A MATHEMATICAL MODEL OF THE SPREAD OF BATRACHOCYTRIUM DENDROBATIDIS IN THE CASCADES FROG (RANA CASCADAE)

By

Kathleen M. Harer

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Committee Membership

Dr. Christopher Dugaw, Committee Chair

Dr. Diane Johnson

Dr. John Reiss

Dr. Christopher Dugaw, Graduate Coordinator

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ABSTRACT

A MATHEMATICAL MODEL OF THE SPREAD OF BATRACHOCYTRIUM DENDROBATIDIS IN THE CASCADES FROG (RANA CASCADAE)

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Over recent decades the chytrid fungus Batrachochytrium dendrobatidis has been the cause of wide-spread disease in many amphibian populations. This study models the long term effects of the related disease, chytridiomycosis, in a population of Cascades frogs (Rana cascadae). Susceptibility and infectiousness were varied to determine the long-term outcome of the population: 1) extinction of the infected frogs and recovery of the frog population, 2) persistence of both the infected and uninfected populations, 3) extinction of both the infected and uninfected. Both the deterministic and stochastic models showed extinction as the most likely long term outcome.
ACKNOWLEDGEMENTS

To my family for all their patience and support. Special acknowledgement to my thesis committee for their wisdom and input: Dr. Christopher Dugaw, Dr. Diane Johnson, and Dr. John Reiss.
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Box plots show the quartiles of surviving adults when susceptibility varies and infectiousness is 0.001. a) Susceptibility of tadpoles, b) Susceptibility of juveniles, c) Susceptibility of adults. 24

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INTRODUCTION

There are many causes suggested for the amphibian decline that has been taking place over the last few decades (Blaustein, 1990; Berger et al., 1998). While this could be part of a natural mass extinction, it seems unlikely due to the other factors that have been studied surrounding amphibian mass mortality (Blaustein et al., 1994). One of the major stressors identified was human impact (Blaustein et al., 1994). Human expansion encroaches on amphibian habitat and can fragment it, breaking up populations and disrupting normal breeding processes. These human activities can also include drought, pesticide drift, and human impacts during conservation activities (Daszak et al., 2004; Carey et al., 1999). Other potential factors include non-native predators, climate change, and increased UV-B radiation (Adams et al., 2001; Lips, 1999).

Chytridiomycosis is a disease characterized by fungal zoospores that feed on the keratinized structures of amphibians (Berger et al., 1998; Speare, 2001; Nichols et al., 2001; Pessier et al., 1999; Fellers et al., 2001). Formally identified in 1998 as a major cause of amphibian decline worldwide (Berger et al., 1998), Green and Sherman referred to it as “the only infectious disease currently associated with population declines of multiple species” (Green and Sherman, 2001). It has been detected in many declining species throughout the United States (Berger et al., 1999; Green and Sherman, 2001; Milius, 1998; Fellers et al., 2001). Archival records indicate two of the earliest appearances of chytridiomycosis in North America were in the California Sierra Nevada and the Colorado Rockies in the 1970s (Carey et al., 1999). The disease has since been recorded worldwide including Africa, America, Europe, Asia and New Zealand (Lips, 1999; Mutschmann et al., 2000; Bosch et al., 2001; Uni et al., 2008; Bradley, 2002; Weldon, 2002), with mass mortality events recorded in Central America (Berger et al., 1998), North America (Muths et al.,
The causative agent of chytridiomycosis, *Batrachochytrium dendrobatidis* (Bd), has been found in many amphibians including salamanders, toads and frogs (Berger et al., 1998; Speare, 2001). It is spread by zoospores in the water, frog to frog contact, and by intermediaries such as rocks (Longcore et al., 1999).

Tadpoles are at a relatively low risk of developing chytridiomycosis, as they have only a small amount of keratin (located primarily in the mouthparts) (Rachowicz and Vredenburg, 2004). However, infected larvae and tadpoles carry the fungus through metamorphosis when the fungus begins feeding on the keratin in the epidermis (Berger et al., 1999; Speare, 2001; Boyle et al., 2004). Tadpoles are thought to be a major vector in the spread of the disease and stress brought on by environmental and infectious factors in the larval stage can lead to increased levels of mortality in adulthood. Although some studies have shown a low prevalence of Bd (Nieto et al., 2007; Green and Sherman, 2001), one study refers to the chytrid fungus as “insidious” and suggests that many outbreaks have gone undetected (Green and Sherman, 2001).

