential role of *Jl* in protecting and recovering the infected frogs. Second, our simulations help understand the role of temperature-dependent effects on the ability of *Jl* interaction with *Bd* and identified crucial parameters that need to be measured accurately in the field. Third, we found that the number of *Bd* infected frogs more drastically reduced when we inoculate *Jl* during breeding than in hibernation.

Our study was consistent with other studies on the prevalence of *Bd* in GTF [8]. Previous results concluded that frogs died within the *Bd* optimal temperature window ($17^{\circ}\text{C} - 25^{\circ}\text{C}$) and recovered above 25°C [40, 11]. Our model demonstrates that temperature could be a major factor influencing frog population susceptibility to *Bd*. Changing climatic patterns may affect which species are exposed to *Bd*.

Frog growth rate was very sensitive to *Bd* levels. In order to attain sexual maturity frogs must reach a certain size (e.g., 3cm for GTF [1]) which in turn affects the breeding rate. The reduction of growth rate on frogs increased time to reproduction which in turn reduces the effective breeding population, leading to local extinction (Figure 5). Quantifying the cost of chytridiomycosis on growth rate of frogs will help us more accurately model disease impacts. The model suggests that the dynamics of *Jl*'s free-living stage is important in understanding the rate of the *Bd* disease transmission and hence frog survival. Thus, it is important to have more accurate information about

the free-living stage and the life history of *Jl* to obtain better understanding of these interactions. The role of temperature on *Jl*, if any, is not known and was not considered. However, we recognize that the natural history of symbiotic bacteria is complex and dependent on various environmental factors [30].

In [16], they suggested that the effects of bacterial inoculation lasts at least 20 weeks. However, additional simulations showed that there were no significant changes in model results (e.g., extinctions, seasonal population levels, etc.) if *Jl* protection was extended beyond 20 weeks.

Our model demonstrated that the timing of inoculation was important. Inoculation was more effective during the breeding season than during hibernation. Transmission of *Bd* between frogs is more likely during breeding and *Jl* inoculation at this time interrupts that. Also, during the breeding season the tadpole and frog stage coexist, increasing the potential for indirect transmission between frogs and tadpoles. Tadpoles acquiring *Bd* are more likely to die as frogs after metamorphosis [23]. Inoculation during the breeding season reduces this type of mortality. One factor affecting the efficacy of inoculation was the *Bd* to *Jl* zoospore ratio (Figure 10). Adding sufficient *Jl* zoospores to protect frogs against *Bd* and chytridiomycosis might have unexpected consequences. For example, one study shows violacein (the compound *Jl* produces that kills *Bd*) is also toxic to bacterivorous nano flagellates [22], and we don't know what the broader ecosystem consequences would be of losing them.

Temperature is important for frog phenology and for disease transmission. We have demonstrated how they interact to protect frogs from chytridiomycosis and how, for frogs that occupy a *Bd* amenable climate, a *Jl* inoculation strategy could be used to mitigate the impact of *Bd*. As climate patterns shift, many amphibian species may find themselves in climatic zones that are favorable for *Bd* [17] necessitating strategies to protect them. We demonstrate that one strategy (inoculation) is plausible if timed to coincide with the breeding season.

Furthermore, environmental factors could either facilitate or hinder the interaction between frogs and *Bd*. In particular, temperature may influence frogs response to infection [31]. It may also influence movement behavior which is an important process in the transmission dynamics of the disease and the dispersal of *Bd* fungus to chytridiomycosis-free areas. Therefore including dispersal mechanisms in our future modeling efforts is a priority. Moreover, there is an increasing concern about what might increase pathogen development and disease transmission in the context of climate change [17]. Our findings demonstrates the complex role of the temperature in the disease dynamics

and control strategies. The approach to explicitly model dependence of parameters on temperature, that we used in this work, can be useful when attempting to couple such disease models to climate change models and capture the potential spread of the disease under climate change conditions.

