Conservation Biology

Contributed Paper

Differential Host Susceptibility to *Batrachochytrium dendrobatidis*, an Emerging Amphibian Pathogen

C. L. SEARLE,*§ S. S. GERVASI,* J. HUA,# J. I. HAMMOND,# R. A. RELYEA,# D. H. OLSON,† AND A. R. BLAUSTEIN*

*Department of Zoology, Oregon State University, Corvallis, OR 97331, U.S.A.
#Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, U.S.A.
†United States Department of Agriculture, Forest Service, Pacific Northwest Research Station, Corvallis, OR 97331, U.S.A.

Abstract: *The amphibian fungal pathogen* Batrachochytrium dendrobatidis *(Bd) has received considerable attention due to its role in amphibian population declines worldwide. Although many amphibian species appear to be affected by Bd, there is little information on species-specific differences in susceptibility to this pathogen. We used a comparative experimental approach to examine Bd susceptibility in 6 amphibian species from the United States. We exposed postmetamorphic animals to Bd for 30 days and monitored mortality, feeding rates, and infection levels. In all species tested, Bd-exposed animals had higher rates of mortality than unexposed (control) animals. However, we found differences in mortality rates among species even though the amount of Bd detected on the different species' bodies did not differ. Of the species tested, southern toads (*Anaxyrus terrestris*) and wood frogs (*Lithobates sylvaticus*) had the highest rates of Bd-related mortality. Within species, we detected lower levels of Bd on individuals that survived longer and found that the relationship between body size and infection levels differed among species. Our results indicate that, even under identical conditions, amphibian species differ in susceptibility to Bd. This study represents a step toward identifying and understanding species variation in disease susceptibility, which can be used to optimize conservation strategies.*

Keywords: amphibian population declines, *Anaxyrus*, chytridiomycosis, *Hyla*, *Lithobates*, *Pseudacris*, *Rana*

Susceptibilidad Diferencial de Huéspedes a *Batrachochytrium dendrobatidis*, un Patógeno de Anfibios Emergente

Resumen: *El patógeno fúngico de anfibios* Batrachochytrium dendrobatidis *(Bd) ha recibido considerable atención debido a su papel en la declinación de poblaciones de anfibios en todo el mundo. Aunque parece que muchas especies de anfibios son afectadas por Bd, existe poca información sobre diferencias específicas en la susceptibilidad a este patógeno. Utilizamos un método experimental comparativo para examinar la susceptibilidad a Bd en 6 especies anfibios de los Estados Unidos. Expusimos a animales postmetamórficos a Bd durante 30 días y monitoreamos las tasas de mortalidad y de alimentación, así como los niveles de infección. En todas las especies probadas, los animales expuestos a Bd tuvieron mayores tasas de mortalidad entre especies aunque la cantidad de Bd detectada sobre los cuerpos de las diferentes especies no difirió. De las especies probadas,* Anaxyrus terrestris *y* Lithobates sylvaticus *tuvieron las mayores tasas de mortalidad relacionada con Bd. Entre especies, detectamos niveles menores sobre indviduos que sobrevivieron más tiempo y encontramos que la relación entre el tamaño del cuerpo y los niveles de infección difirió entre especies. Nuestros resultados indican que, aunque bajo condiciones idénticas, las especies de anfibios difieren en susceptibilidad a Bd y representan un paso hacia la identificación y comprensión de la variación*

§*email searlec@science.oregonstate.edu*
Paper submitted September 13, 2010; revised manuscript accepted February 12, 2011.

Conservation Biology, Volume 25, No. 5, 965–974

DOI: 10.1111/j.1523-1739.2011.01708.x

entre especies de la susceptibilidad a enfermedades, que pueden ser utilizados para optimizar estrategias de conservación.

Palabras Clave: *Anaxyrus*, declinaciones de poblaciones de anfibios, *Hyla*, *Lithobates*, *Pseudacris*, *quitridiomicosis*, *Rana*

Introduction

Understanding patterns of host-pathogen dynamics is essential for mitigating the negative effects of infectious diseases. In many cases, infectious diseases are studied in a one-pathogen, one-host system, and little attention is paid to how pathogens may affect different hosts. However, most pathogens can infect more than one species (Woolhouse et al. 2001), and hosts may differ in susceptibility to a given pathogen. Differences in host susceptibility can drive ecological phenomena such as pathogen dilution or pathogen amplification (Schmidt & Ostfeld 2001; LoGiudice et al. 2003; Keesing et al. 2006). Additionally, some hosts may act as reservoirs for a disease. In the absence of a reservoir host, direct transmission of pathogens is generally density dependent (Nokes 1992). Reservoir hosts, however, allow a pathogen to persist even when the density of susceptible hosts is low, exacerbating the severity of disease epidemics (Haydon et al. 2002). Thus, understanding how different host species respond to infection by a pathogen is essential for predicting its effects. It is clear that disease poses a substantial threat to some taxonomic groups as exemplified by global declines in amphibian populations (Stuart et al. 2004).

Global declines of amphibian populations have been attributed to multiple factors, including infectious diseases (Houlahan et al. 2000; Blaustein & Kiesecker 2002; Stuart et al. 2004). In particular, the emerging fungal pathogen *Batrachochytrium dendrobatidis* (Bd) is associated with amphibian population declines worldwide (e.g., Lips et al. 2006; Skerratt et al. 2007; Vredenburg et al. 2010). This fungus infects over 400 species (Aanensen & Fisher 2011). *Batrachochytrium dendrobatidis* infects keratinized structures of amphibians such that mouthparts of larvae are infected, but postmetamorphic amphibians can be infected over their entire epidermis (Marantelli et al. 2004; Berger et al. 2005). Therefore, rates of mortality from Bd appear to be more pronounced at the postmetamorphic stage (Parris & Cornelius 2004; Berger et al. 2005; Carey et al. 2006), when Bd disrupts various physiological functions (Voyles et al. 2009). The effects of Bd on amphibian populations in the wild appear to vary among species and locations. For example, mass die-offs associated with Bd have occurred in California and Colorado (U.S.A.) (Muths et al. 2003; Vredenburg et al. 2010), Queensland, Australia (Berger et al. 1998), and throughout Panama (Lips et al. 2006). In contrast, in other locations including South Carolina (U.S.A.) (Daszak et al. 2005) and some species in Queensland (Kriger & Hero 2006), populations appear to be persisting in the presence of Bd.

