Ecology, 86(12), 2005, pp. 3149–3159

INVESTIGATING THE POPULATION-LEVEL EFFECTS OF CHYTRIDIOMYCOSIS: AN EMERGING INFECTIOUS DISEASE OF AMPHIBIANS

CHERYL J. BRIGGS,[1,3] VANCE T. VREDENBURG,[1] ROLAND A. KNAPP,[2] AND LARA J. RACHOWICZ[1]

[1]*Department of Integrative Biology, University of California, Berkeley, California 94720-3140 USA*
[2]*Sierra Nevada Aquatic Research Laboratory, University of California, HCR 79, Box 198, Crowley Lake, California 93546 USA*

Abstract. Chytridiomycosis is an emerging infectious disease that has recently been reported in amphibian populations throughout the world. It has been associated with many cases of population declines and extinctions. In some areas of the Sierra Nevada of California the disease appears to be the causal factor in the rapid extinction of local populations of the mountain yellow-legged frog, *Rana muscosa*, within a few years of the first detection of the disease. In other areas, however, *R. muscosa* populations appear to persist for many years, despite high levels of infection in tadpoles. Here we present simple models of the dynamics of the disease within an individual lake and ask whether our current understanding of the disease is consistent with the field survey observations of: (a) extinction due to the disease over a wide range of host population sizes, and (b) persistence of frog populations with the disease at some sites. Despite our laboratory observation of chytridiomycosis being invariably lethal to postmetamorphic frogs, the observed long-term persistence of infected frog populations can only be explained if at least some infected adult frogs survive and reproduce.

Key words: amphibian declines; Batrachochytrium dendrobatidis; *chytrid fungus; demographic stochasticity; model;* Rana muscosa.

INTRODUCTION

In the late 1990s a new disease was noticed in amphibian populations, independently in several widely separated and remote parts of the world. Berger et al. (1998) first identified a chytridiomycete fungus associated with diseased frogs at sites of frog die-offs in Australia and Central America. In 1999, Longcore et al. isolated the fungus, and described the apparent disease-causing organism as a new genus and species of chytrid fungus, *Batrachochytrium dendrobatidis*. (The disease caused by the fungus has been termed chytridiomycosis.) Since that time, *B. dendrobatidis* has been identified in >100 amphibians (e.g., Young et al. 2001, Lips et al. 2003, Berger et al. 2004), and has been implicated in many cases of population declines and possible extinctions throughout the world (e.g., Bosch et al. 2001, Muths et al. 2003, Lips et al. 2004). Whether *B. dendrobatidis* is a new pathogen that has recently spread to all of these parts of the world, or an existing fungus that has recently turned pathogenic, is an area of active current research (Morehouse et al. 2003).

In the few years since the discovery of *B. dendrobatidis*, efforts have been underway to understand its basic biology, genetics, transmission dynamics, mechanism of disease, and impact on individuals. However, at this point there is much that we do not know about the disease. Simple models, such as the one presented here, provide a framework for gathering together the pieces of information that are being assembled from experiments and observations, and for understanding their potential population-level consequences. Models can suggest crucial parameters to measure in the field, and help us prioritize our experimental investigations.

In this paper, we describe one attempt to understand the devastating effect that chytridiomycosis is having on a once common species of frog native to the high-elevation lakes in the California Sierra Nevada. Over the last few years, rapid local extinctions of some of the last remaining large populations of mountain yellow-legged frogs (*Rana muscosa*) have occurred, with the frogs disappearing from some entire watersheds of the Sierra Nevada. Chytridiomycosis appears to be the most likely cause of these die-offs (L. J. Rachowicz, R. A. Knapp, J. Morgan, M. Stice, V. T. Vredenburg, J. Parker, and C. J. Briggs, *unpublished manuscript*). However, at several sites in the northern Sierra Nevada, which were some of the first sites at which the disease was identified in *R. muscosa* (Fellers et al. 2001), the infected frog populations have persisted for as long as we have known about the disease. Here we use a simple stochastic model to ask whether our current understanding of the disease is consistent with the field sur-

Manuscript received 14 September 2004; revised 28 February 2005; accepted 14 March 2005. Corresponding Editor: A. R. Ives. For reprints of this Special Feature, see footnote 1, p. 3137.

[3] E-mail: cbriggs@berkeley.edu

vey observations of: (a) extinction due to the disease over a wide range of host population sizes, and (b) persistence of the frog populations with the disease at some sites.

Study System

The disease: Chytridiomycosis caused by the chytrid fungus Batrachochytrium dendrobatidis

The chytrid fungus *Batrachochytrium dendrobatidis* (Longcore) specifically attacks keratin in amphibians. Tadpoles have keratin in their mouthparts, whereas postmetamorphic frogs have keratin throughout their skin. Therefore, the impact of the disease on individuals is stage specific. We have not detected negative health impacts of the disease on *R. muscosa* tadpoles (Rachowicz and Vredenburg 2004; but see Parris 2004, Parris and Baud 2004). In postmetamorphic animals, the impact of chytridiomycosis is highly species specific. Some widespread species, such as *Rana catesbeiana* (American bullfrog) (Daszak et al. 2004) and *Xenopus laevis* (Parker et al. 2002), appear to be carrier species, in which infected adults are apparently unaffected. However, in other species (including *R. muscosa*) postmetamorphic individuals can die due to acute chytridiomycosis within days or weeks of infection (Nichols et al. 2001, Lips et al. 2004, Rachowicz and Vredenburg 2004; L. J. Rachowicz, R. A. Knapp, J. Morgan, M. Stice, V. T. Vredenburg, J. Parker, and C. J. Briggs, *unpublished manuscript*).

The only known infectious stage of *B. dendrobatidis* is a mobile aquatic zoospore, which is released from infected tissue and targets keratin-containing material (Longcore et al. 1999, Piotrowski et al. 2004). Zoospores live for <24 hours, and no long-lived stage that can persist outside the host has been observed. However, *B. dendrobatidis* can be cultured in tryptone broth (Longcore et al. 1999), and other chytridiomycetes are saprophytes, so there is the potential that it might be able to persist saprophytically (Johnson and Speare 2003).

The host: Rana muscosa, *mountain yellow-legged frogs*

Rana muscosa (Camp) is a native Californian frog that exists almost entirely on protected federal lands at high elevations (up to 3700 m) (Knapp and Matthews 2000). This once common species has become increasingly rare during the past century (Vredenburg et al. 2005). The best-documented cause of the decline during most of the last century was the introduction of nonnative trout for recreational fishing (Bradford 1989, Knapp and Matthews 2000, Vredenburg 2004); however, trout stocking was halted in the national parks in the 1990s. The remaining fragmented populations of *R. muscosa* are now being threatened by chytridiomycosis.