While Bd has negatively impacted many amphibian populations, some frog species appear to be resilient to chytridiomycosis. In 2004, an experimental study was conducted to determine the effects of Bd on American Bullfrogs (Daszak et al., 2004). After one month, 25-50% of post-metamorphic bullfrogs were histologically found to be infected with Bd, with no progression to chytridiomycosis or death even though they were inoculated with a strain of Bd that was known to be fatal to multiple other frog species. Bullfrogs are the most commonly farmed amphibian and have been known to escape captivity and establish feral populations, encroaching on the already struggling native species (Daszak et al., 2004; Garner, 2006). While they do not develop the disease themselves, Bullfrogs have been implicated as an important vector in the transmission of Bd to other amphibians.
One species negatively affected by Bd is the Cascades frog (Rana cascadae). This species had an International Union for Conservation of Nature (IUCN) listing of vulnerable in 1996 and is currently at near-threatened status. The IUCN states that the Cascades frog is probably in decline at a rate of less than or equal to 30% over ten years, which means it will likely be classified as vulnerable once again. Other than disease, threats to the Cascades frog include: (1) The presence of non-native predatory fish (Hayes and Jennings, 1986; Jennings and Hayes, 1994; Adams et al., 2001), (2) Gradual loss of habitat and limited dispersal, (3) Loss of breeding habitat due to drought (Fellers and Drost, 1993), (4) Pesticide drift (Davidson et al., 2001), and (5) UV-B radiation (Adams et al., 2001; Palen et al., 2002).

In 2004, a study showed that larvae of the Cascades frogs did not experience increased mortality after exposure to Bd, however there was an increase in mouthpart abnormalities, which is a symptom of the disease (Blaustein et al., 2005). Just two years later, an increase in mortality of juvenile Cascades frogs due to chytridiomycosis was shown in a laboratory setting (Garcia et al., 2006).

Also in 2006, a study examined the distribution of Bd in the Cascades frog in the Klamath Mountains, California and identified factors associated with Bd infection. Bd was shown to be present in several California Cascades frog populations and in all life stages which suggests the possibility of die-offs in the wild (Pope et al., 2014).

The Cascades Frog Conservation Assessment (Pope et al., 2014) discusses the status of the species and investigates factors related to their decline. The report showed low recruitment of juveniles to adults due to an insufficient amount of water in breeding ponds and a high susceptibility to Chytridiomycosis. The average survival rate of adult Cascades frogs in this report was 66%.
In 2011, Piovia-Scott et al. studied the factors related to the distribution and prevalence of Bd in the Cascades frog at 112 sites in the Klamath Mountains in northwest California. The objective of the study was to: (1) Determine the distribution of Bd, (2) Evaluate changes in the distribution of the Cascades frog, and (3) Assess associations between biotic and abiotic drivers and Bd infection. The study determined that Bd was present in 64% of sites and Cascades frogs were found in 79% of sites, most infected with Bd. The study also showed the prevalence of Bd for each life stage: tadpoles 4%, juveniles 36%, adults 25%. Two things to note are that juveniles had the highest prevalence of Bd, and that the probability of infection decreases during the breeding season for adults. One conclusion is that juveniles are most susceptible to chytridiomycosis due to the high prevalence of Bd in this life stage and the consistent probability of infection.

In 2005, Briggs et al. created a staged-based model of the spread of Bd in a population of mountain yellow-legged frogs (\textit{Rana muscosa}). In it they used a matrix to model the transitions between life and infectious stages, and a set of differential equations to model the spread of the disease. Simulations were run using varied sets of parameters to show different long term behaviors of the \textit{R. muscosa} population including recovery of the uninfected population, persistence of the infected and uninfected populations, and extinction of both populations. The study concluded that there were sets of parameters that supported each of the three scenarios.

In the present study, I investigated the effects of Bd on an \textit{R. cascadae} population by combining components of the Briggs 2005 model with more up-to-date findings to evaluate the effects of susceptibility and infectiousness. I used deterministic and stochastic models to evaluate the long term equilibrium of the disease. Some of results I expected to find were (1) The model predicts the long term equilibrium of infected adults is approximately 10% (2) There are sets of parameters that lead to all of the possible outcomes: recovery
of the uninfected population, persistence of the infected and uninfected populations, and extinction of both populations.
METHODS

Figure 1: The life cycle of a Cascades frog.

Figure 2: The yearly cycle of a Cascades frog in a diseased population.

The model includes tadpole, juvenile, and adult stages, and covers the yearly life cycle of a Cascades frog (figure 1). In the matrix model below, $L_{\tau}$ is the number of uninfected tadpoles, $J_{\tau}$ is the number of uninfected juveniles, and $A_{\tau}$ is the number of uninfected adult frogs, all in year $\tau$. $L_{I\tau}$ is the number of infected tadpoles, $J_{I\tau}$ is the number of infected juveniles, and $A_{I\tau}$ is the number of infected adult frogs, all in year $\tau$. For each cycle (figure 2), a frog will overwinter with yearly survival rates of $\sigma_L$, $\sigma_J$, $\sigma_A$, $\sigma_{LI}$, $\sigma_{JI}$, and $\sigma_{AI}$. This progresses to the breeding season and disease transmission phase with survival rates of $dL$, $dJ$, $dA$, $dLI$, $dJI$ and $dAI$. 