Differential susceptibility to Bd observed in the field may be due to multiple independent or interacting factors, including host physiology, host life history, environmental conditions, and community structure. For example, in organisms other than amphibians, body size has a large influence on pathogen prevalence and diversity in hosts. Larger animals are predicted to have higher pathogen loads because they provide more space for a pathogen to colonize and usually are older and have had more time to acquire pathogens (Kuris et al. 1980; Poulin 1995). In amphibians, smaller postmetamorphic animals can have higher levels of Bd than larger animals (Kriger et al. 2007), but the opposite relation was found in larvae in 2 studies (Smith et al. 2007; Symonds et al. 2007). Seasonal changes in temperature can alter growth rates of pathogens and host responses, creating cyclical patterns in pathogen prevalence (Dowell 2001; Raffel et al. 2006). This may be particularly relevant for Bd because it is a cold-associated pathogen (Longcore et al. 1999; Piotrowski et al. 2004). In the field, there is a greater incidence of Bd in winter months compared with summer months, and experimentally infecting animals at colder temperatures increases the negative effects of Bd (Berger et al. 2004).

Certain life-history strategies may increase rates of pathogen transmission or growth. In a field survey, Kriger and Hero (2007) found that stream-breeding amphibians are more likely to be infected with Bd than pond-breeders and almost all infected animals occur in permanent water bodies. Bielby et al. (2008) analyzed correlates of Bd-associated declines and found that high-elevation species with restricted ranges are most likely to experience Bd-related declines. These patterns provide valuable information for predicting future declines, but it is difficult to consider all factors in one study. In a laboratory experiment, we investigated the degree to which species of amphibians were negatively affected by Bd, which allowed us to address causation in addition to correlation.

The few researchers who have tested amphibian susceptibility to Bd have done so with few species or with larval animals. Larvae of 4 species of anurans (frogs and toads) exposed to Bd had different levels of mortality and mouthpart pigmentation (Blaustein et al. 2005). Susceptibility of juvenile or adult amphibians to Bd has been examined in 3 or fewer species at a time (Nichols et al.

2001; Daszak et al. 2004; Garcia et al. 2006). We tested the susceptibility to Bd of 6 anuran species shortly after metamorphosis. We monitored mortality, infection levels, and feeding rates to determine baseline differences in how these species responded to Bd exposure.

Methods

Animal Husbandry

We collected eggs of upland chorus frogs (*Pseudacris feriarum*) from Seven Island Wildlife Refuge, Tennessee (35°N, 83°W); wood frogs (*Lithobates sylvaticus*) and western chorus frogs (*Pseudacris triseriata*) from the Edwin S. George Reserve, Michigan (42°N, 84°W); gray tree frogs (*Hyla versicolor*) and northern leopard frogs (*Rana pipiens*) from Pymatuning Laboratory of Ecology farm site and Mallard Pond, respectively, Pennsylvania (both 41°N, 80°W); and southern toads (*Anaxyrus terrestris*) from Orange County, Florida (29°N, 81°W). We transported eggs to the Pymatuning Laboratory of Ecology in Pennsylvania, where they hatched in 200-L plastic pools containing aged well water. We moved free-swimming tadpoles to 100-L plastic pools filled with 90 L of well water, approximately 1 L of pond water, 5 g of a commercial brand of rabbit food, and 100 g of dried oak leaves (*Quercus* spp.). Before we introduced tadpoles to the pools, the mixture sat for at least 15 days to allow the algal community to develop. Tadpole density was approximately 25 per pool. We covered all pools with cloth that provided 60% shade, excluded predators, and prevented animals from escaping. When animals reached metamorphosis (Gosner [1960] stages 42–44), we moved them from wading pools to 1-L containers lined with sphagnum moss, where they were kept until full tail absorption. We fed postmetamorphic animals pinhead crickets (*Acheta domestica*) ad libitum for 1–2 weeks before shipping them overnight to a laboratory at Oregon State University, Corvallis, Oregon (U.S.A.).

On arrival, we placed animals in glass terraria held at 21.5–23.3 °C with a 13 h:11 h light to dark photoperiod. Animals acclimated for 24 h before initiation of the experiment. We randomly assigned individuals of each species to either Bd-exposed or unexposed (control) treatments. We used 50 individuals of each species (25 per treatment), except for wood frogs, for which we used 44 animals (22 per treatment). We measured the mass and snout-vent length of individuals and placed them in Petri dishes (140 × 30 mm) with holes in the lid and a thin film of water covering the bottom. Animals could partially climb the walls of the Petri dish, but could not completely lift themselves off the bottom; thus, they were in constant contact with the water. We kept animals in these dishes for the duration of the experiment (30 days) and fed them twice a week with a number of pinhead crickets that we based on the average size of the species (1 cricket/0.1 g body mass). Due to differences in breeding phenology, we did not test all species simultaneously, but all species were treated with identical methods in the same laboratory.

Experimental Procedure

We exposed animals to experimental treatments when they were transferred to individual Petri dishes. We used Bd strain JEL 274 (originally isolated from an *A. boreas* in Colorado [U.S.A.] [Annis et al. 2004]) cultured on 1% tryptone agar plates that were made 5–7 days prior to inoculation and held at approximately 22 °C. To harvest Bd from agar, we flooded plates with 15 mL dechlorinated water for 5 minutes. To standardize inoculation dose among exposed animals, we pooled water from at least 10 Bd-inoculated plates for each inoculation and quantified the number of zoospores in the inoculum with a hemocytometer. After quantifying zoospores, we diluted inoculum to a concentration of 1.7×10^4 zoospores/mL inoculum. Then, we added 15 mL inoculum to each Petri dish. Thus, Bd-exposed animals were exposed to approximately 2.6×10^5 total zoospores. Control animals were exposed to 15 mL inoculum without Bd culture create dusing sterile agar plates. We exposed all species to the same Bd dose at each inoculation, and we conducted each inoculation during the experiment in the same manner. Water in Petri dishes was changed every 7 days, with reinoculation occurring simultaneously. We placed an additional 10 mL of water into each Petri dish at each water change, which combined with the inoculum was sufficient to completely cover the bottom of the dish with a thin film of water. Animals therefore remained in direct contact with water on their ventral side, but could not submerge themselves.

