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Effects of Amphibian Chytrid Fungus on Individual Survival Probability in Wild Boreal Toads

DAVID S. PILLIOD,* ERIN MUTHS,† RICK D. SCHERER,†‡ PAUL E. BARTELT,§ PAUL STEPHEN CORN,** BLAKE R. HOSSACK,**†† BRAD A. LAMBERT,‡‡ REBECCA MCCAFFERY,†† AND CHRISTOPHER GAUGHAN‡‡

*U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Snake River Field Station, 970 Lusk Street, Boise, Idaho 83706, U.S.A., email dpilliod@usgs.gov

†U.S. Geological Survey, Fort Collins Science Center, Fort Collins, Colorado 80526, U.S.A.

‡Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, Colorado 80523, U.S.A.

§Biology Department, Waldorf College, Forest City, Iowa 50436, U.S.A.

**U.S. Geological Survey, Northern Rocky Mountain Science Center, Aldo Leopold Wilderness Research Institute, Missoula, Montana 59801, U.S.A.

††Wildlife Biology Program, College of Forestry and Conservation, University of Montana, Missoula, Montana 59812, U.S.A.

‡‡AECOM, Fort Collins, Colorado 80524, U.S.A.

Abstract: *Chytridiomycosis is linked to the worldwide decline of amphibians, yet little is known about the demographic effects of the disease. We collected capture–recapture data on three populations of boreal toads (*Bufo boreas [Bufo = Anaxyrus]*) in the Rocky Mountains (U.S.A.). Two of the populations were infected with chytridiomycosis and one was not. We examined the effect of the presence of amphibian chytrid fungus* (Batrachochytrium dendrobatidis *[Bd]; the agent of chytridiomycosis) on survival probability and population growth rate. Toads that were infected with Bd had lower average annual survival probability than uninfected individuals at sites where Bd was detected, which suggests chytridiomycosis may reduce survival by 31–42% in wild boreal toads. Toads that were negative for Bd at infected sites had survival probabilities comparable to toads at the uninfected site. Evidence that environmental covariates (particularly cold temperatures during the breeding season) influenced toad survival was weak. The number of individuals in diseased populations declined by 5–7%/year over the 6 years of the study, whereas the uninfected population had comparatively stable population growth. Our data suggest that the presence of Bd in these toad populations is not causing rapid population declines. Rather, chytridiomycosis appears to be functioning as a low-level, chronic disease whereby some infected individuals survive but the overall population effects are still negative. Our results show that some amphibian populations may be coexisting with Bd and highlight the importance of quantitative assessments of survival in diseased animal populations.*

Keywords: amphibian chytrid fungus, apparent survival, *Batrachochytrium dendrobatidis*, *Bufo boreas*, chytridiomycosis, Cormack-Jolly-Seber, mark–recapture, population decline

Efectos del Hongo Quitridio Anfibio sobre la Probabilidad de Supervivencia Individual en Sapos Boreales Silvestres

Resumen: *La quitridiomicosis está ligada a la declinación mundial de anfibios, sin embargo se conoce poco sobre los efectos demográficos de la enfermedad. Colectamos datos de captura-recaptura de 3 poblaciones de sapos boreales(*Bufo boreas [Bufo = Anaxyrus]*) en las Montañas Rocallosas (E.U.A.). Dos de las poblaciones estaban infectados con quitridomicosis y una no. Examinamos el efecto de la presencia del hongo quitridio anfibio* (Batrachochytrium dendrobatidis *[Bd]; el agente de la quitridiomicosis) sobre la probabilidad de supervivencia y la tasa de crecimiento poblacional. Los sapos infectados con Bd tuvieron una menor*

