PROCEEDINGS OF THE ROYAL SOCIETY B

FirstCite® e-publishing

Proc. R. Soc. B
doi:10.1098/rspb.2007.1356
Published online

Persistence of the emerging pathogen *Batrachochytrium dendrobatidis* outside the amphibian host greatly increases the probability of host extinction

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Pathogens do not normally drive their hosts to extinction; however, *Batrachochytrium dendrobatidis*, which causes amphibian chytridiomycosis, has been able to do so. Theory predicts that extinction can be caused by long-lived or saprobic free-living stages. The hypothesis that such a stage occurs in *B. dendrobatidis* is supported by the recent discovery of an apparently encysted form of the pathogen. To investigate the effect of a free-living stage of *B. dendrobatidis* on host population dynamics, a mathematical model was developed to describe the introduction of chytridiomycosis into a breeding population of *Bufo bufo*, parametrized from laboratory infection and transmission experiments. The model predicted that the longer that *B. dendrobatidis* was able to persist in water, either due to an increased zoospore lifespan or saprobic reproduction, the more likely it was that it could cause local *B. bufo* extinction (defined as decrease below a threshold level). Establishment of endemic *B. dendrobatidis* infection in *B. bufo*, with severe host population depression, was also possible, in agreement with field observations. Although this model is able to predict clear trends, more precise predictions will only be possible when the life history of *B. dendrobatidis*, including free-living stages of the life cycle, is better understood.

Keywords: mathematical modelling; epidemiology; *Batrachochytrium dendrobatidis*; chytridiomycosis; *Bufo bufo*; amphibian declines

1. INTRODUCTION

Amphibian chytridiomycosis has been described as 'the worst infectious disease ever recorded among vertebrates in terms of the number of species impacted, and its propensity to drive them to extinction' (Amphibian Conservation Summit 2005). Infection with the chytridiomycete fungus *Batrachochytrium dendrobatidis* (Longcore *et al.* 1999) occurs following exposure to water containing free-living aquatic zoospores (Carey *et al.* 2006), released from mature zoosporangia in the keratinized tissues of infected hosts. Infection is limited to these tissues, which are present in the outer layer of skin of adult amphibians, but only in the mouthparts of early-stage larvae (Marantelli *et al.* 2004). This may explain the observation that tadpoles are largely unaffected by infection, while high mortality occurs following the metamorphosis of infected individuals (Berger *et al.* 1998), although mortality can also occur in larval stages (Blaustein *et al.* 2005).

Many of the Chytridiomycota are adapted to life as freshwater aquatic saprobes (Barr 1990), with some, such as *Allomyces* species, producing thick-walled resting sporangia upon the onset of unfavourable conditions, capable of long-term survival. Recently a new, apparently encysted morphological form of *B. dendrobatidis* was identified, which may be capable of long-term survival in the environment (Di Rosa *et al.* 2007). Whether this is a resting stage or a saprobic form of the chytrid is still to be determined. Previous mathematical modelling has shown that pathogens with a saprobic free-living stage can drive their host population to extinction (Godfray *et al.* 1999). Mathematical theory also suggests that pathogens with a long-lived resting stage can regulate their host population to very low levels, increasing the likelihood of stochastic population extinction (Anderson & May 1981). However, this theoretical work has only considered directly developing host populations with continuous breeding and infection, whereas the life cycles of many indirectly developing amphibian hosts include seasonal and synchronized periods of reproduction and development in water (where infection may be encountered) followed by prolonged terrestrial periods. Given the stage-specific effects of infection with *B. dendrobatidis* and the recent findings of Di Rosa *et al.* (2007), there is a clear need to address the topic of free-living, and potentially saprobic, *B. dendrobatidis* and how its persistence in the environment may impact amphibian host populations.

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2007.1356 or via http://www.journals.royalsoc.ac.uk.