Chytridiomycosis in Rana muscosa

We have learned the following about chytridiomycosis in *R. muscosa* from laboratory and field studies:

1) *R. muscosa* tadpoles can become infected through contact with infected individuals and/or *B. dendrobatidis* zoospores from culture, and carry and transmit the disease (Rachowicz and Vredenburg 2004). *R. muscosa*'s multiyear tadpole stage (up to three years [Zweifel 1955]) provides a potential within-species long-lived reservoir for the disease.
2) *R. muscosa* individuals infected during the tadpole stage die as they pass through metamorphosis (Rachowicz and Vredenburg 2004). L. J. Rachowicz et al. (L. J. Rachowicz, R. A. Knapp, J. Morgan, M. Stice, V. T. Vredenburg, J. Parker, and C. J. Briggs, *unpublished manuscript*) have shown through both laboratory and field experiments that virtually 100% of infected *R. muscosa* tadpoles die within a few weeks of metamorphosis.
3) Postmetamorphic *R. muscosa* individuals can become infected through contact with infected tadpoles, postmetamorphic individuals, and/or *B. dendrobatidis* zoospores. Infected postmetamorphic individuals in the laboratory die ~5 weeks postexposure (L. J. Rachowicz, R. A. Knapp, J. Morgan, M. Stice, V. T. Vredenburg, J. Parker, and C. J. Briggs, *unpublished manuscript*). All stages of *R. muscosa* are highly aquatic, rarely moving >1 m from water (Bradford 1983), keeping them in contact with this water-borne pathogen.

The earliest documented case of chytridiomycosis in *R. muscosa* is from 1998 at several sites in the northern Sierra Nevada in Yosemite National Park (Fellers et al. 2001). However, evidence from museum specimens suggests that *B. dendrobatidis* was present in the Sierra Nevada at least two decades earlier (Green and Sherman 2001). At several sites in the northern Sierra, *R. muscosa* populations appear to be persisting with the fungus. In at least one of the initial sites in which *B. dendrobatidis* was confirmed in 1998 (Summit Meadow, Yosemite National Park [Fellers et al. 2001]), *R. muscosa* populations infected with chytridiomycosis are still present today (at least six years after first identification of the disease). Population data are scarce for these northern Sierra Nevada sites, but one example is shown in Fig. 1b. In other areas, especially in the southern Sierra, R. Knapp (*unpublished data*; L. J. Rachowicz, R. A. Knapp, J. Morgan, M. Stice, V. T. Vredenburg, J. Parker, and C. J. Briggs, *unpublished manuscript*) has documented many cases in which extinction of *R. muscosa* populations occurred within a few years after *B. dendrobatidis* was first observed. In these southern sites, the observed pattern is that dead and dying postmetamorphic individuals are noticed, fol-

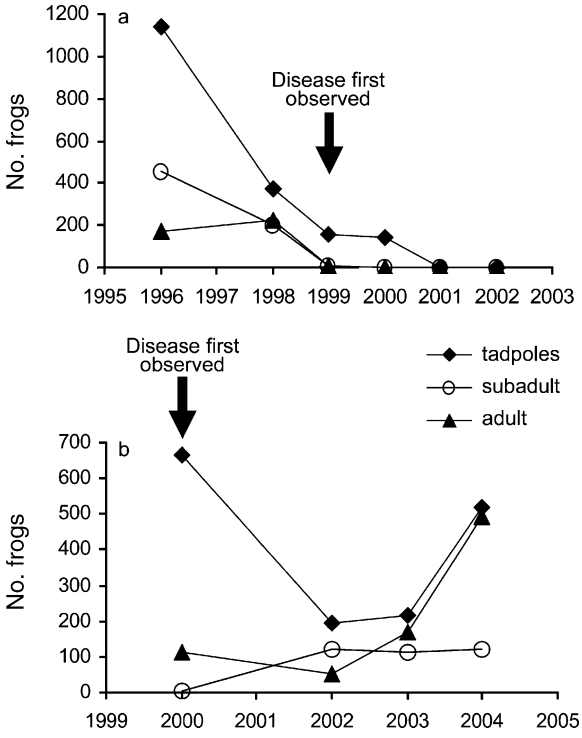

FIG. 1. Field data from an example of a frog die-off site and an example of a ''persistent'' site. (a) Rapid die-off within two years of initial disease detection in Frog Lake, Humphreys Basin, Sierra National Forest, California, USA, 1996–2003. (b) Apparent persistence with the disease at ''Conness Pond,'' Yosemite National Park, California. Averages of visual survey counts each year are shown.

lowed by infected tadpoles. The tadpoles may persist for a few years, but as each age cohort passes through metamorphosis they die of chytridiomycosis (Fig. 1a). Rapid die-offs of *R. muscosa* populations consistent with chytridiomycosis have occurred in >50 southern Sierran water bodies in recent years (R. Knapp, *unpublished data*).

Theoretical Approach

The model that we present here describes the dynamics of the frog population in a single lake. In the long term, mountain yellow-legged frogs probably exist as a metapopulation, with populations going extinct and being recolonized. Spatial processes and dispersal are, of course, likely to be important in the invasion of the disease into new lakes and in the spread of the disease through the Sierra Nevada. The model described here, however, considers only what happens after the disease invades a new lake. The pattern that we have observed in the southern Sierra Nevada is that the frog population is driven extinct within a couple of years following disease invasion. *R. muscosa* has very limited dispersal (V. T. Vredenburg, *unpublished data*), so over this short time scale, immigration and emigration of frogs are probably not that important to the dynamics within a single lake.

Here we develop a stage-structured stochastic simulation model. It was important to include demographic stochasticity in the model because at many of the sites where the frogs appear to be persisting with the disease, the frog populations are very small, consisting of only tens to hundreds of postmetamorphic individuals. Therefore, we developed an individual-based model, with integer numbers of individuals in each stage class. The transitions between classes, fecundity, and the transmission process are all treated as probabilistic events, rather than rates or fractions of the population as in deterministic models. Environmental stochasticity can also be very important for these populations, with rainfall and the number of ice-free days in a given year affecting the demographic parameters. Therefore, we also performed simulations including environmental stochasticity in the parameter that specifies how many adult frogs a particular lake can support. Although fecundity, survival, and maturation can be approximated satisfactorily by a discrete-time model, disease transmission and production of new infected individuals cannot. These disease-related processes occur continually during the ice-free summer months (and possibly also during the months when the frogs and tadpoles are under the ice). Therefore we developed a hybrid model (e.g., Rohani et al. 1994, Briggs and Godfray 1996) that combines discrete-time between-year dynamics with continuous-time within-year dynamics.

Model Description

Basic matrix model

The model includes the stage structure of the *R. muscosa* system (Fig. 2a), in which individuals spend up to three years (depending on temperature and number of ice-free days) as a tadpole before metamorphosis. Following metamorphosis, individuals are juveniles (or subadults) for two years before they can reproduce, and they can remain as reproductive adults for many years. Breeding occurs during a short window of time immediately after the lakes thaw in the spring, and distinct age cohorts are observable in the frog populations. A deterministic version without disease would take the form of a standard matrix model (Eq. 1, at the bottom of the following page) where $L_1(\tau)$, $L_2(\tau)$, and $L_3(\tau)$ are the number of first-, second-, and third-year tadpoles, respectively, $J_1(\tau)$ and $J_2(\tau)$ are the number of first- and second-year postmetamorphic juveniles, respectively, and $A(\tau)$ is the number of adult frogs, all in year τ. Variable σ_x is the yearly survival probability for each stage x, p_F is the proportion of adults reproducing in a given year, and F is the annual fecundity of reproducing adults. A fraction p_{L1} of individuals in the first-year tadpole stage remain as tadpoles for a second year. The remaining $(1 - p_{L1})$ metamorphose after the first year and enter the J_1 stage. Additional mortality may occur