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probabilidad de supervivencia promedio anual que los individuos no infectados en sitios en lo que se detectó Bd, lo que sugiere que la quitridiomicosis puede reducir la supervivencia en 31–42% en sapos boreales silvestres. Los sapos negativos a Bd en sitios infectados tuvieron probabilidades de supervivencia comparables a las de sapos que fueron negativos a Bd en sitios no infectados. La evidencia de que las covariables ambientales (particularmente las temperaturas frías durante la época de reproducción) influyeron en la supervivencia de sapos fue débil. El número de individuos en poblaciones enfermas declinó en 5–7%/año a lo largo de los 6 años del estudio, mientras que la población no infectada tuvo un crecimiento poblacional comparativamente estable. Nuestros datos sugieren que la presencia de Bd en estas poblaciones de sapos no está causando declinaciones poblacionales rápidas. Más bien, la quitridiomicosis parece estar funcionando como una enfermedad crónica, de bajo nivel, por lo cual algunos individuos infectados sobreviven pero los efectos sobre la población son negativos. Nuestros resultados muestran que algunas poblaciones de anfibios pueden estar coexistiendo con Bd y resaltan la importancia de evaluaciones cuantitativas de la supervivencia de poblaciones con animales enfermos.

Palabras Clave: *batrachochytrium dendrobatidis*, *Bufo boreas*, Cormack-Jolly-Seber, declinación poblacional, hongo quitridio anfibio, marca-recaptura, quitridiomicosis, supervivencia aparente

Introduction

The amphibian chytrid fungus (*Batrachochytrium dendrobatidis* [Bd]) is a widespread pathogen that is hypothesized to be the cause of mass mortality in some amphibian populations (Daszak et al. 2003). Chytridiomycosis results when Bd invades keratinized tissue of an amphibian and causes hyperkeratosis (Longcore et al. 1999; Pessier et al. 1999). Hyperkeratosis disrupts cutaneous function (Voyles et al. 2009) and may compromise the host's immune system (Rosenblum et al. 2008). Laboratory results confirm the pathogenicity of Bd in amphibians (e.g., Longcore et al. 1999), and field studies show chytridiomycosis has played a significant role in some declines of amphibian populations around the world (e.g., Berger et al. 1998; Bosch et al. 2001; Lips et al. 2006).

Despite the apparent lethality of Bd, the etiology of the disease is not completely understood. Information is still needed on factors that influence susceptibility of individuals and populations to chytridiomycosis, variability in pathogenicity of Bd, and the environmental conditions that may influence the host–pathogen dynamic. Innate defenses involved in resistance to chytridiomycosis occur in some amphibians (Woodhams et al. 2007), and sick animals may alter their behavior to raise their body temperature to combat the disease (Richards-Zawacki 2009). Consequently, some populations persist with Bd (Retallick et al. 2004; Briggs et al. 2005; Kriger & Hero 2006), whereas others are extirpated (Lips et al. 2006; Skerratt et al. 2007; Ryan et al. 2008).

Understanding the variability of the responses of populations to Bd requires information on demographic parameters, especially mortality rates of infected individuals in the wild. Despite the abundance of recent research on Bd, differences in demographic parameters, such as survival, between infected and uninfected individuals have been compared in only three studies. Retallick et al. (2004) were the first to examine the effects of Bd on anuran survival. They examined infected and uninfected individuals of *Taudactylus eungellensis* and *Litoria wilcoxii/jungguy* over 4 years and found no evidence that survival differed between infected and uninfected frogs. Kriger and Hero (2006) also found no evidence that Bd infection affects survivorship. In both these studies, researchers used return rate (i.e., proportion of marked individuals captured in subsequent surveys) as the response variable. Return rate tends to be a biased estimator of survival because it assumes the probability of detection is constant over years, which is rarely the case (Mazerolle et al. 2007).

Using multistate, capture–recapture models of adult male *Litoria pearsoniana*, Murray et al. (2009) conducted the first robust study of the probability of surviving chytridiomycosis. They found that uninfected frogs had higher survival than infected frogs. Murray et al. (2009) studied a single population over a relatively short period of time (6 months), but their robust analysis provided much needed information on variation of population susceptibility and responses to chytridiomycosis in the wild. Similar studies conducted over longer periods of time that span multiple populations across the range of a species could greatly improve our understanding of this host–pathogen relationship.