6
\[
\begin{bmatrix}
L \\
LI \\
J \\
JI \\
A \\
AI
\end{bmatrix}
\tau+1 = 
\begin{bmatrix}
0 & 0 & 0 & 0 & \sigma_A \cdot pF & 0 \\
0 & 0 & 0 & 0 & 0 & 0 \\
\sigma_L & 0 & 0 & 0 & 0 & 0 \\
0 & \delta \cdot \sigma_{LI} & 0 & 0 & 0 & 0 \\
0 & 0 & \sigma_J \cdot \eta & 0 & \sigma_A & 0 \\
0 & 0 & 0 & \delta \cdot \sigma_{JI} \cdot \eta & 0 & \sigma_{AI}
\end{bmatrix}
\begin{bmatrix}
L \\
LI \\
J \\
JI \\
A \\
AI
\end{bmatrix}
\tau
\]

\[\eta = \exp((AI_\tau + A_\tau))/K\] where \(AI_\tau + A_\tau\) refers to the number of infected and non-infected adults. \(K = A^*/\ln[\psi/(1-\sigma_A)]\) where \(\psi = (\sigma_A pF \cdot \sigma_J) [m_L \sigma_L (1-p_L) + \sigma_L p_L]\). The parameter \(A^*\) represents the adult density equilibrium in the deterministic model and \(K\) is the strength of density dependence and decides the average density of adults in a disease free population.

There were several assumptions made in the model. It was assumed the infection continues through metamorphosis, and infected adults do not give birth to infected tadpoles. It was also assumed the infection could spread within and between all life stages, which has been supported in experiments involving \(R.\ muscosa\) (Rachowicz and Vredenburg, 2004). Although \(Bd\) is spread through many means, this model assumes the zoospores were spread only by frog to frog contact.

There are reasonable parameter estimates from many sources as to the overwinter survival rates, probability of surviving metamorphosis, probability of an adult reproducing, and average fecundity. The parameters and their estimates are summarized in tables 1 & 2.
Table 1: Definitions for model parameters and the default values for uninfected frogs used in model simulations.

<table>
<thead>
<tr>
<th>Parameter description</th>
<th>Symbol</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overwinter survival probability, tadpoles</td>
<td>$\sigma_L$</td>
<td>0.00625</td>
<td>Doubledee et al. 2003</td>
</tr>
<tr>
<td>Overwinter survival probability, juvenile</td>
<td>$\sigma_J$</td>
<td>0.4</td>
<td>Licht 1974</td>
</tr>
<tr>
<td>Overwinter survival probability, adult</td>
<td>$\sigma_A$</td>
<td>0.66</td>
<td>Pope 2014</td>
</tr>
<tr>
<td>Probability of surviving metamorphosis</td>
<td>$m_L$</td>
<td>0.9</td>
<td>Briggs et al. 2005</td>
</tr>
<tr>
<td>Probability an adult reproduces in a given year</td>
<td>$pF$</td>
<td>0.5</td>
<td>Briggs et al. 2005</td>
</tr>
<tr>
<td>Average fecundity of reproducing adult</td>
<td>$F$</td>
<td>350</td>
<td>Pope pers. com.</td>
</tr>
<tr>
<td>Death rate of tadpoles over the summer</td>
<td>$d_L$</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>Death rate of juveniles over the summer</td>
<td>$d_J$</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Death rate of adults over the summer</td>
<td>$d_A$</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Susceptibility of tadpoles, juveniles, adults</td>
<td>$\phi_L \phi_J \phi_A$</td>
<td>varies</td>
<td></td>
</tr>
<tr>
<td>Infectiousness of tadpoles, juveniles, adults</td>
<td>$\gamma$</td>
<td>varies</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Definitions for model parameters and the default values for infected frogs used in model simulations.

<table>
<thead>
<tr>
<th>Parameter description</th>
<th>Symbol</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overwinter survival probability, tadpoles</td>
<td>$\sigma_{LI}$</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Overwinter survival probability, juvenile</td>
<td>$\sigma_{JI}$</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Overwinter survival probability, adult</td>
<td>$\sigma_{AI}$</td>
<td>0.3</td>
<td>Briggs et al. 2005</td>
</tr>
<tr>
<td>Probability of surviving metamorphosis</td>
<td>$m_{LI}$</td>
<td>0.5</td>
<td>Briggs et al. 2005</td>
</tr>
<tr>
<td>Probability an adult reproduces in a given year</td>
<td>$pF_I$</td>
<td>0</td>
<td>Briggs et al. 2005</td>
</tr>
<tr>
<td>Average fecundity of reproducing adult</td>
<td>$F_I$</td>
<td>0</td>
<td>Briggs et al. 2005</td>
</tr>
<tr>
<td>Death rate of tadpoles over the summer</td>
<td>$d_{LI}$</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>Death rate of juveniles over the summer</td>
<td>$d_{JI}$</td>
<td>0.2</td>
<td>Briggs et al. 2005</td>
</tr>
<tr>
<td>Death rate of adults over the summer</td>
<td>$d_{AI}$</td>
<td>0.01</td>
<td>Briggs et al. 2005</td>
</tr>
</tbody>
</table>
The disease transmission process is represented by the following set of differential equations:

\[
\begin{align*}
\frac{dL(t, \tau)}{dt} &= -v_L L(t, \tau) - d_L L(t, \tau) \\
\frac{dL_I(t, \tau)}{dt} &= v_L L(t, \tau) - d_{LI} L_I(t, \tau) \\
\frac{dJ(t, \tau)}{dt} &= -v_J J(t, \tau) - d_J J(t, \tau) \\
\frac{dJ_I(t, \tau)}{dt} &= v_J J(t, \tau) - d_{JI} J_I(t, \tau) \\
\frac{dA(t, \tau)}{dt} &= -v_A A(t, \tau) - d_A A(t, \tau) \\
\frac{dA_I(t, \tau)}{dt} &= v_A A(t, \tau) - d_{AI} A_I(t, \tau)
\end{align*}
\]

with \( v_L = v_J = v_A = \sigma_L [\gamma_L L_I(t, \tau) + \gamma_J J_I(t, \tau) + \gamma_A A_I(t, \tau)] \)

\( L(t, \tau), J(t, \tau), \) and \( A(t, \tau) \) are the number of non-infected Cascades frogs in each stage at time \( t \) and year \( \tau \), and \( L_I(t, \tau), J_I(t, \tau), \) and \( A_I(t, \tau) \) are the number of infected Cascades frogs in the same time and year and for each stage respectively. \( d_L, d_J, \) and \( d_A \) are the death rates of uninfected tadpoles, juveniles, and adults, respectively, and \( d_{LI}, d_{JI}, \) and \( d_{AI} \) are the death rates of infected individuals for the same stages.

The rate of disease transmission is equal to the overwinter survival probability of tadpoles (\( \sigma_L \)) multiplied by the sum of the infected individuals (\( \gamma_L L_I(t, \tau) + \gamma_J J_I(t, \tau) + \gamma_A A_I(t, \tau) \)) and the number of healthy frogs in that stage (\( L(t, \tau), J(t, \tau), \) or \( A(t, \tau) \)). From this subtract the death rate multiplied by the number of infected frogs (\( L_I(t, \tau), J_I(t, \tau), \) or \( A_I(t, \tau) \)).

The deterministic model begins with the initial count of frogs \( x_0 \) in each life and infectious stage. There is no disease transmission during the overwinter or breeding phases and \( x_0 \) is multiplied by the transmission matrix sub-model \( M \). This yields the number of frogs \( Mx_0 \) that will enter the disease transmission phase. We solve the differential equations that model this phase for \( t = 0 \) to \( T \) where \( T=120 \) days. A solution of the differential equations
yields the number of frogs following the disease transmission mortality phase of $T$ days in year $\tau$, which is also the number of frogs in each life and infectious stage in year $\tau + 1$.

The stochastic model follows the method outlined in section 15.1.3 of Caswell 2001. This method uses the transmission matrix $M$ and the fertility matrix $R$. For all stages in $x_0$, a random vector was generated from a multinomial distribution with parameters $x_o$ and $M$. The addition of these vectors gives the number of frogs produced at $t + 1$ by transition. In the event that $x(i) > 0$ and $r(1, i) > 0$ a vector is drawn from a binomial distribution with parameters $x(5)$ and $\sigma_A * pF * F$, where $F$ is drawn from a Poisson distribution with a mean fecundity of 350 eggs. The resulting vector contains the number of frogs at $t + 1$ by births. Addition of these two vectors gives the number of frogs in each life and infectious stage for time $t + 1$.

The stochastic model begins with the same initial phases of the deterministic model. They use the same initial count of frogs $x_0$ in each life and infectious stage, and start with the same transition matrix $M$. For each column entry in the resulting $Mx_0$, create a random $1 \times 6$ matrix $p$. The entry $p(1)$ is drawn from a binomial distribution with parameters $Mx_0(:\endtype)$ and $\sigma_A * pF$, where $\sigma_A$ is the overwinter survival probability for uninfected adults and $pF$ is the probability an uninfected adult reproduces. Add to $p(1)$ another random number drawn from a binomial distribution with parameters $p(5)$ and $\sigma_A * pF * F$, where $F$ is drawn from a Poisson distribution with a mean fecundity of 350 eggs. This yields the number of frogs that will enter the disease transmission phase. Instead of using the differential equations from the deterministic model we use 12 transitions and their rates to model the disease transmission phase (Table 3). Although amphibian species have been shown to clear infection at times, this transition was not included in this model.