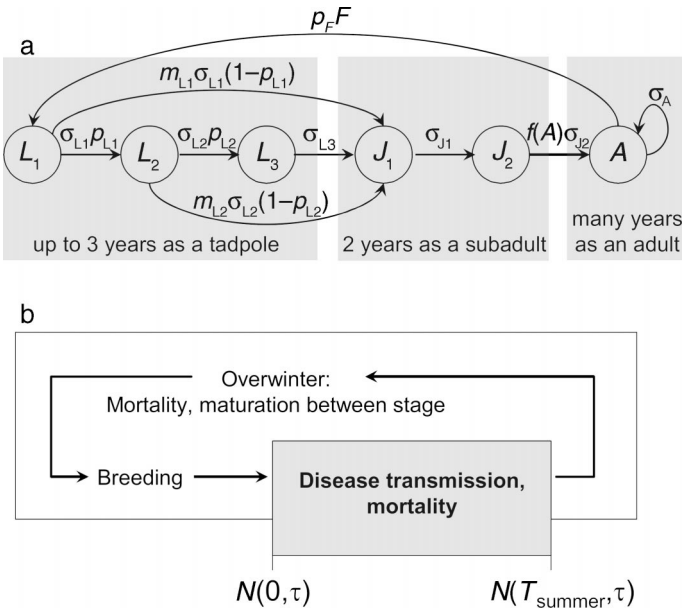

FIG. 2. Diagrams of the model structure. (a) Stage structure of the disease-free population. (b) Sequence of events during the year, combining a discrete-time overwinter model of maturation, survival, and fecundity, with continuous-time within-summer dynamics of disease transmission and production of infected individuals. Both parts of the model are implemented as integer-based stochastic models. Refer to Table 1 for an explanation of terms. L (L_1, L_2, L_3) is the larval frog stage.

during metamorphosis, which is included in $m_{L1} \leq 1$, the survival probability of first-year tadpoles through metamorphosis. The variables p_{L2} and m_{L2} are defined in an analogous way for second-year tadpoles. Variable σ_{L3} includes the probability of both surviving through the L_3 stage and surviving through metamorphosis for third-year tadpoles.

Inclusion of density dependence

Density dependence in the model is included in the recruitment from the juvenile stage to the adult stage. In many disease-free and fish-free populations in the Sierra Nevada, the apparent carrying capacity for adult frogs is one frog for every 2 m of shoreline (see Vredenburg 2004: Fig. 3a; V. Vredenburg and R. Knapp, *unpublished data*). We have assumed that recruitment of juveniles to the adult stage is a decreasing function of current adult density. Juveniles that cannot find a site are assumed to leave the pond and attempt to find another pond. The parameter K represents the strength of density dependence, and determines the average density of adults in a disease-free population, such that $K = A^*/\ln[\psi/(1 - \sigma_A)]$, where A^* is the equilibrium adult density in the deterministic model, and $\psi = (\sigma_A p_A F \sigma_{J1}\sigma_{J2})[m_{L1}\sigma_{L1}(1 - p_{L1}) + m_{L2}\sigma_{L2}(1 - p_{L2})\,\sigma_{L1}p_{L1} + \sigma_{L3}\sigma_{L2}p_{L2}\sigma_{L1}p_{L1}]$.

Inclusion of demographic stochasticity

In order to allow for the effects of demographic stochasticity, the matrix model described above was converted into an individual-based model, with integer numbers of individuals in each stage class. We followed the technique outlined in Caswell (2001: Section 15.1.3) to perform stochastic simulations of the model. The probability of survival and/or maturation for each individual can be thought of as a flip of a weighted coin, with the probability of ''heads'' determined by the specific entry in the matrix above. For example, each individual in the J_1 stage in year τ has probability $\sigma_{L1}p_{L1}$ of surviving and entering the J_2 stage to start year $\tau + 1$. Therefore, the number of individuals making the transition from $J_1(\tau)$ to $J_2(\tau + 1)$ in each year τ is a draw from a binomial distribution with parameters $p = \sigma_{L1}p_{L1}$ and $n = J_1(\tau)$. Similarly, the yearly survival of adult frogs is σ_A and the probability that each adult frog reproduces in year τ is p_F, so the number of reproducing frogs in each year is a draw from a binomial distribution with parameters $p = \sigma_A p_F$ and $n = A(t)$. Each reproducing frog produces a number of offspring drawn from a Poisson distribution with mean F.

$$\begin{pmatrix} L_1 \\ L_2 \\ L_3 \\ J_1 \\ J_2 \\ A \end{pmatrix}(\tau + 1) = \begin{Bmatrix} 0 & 0 & 0 & 0 & 0 & \sigma_A p_F F \\ \sigma_{L1}p_{L1} & 0 & 0 & 0 & 0 & 0 \\ 0 & \sigma_{L2}p_{L2} & 0 & 0 & 0 & 0 \\ m_{L1}\sigma_{L1}(1 - p_{L1}) & m_{L2}\sigma_{L2}(1 - p_{L2}) & \sigma_{L3} & 0 & 0 & 0 \\ 0 & 0 & 0 & \sigma_{J1} & 0 & 0 \\ 0 & 0 & 0 & 0 & \sigma_{J2}\exp[-A(t)/K] & \sigma_A \end{Bmatrix} \begin{pmatrix} L_1 \\ L_2 \\ L_3 \\ J_1 \\ J_2 \\ A \end{pmatrix}(\tau) \qquad (1)$$

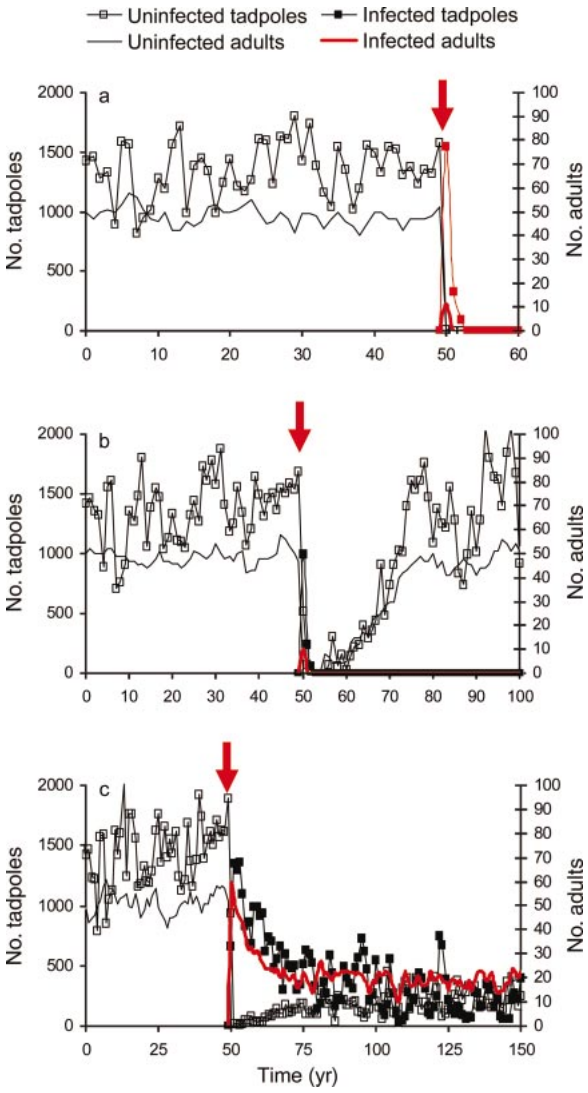

FIG. 3. Runs of the simulation model. Each simulation starts with a disease-free population, and 10 infected adults are introduced on day 50 (red arrow = disease introduced). (a) Rapid disease-caused extinction of the frog population with $\phi_L = 0.001$, $\phi_J = \phi_A = 1$, $\gamma_L = 0.006$ d^{-1}, $\gamma_J = \gamma_A = 0.8$ d^{-1}. (b) Extinction of the disease and recovery of the frog population with $\phi_L = 0.05$, $\phi_J = \phi_A = 1$, $\gamma_L = 2 \times 10^{-4}$ d^{-1}, $\gamma_J = \gamma_A = 0.002$ d^{-1}. (c) Persistence with the disease if adult frogs can potentially survive with the disease, but all infected tadpoles die at metamorphosis with $c_A = 0.9$, $\phi_L = 0.001$, $\phi_J = 2 \times 10^{-4}$, $\phi_A = 1$, $\gamma_L = 0.045$ d^{-1}, $\gamma_J = 1 \times 10^{-6}$, $\gamma_A = 8.6 \times 10^{-4}$ d^{-1}, $d_{LI} = d_{JI} = d_{AI} = 0$. In all simulations, A* = 50, there is no environmental stochasticity, and all other parameters are as in Table 1.