We monitored mortality daily and removed dead animals from their dishes and preserved them in 95% ethanol. We monitored feeding rates by recording the number of crickets left in each dish 24 h after each feeding.

After 30 days, we weighed, measured, and then euthanized all surviving animals in MS-222 and preserved them in 95% ethanol. We then determined Bd-infection levels with quantitative polymerase chain reaction (qPCR) for all Bd-exposed animals and 4 randomly selected control individuals from each species. With a sterile fine-tip swab (Medical Wire and Equipment, Corsham, United Kingdom), we swabbed the left ventral surface of each animal 10 times, covering the legs, feet, and drink patch. We placed each swab in a sterile vial and capped the vial. We swabbed animals after they had been preserved, so animals that died during the experiment and those that were euthanized after 30 days were tested for Bd. To extract DNA, we added 60 μL Prepman Ultra (Applied

Biosystems, Carlsbad, California), heated the vial to 100 °C for 10 minutes, cooled the vial for 2 minutes, and then extracted the supernatant. Before conducting qPCR, we diluted each sample to a 10% solution. We conducted qPCR on an ABI PRISM 7500 (Applied Biosystems) according to methods of Boyle et al. (2004). We analyzed each sample in triplicate and calculated the average number of genome equivalents per individual. If a sample tested positive in only 1 or 2 replicates, we reanalyzed the sample. An individual was considered Bd-positive if all 3 samples (run once) were positive or if 4 out of 6 samples (run twice) were positive. Additionally, we swabbed Bd-exposed animals that tested negative a second time on their right side and reanalyzed the sample.

Statistical Analyses

We performed statistical analyses in *R* statistical computing environment (version 2.9.0, Institute for Statistics and Mathematics, Vienna) with the Survival package for survival analyses. To test whether the initial size differed among species, we performed 1 way analysis of variance (ANOVA) followed by a Tukey's honestly significant difference test on initial mass and length.

We used a Cox's proportional hazards model to compare rates of survival among species and treatments. This is a method of survival analysis that allows one to compare the probability of mortality from multiple factors through differences in survival curves (Cox 1972; Parmar & Machin 1995). We used Cox's proportional hazards model to determine a hazard ratio, which indicates the association of a factor with the probability of mortality (hazard ratio >1, increase in the probability of mortality; hazard ratio <1, decrease in probability of mortality). Our initial among-species model included Bd treatment, species, and the interaction between these factors. We considered the model with the lowest Akaike's information criterion (AIC) the best model (threshold ΔAIC of one to distinguish among models). Additionally, we applied Cox's proportional hazard models to individual species so we could accurately estimate hazard functions for Bd treatment, mass, and length within each species.

We transformed infection levels (log-average genome equivalents per individual +1) in an among-species general linear model with predictors of species, mass, length, days to death, and all 2-way interactions between these factors. We then selected the model with the lowest AIC. Additionally, to understand within species patterns, we applied general linear models to individual species to test whether mass, length, and time to death were associated with infection levels.

Feeding rate was the average number of crickets eaten 24 h after feeding. We used a 2-tailed Mann–Whitney *U* test to compare differences in feeding between control and Bd-exposed animals within each species. We did not compare feeding rates among species because not all species were fed the same number of crickets.

Results

Among-Species Comparisons

Initial body mass and length differed among species (mass: $F_{5,288} = 580.9$, $p < 0.01$; length: $F_{5,288} = 844.0$, $p < 0.01$) (Table 1). A Tukey's test revealed that gray tree frogs and wood frogs did not differ significantly in mass or length. Additionally, western chorus frogs, upland chorus frogs, and southern toads did not differ significantly in mass and upland chorus frogs and western chorus frogs did not differ significantly in length. These parameters differed significantly in all other comparisons among species ($p < 0.05$).

There was greater mortality of animals in the Bd-treatments than in controls (Fig. 1), but the effects of Bd differed among species. The best Cox's proportional model of survival included Bd treatment and the Bd treatment by species interaction (Table 2). A model including species had a slightly higher AIC value (ΔAIC = 0.5). Exposure to Bd increased mortality across species by a factor of 3.63 (95% CI: 1.99–6.63, $P < 0.01$). However, the effects of Bd differed among species (Bd-treatment by species interaction; $p < 0.01$).

Both time to death and mass were associated with Bd infection. We did not detect Bd on any control animals. In contrast, all but 2 Bd-exposed animals (both western chorus frogs) tested positive for Bd infection. When samples from these 2 uninfected animals were reanalyzed with a second swabbing, Bd was not detected in any of the 6 total qPCR wells of these 2 individuals. Our all-species general linear model for infection included species, mass, time to death, species by mass interaction, and species by time-to-death interaction (Table 2). Animals that survived longer had significantly lower infection levels than those that died earlier in the experiment ($F_{1,289} = 42.94$, $p < 0.01$) and heavier animals had significantly lower levels of infection ($F_{1,289} = 4.25$, $p = 0.04$). There was also an interaction between species and mass ($F_{1,289} = 4.09$, $p = 0.05$; Fig. 2), but species did not explain significant variance in infection levels ($F_{1,289} = 1.43$, $p = 0.23$). Average infection levels (in genome equivalents Bd [ge]) were 174.1 ge (SE 31.2) for southern toads, 133.3 ge (31.9) for wood frogs, 72.6 ge (23.5) for western chorus frogs, 98.6 ge (18.2) for northern leopard frogs, 281.3 ge (54.8) for upland chorus frogs, and 57.4 ge (14.6) for gray tree frogs.

Within-Species Comparisons

In Cox's proportional model (Fig. 1), the probability of mortality increased significantly for all species that were exposedto Bd, but each species had a different hazard

Table 1. Summary information for each amphibian species tested for susceptibility to *Batrachochytrium dendrobatidis* (Bd) in order of descending hazard ratio,[a] indicating the association between Bd exposure and probability of mortality.