Simulations were run by varying a frog’s susceptibility ($\phi_L, \phi_J, \phi_A$) and infectiousness ($\gamma$) to determine the long term effects of a Cascades frog population with Bd. The level
Table 3: 12 possible events with transitions and rates

<table>
<thead>
<tr>
<th>Event</th>
<th>Transition</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected tadpole dies</td>
<td>L → L-1</td>
<td>( v_L L(t, \tau) )</td>
</tr>
<tr>
<td>Tadpole gets infected</td>
<td>LI → LI+1</td>
<td>( v_L L(t, \tau) )</td>
</tr>
<tr>
<td>Infected tadpole dies</td>
<td>LI → LI-1</td>
<td>( d_{LI} L(t, \tau) )</td>
</tr>
<tr>
<td>Uninfected tadpole dies</td>
<td>L → L-1</td>
<td>( d_L L(t, \tau) )</td>
</tr>
<tr>
<td>Uninfected juvenile dies</td>
<td>J → J-1</td>
<td>( v_J J(t, \tau) )</td>
</tr>
<tr>
<td>Juvenile gets infected</td>
<td>JI → JI+1</td>
<td>( v_J J(t, \tau) )</td>
</tr>
<tr>
<td>Infected juvenile dies</td>
<td>JI → JI-1</td>
<td>( d_{JI} J(t, \tau) )</td>
</tr>
<tr>
<td>Uninfected juvenile dies</td>
<td>J → J-1</td>
<td>( d_J J(t, \tau) )</td>
</tr>
<tr>
<td>Uninfected adult dies</td>
<td>A → A-1</td>
<td>( v_A A(t, \tau) )</td>
</tr>
<tr>
<td>Adult gets infected</td>
<td>AI → AI+1</td>
<td>( v_A A(t, \tau) )</td>
</tr>
<tr>
<td>Infected adult dies</td>
<td>AI → AI-1</td>
<td>( d_{AI} A(t, \tau) )</td>
</tr>
<tr>
<td>Uninfected adult dies</td>
<td>A → A-1</td>
<td>( d_A A(t, \tau) )</td>
</tr>
</tbody>
</table>

of infectiousness is equal for all stages \( \gamma_L = \gamma_J = \gamma_A \) (there is no evidence to support a difference in the level of infectiousness) (Pope et al., 2014). Juvenile frogs are the most susceptible to Bd and their level of infectiousness is set to 1. Lab experiments have also revealed that post metamorphic individuals are easier to infect with the disease than tadpoles and \( \phi_L \) and \( \phi_A \) are set relative to \( \phi_J \). It is assumed that tadpole susceptibility is approximately one tenth of juvenile susceptibility and adult susceptibility is approximately one half of juvenile susceptibility.

The simulation starts with an initial population vector that includes 50 individuals in each of the healthy frog stages, 1 infected tadpole, 0 infected juveniles, and 5 infected adults. Infectiousness \( \gamma \) begins at 0.00001 and varies by magnitude up to 1.0. For each \( \gamma \), we vary \( \phi_L \) for the following numbers: 0.00001, 0.0001, 0.001, 0.01, 0.1, and 1. Then we will similarly vary \( \phi_J \) and \( \phi_A \). After this, we increase \( \gamma \) by one order of magnitude and vary \( \phi_L, \phi_J, \) and \( \phi_A \) as before.
RESULTS

Deterministic

Parameter graphs were used to show the relationship of the two sub-populations with varying levels of susceptibility and infectiousness. Each life stage has a different level of susceptibility, and there is only one level of infectiousness. There is no evidence to show there are different levels of infectiousness but there is evidence that susceptibility can vary (Garcia et al., 2006).

In the deterministic model, the most frequent long-term outcome is extinction of both populations. Long term extinction of both the uninfected and infected population occurs in approximately 61% of all outcomes. About 18% of outcomes resulted in extinction of the infected population and recovery of the uninfected population, and 21% of outcomes resulted in persistence of both populations. In the deterministic model susceptibility did not appear to be a factor but infectiousness did. In the graph where infectiousness is varied, it reflects the same pattern of long term outcomes demonstrated by the graphs that varied susceptibility. When infectiousness is low there is a recovery of the uninfected population. As infectiousness rises there is a persistence of all frogs regardless of life stage. When infectiousness is greater than 0.01 there is an extinction of all frogs (figs. 4,5,6,7).

Regardless of the life stage, when infectiousness is 0.0001 generally the infected population goes extinct and the uninfected population recovers to approximately 45 individuals. The exception is when susceptibility is higher than 0.1 for tadpoles and adults (fig. 4). Both populations persist when infectiousness is increased to 0.001. The infected population persists at approximately 30%, 15%, and 2% for tadpoles, juveniles and adults respectively, with an average of 16% infected (fig. 5). When infectiousness is greater than 0.01 both populations become extinct regardless of life stage (fig. 6).
Figure 3: Representation of deterministic long term outcomes: a) Extinction of the infected population and recovery of the uninfected population, b) Persistence of the infected and uninfected populations, c) Both populations become extinct.
Figure 4: Parameter graphs show the effect of varying susceptibility levels by life stage when infectiousness is 0.0001. a) Varying susceptibility of tadpoles, b) Varying susceptibility of juveniles, c) Varying susceptibility of adults.
When infectiousness is 0.001 the most common outcome is persistence of both the uninfected and infected adult populations. As tadpole susceptibility increases, the infected individuals persist at 30% and slowly decline to extinction (fig. 5a). Any level of juvenile susceptibility leads to persistence of both the diseased and the uninfected individuals (fig. 5b). As adult susceptibility rises, the uninfected population persists and the infected population grows to only 1 individual when it is at its highest (fig. 5c). When infectiousness is 0.001, any level of susceptibility for tadpoles and juveniles yields persistence of both the infected and uninfected populations (fig 5ab). As tadpole susceptibility increases, the infected population persists at approximately 30%, and as juvenile susceptibility varies, the infected population persists at approximately 25%. Any level of adult susceptibility leads to persistence of the infected population at approximately 2% (fig. 5c). When infectiousness is high (> 0.01), any level of susceptibility and life stage leads to extinction of both populations (fig. 6).