Received 2 October 2007
Accepted 7 November 2007

The world trade in amphibians is implicated in the emergence of chytridiomycosis, by introducing infected animals into naive populations (Fisher & Garner 2007). This is likely to have occurred in the UK where introduced infected *Rana catesbeiana* occurred in ponds used for breeding by *Rana temporaria*, *Lissotriton vulgaris*, *Triturus cristatus* and *Bufo bufo* (Cunningham *et al.* 2005, http://www.spatialepidemiology.net/bd). Of these four species, *B. bufo* (European common toad) is known to be susceptible to infection and *B. dendrobatidis*-related declines of this species have been recorded (Bosch & Martinez-Solano 2006). We have used experimental infections of *B. bufo* with *B. dendrobatidis* to study the infection dynamics in this host, and used data from these experiments to parametrize a deterministic mathematical model that describes the population dynamics of *B. bufo* following an introduction of *B. dendrobatidis*, modelled as a free-living stage that may reproduce saprobically. This model is used to develop insights into the critical factors determining the ability of *B. bufo* populations to persist following the introduction of *B. dendrobatidis*.

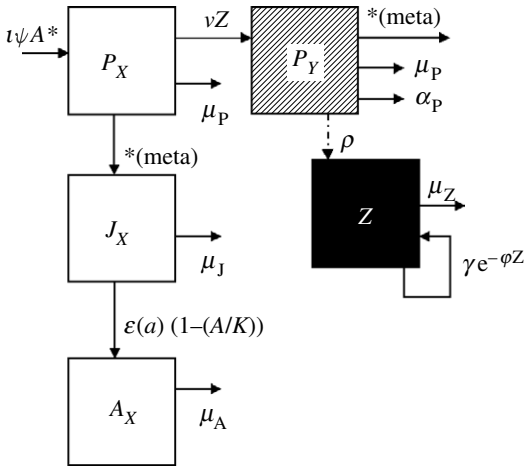

Figure 1. Schematic showing a model structure, including uninfected tadpoles, juveniles and adult *B. bufo* (P_X, $\mathcal{J}_X$ and A_X, respectively), infected tadpoles (P_Y) and the free-living zoospore population (Z). All the rates are fully defined in table 1. Asterisk, change at single time point; meta, metamorphosis; dot-dashed arrow, zoospore release.

2. MATERIAL AND METHODS

(a) *Experimental procedures*

An infection experiment, in which singly contained tadpoles were exposed to repeated doses of *B. dendrobatidis* and assessed for infection by qPCR following metamorphosis, and a transmission experiment, in which groups of tadpoles were housed at different densities with different numbers of 'seed' infected tadpoles, with final infection status assessed by qPCR, are described fully in the electronic supplementary material (§A). Infectious material (water and animals) was decontaminated by exposure to Virkon (Johnson *et al.* 2003) or by autoclaving.

(b) *Mathematical model*

The model is a system of differential and partial differential equations describing chytridiomycosis in a *B. bufo* population at a single pond site over time. Three toad stages are represented: tadpoles, $P_i(t)$; juveniles, $\mathcal{J}_i(t,a)$; and adults, $A_i(t)$, with subscript i indicating whether the stage is uninfected ($i=X$) or infected ($i=Y$) with *B. dendrobatidis*. The number of free-living *B. dendrobatidis* zoospores at time t is given by $Z(t)$, based upon model G proposed by Anderson & May (1981). A schematic of the model is shown in figure 1 and the full set of equations is given as follows:

$$\frac{\mathrm{d}P_X(t)}{\mathrm{d}t} = \delta(T-t_1)\nu\psi A_X(t) - [\mu_P + \nu Z(t)]P_X(t) - \delta(T-t_2)P_X(t), \tag{2.1}$$

$$\frac{\mathrm{d}P_Y(t)}{\mathrm{d}t} = \nu Z(t)P_X(t) - (\mu_P + \alpha_P)P_Y(t) - \delta(T-t_2)P_Y(t), \tag{2.2}$$