Species	*Average mass in mg (SE)*[b]	*Average length in mm (SE)*[b]	*Hazard ratio (SE)*	p *for the Cox PH model*[c]
Southern toad	146.8 (2.2)	11.45 (0.08)	171.27 (1.11)	<0.01
Wood frog	303.7 (9.4)	15.16 (0.13)	77.80 (0.74)	<0.01
Western chorus frog	173.1 (3.8)	13.20 (0.12)	23.18 (0.66)	<0.01
Northern leopard frog	997.2 (30.4)	23.39 (0.26)	16.69 (0.51)	<0.01
Upland chorus frog	199.1 (3.8)	13.14 (0.11)	4.92 (0.36)	<0.01
Gray tree frog	267.1 (5.5)	14.61 (0.11)	2.99 (0.32)	= 0.01

[a] *A numerical comparison of the mortality rates between treatments.*
[b] *Determined with initial measurements recorded before initiation of the experiment.*
[c] *Cox proportional hazard models indicating the effect of Bd on survival of each species.*

ratio (Table 1). In 4 species mortality and mass were significantly related; individuals with a greater mass had lower hazard ratios (southern toads: hazard ratio = 0.98, $p = 0.03$; wood frogs: hazard ratio = 0.98, $p = 0.01$; western chorus frogs: hazard ratio = 0.97, $p = 0.01$; northern leopard frogs: hazard ratio = 0.99, $p < 0.01$). The hazard ratio for the other 2 species did not differ significantly as a function of mass (upland chorus frogs:

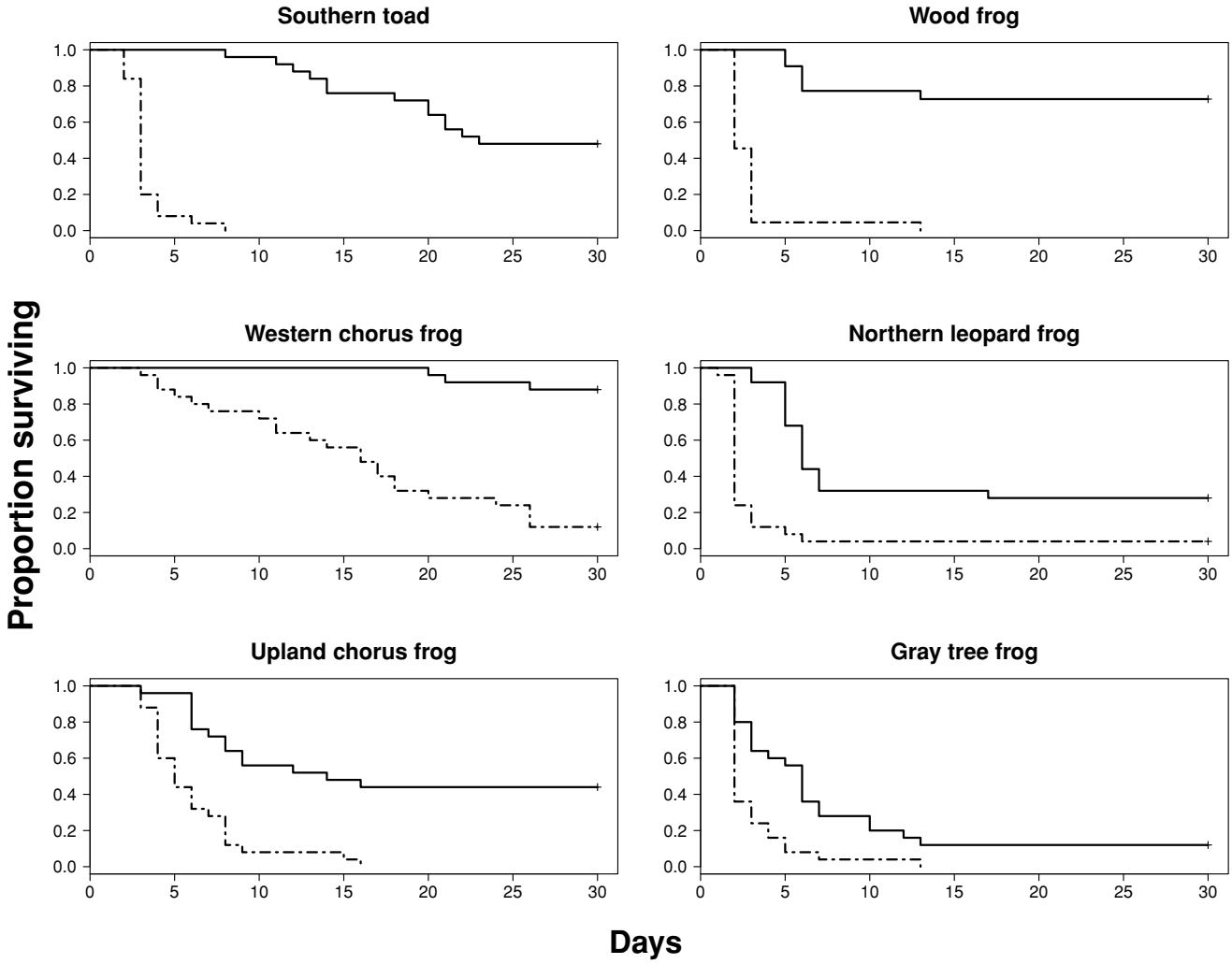

Figure 1. Thirty-day survival of Batrachochytrium dendrobatidis (Bd)-exposed (dashed lines) and control groups (solid lines) of amphibians for each species, half the animals were exposed to Bd and the other half were not.

Table 2. Candidate models of mortality and infection with *Batrachochytrium dendrobatidis* (Bd) among amphibian species.

Model covariates[a]	*Akaike's information criterion*	Δ_i[b]
Among species mortality		
Bd + Bd* species	2120.5	0.0
Bd + species +Bd* species	2121.0	0.5
Bd + species	2121.5	1.0
Bd* species	2137.1	16.6
species + Bd* species	2137.1	16.6
Bd	2134.5	14.0
species	2255.4	134.9
Among species infection[c]		
Species + mass + days + species*mass + species*days	520.3	0.0
Mass + days + species*mass + species*days	521.6	1.3
Species + mass +days + species*mass + species*days + mass*days	521.8	1.5
Mass + days +species*mass	522.0	1.7
Mass + days + species*days	522.0	1.7
Species + mass + length + days + species*mass + species*days	522.5	2.2
Species + mass + length + days + species*mass + species*length + species*days	522.6	2.3

[a] *Key: Bd, Bd treatment (exposed or unexposed); species, species identity; mass (mg) and length (mm), measurements recorded at the initiation of the experiment; days, number of days until death for each individual.*
[b] *Difference in Akaike's information criterion (AIC) values between the selected model and the best (lowest AIC) model. Smaller values indicate models that are more similar to the best model.*
[c] *Only the 7 models with the lowest Akaike's information criterion values are shown.*

hazard ratio = 0.98, $p = 0.09$; gray tree frogs: hazard ratio = 0.99, $p = 0.49$). Length did not explain significant variance in mortality for any species.