When holding the susceptibility levels constant and varying infectiousness, the trend suggested in figures 4, 5, and 6 was confirmed. When infectiousness equals 0.0001, the infected population becomes extinct and the uninfected population recovers (fig 7). When infectiousness is 0.001, both populations persist at different levels based on the susceptibility of the life stage, and when infectiousness is greater than or equal to 0.01 both populations become extinct (fig 7).
Figure 5: Parameter graphs show the effect of varying susceptibility levels by life stage when infectiousness is 0.001. a) Varying susceptibility of tadpoles, b) Varying susceptibility of juveniles, c) Varying susceptibility of adults.
Figure 6: Parameter graphs show the effect of varying susceptibility levels by life stage when infectiousness is 0.01. a) Varying susceptibility of tadpoles, b) Varying susceptibility of juveniles, c) Varying susceptibility of adults.
Figure 7: Parameter graph for infectiousness ($\gamma$)

Table 4: Summary of results from the parameter graphs shown in the results section. The column that contains bold face values correspond to a range in one particular life stage (tadpoles, juveniles, or adults). The infectiousness ($\gamma$) is the same for all life stages, and increases for each life stage from low to high. The default values are: $\phi_L=0.01$, $\phi_J=1$, $\phi_A=0.5$

<table>
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<th>$\gamma$</th>
<th>$\phi_L$</th>
<th>$\phi_J$</th>
<th>$\phi_A$</th>
<th>Long-term outcome</th>
</tr>
</thead>
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</tr>
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</tr>
<tr>
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<td><strong>0.00001:1</strong></td>
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</tr>
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<tr>
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<td>0.01</td>
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<td><strong>0.00001:1</strong></td>
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</tr>
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</tr>
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<td><strong>0.1:1</strong></td>
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</tr>
<tr>
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<td><strong>0.00001:1</strong></td>
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</tr>
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</table>
Figure 8: Representation of stochastic long term outcomes: a) Extinction of the infected population and recovery of the uninfected population, (b) Both populations become extinct.

The two long term outcomes realized through stochastic simulations were extinction of the infected population and recovery of the uninfected, and total extinction (fig. 8). There were no simulations that resulted in persistence of the infected population, regardless of the outcome of the uninfected population. Parameter graphs were used to show the relationship of the infected and uninfected populations with varying levels of susceptibility and infectiousness. Each life stage has a different level of susceptibility and there is one level of
infectiousness. Simulations were run 1000 times for 300 years each.

About 23% of the tadpole simulations resulted in a long term recovery of the uninfected population and the number of the uninfected adults is about 15 regardless of life stage. Juvenile simulations resulted in recovery of about 22% of the time, and adult simulations resulted in recovery about 21% of the time. The only other outcome is an extinction of frogs regardless of life stage and susceptibility.

Recovery was the only long term outcome in all simulations where susceptibility varied and infectiousness was 0.0001 regardless of the life stage (fig. 9). When infectiousness is 0.001 the average number of uninfected individuals is around 15 (fig. 10). The life stage and level of susceptibility did not seem to have as high an impact as infectiousness, where an extinction occurred when infectiousness is greater than 0.01. In the stochastic model, susceptibility does not seem to be a factor in long term outcome in any life stage. However infectiousness does seem to be a factor, as low infectiousness gives way to a recovery of the uninfected population and high levels cause an extinction.

The deterministic and stochastic graphs that vary infectiousness show similar patterns (fig. 7,13). When infectiousness is low the uninfected population recovers, and when infectiousness is high there is an extinction. The levels of susceptibility in which there is a significant change is when infectiousness is 0.001 and 0.01. In the deterministic model there is a decline in uninfected adults and a re-introduction of the infected population followed by an extinction at higher levels of infectiousness (fig. 7). In the stochastic model there is a sharp decline in the uninfected population at 0.001 and extinction at 0.01 (fig. 13).
Figure 9: Parameter graphs show the effect of varying susceptibility levels by life stage when infectiousness is 0.0001. a) Varying susceptibility of tadpoles, b) Varying susceptibility of juveniles, c) Varying susceptibility of adults.
Figure 10: Parameter graphs show the effect of varying susceptibility levels by life stage when infectiousness is 0.001. a) Varying susceptibility of tadpoles, b) Varying susceptibility of juveniles, c) Varying susceptibility of adults.
Figure 11: Box plots show the quartiles of surviving adults when susceptibility varies and infectiousness is 0.0001. a) Susceptibility of tadpoles, b) Susceptibility of juveniles, c) Susceptibility of adults.
Figure 12: Box plots show the quartiles of surviving adults when susceptibility varies and infectiousness is 0.001. a) Susceptibility of tadpoles, b) Susceptibility of juveniles, c) Susceptibility of adults.
Table 5: Summary of results from the parameter graphs shown in the results section. The column that contains bold face values correspond to a range in one particular life stage that is being varied (tadpoles, juveniles, or adults). The infectiousness ($\gamma$) is the same for all life stages, and increases for each life stage from low to high. The default values are: $\phi_L=0.01$, $\phi_J=1$, $\phi_A=0.5$