A recent study in [36] revealed that both environmental and genetic factors have profound impact on the dynamics of *Bd* infection in a lowland leopard frog (*Lithobate Yavapaiensis*) population. Specifically, the authors found that the integration of environmental and genetic factors predict *Bd* infection prevalence, whereas genetic diversity predict mortality. Another recent study [35] showed that frog species rely on different behavior strategies (diurnal versus nocturnal) to persist under environmental variations. Therefore, future research efforts should focus on integrating environmental and genetic variations, behavior strategies and potential climate change effects to gain a more comprehensive understanding of *Bd* spatial epidemiology and frog dynamics through mathematical modeling and simulations.

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Table 1: *Model parameter descriptions and units*

Parameter	Description	Unit
$g_s/g_{Bd}/g_{Jl}$	growth rate of susceptible frogs/frogs with Bd /frogs with Jl	$\frac{size}{[time]}$
$\mu_s/\mu_{Bd}/\mu_{Jl}$	mortality rate of susceptible tadpoles/tadpoles with Bd /tadpoles with Jl	$\frac{1}{[time]}$
$m_s/m_{Bd}/m_{Jl}$	mortality rate of susceptible frogs/frogs with Bd /frogs with Jl	$\frac{1}{[time]}$
$\nu_{TBd} (\nu_{FBd})$	rate at which tadpoles (frogs) get Bd from the environment	$\frac{1}{[zspre][time]}$
$\nu_{TJl} (\nu_{FJl})$	rate at which tadpoles (frogs) get Jl from the environment	$\frac{1}{[zspre][time]}$
$\rho^{TBd} (\rho^{FBd})$	rate at which tadpoles (frogs) with Bd release zoospores	$\frac{1}{[time]}$
$\rho^{TJl} (\rho^{FJl})$	rate at which tadpoles (frogs) with Jl release zoospores	$\frac{1}{[time]}$
$\gamma^{Bd} (\gamma^{Jl})$	growth rate of Bd (Jl)	$\frac{1}{[time]}$
η_{FJl}	clearing rate of Jl	$\frac{1}{[time]}$
$\xi^{Bd} (\xi^{Jl})$	constant determining severity of density-dependent regulation of Bd (Jl) on population growth	$\frac{1}{[zspre]}$
$\psi_{Bd} (\psi_{Jl})$	mortality rate of Bd (Jl)	$\frac{1}{[time]}$

Table 2: *Model parameter values and their corresponding source*

Parameter	Value	Source
$g_s(x, Q)$	$0.489(6 - x)$	[1]
$g_{J1}(x, Q)$	$0.489(6 - x)$	-
$g_{Bd}(x, Q)$	$0.9 * 0.489(6 - x)$	-
μ_s	28	[1]
μ_{J1}	28	-
μ_{Bd}	30	-
$m_s(x, Q)$	$(3.400 \phi_0 + 0.232 \phi_1 + 2.908 \phi_2 + 3.093 \phi_3)(1.0 + 0.00343 Q)$	[1]
$m_{Bd}(x, Q)$	$1.2 * (3.400 \phi_0 + 0.232 \phi_1 + 2.908 \phi_2 + 3.093 \phi_3)(1.0 + 0.00343 Q)$	-
$m_{J1}(x, Q)$	$(3.400 \phi_0 + 0.232 \phi_1 + 2.908 \phi_2 + 3.093 \phi_3)(1.0 + 0.00343 Q)$	-
ν_{TBd}	6×10^{-9}	[21]
ν_{FBd}	4×10^{-8}	-
ν_{TJ1}	6×10^{-9}	[21]
ν_{FJ1}	4×10^{-8}	-
ρ^{TBd}	1×10^4	[21]
ρ^{FBd}	1×10^4	[14, 21]
ρ^{TJ1}	1×10^4	-
ρ^{FJ1}	1×10^4	-
γ^{Bd}	$0 - 9000$	[5]
γ^{J1}	$0 - 9000$	-
η_{FJ1}	$5.9 - 11.9$	[16]
ξ^{Bd}	10^{-9}	[21]
ξ^{J1}	10^{-9}	-
ψ_{Bd}	15128, at $37^\circ C$ 630, at $32^\circ C$ 32, at $30^\circ C$ (temperature/30)*32, when temperature is between $27^\circ C$ to $30^\circ C$ 13.7, else	[3] [18] [28] - [21]
ψ_{J1}	13.7	-