We conducted a 6-year capture–recapture study on three populations of boreal toads (*Bufo boreas* [*Bufo* = *Anaxyrus*]) in the Rocky Mountains (U.S.A.) to examine the factors that influence survival and annual rate of population growth at two sites where Bd was detected (hereafter, infected sites) and one site where Bd was not detected (hereafter, uninfected site). The boreal toad is a good model for studies of chytridiomycosis effects because the species is susceptible to chytridiomycosis (Blaustein et al. 2005; Carey et al. 2006; Garcia et al. 2006), Bd has been found in boreal toad populations throughout the species' range (Pearl et al. 2007; Muths et al. 2008), and populations of boreal toads have declined coincident with the detection of Bd in populations (Muths et al. 2003; Scherer et al. 2005).

Methods

Host Ecology

Boreal toads are the latest breeders among amphibians in the Rocky Mountains, and peak breeding activity for the species usually occurs during the latter stages of snowmelt (Vertucci & Corn 1996). Toads breed at night when temperatures are still cold, sometimes near freezing. Males congregate at the breeding site before females and compete for arriving females. This breeding behavior leads to much contact among individuals and may facilitate disease transmission in some amphibians (Rowley & Alford 2007). Additionally, the motile zoospores of Bd can move from infected to healthy toads through the water (Carey et al. 2006). Probably all females do not breed each year, but each female may lay over 5000 eggs in years she oviposits (Carey et al. 2005). The number of females at breeding sites is much lower than the number of males (e.g., at our sites approximately one female to 25 males). Survival to sexual maturity is low, but this has minimal effect on population growth rate, which is most sensitive to adult survival (Biek et al. 2002; Vonesh & De la Cruz 2002). Boreal toads may live ≥9 years (Carey et al. 2005), and adult survival can be high and stable among years (Scherer et al. 2008). Adult boreal toads are active for 5–6 months of the year and may have body temperatures of ≥30 °C during their active period (Carey 1978; Bartelt & Peterson 2005).

Selection and Characteristics of Sample Sites

We selected three sites along an 11° latitudinal gradient from Montana to Colorado (Fig. 1): a group of six ponds at Lost Trail National Wildlife Refuge, Montana (LT) (1090 m elevation), an oxbow pond near the Blackrock Ranger Station, Bridger-Teton National Forest, Wyoming (BR) (2082 m), and a group of three ponds at Denny Creek, San Isabel National Forest, Colorado (DC) (3360 m). We selected sites on the basis of existing information about toad populations (historical surveys indicated consistent annual reproduction) and logistical considerations. We first detected Bd at LT in 2005 and at BR in 2003. Bd has not been detected at DC. Other species of amphibians occur at these sites, but we did not test them for Bd.

We selected the environmental covariates in our demographic models a priori. We recorded maximum and minimum daily temperatures (SNOTEL 2009) and humidity (RAWS 2009) taken at weather stations that were 2–37 km away from the sites. We summarized weather data separately for the breeding season and active season at each site in each year. We defined the breeding season as 1 week prior to our first observation of a toad at each site (prior to egg laying) plus 28 days after the first observation. To examine the influence of stressors that occur during the breeding season (e.g., cold temperatures) on toad survival, we calculated the average daily minimum air temperature, counted the number of days of killing frost (temperatures ≤−4.4 °C [24 °F]; Natural Resources Conservation Service), and recorded the ordinal day of the last killing frost.