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<th>$\phi_J$</th>
<th>$\phi_A$</th>
<th>Long-term outcome</th>
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Figure 13: Parameter graph for infectiousness ($\gamma$)
DISCUSSION

In agreement with many studies mentioned in this paper (Jennings and Hayes, 1994; Fellers et al., 2008; Pope et al., 2014), this model confirms that the Cascades frog population is heading towards extinction when *Batrachochytrium dendrobatidis* is located in or near their habitat. The deterministic model shows approximately 45 uninfected adults recover when the diseased adults go extinct, and the equilibrium for the stochastic model reaches about 70 uninfected individuals.

When the outcome is long term persistence of the disease and frog population approximately 17% of infected adults persist. This is greater than the hypothesized 10%, which could be caused by many factors. This model originated from ideas from a 2005 study by Briggs et al., which focused on the mountain yellow-legged frog. These species could have different levels of susceptibility and infectiousness than the Cascades frog. Many things have been changed to reflect the latest information, which will cause it to have different results.

The most common long term outcome for both the deterministic and stochastic models was extinction, especially when infectiousness was greater than 0.01, which is similar in both models. The stochastic model shows that extinction of the disease and persistence of the uninfected adults happened approximately 13% of the time and a long term extinction of the disease and the frogs happened the other 87% of the time. There were no stochastic simulations where the infected adults persisted which shows many factors need to be taken into account.

Tadpoles have not been exposed to the pathogen for as long as adults, which some say may reduce the probability of developing detectable infections. Bd infects keratinized epidermal cells in amphibians (Pessier et al., 1999; Piotrowski et al., 2004), which suggests
tadpoles are the least at risk for chytridiomycosis. Tadpoles have only a small amount of keratin on their bodies that is located primarily on the mouthparts (Rachowicz and Vredenburg, 2004) (as opposed to their skin), and there is no keratin available for the fungus to feed on. Juveniles and adults have keratin located all throughout their skin. As infected tadpoles pass through metamorphosis the fungus generally begins feeding on the keratin in the epidermis (Berger et al., 1999; Speare, 2001; Boyle et al., 2004). A 2005 study showed three Bd positive *R. cascadae* tadpoles survived through metamorphosis until the end of the 30 day study (Blaustein et al., 2005), but the long term outcomes of these frogs and the impact they could have on their population are unknown.

A 2011 study investigated the dynamics of predator prey interactions when salamanders are the predators and *R. cascadae* tadpoles are the prey (along with three other frog species) (Han et al., 2011). Salamanders are thought to be one of the most common predators in amphibian habitats and are also susceptible to Bd. The study found the predation risk for another species, *Anaxyrus boreas*, decreased due to more active swimming and greater likelihood they would take refuge. However *R. cascadae* tadpoles were unaffected by Bd, possibly because *R. cascadae* tadpoles require more information before responding with anti-predator behavior. Salamanders were not selective in their predation based on infection status, which could affect the long term survival of salamanders and frogs in co-habitation.

Juveniles have been shown to be more likely than other life stages to be infected with Bd and their probability of infection does not change over the summer (Piovia-Scott et al., 2011). Juvenile survival has a strong influence on the population dynamics of many anuran species relative to larval survival, which suggests the susceptibility of juveniles to Bd could have significant consequences on declining amphibian populations. A 2006 study found juvenile Cascades frogs were highly susceptible to Bd and died in large numbers within one week of exposure (Garcia et al., 2006).
Even though the juvenile stage is shown to be the most susceptible to Bd, in both the deterministic and stochastic models juvenile susceptibility did not appear to be a factor in the long term outcome of either population, which could be because the juvenile stage is much shorter than both the tadpole and adult stages. Tadpole and adult susceptibility seem to have similar effects on the survival of the adult population. They both respond similarly to changes in susceptibility and infectiousness (at different levels of susceptibility cover all three long term outcomes).

The possibility that Bd exists between outbreaks along with the possibility of non-amphibian vectors should be considered (Daszak et al., 2003; Johnson and Speare, 2003). A saprobe is an organism (fungus, bacteria) that lives on dead organic matter and if Bd persisted similarly, the fungus could remain in the habitat year round (Garcia et al., 2006). The possibility that Bd acts as a saprobe or host reservoir was not included in this model, but previous models which included this possibility have shown this leads to extinction of the uninfected population and persistence of the infected population (Godfray et al., 1999). This particular outcome was the only long-term outcome not seen in any simulations run in this study.