Inclusion of environmental stochasticity

The model was set up such that environmental stochasticity could be included in the adult "carrying capacity," $\hat{A}(\tau)$, which represents how many adult frogs the pond could support in a given year τ. For runs with environmental stochasticity, $\hat{A}(\tau)$ is drawn from a symmetrical beta distribution with mean A^* (the equilibrium density for the deterministic model), and the parameter K is set equal to $\hat{A}(\tau)/\ln[\psi/(1 - \sigma_A)]$.

Inclusion of the disease submodel

The disease-related processes of transmission and production of infected individuals occur continually during the ice-free summer months (and possibly also under the ice during the rest of the year). Fig. 2b illustrates the sequence of events as they are assumed to occur during a year in our hybrid model. $N(t, \tau)$ now represents the number of individuals in each stage N on day t in year τ. Mortality and maturation between stages occur over the winter. Immediately after ice thaw, breeding occurs. The discrete-time stochastic model outlined above describes these processes, and calculates the numbers in each stage on day 0 in year $\tau + 1$ [that is, $N(0, \tau + 1)$] from the numbers of individuals in each stage at the end of the disease transmission period in year τ [that is, $N(T_{end}, \tau)$]. T_{end} could be taken as the length of the summer (~100 days), or if transmission occurs year-round, T_{end} could be set equal to 365 days. The continuous-time disease submodel, described next, calculates the numbers of individuals in each stage at the end of the transmission period in year τ [$N(T_{end}, \tau)$] from the numbers at the start of the transmission period in that same year [$N(0, \tau)$].

Most simple disease models are formulated as systems of differential equations, with rates of movement of susceptible hosts to the infected stage following transmission. For the frog system, with transmission possible within and between each stage, such a set of differential equation models over a single summer would look like the following:

Tadpoles in each year class $i = 1, 2, 3$:

$$\begin{aligned} dL_i(t, \tau)/dt &= -\nu_L L_i(t, \tau) - d_L L_i(t, \tau) \\ dL_{Ii}(t, \tau)/dt &= \nu_L L_i(t, \tau) - d_{LI} L_{Ii}(t, \tau) \end{aligned} \qquad (2)$$

with

$$\begin{aligned} \nu_L = \phi_L[&\gamma_L L_{I1}(t, \tau) + \gamma_L L_{I2}(t, \tau) + \gamma_L L_{I3}(t, \tau) \\ &+ \gamma_J J_{I1}(t, \tau) + \gamma_J J_{I2}(t, \tau) + \gamma_A A_I(t, \tau)] \end{aligned}$$

Juveniles, in each year class $j = 1, 2$:

$$\begin{aligned} dJ_j(t, \tau)/dt &= -\nu_J J_j(t, \tau) - d_J J_j(t, \tau) \\ dJ_{Ij}(t, \tau)/dt &= \nu_J J_j(t, \tau) - d_{JI} J_{Ij}(t, \tau) \end{aligned} \qquad (3)$$

with

$$\begin{aligned} \nu_J = \phi_J[&\gamma_L L_{I1}(t, \tau) + \gamma_L L_{I2}(t, \tau) + \gamma_L L_{I3}(t, \tau) \\ &+ \gamma_J J_{I1}(t, \tau) + \gamma_J J_{I2}(t, \tau) + \gamma_A A_I(t, \tau)] \end{aligned}$$

Adults:

$$dA(t, \tau)/dt = -\nu_A A(t, \tau) - d_A A(t, \tau)$$

$$dA_I(t, \tau)/dt = \nu_A A(t, \tau) - d_{AI} A_I(t, \tau) \quad (4)$$

with

$$\nu_A = \phi_A[\gamma_L L_{I1}(t, \tau) + \gamma_L L_{I2}(t, \tau) + \gamma_L L_{I3}(t, \tau) + \gamma_J J_{I1}(t, \tau) + \gamma_J J_{I2}(t, \tau) + \gamma_A A_I(t, \tau)]$$

where $L_1(t, \tau)$, $L_2(t, \tau)$, $L_3(t, \tau)$, $J_1(t, \tau)$, $J_2(t, \tau)$, and $A(t, \tau)$ are the numbers of uninfected frogs in each class at time t in year τ, as before. $L_{I1}(t, \tau)$, $L_{I2}(t, \tau)$, $L_{I3}(t, \tau)$, $J_{I1}(t, \tau)$, $J_{I2}(t, \tau)$, and $A_I(t, \tau)$ are now the number of infected frogs in each of those classes, where the subscript I indicates infected animals. The values d_L, d_J, and d_A are the per-capita death rates of uninfected tadpoles, juveniles, and adults, respectively, and d_{LI}, d_{JI}, and d_{AI} are the per capita death rates of infected individuals in those same stages.

We have made several assumptions about the transmission process. First, we have assumed that the rate of disease transmission is proportional to the rate of contact between uninfected and infected individuals. This form of mass action or density-dependent disease transmission function has been called into question (McCallum et al. 2001) for diseases in which the rate of contact does not necessarily increase linearly with the population size or population density (e.g., sexually transmitted diseases, or systems in which there are particular forms of social structure). However, we would argue that tadpoles in ponds represent a system that is most likely to meet the density-dependent transmission process assumptions: more frequent contact at high densities than at low densities. Density-dependent transmission may not be the most appropriate for transmission between adult frogs if they have somewhat fixed feeding territories along the shoreline. We also tried different transmission functions, including frequency-dependent transmission between adult frogs. These did not qualitatively change the predictions of our model, and therefore we report only the results of the density-dependent transmission version here.

Second, we have allowed disease transmission to occur within and between all developmental stages of the frogs. This assumption has been supported in *R. muscosa* by laboratory and field experiments (L. J. Rachowicz, R. A. Knapp, J. Morgan, M. Stice, V. T. Vredenburg, J. Parker, and C. J. Briggs, *unpublished manuscript*).

Third, although disease transmission actually occurs through zoospores released from infected individuals coming into contact with susceptible hosts, we have approximated it with direct contact between infected and susceptible individuals. This is a reasonable approximation because zoospores are thought to be infectious for less than a day, and therefore the number of infectious zoospores at any point in time correlates extremely well with the weighted number of infectious individuals. This approximation would not work well for a longer-lived free-living infectious stage (e.g., the hypothesized, but never observed, resistant sporangium stage).