$$\frac{\partial \mathcal{J}_X(t,a)}{\partial t} + \frac{\partial \mathcal{J}_X(t,a)}{\partial a} = \left\{ \begin{array}{ll} \delta(T-t_2)P_X(t) & a=0 \\ -\mu_J \mathcal{J}_X(t,a) & 0<a<\omega \\ -(\mu_J+\varepsilon)\mathcal{J}_X(t,a) & a\geq\omega \end{array} \right\}, \tag{2.3}$$

$$\frac{\mathrm{d}A_X(t)}{\mathrm{d}t} = \int_\omega^\infty \varepsilon \mathcal{J}_X(t,a)\mathrm{d}a\left[1-\frac{A_X(t)}{K}\right] - \mu_A A_X(t), \tag{2.4}$$

$$\frac{\mathrm{d}Z(t)}{\mathrm{d}t} = \rho P_Y + \gamma Z(t)\exp[-\varphi Z(t)] - \mu_Z Z(t). \tag{2.5}$$

T denotes the time since the start of the year (which can be described using the floor function $T=t-\lfloor t \rfloor$). Tadpoles appear at a single time point t_1 (day 60) every year (modelled as a Dirac delta function), with number $\iota\psi A_X(t)$, where $\iota A_X(t)$ is the number of breeding partnerships and ψ is the number of tadpoles produced by each partnership. Tadpoles are infected by free-living zoospores in the pond (at a rate determined by the transmission parameter, ν) and move into the infected tadpole class, $P_Y(t)$. All tadpoles die at a constant *per capita* rate μ_P, with infected tadpoles suffering an additional disease-related increase in mortality (α_P). Infected tadpoles release zoospores into the pond at a constant rate, ρ. At time t_2 (day 155), all tadpoles metamorphose into juveniles. It is assumed that all tadpoles maintain their infection status through metamorphosis, and that juveniles leave the pond immediately after metamorphosis, so that uninfected tadpoles become uninfected juveniles, $\mathcal{J}_X(t)$. The infected juvenile stage is not included in this model, as high experimental death rates (table S1 in the electronic supplementary material) mean that they are all predicted to die before they could return to the pond to contribute further to infection or population growth. Juveniles die at a constant *per capita* rate μ_J and cannot develop into adults for at least 500 days after metamorphosis (denoted by ω), but then mature at a constant rate ε. Consequently, individuals do not contribute to the birth of tadpoles until at least the second breeding season following their metamorphosis. Growth of the adult toad population is regulated by the density-dependent function $[1-(A_X(t)/K)]$, where K determines the strength of the population regulation. Excess juveniles are assumed to leave the population in search of a new breeding site. Adults die at a constant *per capita* rate μ_A. It is assumed that infected adult toads do not contribute to the transmission dynamics of *B. dendrobatidis*. The model allows free-living zoospores to reproduce saprobically, at a *per capita* rate determined by the parameter γ. For analysis of the effect of a non-replicating resting stage, γ is set to 0. The size of the free-living *B. dendrobatidis* population in the pond is limited by the function $\exp[-\varphi Z(t)]$, which reduces saprobic reproduction as zoospore numbers increase, with φ denoting the severity of density-dependent regulation (see Godfray *et al.* (1999), who use this function to represent the population dynamics

Table 1. Model parameters with full definitions, estimated values, ranges explored and source for each parameter estimate.