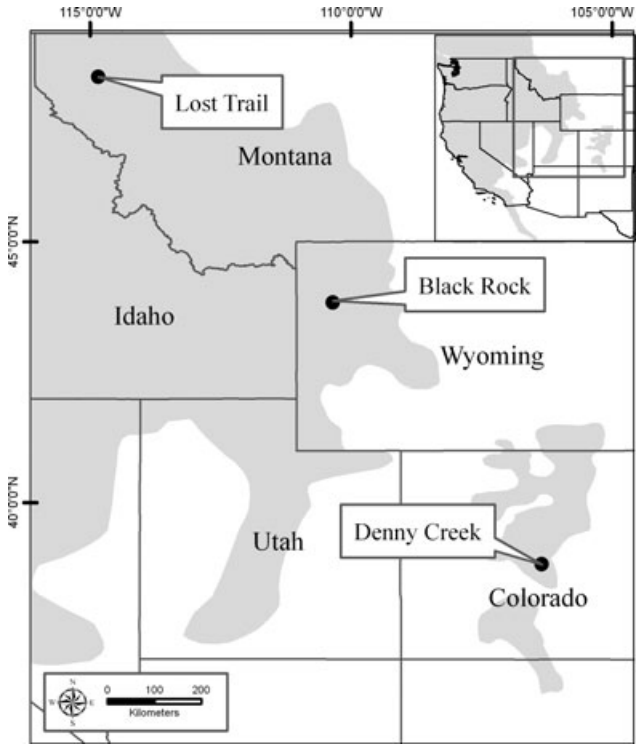

Figure 1. Map of study sites at Lost Trail National Wildlife Refuge, Montana, Blackrock, Wyoming, and Denny Creek, Colorado. Gray area is range of the boreal toad (Bufo boreas [Bufo = Anaxyrus]) *across the Rocky Mountains and western United States (inset) as described by Goebel et al. (2009).*

We defined the active season as the interval between the last day of killing frost in the spring and the first day of killing frost in autumn. Because warm temperatures during the active season may influence toad immune responses and Bd pathogenicity, we included two related covariates: average daily maximum air temperature during the hottest months (July and August) of the active season and a covariate representing the potential for toads to raise their body temperature above ambient air temperatures during the day. We calculated the latter covariate as the number of hours available to a toad during the active season when the animal could raise its body temperature to ≥25 °C by basking. Basking hour estimates were calculated from a combination of known thermal associations of toads (Bartelt 2000) and results from a physiological modeling approach that uses first principles of environmental biophysics. Measures of air temperature, relative humidity, zenith angle of the sun, topographic features, and vegetative structure were used to estimate hourly

body temperatures of toads created by heat transfer and evaporative cooling at each study site over the active season (P.E.B., W.P. Porter, and R.W. Klaver, unpublished data).

Collection and Analyses of Samples

From 2003 through 2008 we sampled each population during the first 2–3 weeks of the breeding season (May at LT and BR, June at DC) in multiple capture sessions. During capture sessions, 1–6 workers used headlamps to search sites and adjacent wetlands and terrestrial areas for toads. Sampling began shortly after dark and continued for at least 30 min after the last toad was observed (2 h 30 min minimum search time per capture session). We captured toads individually with plastic bags. We injected a passive integrated transponder (PIT) tag into the dorsal subcutaneous tissue of newly captured individuals. We recorded, measured, and released all captured animals.

Before we measured or tagged toads, we tested a subset of them for Bd presence by swabbing the ventral skin of the body and hind feet with a sterile swab (Hyatt et al. 2007). We sealed each sample swab in a vial and placed each vial in an individual plastic bag. Sample swabs were then sent to Pisces Molecular (Boulder, Colorado) for analysis with polymerase chain reaction to detect Bd (Annis et al. 2004 as modified by J. Wood, personal communication). This technique reliably detects Bd in individuals over time (Hyatt et al. 2007).

Because we captured many fewer females than males, we used only males in this analysis. To avoid the potential bias of changes in Bd prevalence over time (e.g., seasons; Murray et al. 2009), we tested for Bd for 1–2 weeks near the beginning of the breeding season each year. To avoid potential contamination of the collected tissue and disease transmission among sites, we adhered scrupulously to clean procedures in the field (sensu Muths et al. 2008).