Fourth, we have divided the transmission coefficient, which is frequently denoted as β in standard disease models, into two components, $\beta = \phi_x\gamma_y$ for the interaction between a given susceptible stage x and infectious stage y. Parameter ϕ_x is the relative susceptibility of stage x, which is a measure of how easy it is for an individual to get infected when it comes in contact with an infected individual. Parameter γ_y is the relative infectiousness of stage y, which relates to the rate of release of zoospores from infected individuals of that stage. For simplicity we have assumed that values of ϕ and γ do not change between the three tadpole year classes, nor between the two juvenile year classes.

For the stochastic simulations, we do not use the differential equations above, but instead use a stochastic approximation thereof, which follows the fate of integer numbers of individuals in each age class as they pass through a sequence of discrete infection and mortality events. We used Gillespie's direct method (Gillespie 1977, described in Wearing et al. 2004), in which the time trajectory over the course of the summer (i.e., through the period when disease transmission occurs) can be efficiently described by a sequence of times between events, and a list of the type of events (e.g., infection of first-year tadpole, mortality of infected adult, etc.). The time between events follows an exponential distribution, and events are independent. Eighteen distinct types of events can occur in any point in time (which correspond to the different terms on the right-hand sides of the differential equations in Eqs. 2–4): six involve infection of the six frog stages, with $a_1 = \nu_L L_1(t, \tau)$, $a_2 = \nu_L L_2(t, \tau)$, $a_3 = \nu_L L_3(t, \tau)$, $a_4 = \nu_J J_1(t, \tau)$, $a_5 = \nu_J J_2(t, \tau)$, $a_6 = \nu_A A(t, \tau)$, six involve death of an uninfected host, with $a_7 = d_L L_1(t, \tau)$, $a_8 = d_L L_2(t, \tau)$, $a_9 = d_L L_3(t, \tau)$, $a_{10} = d_J J_1(t, \tau)$, $a_{11} = d_J J_2(t, \tau)$, $a_{12} = d_A A(t, \tau)$, and six involve death of an infected host, with $a_{13} = d_{LI} L_{I1}(t, \tau)$, $a_{14} = d_{LI} L_{I2}(t, \tau)$, $a_{15} = d_{LI} L_{I3}(t, \tau)$, $a_{16} = d_{JI} J_{I1}(t, \tau)$, $a_{17} = d_{JI} J_{I2}(t, \tau)$, $a_{18} = d_{AI} A_I(t, \tau)$.

The model proceeds as follows. Starting from time $t = 0$ in year τ, two independent random numbers, U_1 and U_2 are drawn from a uniform distribution on the interval [0, 1]. The time until the event will be: $\tau = -\ln(U_1)/a_{tot}$, where $a_{tot} = \Sigma_{i=1}^{18} a_i$. Time is incremented to $t + \tau$. U_2 is used to determine the identity of the next event. The probability that the event is type i (for $i = 1$ to 18, as defined above) is a_i/a_{tot} (e.g., if $U_2 \leq a_1/a_{tot}$, then the event is the infection of a first-year tadpole, if $a_1/a_{tot} < U_2 \leq (a_1 + a_2)/a_{tot}$, then the event is the infection of a second-year tadpole, etc.). If the event is an infection, then the number in the affected susceptible host class is decremented by one, and the corresponding infected host stage is incremented by one. If the event is the death of an uninfected or infected

TABLE 1. Model parameter descriptions, symbols, and default values.

Parameter description	Uninfected hosts		Infected hosts	
	Symbol	Default value	Symbol	Default value
Overwinter survival probability, first-year tadpoles	σ_{L1}	0.2	σ_{LI1}	0.2
Overwinter survival probability, second-year tadpoles	σ_{L2}	0.7	σ_{LI2}	0.7
Overwinter survival probability, third-year tadpoles	σ_{L3}	0.7	σ_{LI3}	0
Overwinter survival probability, first-year juveniles	σ_{J1}	0.7	σ_{JI1}	0
Overwinter survival probability, second-year juveniles	σ_{J2}	0.7	σ_{JI2}	0
Overwinter survival probability, adults	σ_{A}	0.9	σ_{AI}	0
Probability of a first-year staying as a tadpole for the second year	p_{L1}	1	p_{IL1}	1
Probability of a second-year staying as a tadpole for the third year	p_{L2}	0.5	p_{IL2}	0.5
Probability of surviving metamorphosis after the first year	m_{L1}	0.9	m_{IL1}	0
Probability of surviving metamorphosis after the second year	m_{L2}	0.9	m_{IL2}	0
Probability that an adult reproduces in a given year	p_{F}	0.25	p_{FI}	0
Average fecundity of reproducing adult (Poisson distributed)	F	100	F_{I}	0
Death rate of tadpoles during summer	d_{L}	0	d_{LI}	0
Death rate of juveniles during summer	d_{J}	0	d_{JI}	0.1
Death rate of adults during summer	d_{A}	0	d_{AI}	0.02
Relative susceptibility of tadpoles, juveniles, adults	ϕ_{L}, ϕ_{J}, ϕ_{A}		varied	
Infectiousness of tadpoles, juveniles, adults	γ_{L}, γ_{J}, γ_{A}		varied	

host, then the number in the affected stage class is decremented by one. The process starts again at the updated time with the drawing of two new uniform random numbers, and is repeated until the end of the summer period for that year (that is, until $t = T_{end}$ is reached).

In order to incorporate the disease submodel into the full model, assumptions need to be made about what happens to each class of infected frogs over the winter. The default assumptions are: (1) individuals in the tadpole stage survive and mature in the same way as uninfected tadpoles; (2) infected tadpoles die as they pass through metamorphosis; and (3) all infected postmetamorphic animals do not survive overwinter. However, in variants of the model investigated below, we allow assumptions (2) and (3) to be violated, and therefore a version of the model with survival through metamorphosis and after metamorphosis was also developed. In that full model, all survival, maturation, and fecundity parameters are defined for the infected classes in the same way as for the uninfected classes (see Table 1). The parameter c_{J1} describes the probability that an infected tadpole retains the infection through metamorphosis.

Parameter estimates

For the disease-free frog model, we have reasonable estimates of all of the demographic parameters from the work on the system by Vance Vredenburg and Roland Knapp (*unpublished data,* see Table 1). Our laboratory and field observations suggest that tadpole survival is unaffected by chytridiomycosis, and therefore we set the infected tadpole parameters equal to the values for uninfected tadpoles. Laboratory experiments have shown that subadults die on average ~10 days after exposure to chytrid zoospores, and adults die ~50 days post exposure (L. J. Rachowicz, *personal communication*). Therefore, the default values of these parameters have been set equal to $d_{JI} = 1/10\ d^{-1}$ and $d_{AI} = 1/50\ d^{-1}$.

Estimates for the disease transmission parameters are more difficult to obtain, and although experiments are currently underway, we do not yet have reliable parameter values. Therefore, we carried out Monte Carlo simulations in which random combinations of parameters were chosen repeatedly over the feasible range of values, and simulations were performed to determine the types of dynamics that are possible from the model. We fixed the value of ϕ_A at 1, such that the susceptibilities of the other stages are measured relative to the susceptibility of adult frogs. From our laboratory experiments we know that postmetamorphic frogs are much more susceptible to the disease than tadpoles. In a recent experiment, tadpoles exposed to 5×10^6 zoospores did not become infected, but postmetamorphic frogs exposed to only 10^4 zoospores did (C. J. Briggs, M. Stice, T. Tunstall, V. T. Vredenburg, and D. C. Woodhams, *unpublished manuscript*). Therefore, in our simulations we investigated the effects of $\phi_L < \phi_J$ and ϕ_A.