Time to death explained significant variance in observed levels of Bd infection in all species; animals that survived longer had lower levels of infection (southern toads: $F_{1,22} = 5.69$, $p = 0.03$; wood frogs: $F_{1,19} = 4.75$, $p = 0.03$; western chorus frogs: $F_{1,22} = 5.22$, $p = 0.03$; northern leopard frogs: $F_{1,22} = 19.68$, $p < 0.01$; upland chorus frogs: $F_{1,22} = 17.26$, $p < 0.01$; gray tree frogs: $F_{1,22} = 11.54$, $p < 0.01$). Mass explained significant variance in infection in northern leopard frogs ($F_{1,22} = 21.35$, $p < 0.01$) and gray tree frogs ($F_{1,22} = 10.79$, $p < 0.01$). Larger animals had lower infection levels in northern leopard frogs, and smaller animals had lower infection levels in gray tree frogs (Fig. 2). Body length explained significant variance in observed infection in wood frogs ($F_{1,19} = 7.30$, $p = 0.03$); longer animals had lower infection levels. Mass and length were not significantly associated with infection for any other species ($p > 0.05$).

In all species, Bd-exposed animals on average had lower feeding rates than control animals (Fig. 3). This effect was significant only for western chorus frogs (Mann–Whitney: $p < 0.01$)

Discussion

In all 6 amphibian species we examined, exposure to Bd was associated with increased mortality, but the magnitude of this effect differed among species. Infections levels did not differ among species, which suggests physiological or immunological differences in how these species are infected by Bd or respond to Bd infection. When an organism is infected by a pathogen, it can use 2 strategies to defend itself: resistance or tolerance (Schneider & Ayres 2008; Raberg et al. 2009). Resistance is the ability of an organism to limit pathogen burden, and tolerance is the ability of an organism to limit harm caused by the pathogen (Raberg et al. 2009). We found that species had similar Bd infection levels but different mortality levels, which suggests that species have a similar resistance but different tolerance to Bd. Because we did not measure infection throughout the experiment, it is also possible that species differed in infection levels at different times during the experiment. Additionally, we found 2 Bd-exposed western chorus frogs that were not infected with Bd even though these individuals were alive for the duration of the experiment and were inoculated with Bd 4 separate times. These individuals may have a genotype that is resistant to acquiring Bd or can quickly recover from a Bd infection. In previous laboratory experiments, the American bullfrog (*Lithobates catesbeianus*) was unaffected by Bd infection (Daszak et al. 2004), and in field studies some species of amphibians have been identified that appear to survive in the wild with Bd infections (Daszak et al. 2005; Kriger & Hero 2006, Goka et al. 2009). Thus, there may be multiple mechanisms by which amphibians resist or tolerate Bd infection.

Southern toads and wood frogs had the highest rates of Bd-related mortality, whereas upland chorus frogs and gray tree frogs had the lowest. Results of other studies suggest that toads such as western toads (*A. boreas*) may be more susceptible to Bd than other species (Blaustein et al. 2005; Garner et al. 2009), but these conclusions are only possible with comparative studies in which species are tested under the same experimental protocol. Comparative studies help predict which species are likely to suffer the most from Bd. Researchers have developed models that predict sites of Bd epidemics and declines (Ron 2005; Bielby et al. 2008; Rödder et al. 2009). However, these models generally focus on environmental factors that affect Bd growth, not on host characteristics that lead to declines. Bielby et al. (2008) and Rödder et al. (2009) included life-history characteristics of amphibian hosts, but did not incorporate differences in species susceptibility to Bd, likely because these data do not exist for many species.