parameter	definition	estimated value and units	range	reference
t_1	time point at which yearly tadpole cohort born	day 60	—	Smith (1951) and Reading (2003)
t_2	time point at which metamorphosis occurs	day 155	day 125–180	Smith (1951) and Reading (2003)
ω	minimum time since metamorphosis before juveniles start to mature into adults	500 days	—	Reading (1991) and Beebee & Griffiths (2000)
ι	ratio of successfully mating couples to total number of adults	0.14 (no units)	0.10–0.17	Reading (2001)
ψ	fecundity (eggs per mated couple)	2000 mated couple^{-1}	400–5000	Reading (1986) and Beebee & Griffiths (2000)
ε	rate of *per capita* maturation of juveniles into adults	0.16 yr^{-1}	0.1–15.0 yr^{-1}	Beebee & Griffiths (2000)
μ_P	tadpole *per capita* natural death rate	7.55 yr^{-1}	6.19–9.71 yr^{-1}	Smith (1951) and Reading (2003)
μ_J	juvenile *per capita* natural death rate	0.73 yr^{-1}	0.30–4.00 yr^{-1}	Gittins (1983)
μ_A	adult *per capita* natural death rate	0.73 yr^{-1}	0.30–2.25 yr^{-1}	Gittins (1983)
K	constant limiting the maximum adult toad population size	14 000	10 000–40 000	Beebee & Griffiths (2000)
α_P	additional *per capita* death rate in tadpoles due to infection	1.62 yr^{-1}	0–3.25 yr^{-1}	this work
ν	transmission parameter determining *per capita* rate of infection of tadpoles	6×10^{-9} zoospore^{-1} yr^{-1}	2×10^{-9}–5×10^{-8} zoospore^{-1} yr^{-1}	this work
ρ	*per capita* rate of zoospore release from infected tadpoles	1×10^{5} yr^{-1}	1×10^{4}–6.6×10^{6} yr^{-1}	this work
μ_Z	*per capita* death rate of free-living *B. dendrobatidis*	13 yr^{-1}	1–365 yr^{-1}	Johnson & Speare (2003)
γ	*per capita* growth rate of free-living *B. dendrobatidis*	0 yr^{-1}	0–9000 yr^{-1}	Berger *et al.* (2005)
φ	constant determining severity of density-dependent regulation of *B. dendrobatidis* population growth	10^{-6} zoospore^{-1}	10^{-10}–10^{-4} zoospore^{-1}	—

of saprophytic bacteria). Free-living zoospores die at a constant *per capita* rate, μ_Z.

To enable population extinctions to be registered by the deterministic model, the toad and *B. dendrobatidis* populations were set to 0 (extinction) if they fell below their respective thresholds of 50 adults and 100 zoospores. The model was run without infection until the toad population reached equilibrium. *B. dendrobatidis* was then introduced into the model as a pulse of 10 000 zoospores in the free-living state, $Z(t)$. The model was run in Berkeley Madonna v. 8.0.1, and equations were solved numerically using a fourth-order Runge–Kutta method, with a time step of 0.0005 years.

(c) *Parameter estimation*

Parameters are fully defined, with initial estimated values, explored ranges and associated references, in table 1. Parameters for the uninfected *B. bufo* population dynamics were obtained from the literature reporting extensive studies of *B. bufo* populations in the UK (Gittins 1983; Reading 2003). Large ranges of values for the maturation and natural death rates of juvenile toads were used to reflect the uncertainty in these parameter estimates.

Infection-specific death rates for tadpoles and juveniles, the transmission parameter and the rate of zoospore release by infected tadpoles were calculated from the results of the laboratory experiments, detailed fully in the electronic supplementary material (§B).

Sensitivity analysis was undertaken in the full model of all estimated parameters over their full range of values. Parameters of interest were varied together to assess their combined effect. The key outcome measured was the occurrence of extinction of both the *B. bufo* and *B. dendrobatidis* populations.

3. RESULTS

The three possible outcomes of disease emergence are as follows: (i) local extinction of the host, (ii) local extinction of the pathogen, and (iii) persistence of both the host and the pathogen. Model outcomes for our system indicate that all three of these scenarios are possible within the parameter value ranges outlined in table 1, although the likelihood of each depends on the assumptions made regarding the systems biology and the exact parameter combinations.