The Monte Carlo simulations investigated parameter values over several orders of magnitude. The parameter values were chosen to give each order of magnitude equal representation. For example, if ϕ_L values over x orders of magnitude are being investigated, with a minimum ϕ_L value of 10^{-y}, for each run, $\phi_L = 10^{(U*x-y)}$, where U is a uniform deviate in [0,1]. For each set of assumptions that we investigated below, Monte Carlo simulations of 10^6 randomly chosen combinations of parameters were performed. For each parameter combination, a single realization of the stochastic model was run. In order to determine the effect of invasion of the chytrid into a disease-free frog population, each simulation was started with the number of frogs in each stage set to its disease-free (deterministic) equilibrium

density, and the stochastic simulation was allowed to run for 50 years without the disease. In year 50, 10 infected adult frogs were introduced into the population, and the simulation was continued for an additional 100 years. The time from disease invasion until the extinction of the frog population and/or the disease was recorded for each run. The fraction of runs in which the frogs or the disease persist for a given length of time will obviously depend on the range of parameters that we chose to investigate. Therefore, these fractions should be used only for comparisons between runs, and not interpreted as the *probability* of persistence.

Results

Frog populations persist without the disease

In the absence of the disease, frog populations with the default *R. muscosa* demographic parameter values have a very low annual risk of extinction. Without environmental stochasticity, even populations in lakes that can support only low numbers of adults ($A^* = 10$ adult frogs) can persist indefinitely (only 1 out of 10^4 runs of the model without environmental stochasticity, went extinct in $<10^4$ years). Even very high levels of environmental stochasticity increased the risk of extinction only slightly (5 out of 10^4 runs of the model with A^* drawn from a symmetrical beta distribution, with mean = 10 and range 0–20 went extinct in $<10^4$ years). The low risk of extinction is likely due to the relatively high annual multiplicative growth rate from low densities ($\lambda = 1.54$ = the dominant eigenvalue of the deterministic model at low densities). This high rate of increase from low densities is substantiated by the observed rate of increase of frogs reintroduced into lakes following trout removal (R. A. Knapp, *unpublished data*).

If chytridiomycosis is invariably fatal, persistence with disease is highly unlikely

With our default demographic parameters for *R. muscosa*, and with our current working understanding of the effects of chytridiomycosis on tadpoles (i.e., no effect until metamorphosis) and postmetamorphic frogs (i.e., fairly rapid death), the only two likely outcomes following disease invasion are (1) rapid extinction of the frog population and the disease (Fig. 3a), or (2) some level of initial impact of the disease on the frog population (depending on the transmission parameters), followed by extinction of the disease and recovery of the frog population (Fig. 3b). Persistence of the frog population with the disease is highly unlikely. Unsurprisingly, high values of the transmission parameters (ϕ's and γ's) are more likely to lead to extinction of the frog population, and low values are more likely to lead to failure of the disease to invade the population, or to have only a small impact on the population before extinction of the disease.

With our default assumptions, the multiyear tadpole stage is the only stage carrying the pathogen from one year to the next, and therefore it is absolutely crucial for the persistence of the disease. However, the ''reservoir'' for the disease in the tadpole stage is not sufficient to allow for sustained persistence of the disease in the population. Some of the model results with the default assumptions are tabulated in Table 2a. A simplified version of the model was investigated first in which $\phi_J = \phi_A = 1$ and $\gamma_J = \gamma_A$, that is, differences between tadpoles and postmetamorphic stages were investigated, but all postmetamorphic stages were assumed identical in transmission parameters. The persistence of the disease depends greatly on the susceptibility of the tadpole stage relative to the susceptibility of the postmetamorphic stages. With low levels of susceptibility of tadpoles, the disease cannot persist, even for a short while. (With $\phi_L < 0.01$ the disease was never found to persist longer than five years; i.e., 0 out of 1 million runs, for any values of γ_L or γ_A.) As ϕ_L is increased to values at which the tadpoles and postmetamorphic stages are similar in susceptibility, the likelihood of disease persistence is increased somewhat, but it remains difficult to find parameter combinations for which the disease can persist for longer than 10 years. As ϕ_L is increased further, it becomes more likely that the frog population will be driven extinct by the disease.

Increasing either the average number of adult frogs that a pond can support (A^*), or increasing the average fecundity of adults (F), has the same effects on persistence. Both increase the potential rate of input of new susceptible individuals to the population, and therefore both increase the likelihood of persistence of a highly virulent disease (provided the frog population also persists). However, even for high levels of fecundity or moderately high adult ''carrying capacities'' it was difficult to find parameter values for which an invariably lethal disease could persist in the frog population (see Table 2a). Increasing either adult carrying capacity or fecundity also made it more likely that the disease would cause extinction of the frog population.

If some infected tadpoles lose disease at metamorphosis, there is no persistence with disease

Our laboratory and field experiments suggest that tadpoles retain the infection through metamorphosis and then die due to the disease (L. J. Rachowicz, R. A. Knapp, J. Morgan, M. Stice, V. T. Vredenburg, J. Parker, and C. J. Briggs, *unpublished manuscript*). However, because keratin is present in different parts of the body of tadpoles and postmetamorphic individuals, it has been suggested that in some species tadpoles might lose the infection as they go through metamorphosis, and have to be re-infected as subadults or adults. We found that loss of infection at metamorphosis in the model reduces the likelihood that the disease will cause extinction of the frog population, and reduces the potential for the disease to persist in the frog population (Table 2b). If tadpoles retain the in-

TABLE 2. Summary of results on persistence.

ϕ_L range (d^{-1})	ϕ_J range (d^{-1})	A^*, carrying capacity (no. frogs)	F, fecundity (no. offspring produced/yr)	c_{J1}, fraction retaining infection	c_A, fraction surviving	Percentage of runs: Frogs persist >100 yr	Disease persists >5 yr	Disease persists >10 yr	Disease persists >100 yr
a) Disease invariably fatal to postmetamorphic frogs									
Investigating effect of susceptibility of tadpoles ϕ_L (with $\phi_J = \phi_A$, $\gamma_J = \gamma_A$)									
10^{-7} to 0.01	1	50	100			87	0	0	0
0.01 to 0.1	1	50	100			48	0.21	0.021	0
0.1 to 1	1	50	100			32	1.6	0.48	0.001
1 to 10	1	50	100			20	5.4	2.5	0.15
10 to 100	1	50	100			12	11	7.5	0.91
100 to 10^3	1	50	100			6.7	13	9.3	0.69
10^3 to 10^4	1	50	100			2.5	13	9.2	0.002
Investigating effect of adult ''carrying capacity,'' A^*									
10^{-7} to 1	1	*10*	100			79	0.083	0.006	0
10^{-7} to 1	1	*50*	100			74	0.28	0.075	0
10^{-7} to 1	1	*100*	100			71	0.38	0.13	0.007
10^{-7} to 1	1	*200*	100			68	0.42	0.15	0.036
Investigating effect of adult fecundity, F									
10^{-7} to 1	1	50	*100*			74	0.28	0.075	0
10^{-7} to 1	1	50	*200*			71	0.37	0.13	0.01
10^{-7} to 1	1	50	*500*			67	0.56	0.28	0.07
b) Some tadpoles lose infection at metamorphosis									
10^{-7} to 1	10^{-4} to 10^3	50		*1.0*		72	0.36	0.093	0
10^{-7} to 1	10^{-4} to 10^3	50		*0.5*		100	0.32	0.093	0.002
10^{-7} to 1	10^{-4} to 10^3	50		*0*		100	0.34	0.088	0
Tadpoles survive metamorphosis, but retain infection									
10^{-7} to 1	10^{-4} to 10^3	50		*1.0*		62	0.67	0.21	0.002
c) Tadpoles die at metamorphosis, but some infected subadult and adult frogs survive									
10^{-7} to 1	10^{-4} to 10^3	50			*0*	67	0.28	0.064	0.002
10^{-7} to 1	10^{-4} to 10^3	50			*0.1*	57	23	0.072	0
10^{-7} to 1	10^{-4} to 10^3	50			*0.5*	67	86	50	0.22
10^{-7} to 1	10^{-4} to 10^3	50			*0.9*	82	100	98	57
10^{-7} to 1	10^{-4} to 10^3	50			*1.0*	85	100	100	60