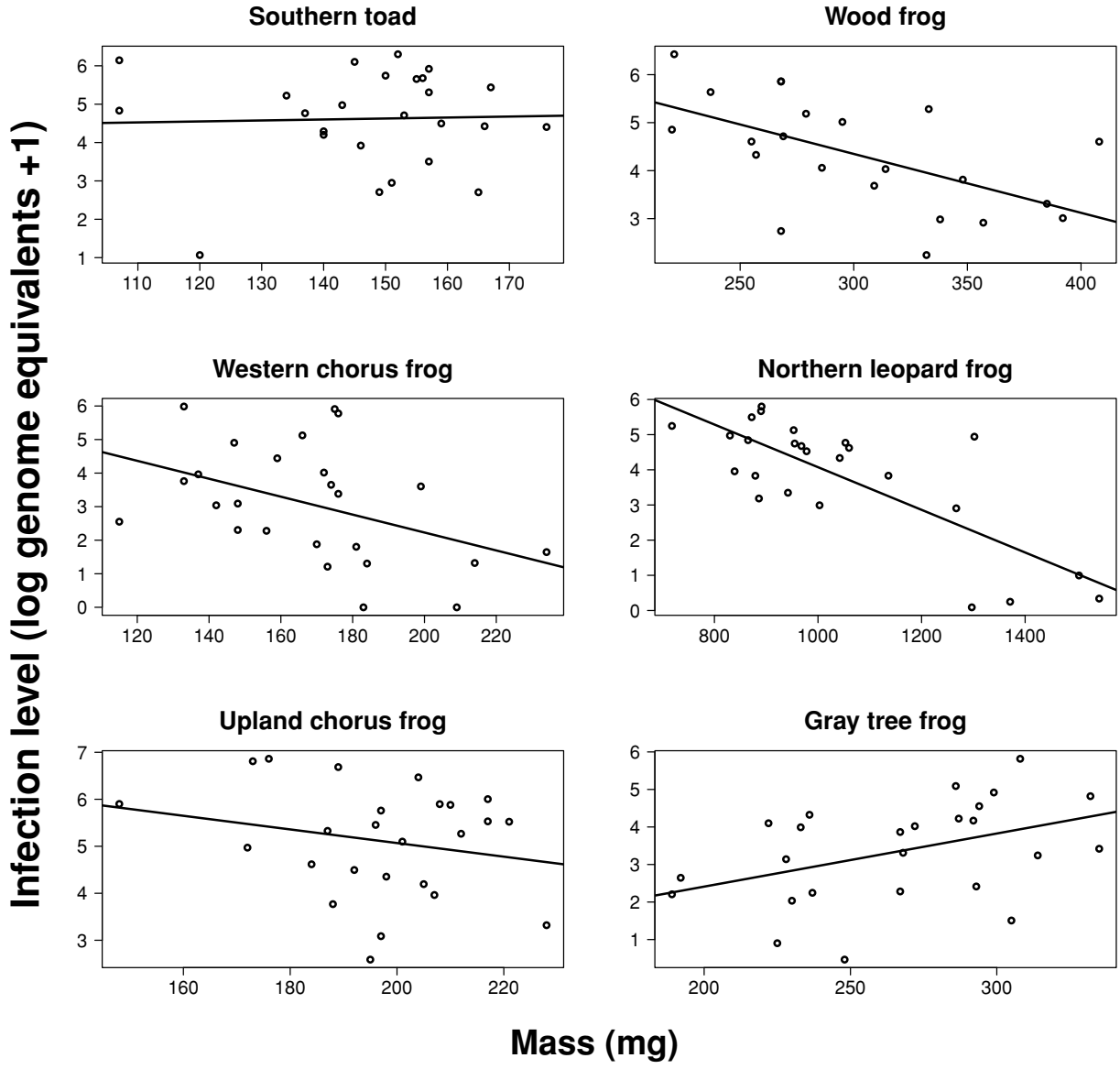

Figure 2. Batrachochytrium dendrobatidis infection levels (log-average genome equivalents per individual + 1) of all amphibian species in relation to body mass.

Within species we found that animals with relatively higher levels of Bd died sooner than those with lower infection levels. Infection in postmetamorphic amphibians covers the skin and reduces an amphibian's ability to osmoregulate, which leads to electrolyte imbalance and death (Voyles et al. 2009). With higher Bd loads, more skin is covered by the pathogen, which could lead to electrolyte imbalance occurring sooner. Additionally, animals may reach a threshold level of infection after which point physiological functions that normally prevent infection or aid in recovery from infection are overwhelmed, resulting in mortality. Vredenburg et al. (2010) suggest that mass die-offs of amphibians occur when infection intensity reaches a threshold. If highly infected animals die quickly, it may take longer to achieve this threshold. Thus, understanding levels of infection, rather than simple presence or absence, is relevant when studying Bd dynamics in the laboratory and field.

The size of a host can have a dramatic effect on the degree to which it is infected by a pathogen (Kuris et al. 1980; Poulin 1995). We found that the magnitude and direction of the relation between mass and infection levels varied among species. Larger animals may acquire greater amounts of Bd, but may also be healthier and better able to resist infection (Carey et al. 1999), which could lead to longer survival. In previous studies with different species than the ones we studied here, both positive and negative relations have been found between body size and Bd infection (Kriger et al. 2007; Smith et al. 2007; Symonds et al. 2007). We found a positive correlation

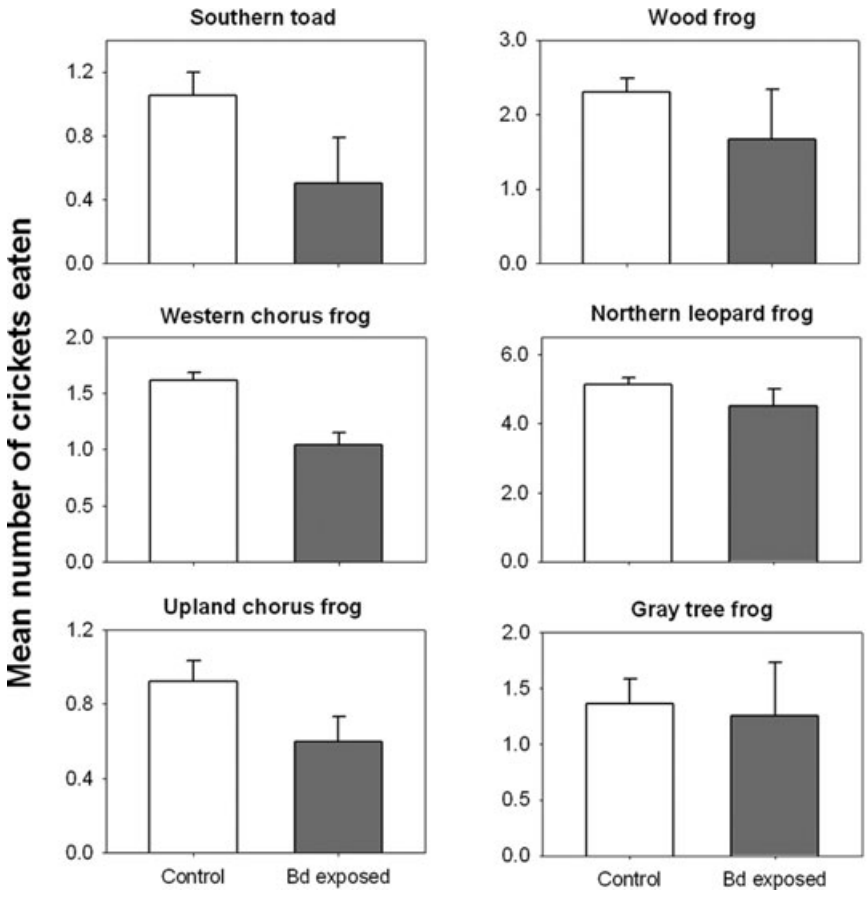

Figure 3. Mean (SE) number of crickets eaten at each feeding event for each species of amphibian exposed to Batrachochytrium dendrobatidis (Bd) (shaded bars) and not exposed to Bd (unshaded bars).

between mass and infection levels in gray tree frogs, but in other species the correlation was either negative or neutral.