(a) *Without saprobic reproduction* ($\gamma=0$)

Sensitivity analysis indicates that the system is highly sensitive to the transmission parameter and the rate of zoospore release from infected tadpoles. Figure 2*a* shows the population outcomes 35 years after the introduction of infection over the full ranges of both the transmission

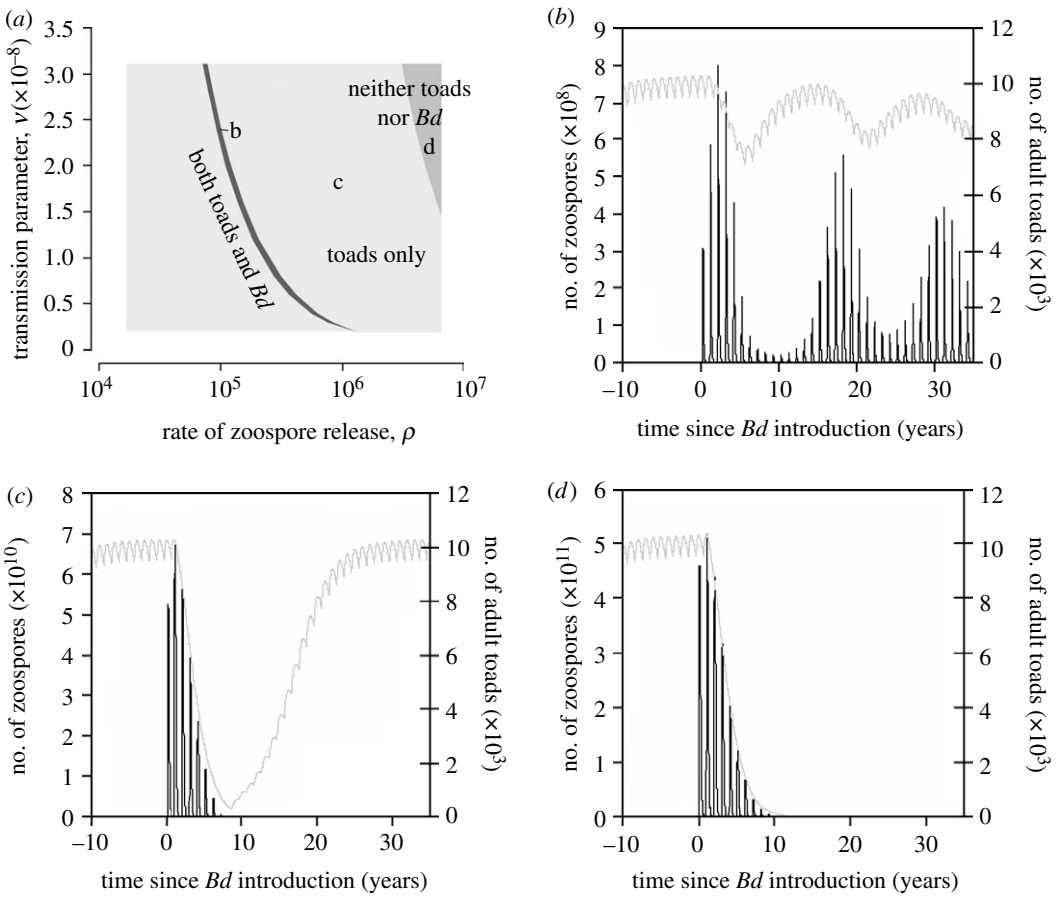

Figure 2. Stability and dynamics of the *B. bufo* and *B. dendrobatidis* populations, with an average zoospore lifespan of four weeks ($\mu_Z = 13$ yr^{-1}) and no saprobic reproduction ($\gamma = 0$). (*a*) Status of each population 35 years after the introduction of *B. dendrobatidis*, across the full ranges of the transmission parameter, ν, and the rate of zoospore release, ρ. Note that ρ is on a log scale. (*b–d*) Population dynamics of adult toads and the free-living zoospore population at each of three points 'b', 'c' and 'd' indicated in (*a*) black line, zoospores; grey line, adult toads. (*b*) $\rho = 1\times10^5$, $\nu = 2.4\times10^{-8}$, (*c*) $\rho = 1\times10^6$, $\nu = 1.8\times10^{-8}$ and (*d*) $\rho = 6\times10^6$, $\nu = 2.3\times10^{-8}$. All the other parameters are the same as those given in table 1. Note the different left *y*-axis scales in (*b–d*). *Bd*, *B. dendrobatidis*.

parameter and the zoospore release rate, without saprobic zoospore reproduction ($\gamma = 0$), and an average zoospore infectious lifetime of four weeks. The most likely outcome over this parameter space was the extinction of the *B. dendrobatidis* population with toad population recovery after the initial epidemic (figure 2*c*). Extinction of the toad species (with *B. dendrobatidis* extinction following shortly behind) only occurred for the highest values of transmission and zoospore release (figure 2*d*). Coexistence of both species was possible over a narrow band of parameter combinations (figure 2*b*).