Notes: Each row represents a Monte Carlo simulation of 10^6 parameter combinations in the specified range of disease transmission parameters. Unless otherwise specified, $\phi_A = 1$; γ_L, γ_J, and γ_A were all chosen independently from the range 10^{-7} to 10. Numbers in italics highlight the input variable that is changed between rows.

fection through metamorphosis, but infected individuals do not die immediately upon metamorphosis, then this has the reverse effect, allowing for somewhat longer persistence of the disease in a small fraction of the parameter combinations.

If some infected adults can survive with disease, disease persistence is possible

If the disease is not invariably lethal to postmetamorphic individuals, then it becomes relatively easy to find combinations of transmission parameters that allow for long-term persistence of the disease in an infected frog population. In Table 2c, we assume that infected tadpoles die at metamorphosis, but that individuals that become infected during the subadult or adult stage suffer only an increased overwinter mortality. The expression $(1 - c_A)$ is the factor by which the overwinter survival of the postmetamorphic stages is reduced due to the disease. With $c_A = 1$ (i.e., the only impact of the disease is on mortality of tadpoles at metamorphosis), the disease persisted for over 10 years for virtually all of the transmission parameter combinations, and persisted for over 100 years for 60% of the parameters. With $c_A = 0.9$ (i.e., a 10% reduction in overwinter survival of subadults and adults), more than half of the transmission parameter combinations lead to persistence with the disease for >100 years. Fig. 3c shows one simulation run with long-term persistence of the disease through survival of infected adults. With relatively high transmission parameters, it is possible to have the frog population persisting with virtually all of the postmetamorphic individuals infected with the disease.

DISCUSSION

Our current working understanding of chytridiomycosis in *R. muscosa* is that it is invariably lethal, because laboratory and field experiments have shown that infected tadpoles die as they pass through metamorphosis (L. J. Rachowicz, R. A. Knapp, J. Morgan, M.

Stice, V. T. Vredenburg, J. Parker, and C. J. Briggs, *unpublished manuscript*). Laboratory experiments have also revealed that postmetamorphic animals are easier to infect with the disease than tadpoles, and that when infected they die within weeks of infection (C. Briggs et al., *unpublished data*). Our simple simulation model suggests that these assumptions are consistent with what we are observing in the southern parts of the California Sierra Nevada (Sequoia and Kings Canyon National Parks). In those areas, the frog populations in many lakes have gone extinct within a few years after *B. dendrobatidis* was first detected (L. J. Rachowicz, R. A. Knapp, J. Morgan, M. Stice, V. T. Vredenburg, J. Parker, and C. J. Briggs, *unpublished manuscript*; R. Knapp, *unpublished data*). The pattern of die-off in the model is consistent with that observed in the field: postmetamorphic animals disappear first; tadpoles remain until individuals in each cohort die at metamorphosis.

Our current working understanding of the disease is not, however, consistent with the apparent persistence with *B. dendrobatidis* at sites in the northern part of the Sierra Nevada (Yosemite National Park, and National Forest lands to the north). The model suggests that the differences in the north vs. south cannot be explained purely by differences in transmission parameters between the different areas, either in susceptibility to infection (ϕ's), or in infectiousness of infected individuals (γ's). With an invariably lethal disease, no combinations of susceptibility or infectiousness resulted in long-term persistence of the disease in the frog population. Similarly, the model suggests that differences in the size of populations in the north vs. south do not explain differences in persistence with the disease. In the model, it is slightly easier to find transmission parameters resulting in long-term disease persistence in large populations; however, this effect was relatively small. In contrast, the persisting frog populations in the northern Sierra Nevada tend to be smaller populations than the southern populations prior to disease invasion.

The model suggests that the key to long-term persistence with *B. dendrobatidis* is survival of at least some fraction of infected postmetamorphic individuals. This implies that we should be concentrating our efforts on (a) determining how long infected subadults and adults in the northern areas are surviving with the disease, and (b) investigating factors that might allow infected postmetamorphic individuals to survive at the northern sites. Recent developments in PCR (polymerase chain reaction) techniques for nonlethal detection of the disease (Boyle et al. 2004) has made proposal (a) possible this summer for the first time. A project involving tagging and repeat captures of infected individuals has suggested that infected adults can survive at least over the course of a single summer (C. Briggs et al., *unpublished data*); however, overwinter survival is yet to be measured. Efforts are underway to understand the physiological mechanism by which chytridiomycosis kills infected adults, which will greatly help with effort (b). In other amphibian species, high temperature and variability in temperature have been shown to be detrimental to the growth of *B. dendrobatidis*, reducing frog mortality due to chytridiomycosis (Woodhams et al. 2003, Berger et al. 2004, Piotrowski et al. 2004). There are observable differences in the habitat characteristics between our two types of sites that may allow frogs at the persistent sites to experience higher and more variable temperatures than those at die-off sites. Many of the sites at which frog populations have persisted with *B. dendrobatidis* consist of marsh and stream areas with emergent vegetation, in addition to lakes, while most of the die-offs have occurred in deeper lakes surrounded by granite bedrock. We are currently investigating how these different habitat characteristics translate into different temperature profiles experienced by the postmetamorphic frogs. Another factor to investigate is regional differences in antimicrobial peptides in *R. muscosa* skin, which have been shown in other species to be a defense against chytridiomycosis (Rollins-Smith et al. 2002, 2003).

The model presented here includes only a single host and no saprophytic growth by *B. dendrobatidis*. Regional persistence of the pathogen could be explained if other reservoir host species are present that are not as strongly affected as *R. muscosa* by the disease. Screening for other amphibian or nonamphibian reservoir hosts is underway, but none have yet been found at our Sierran sites (J. Morgan, *unpublished data*). *R. muscosa* is the most abundant amphibian species at most sites, but *Pseudacris regilla*, *Bufo canorus*, *Bufo boreas*, and *Ambystoma macrodactylum* are also present (although these species are either less aquatic or much less abundant than *R. muscosa*). A lethal emerging infectious disease that can also grow saprophytically would be a tremendous threat, as it would have the potential to persist even in the absence of any living host, and potentially render a site unsuitable for reintroductions following host extinction. We have not considered the possibility of saprophytic growth in the model here, but previous models have suggested that it readily leads to host extinction and disease persistence (Godfray et al. 1999).

Acknowledgments

This work was supported by NIH/NSF Ecology of Infectious Disease Program grant R01 ES12067 from the National Institute of Environmental Health Sciences, and a University of California Water Resources grant. We would like to thank Rob Bingham, Martha Hoopes, Craig Moritz, Jess Morgan, John Parker, Mary Stice, John Taylor, and Tate Tunstall for insightful discussion, and John Latto for valuable comments on the manuscript.