Statistical Analyses

We used MARK (White & Burnham 1999) to analyze the capture–recapture data. Because our focus was on survival probability and the effect of disease on that parameter, we used the Cormack-Jolly-Seber (CJS) model (Lebreton et al. 1992). The CJS model contains two parameters: apparent survival (Φ) and capture probability (p). Apparent survival over interval i (Φ_i) is the probability that a marked individual in the population during the sampling period at time i survives and remains in the population until the sampling period at time $i + 1$. This parameter is referred to as apparent survival, rather than true survival, because permanent emigration cannot be distinguished from death. Although permanent emigration can bias estimates of survival probability, we considered its effect negligible because earlier studies indicate it is near zero in male boreal toads (Muths et al. 2006). Hereafter, we refer to this parameter as survival or Φ.

Whereas Φ and the covariates that affect Φ were the primary parameters of interest, we first evaluated models of p to avoid unnecessary bias and imprecision in survival estimates (Lebreton et al. 1992). We identified the best model of p by pairing the following five models with the global model for Φ (site $\times$ time): (1) p was constant across years and sites (null model), (2) p varied across years only, (3) p varied among sites only, (4) p varied across years and among sites, and (5) p varied across individuals of different disease state.

We hypothesized Φ is lower in individuals that test positive for Bd than in individuals that test negative. We also hypothesized that the effects of environmental covariates on Φ differ between individuals of different disease states. To address these hypotheses, we evaluated 13 models (see Supporting Information). We assigned each captured individual to one of three possible disease states each year: tested and Bd positive, tested and Bd negative, and untested. We assigned a disease state for each marked individual in each year of the study, even if we did not recapture an individual in a given year. We assigned as untested the disease state of toads that were not captured, or were captured but not tested, in a particular year. We allowed each individual's disease state to change between years; thus, disease state was modeled as a covariate that varied over time.

We then assessed five models of how environmental covariates interacted with disease to affect Φ in individuals. We designed each model so that the effect of the environmental covariate on Φ depended on the disease state of the toad (i.e., an interactive effect of the environmental covariate and disease state; see Supporting Information for details of CJS models) and assumed the effect of the environmental covariate would be similar among populations.

Finite Models of Population Growth

We used a reverse-time, capture–recapture model to estimate the annual rate of population growth (Pradel 1996). The annual rate of population growth (λ) can be defined as population size at time $t + 1$ divided by the population size at time t (N_{t+1}/N_t). We modeled the average λ directly with the λ parameterization of the model. In addition to λ, the λ parameterization included the same parameters in the CJS model, p and Φ. Because we had modeled these parameters already in our evaluation of models of survival probability, we used the structure for p and Φ from the best model of that analysis (i.e., the model with the lowest AIC_c value). We evaluated three structures for λ: λ is the same at the sites that were positive for Bd but different from λ at DC; λ is different at all three sites; and λ is the same across sites.

Model Evaluation and Parameter Estimation

Using program RELEASE (Lebreton et al. 1992), we tested the fit of the capture data to the CJS model with the most

parameters [p(site × time), Φ(site × time)]. We used $\Delta QAIC_c$ (delta quasi-Akaike information criterion) values and Akaike weights (w_i) to determine which model(s) had the most support, given the data. The $QAIC_c$ is a modification of AIC that accounts for small sample size and overdispersion (Burnham & Anderson 2002). The w_i quantifies the strength of evidence in support of a particular model, i, and can be interpreted as the probability that the model is the best model of those in the candidate set (Burnham & Anderson 2002). We considered models within two $QAIC_c$ units of the best model competitive models (Burnham & Anderson 2002). In some analyses, no single model was clearly better than other models that were evaluated, and inference from a single model was not robust. To address this issue, we used model averaging to derive estimates of parameters. To calculate these estimates, we extracted the estimate for a particular parameter from each model and computed a weighted average in which each estimate's weight was the w_i of the model from which it came (Burnham & Anderson 2002).

Results

Prevalence and Capture Probability

From 2003 to 2008 we captured 2917 male toads from the three populations and tested 353 of them for Bd (Table 1). We did not detect Bd in the 140 samples taken at DC. The average annual naïve estimate of Bd prevalence was 62% at LT and 53% at BR.