We also found that exposure to Bd was associated with lower feeding rates in western chorus frogs. Reduced feeding could reduce an animal's size, which may change how it responds to Bd infection. In the field, amphibian size may be reduced by stressors such as climate change and habitat alteration (Karraker & Welsh 2006; Reading 2006). Such stressors could lead to a chain of events in which environmental stressors and Bd alter behavior (such as feeding rates). Altered behavior in turn can affect body size and Bd infection rates.

Exposing multiple species of amphibians to Bd under identical conditions is essential for understanding basic differences in species susceptibility. However, there are limitations to interpreting the results of our controlled laboratory experiments. For example, we could not account for differences in habitat or behavior among species that may influence susceptibility of amphibians to Bd in the field. Bd had the greatest negative association with southern toads in the laboratory. However, in the wild, southern toads often occur in dry areas that are suboptimal for Bd growth (Piotrowski et al. 2004), which may decrease their susceptibility to Bd. Additionally, habitats of these species may differ in humidity and temperature, which also affect Bd growth. Therefore, when incorporating the ability of species to choose habitat into predictions of susceptibility, the effect of Bd on each species may change. Other factors, such as bacteria living on amphibian skin in the field but not in our mesocosm-reared animals, may provide protection from Bd (Woodhams et al. 2006; Becker & Harris 2010). Additionally, we used only one strain of Bd to inoculate all species, but sensitivity to Bd strains may differ among species (Retallick & Miera 2007). Investigating all these factors simultaneously in models or experiments is difficult.

Bd has been found in the field on all of the species we tested, except for upland chorus frogs (Longcore et al. 2007; Rothermel et al. 2008; Rizkalla 2010). Thus, Bd likely affects these species in the field. Comparisons of susceptibility among large numbers of amphibians could identify reservoir hosts and help predict patterns of Bd prevalence in different amphibian communities. Additionally, experimental exposures could elucidate patterns of susceptibility that can be predicted as a function of taxonomic classification, body size, or geographic location. Although others have attempted to do this with field surveys and predictive modeling, experimental manipulation of Bd infections is the only reliable way to test differences in susceptibility. On a large scale, comparative

susceptibility studies could identify factors correlated with infection patterns and inform conservation efforts that go beyond work aimed at individual species. Susceptibility data generated from controlled experiments complement field surveys, correlational studies, and modeling efforts and can help optimize conservation strategies.

Acknowledgments

We thank J. Adams, L. Cha, L. Yeo, E. Borer, W. Stubblefield, J. Bartholomew, J. Crockett, B. Garrington, P. Smith, and M. Christie for their assistance with this project. Additionally, J. Spatafora and the Center for Genome Research and Biocomputing at Oregon State University kindly provided laboratory space for conducting qPCR. Support for this project was provided by the U.S. National Science Foundation and U.S. Forest Service Pacific Northwest Research Station, Corvallis, Oregon.

Literature Cited

Aanensen, D. M., and M. C. Fisher. 2011. Global *Bd*-mapping project. Imperial College, London. Available from http://www.bd-maps.net/ (accessed April 2011).

Annis, S. L., F. P. Dastoor, H. Ziel, P. Daszak, and J. E. Longcore. 2004. A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. Journal of Wildlife Diseases **40:**420–428.

Becker, M. H., and R. N. Harris. 2010. Cutaneous bacteria of the redback salamander prevent morbidity associated with a lethal disease. Public Library of Science ONE 5(6):e10957. DOI:10.1371/journal.pone.0010957.

Berger, L., G. Marantelli, L. F. Skerratt, and R. Speare. 2005. Virulence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* varies with the strain. Diseases of Aquatic Organisms **68:** 47–50.

Berger, L., et al. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences **95:**6031–9036.

Berger, L., et al. 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Australian Veterinary Journal **82:**31–36.

Berger, L., R. Speare, and L. F. Skerratt. 2005. Distribution of *Batrachochytrium dendrobatidis* and pathology in the skin of green tree frogs *Litoria caerulea* with severe chytridiomycosis. Diseases of Aquatic Organisms **68:**65–70.

Bielby, J., N. Cooper, A. Cunningham, T. Garner, and A. Purvis. 2008. Predicting susceptibility to future declines in the world's frogs. Conservation Letters **1:**82–90.

Blaustein, A. R., and J. M. Kiesecker. 2002. Complexity in conservation: lessons from the global decline of amphibian populations. Ecology Letters **5:**597–608.

Blaustein, A. R., J. M. Romansic, E. A. Scheessele, B. A. Han, A. J. Pessier, and J. E. Longcore. 2005. Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. Conservation Biology **19:**1460–1468.

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.

Carey, C., J. E. Bruzgul, L. J. Livo, M. L. Walling, K. A. Kuehl, B. F. Dixon, A. P. Pessier, R. A. Alford, and K. B. Rogers. 2006. Experimental exposures of boreal toads (*Bufo boreas*) to a pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*). EcoHealth **3:**5–21.

Carey, C., N. Cohen, and L. Rollins-Smith. 1999. Amphibian declines: an immunological perspective. Developmental and Comparative Immunology **23:**459–472.

Cox, D. 1972. Regression models and life-tables. Journal of the Royal Statistical Society. Series B (Methodological) **34:**187–220.

Daszak, P., D. E. Scott, A. M. Kilpatrick, C. Faggioni, J. W. Gibbons, and D. Porter. 2005. Amphibian population declines at Savannah River site are linked to climate, not chytridiomycosis. Ecology **86:**3232–3237.

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:**201–208.

Dowell, S. F. 2001. Seasonal variation in host susceptibility and cycles of certain infectious diseases. Emerging Infectious Diseases **7:**369–374.

Garcia, T. S., J. M. Romansic, and A. R. Blaustein. 2006. Survival of three species of anuran metamorphs exposed to UV-B radiation and the pathogenic fungus *Batrachochytrium dendrobatidis*. Diseases of aquatic organisms **72:**163–169.

Garner, T. W. J., S. Walker, J. Bosch, S. Leech, J. M. Rowcliffe, A. A. Cunningham, and M. C. Fisher. 2009. Life history tradeoffs influence mortality associated with the amphibian pathogen, *Batrachochytrium dendrobatidis*. Oikos **118:**783–791.