Cascades frogs in the Pacific Northwest are grouped as one taxonomic unit, but some suggest this designation may be changing since evidence has been shown there are multiple classes of Cascades frogs in California. Cascades frogs in this area occupy a range of aquatic habitats and meadows, they breed in still or very slow-flowing water, and their larvae metamorphose in the same season they develop from eggs. A 2008 study documented that dramatic declines have occurred in *R. cascadae* populations in many parts of California (Fellers et al., 2008). In the Sierra Nevada, populations were shown to have become extinct only a few years after Bd was detected. Although they became extinct within a few years of detection, it is unknown exactly how long the population was infected before detection.
This is consistent with the results from the model which gave a mean time to extinction of 4.6 years, which is even greater than that of the stochastic simulations. The pattern of die-off in the model is as follows: infected frogs die first, followed by uninfected juveniles, then tadpoles and adults.

The ecological parameters influencing susceptibility of amphibians to Bd in nature are largely unknown (Garcia et al., 2006). Bd can be reduced in efficacy due to high temperatures and variability in temperature. Some frog populations show an increase in survival with either of these factors. Amphibian immune capacity can also be temperature sensitive (Raffel et al., 2006). The probability that an adult becomes infected with Bd decreases as the temperature increases. Through the summer, adults have a greater ability to resist or clear the infection, but that was not included in this model. This could lead to reduced prevalence of Bd in adults in the summer (Piovia-Scott et al., 2011). Studies have shown adults have an elevated mortality within one week of exposure and die within weeks of infection (Briggs et al., 2005). Variability in temperature could also lead to increased immune function in juveniles, which increases their ability to clear infections as effectively as adults (Fisher et al., 2009).

Many studies have implicated climate change as a factor in long term survival and one study suggests that climate change is the biggest growing threat to amphibian populations (Blaustein et al., 2001). Changing temperature may interfere with breeding, hibernation and ability to find food. This could lead to a change in long-term population structure. Climate change could also affect amphibian immune capacity by increasing the spread of disease, increasing pollution, acidification and UV radiation (Blaustein et al., 2001)

A 2011 study showed Bd prevalence was low in tadpoles (Piovia-Scott et al., 2011), and in laboratory trials Bd did not increase mortality in tadpoles (Blaustein et al., 2005; Han et al., 2008, 2011; Searle et al., 2010). Another study showed no tadpoles infected with Bd,
however there were abnormalities of the tooth and jaw which included missing or complete loss of the keratinized structures of the mouth parts (Blaustein et al., 2005). *R. cascadae* showed greater persistence of the infected frogs than the uninfected frogs. Another thing to note is that three Bd+ *R. cascadae* and four controls (which were Bd-) completely metamorphosed by the end of the 30 day study, which is in contrast to studies involving other anuran species in which all Bd+ tadpoles died while passing through metamorphosis. In the Piovia-Scott study, approximately 14% of tadpoles survived metamorphosis.

It is unknown why some anuran species are more vulnerable to mass mortality events due to Bd, and other infected populations thrive. A current isolated strain of Bd is thought to be a recently emerged clone (Morehouse et al., 2003). Different strains of Bd are genetically similar but they may affect amphibian species differently. Some species may not have been exposed to Bd for as long as others and may show signs of infection in the future. The environment may be a factor due to its ability to act as a host reservoir, and may explain how different communities of the same species may respond differently to Bd. The environment also provides several predators such as non-native fish, birds, snakes and otters.

This model approaches the Cascades frog as a single population in a single pond, as opposed to a metapopulation where there are multiple frog populations in different ponds connected by dispersal (Hanski and Gilpin, 1997). The dynamics of this type of system include movement within each pond and among all the populations. While many metapopulation models show that increasing movement among populations reduces the chance of metapopulation extinction, epidemiological models show this can lead to an epidemic and total extinction (Hess, 1996). A fusion of these types of models was merged in 1996 and showed highly contagious diseases of moderate severity spread widely and led to total extinction (Hess, 1996). The key is whether or not there are enough infected frogs dispersing
and infecting other populations. The current study shows that infectiousness is a greater factor than susceptibility. At low levels of infectiousness the uninfected frogs persist at a low level and may not have the number of individuals needed in order to spread the disease to other populations. However, when infectiousness is moderate or high the single population dies out and there may be greater numbers of infected frogs to spread the disease and lead to a total extinction when extended to a metapopulation.

Future work

- To continue the approach of this study:
  - Examine the outcome when infectiousness varies by life stage.
  - Account for differences in rates of infection throughout the summer.
  - Extend the model to include multiple ponds and populations.

- Investigate the regional differences in antimicrobial peptides in *Rana cascadae* skin, which have been shown in other species to be a defense against Chytridiomycosis.

- The differences in susceptibility would suggest that we should examine different species and life stages to fully determine how Bd affects amphibians.

- Examine the effects of the length of time infected tadpoles, juveniles and adults are surviving with the disease.

- Investigate factors that might allow infected post-metamorphic individuals to survive.

- Continued Conservation Assessment in the coming years - this species should be watched.
BIBLIOGRAPHY