Literature Cited

Berger, L., et al. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain

forests of Australia and Central America. Proceedings ofthe National Academy of Sciences (USA) **95**:9031–9036.
Berger, L., et al. 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Australian Veterinary Journal **82**:434–439.
Bosch, J., I. Martinez-Solano, and M. Garcia-Paris. 2001. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. Biological Conservation **97**:331–337.
Boyle, D. G., D. B. Boyle, V. Olsen, J. A. T. Morgan, and A. D. Hyatt. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Diseases of Aquatic Organisms **60**:141–148.
Bradford, D. F. 1983. Winterkill, oxygen relations, and energy metabolism of a submerged dormant amphibian, *Rana muscosa*. Ecology **64**:1171–1183.
Bradford, D. F. 1989. Allotopic distribution of native frogs and introduced fishes in high Sierra Nevada lakes of California: implication of the negative effect of fish introductions. Copeia 1989:775–778.
Briggs, C. J., and H. C. J. Godfray. 1996. The dynamics of insect–pathogen interactions in seasonal environments. Theoretical Population Biology **50**:149–177.
Caswell, H. 2001. Matrix population models: construction, analysis and interpretation. Sinauer, Sunderland, Massachusetts, USA.
Daszak, P., A. Strieby, A. A. Cunningham, J. E. Longcore, C. C. Brown, and D. Porter. 2004. Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herpetological Journal **14**:201–207.
Fellers, G. M., D. E. Green, and J. E. Longcore. 2001. Oral chytridiomycosis in the mountain yellow-legged frog (*Rana muscosa*). Copeia 2001:945–953.
Gillespie, D. T. 1977. Exact stochastic simulation of coupled chemical reactions. Journal of Physical Chemistry **81**: 2340–2361.
Godfray, H. C. J., C. J. Briggs, T. A. Jackson, M. O'Callaghan, T. R. Glare, and N. D. Barlow. 1999. A model of insect–pathogen dynamics in which a pathogenic bacterium can also reproduce saprophytically. Proceedings of the Royal Society of London, Series B **266**:233–240.
Green, D. E., and C. K. Sherman. 2001. Diagnostic histological findings in Yosemite toads (*Bufo canorus*) from a die-off in the 1970s. Journal of Herpetology **35**:92–103.
Johnson, M. L., and R. Speare. 2003. Survival of *Batrachochytrium dendrobatidis* in water: quarantine and disease control implications. Emerging Infectious Diseases **9**:922–925.
Knapp, R. A., and K. R. Matthews. 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. Conservation Biology **14**:428–438.
Lips, K. R., D. E. Green, and R. Papendick. 2003. Chytridiomycosis in wild frogs from Southern Costa Rica. Journal of Herpetology **37**:215–218.
Lips, K. R., J. R. Mendelson, A. Munoz-Alonso, L. Canseco-Marquez, and D. G. Mulcahy. 2004. Amphibian population declines in montane southern Mexico: resurveys of historical localities. Biological Conservation **119**:555–564.
Longcore, J. E., A. P. Pessier, and D. K. Nichols. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia **91**:219–227.
McCallum, H., N. Barlow, and J. Hone. 2001. How should pathogen transmission be modelled? Trends in Ecology and Evolution **16**:295–300.
Morehouse, E. A., T. Y. James, A. R. D. Ganley, R. Vilgalys, L. Berger, P. J. Murphy, and J. E. Longcore. 2003. Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. Molecular Ecology **12**:395–403.
Muths, E., P. S. Corn, A. P. Pessier, and D. E. Green. 2003. Evidence for disease-related amphibian decline in Colorado. Biological Conservation **110**:357–365.
Nichols, D. K., E. W. Lamirande, A. P. Pessier, and J. E. Longcore. 2001. Experimental transmission of cutaneous chytridiomycosis in dendrobatid frogs. Journal of Wildlife Diseases **37**:1–11.
Parker, J. M., I. Mikaelian, N. Hahn, and H. E. Diggs. 2002. Clinical diagnosis and treatment of epidermal chytridiomycosis in African clawed frogs (*Xenopus tropicalis*). Comparative Medicine **52**:265–268.
Parris, M. J. 2004. Hybrid response to pathogen infection in interspecific crosses between two amphibian species (Anura: Ranidae). Evolutionary Ecology Research **6**:457–471.
Parris, M. J., and D. R. Baud. 2004. Interactive effects of a heavy metal and chytridiomycosis on Gray Treefrog larvae (*Hyla chrysoscelis*). Copeia 2004:344–350.
Piotrowski, J. S., S. L. Annis, and J. E. Longcore. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia **96**:9–15.
Rachowicz, L. J., R. A. Knapp, J. A. T. Morgan, M. J. Stice, V. T. Vredenburg, J. M. Parker, and C. J. Briggs. *In preparation.* Emerging infectious disease as a proximate cause of amphibian mass mortality in California's Sierra Nevada Mountains.
Rachowicz, L. J., and V. T. Vredenburg. 2004. Transmission of an emerging fungal disease within and between amphibian life stages. Diseases of Aquatic Organisms **61**:75–83.
Rohani, P., H. C. J. Godfray, and M. P. Hassell. 1994. Aggregation and the dynamics of host–parasitoid systems: a discrete-generation model with within-generation redistribution. American Naturalist **144**:491–509.
Rollins-Smith, L. A., C. Carey, J. M. Conlon, L. K. Reinert, J. K. Doersam, T. Bergman, J. Silberring, H. Lankinen, and D. Wade. 2003. Activities of temporin family peptides against the chytrid fungus (*Batrachochytrium dendrobatidis*) associated with global amphibian declines. Antimicrobial Agents and Chemotherapy **47**:1157–1160.
Rollins-Smith, L. A., C. Carey, J. Longcore, J. K. Doersam, A. Boutte, J. E. Bruzgal, and J. M. Conlon. 2002. Activity of antimicrobial skin peptides from ranid frogs against *Batrachochytrium dendrobatidis*, the chytrid fungus associated with global amphibian declines. Developmental and Comparative Immunology **26**:471–479.
Vredenburg, V. T. 2004. Reversing introduced species effects: experimental removal of introduced fish leads to rapid recovery of declining frogs. Proceedings of the National Academy of Sciences (USA) **101**:7646–7650.
Vredenburg, V. T., G. Fellers, and C. Davidson. 2005. The mountain yellow-legged frog *Rana muscosa* (Camp 1917). *In* M. Lanoo, editor. Status and conservation of U.S. amphibians. University of California Press, Berkeley, California, *in press.*
Wearing, H. J., P. Rohani, T. C. Cameron, and S. M. Sait. 2004. The dynamical consequences of developmental variability and demographic stochasticity for host–parasitoid interactions. American Naturalist **164**:543–558.
Woodhams, D. C., R. A. Alford, and G. Marantelli. 2003. Emerging disease of amphibians cured by elevated body temperature. Diseases of Aquatic Organisms **55**:65–67.
Young, B. E., K. R. Lips, J. K. Reaser, R. Ibanez, A. W. Salas, J. R. Cedeno, L. A. Coloma, S. Ron, E. La Marca, J. R. Meyer, A. Munoz, F. Bolanos, G. Chaves, and D. Romo. 2001. Population declines and priorities for amphibian conservation in Latin America. Conservation Biology **15**: 1213–1223.
Zweifel, R. G. 1955. Ecology, distribution and systematics of frogs of the *Rana boylii* group. University of California Publications in Zoology **54**:207–292.