Goka, K., J. Yokoyama, Y. Une, T. Kuroki, K. Suzuki, M. Nakahara, A. Kobayashi, S. Inaba, T. Mizutani, and A. Hyatt. 2009. Amphibian chytridiomycosis in Japan: distribution, haplotypes and possible route of entry into Japan. Molecular Ecology **18:**4757–4774.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16:**183–190.

Haydon, D. T., S. Cleaveland, L. H. Taylor, and M. K. Laurenson. 2002. Identifying reservoirs of infection: a conceptual and practical challenge. Emerging Infectious Diseases **8:**1468–1473.

Houlahan, J. E., C. S. Findlay, B. R. Schmidt, A. H. Meyer, and S. L. Kuzmin. 2000. Quantitative evidence for global amphibian population declines. Nature **404:**752–755.

Karraker, N. E., and H. H. Welsh Jr. 2006. Long-term impacts of even-aged timber management on abundance and body condition of terrestrial amphibians in Northwestern California. Biological Conservation **131:**132–140.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters **9:**485–498.

Kriger, K. M., and J. M. Hero. 2007. The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. Diversity and Distributions **13:**781–788.

Kriger, K. M., and J. Hero. 2006. Survivorship in wild frogs infected with chytridiomycosis. EcoHealth **3:**171–177.

Kriger, K. M., F. Pereoglou, and J. Hero. 2007. Latitudinal variation in the prevalence and intensity of chytrid (*Batrachochytrium dendrobatidis*) infection in eastern Australia. Conservation Biology **21:**1280–1290.

Kuris, A., A. R. Blaustein, and J. Javier Alió. 1980. Hosts as islands. The American Naturalist **116:**570–586.

Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proceedings of the National Academy of Sciences of the United States of America **103:**3165–3170.

LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academy of Sciences **100:**567–571.

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.

Longcore, J. R., J. E. Longcore, A. P. Pessier, and W. A. Halteman. 2007. Chytridiomycosiswidespread in anurans of northeastern United States. Journal of Wildlife Management **71:**435–444.

Marantelli, G., L. Berger, R. Speare, and L. Keegan. 2004. Distribution of the amphibian chytrid *Batrachochytriu mdendrobatidis* and keratin during tadpole development. Pacific Conservation Biology **10:**173–179.

Muths, E., P. Stephen Corn, A. P. Pessier, and D. Earl 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.

Nokes, D. 1992. Microparasites: viruses and bacteria. Pages 349–374 in Natural enemies: the population biology of predators, parasites and disease. Blackwell Scientific, Boston.

Parmar, K., and D. Machin. 1995. Survival analysis: a practical approach. 1st edition. John Wiley and Sons, New York.

Parris, M. J., and T. O. Cornelius. 2004. Fungal pathogen causes competitive and developmental stress in larval amphibian communities. Ecology **85:**3385–3395.

Piotrowski, J. S., S. L. Annis, and J. E. Longcore. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia **96:**9–15.

Poulin, R. 1995. Phylogeny, ecology, and the richness of parasite communities in vertebrates. Ecological Monographs **65:**283–302.

Raberg, L., A. L. Graham, and A. F. Read. 2009. Decomposing health: tolerance and resistance to parasites in animals. Philosophical Transactions of the Royal Society B: Biological Sciences **364:**37–49.

Raffel, T. R., J. R. Rohr, J. M. Kiesecker, and P. J. Hudson. 2006. Negative effects of changing temperature on amphibian immunity under field conditions. Functional Ecology **20:**819–828.

Reading, C. J. 2006. Linking global warming to amphibian declines through its effects on female body condition and survivorship. Oecologia **151:**125–131.

Retallick, R. W. R., and V. Miera. 2007. Strain differences in the amphibian chytrid *Batrachochytrium dendrobatidis* and nonpermanent, sub-lethal effects of infection. Diseases of aquatic organisms **75:**201–207.

Rizkalla, C. E. 2010. Increasing detections of *Batrachochytrium dendrobatidis* in central Florida, USA. Herpetological Review **41:**180–181.

Rödder, D., J. Kielgast, J. Bielby, S. Schmidtlein, J. Bosch, T. W. J. Garner, M. Veith, S. Walker, M. C. Fisher, and S. Lötters. 2009. Global amphibian extinction risk assessment for the panzootic chytrid fungus. Diversity **1:**52–66.

Ron, S. R. 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. Biotropica **37:**209–221.

Rothermel, B., S. Walls, J. Mitchell, C. Dodd, L. Irwin, D. Green, V. Vazquez, J. Petranka, and D. Stevenson. 2008. Widespread occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the southeastern USA. Diseases of Aquatic Organisms **82:**3–18.

Schmidt, K. A., and R. S. Ostfeld. 2001. Biodiversity and the dilution effect in disease ecology. Ecology **82:**609–619.

Schneider, D. S., and J. S. Ayres. 2008. Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. Nature Reviews Immunology **8:**889–895.

Skerratt, L. F., L. Berger, R. Speare, S. Cashins, K. R. McDonald, A. D. Phillott, H. B. Hines, and N. Kenyon. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth **4:**125–134.

Smith, K. G., C. Weldon, W. Conradie, and L. H. du Preez. 2007. Relationships among size, development, and *Batrachochytrium dendrobatidis* infection in African tadpoles. Diseases of Aquatic Organisms **74:**159–164.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science **306:**1783–1786.

Symonds, E. P., H. B. Hines, P. S. Bird, J. M. Morton, and P. C. Mills. 2007. Surveillance for *Batrachochytrium dendrobatidis* using mixophyes (anura: myobatrachidae) larvae. Journal of Wildlife Diseases **43:**48–60.

Voyles, J., et al. 2009. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. Science **326:**582–585.

Vredenburg, V. T., R. A. Knapp, T. S. Tunstall, and C. J. Briggs. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. Proceedings of the National Academy of Sciences **107:**9689–9694.

Woodhams, D. C., L. A. Rollins-Smith, C. Carey, L. Reinert, M. J. Tyler, and R. A. Alford. 2006. Population trends associated with skin peptide defenses against chytridiomycosis in Australian frogs. Oecologia **146:**531–540.

Woolhouse, M. E. J., L. H. Taylor, and D. T. Haydon. 2001. Population biology of multihost pathogens. Science **292:**1109–1112.