Increasing the juvenile maturation rate reduced the time taken for the toad population to go extinct, but model outcomes were relatively insensitive to variations in the rate of *B. dendrobatidis*-induced tadpole and juvenile mortality. Increasing toad fecundity and increasing the length of the tadpole stage (making t_2 later) both increased the likelihood of toad extinction occurring (data not shown). The outcomes were not sensitive to variation in the *B. dendrobatidis* extinction limit between 1 and 1000 zoospores, but varying the toad extinction limit (between 1 and 100 individuals) did affect whereabouts in the parameter space *B. bufo* extinction was deemed to have occurred.

Changing the zoospore lifespan (without any saprobic reproduction) had a large impact on the population patterns of both toads and *B. dendrobatidis*. Across many different transmission and zoospore release parameter combinations, it was seen that longer zoospore lifespans caused larger outbreaks, and the longest lifespans we tested proved sufficient to drive the toad population extinct. Longer lifespans also led to larger regions of coexistence within the parameter space, where the total number of toads cycled in damped oscillations until it reached a new lower level (figure S2, electronic supplementary material). The extent of population depression was increasingly severe with longer zoospore lifespans and with higher rates of transmission and zoospore release.

(b) *Model incorporating saprobic reproduction*

Introducing zoospore saprobic reproduction, with $0 < \gamma < \mu_Z$, increases the region of host extinction slightly, but greatly increases the region of parameter space over which coexistence of the two species is seen (see figure S3 in the electronic supplementary material, in comparison with figure 2*a*). When $\gamma > \mu_Z$, *B. dendrobatidis* is able to persist in the pond indefinitely, regardless of whether any hosts are present or not (figure S3 in the electronic supplementary material), and the likelihood of host extinction increases with increasing saprobic reproduction.

For the parameter ranges presented in table 1, the majority of zoospores are produced by infected tadpoles

and not through saprobic reproduction, and saprobic reproduction alone does not generate enough zoospores to cause *B. bufo* extinction. As a result, *B. bufo* can be temporarily reintroduced into an endemic *B. dendrobatidis* pond, but this reintroduced toad population may only survive a number of generations, as infected tadpoles will quickly boost the zoospore density which can push the reintroduced *B. bufo* population to extinction.

4. DISCUSSION

Mathematical modelling can provide valuable insight into host–pathogen dynamics, drawing together data from a wide range of sources to investigate the probable consequences of the introduction of an infectious disease. Owing to uncertainty regarding the population dynamics of *B. dendrobatidis* and *B. bufo*, and the difficulties of predicting extinction events using deterministic frameworks, the model described here is better able to suggest qualitative trends rather than to make specific predictions about the emergence of chytridiomycosis. Nevertheless, the model has indicated key processes that have the greatest influence over the transmission dynamics of *B. dendrobatidis*, and therefore should be the priority for future research.

Our model confirms the importance of an abiotic reservoir in determining host outcomes, and the recent discovery of a 'resting' stage of *B. dendrobatidis* (Di Rosa *et al.* 2007) supports our conclusion that long-term persistence of the pathogen may be at least partly responsible for driving observed host declines. The magnitude of the transmission parameter, ν, was crucial in determining whether host extinction was possible. Estimates for the minimum zoospore lifespan and reproduction rate needed to cause toad extinction varied widely depending upon the assumed transmission and zoospore release rates. However, longer zoospore lifespans and/or higher zoospore reproduction rates clearly increased the likelihood of *B. bufo* extinction. The inclusion of saprobic growth allowed *B. dendrobatidis* to persist in the absence of *B. bufo*, and the existence of such a life-history stage could render amphibian reintroduction programmes ultimately ineffective, an important consideration given the widespread advocacy for 'amphibian salvage' programmes (Mendelson *et al.* 2006).