Figure Legends

Figure 1. Flow diagram of the mathematical model. Transmission is simulated among two stages (tadpoles and frogs) with *Bd* fungus and *Jl* bacteria. See text for model equations and Table 1 for model parameter descriptions and units. Solid lines represent vital rates; dashed lines represent transmission rates; dashed-dotted lines represent zoospore release rates; dotted line represent the shedding rate.

Figure 2. Average daily temperature of Phoenix, AZ, Lafayette, LA, and Los Angeles, CA for a six-year period beginning from 2004 to the end of 2009.

Figure 3. Frog population dynamics without *Bd* and *Jl* in Phoenix, AZ, Lafayette, LA, and Los Angeles, CA. (a) Frog population dynamics applying 27° C as the critical breeding temperature; (b) Frog population dynamics applying 18°C as the critical breeding temperature.

Figure 4. Frog population dynamics with *Bd* infection for different temperatures. Here $\gamma^{Bd} = 19$, $\gamma^{Jl} = 0$, and $\eta_{FJl} = 0$. All other parameter values are as in Table 2.

Figure 5. Influence of cost of *Bd* in frog growth rate using Los Angeles, CA temperature profile. Here $\gamma^{Bd} = 20$, $\gamma^{Jl} = 0$, and $\eta_{FJl} = 0$. All other parameter values are as in Table 2.

Figure 6. Influence of cost of *Bd* in the rate at which infected frogs die using Los Angeles, CA temperature profile. Here $\gamma^{Bd} = 20$, $\gamma^{Jl} = 0$, and $\eta_{FJl} = 0$. All other parameter values are as in Table 2.

Figure 7. Influence of cost of *Bd* in the reproduction rate of infected frogs using Los Angeles, CA temperature profile. Here $\gamma^{Bd} = 20$, $\gamma^{Jl} = 0$, and $\eta_{FJl} = 0$. All other parameter values are as in Table 2.

Figure 8. Influence of cost of *Bd* in the mortality rate for tadpoles infected with *Bd* using Los Angeles, CA temperature profile. Here $\gamma^{Bd} = 20$, $\gamma^{Jl} = 0$, and $\eta_{FJl} = 0$. All other parameter values are as in Table 2.

Figure 9. Influence of *Jl* inoculation rate ν_{TJl} and ν_{FJl} using Los Angeles, CA temperature profile. Here $\gamma^{Bd} = 20$, $\gamma^{Jl} = 20$, and $\eta_{FJl} = 0$. All other parameter values are as in Table 2.

Figure 10. Influence of free living stage γ^{Jl} growth rate using Los Angeles, CA temperature profile. Here $\gamma^{Bd} = 20$, $\gamma^{Jl} = 0, 20, 40, 100$, and $\eta_{FJl} = 0$. All other parameter values are as in Table 2.

Figure 11. Influence of Jl shedding rate η_{FJl} using Los Angeles, CA temperature profile. Here $\gamma^{Bd} = 20$, $\gamma^{Jl} = 20$, and $\eta_{FJl} = 0, 5.9, 7.9, 11.9$. All other parameter values are as in Table 2.

Figure 12. (a) Difference between dumping Jl during breeding and hibernation; (b) Effectiveness of inoculation depending on number of Jl zoospores.

Figure 1

Figure 2

(a)

(b)

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

(a)

(b)

Figure 12