To better understand the amphibian–*B. dendrobatidis* dynamics, more comprehensive measurement of infection rates and direct measurement of rates of zoospore release by infected hosts at all stages will be essential. Further study of *B. dendrobatidis* aquatic life-history stages, particularly in vital rates such as birth/death rates and persistence in the presence of different limnological backgrounds, organic substrates and potential competitors (Harris *et al.* 2006), is necessary to predict *B. dendrobatidis* environmental survival. Future modelling may need to take into account spatial heterogeneities in the distribution of *B. dendrobatidis* zoospores within the water body, and a recently developed protocol that is sensitive to detecting single zoospores in their aqueous environment will be extremely helpful in quantifying the density of infectious stages in the environment (Walker *et al.* 2007).

A role for infected adults in infection dynamics was not considered here; few infected adult *B. bufo* have been observed in the field and there is no laboratory data available on their rates of infection, mortality or zoospore release. The likelihood of individuals infected as tadpoles returning as infected adults is thought to be small, due to the long maturation period and high mortality in early life-history stages, which is substantially increased by infection. However, if this did occur, infected adults could be an important reservoir.

In comparison with a previous mathematical model of *B. dendrobatidis* infection in *R. muscosa*, which assumed that infection transmission only occurred by direct contact (Briggs *et al.* 2005), the inclusion of a free-living stage in our model made host–pathogen coexistence far more likely. Given the right parameters, *B. dendrobatidis* was able to permanently depress the toad population. This supports analyses from the field, where *B. bufo* populations are persisting at reduced levels following sharp declines in ponds where *B. dendrobatidis* has been detected (S.F. Walker 2007, unpublished data). Our model demonstrates that permanent host population depression can occur without a change in pathogen virulence or host susceptibility, which has been suggested as an explanation for these dynamics (Retallick *et al.* 2004).

Host vital rates may play a role in determining the outcome of disease emergence. Our model showed that increased fecundity increased the likelihood of host population extinction, a conclusion that we share with Briggs *et al.* (2005). While low species fecundity has been identified as a risk factor for extinction caused by *B. dendrobatidis* (Daszak *et al.* 2003), this phenomenon has previously been reported and occurs because higher densities of tadpoles lead to increased transmission rates, and hence an increased level of disease (Boots & Sasaki 2002). Amphibian growth rates and fecundity can be remarkably labile, and recent research has shown how both growth trajectories and egg production may alter in response to climate change (Reading 2007). Climate change and the emergence of chytridiomycosis have also been linked as local temperatures shift towards the growth optimum of *B. dendrobatidis* (Pounds *et al.* 2006; Bosch *et al.* 2007). In this case, understanding temperature-dependent effects on (i) the ability of *B. bufo* to resist infection and (ii) the growth and survival of *B. dendrobatidis* will be essential for parametrizing future models that incorporate the effects of future climate change scenarios on the vital rates of both the host and the pathogen.

This model has demonstrated important trends and identified parameters that need to be measured more accurately in the laboratory and the field. If epidemics of chytridiomycosis are observed in populations of *B. bufo*, mathematical models will be crucial in helping to formulate intervention strategies, since these can readily be applied to models to assess the long-term and widespread impact of different strategies in the fight against chytridiomycosis.

All experiments were performed under a British Home Office licence following full ethical review.

We thank Susan Walker, Jaime Bosch and Melissa Kyriacou for providing access to unpublished data, Ian Handel for help with preparing the figures, Nick Savill for help with parameter estimation and Deirdre Hollingsworth for helpful comments on the model. This work was supported by a UK Natural

Environmental Research Council (NERC) project grant. T.S.C. and K.M.M. are supported by the Medical Research Council, UK.

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