The goodness-of-fit test indicated moderate overdispersion in the data ($\hat{c} = 1.75$). Of the evaluated models of capture probability (p), results of model selection indicated strongly that p varied across years and among sites. Therefore, we used this p structure in all subsequent models that focused on factors influencing Φ. Capture probability in one of the infected sites (BR) (range 0.27–0.53) and the uninfected site (DC) (range 0.36–0.49) were similar. Capture probability at the other infected site (LT) was low (range 0.01–0.26).

Effects of Bd on Survival

Survival varied as a function of disease state of the individual (Table 2). The model-averaged estimate of the regression coefficients for the effect of being untested and the effect of being positive for Bd were both negative and 95% CIs did not include zero ($\hat{\beta}_{\text{Bd-untested}} = -1.02$ [95% CI −1.46 to −0.57] and $\hat{\beta}_{\text{Bd-positive}} = -1.47$ [95% CI −2.57 to −0.36]). Toads that were infected with Bd had lower average annual Φ (0.42, 0.53) than toads that were uninfected (0.73, 0.77) at the infected sites (Fig. 2). Average annual Φ for toads that were negative for Bd at the uninfected site (DC) was comparable (0.76) to Φ of uninfected toads at the infected sites (BR, LT). Untested toads at the infected sites had Φ values intermediate (0.53, 0.61) to toads that were negative and positive for Bd, whereas Φ values for untested toads (0.77) at the uninfected site were indistinguishable from Φ values for toads that were negative at that site (Fig. 2).

Table 1. Number of male toads captured, swabbed, and tested for *Batrachochytrium dendrobatidis* (Bd) and the naïve estimate of Bd prevalence at each site from 2003 to 2008.

Site[a], year	*Number of toads caught*	*Number of toads recaptured*	*Number of toads tested*	*Number of toads positive for Bd*	*Naïve prevalence[b] (%)*
LT					
2003	306	0	0	0	–
2004	7	1	0	0	–
2005	218	21	22	2	9.1
2006	371	41	31	29	93.5
2007	50	19	20	17	85.0
2008	42	10	28	17	60.7
BR					
2003	259	0	28	12	42.9
2004	249	42	41	17	41.5
2005	266	64	6	5	83.3
2006	216	76	28	16	57.1
2007	239	59	4	2	50.0
2008	170	24	22	10	45.5
DC					
2003	145	0	14	0	0.0
2004	80	59	24	0	0.0
2005	71	29	25	0	0.0
2006	88	26	37	0	0.0
2007	86	30	13	0	0.0
2008	55	24	10	0	0.0

[a] *Abbreviations: LT, Lost Trail National Wildlife Refuge, Montana; BR, Blackrock, Wyoming; DC, Denny Creek, Colorado.*
[b] *The number of individuals that tested positive for Bd divided by the total number of individuals tested. Population at LT was first tested in 2005.*

Table 2. Set of models used to estimate the survival probability (Φ) of male boreal toads of different disease states captured at 3 study sites between 2003 and 2008.[a]

Model rank	*Model name*[b]	*QAIC$_c$*	*ΔQAIC$_c$*	w	*Model likelihood*	K	*Qdeviance*
1	Φ (disease state)	2114.78	0.00	0.20	1.00	18	2078.52
2	Φ (LT, BR + disease state)	2115.05	0.27	0.18	0.88	20	2074.73
3	Φ (LT, BR × disease state)	2115.46	0.68	0.14	0.71	20	2075.14
4	Φ (LT/BR + disease state)	2116.45	1.66	0.09	0.44	19	2078.16
5	Φ (disease state × KFdays)	2116.67	1.89	0.08	0.39	21	2074.32
6	Φ (Bd(0) BR, LT, Bd(+); Bd(0) at DC, Bd(−))	2116.68	1.89	0.08	0.39	19	2078.39
7	Φ (disease state × TMINbrd; DC)	2116.79	2.00	0.07	0.37	21	2074.44
8	Φ (LT, BR × Bd(0), Bd(+); Bd(0), DC)	2117.39	2.61	0.05	0.27	21	2075.04
9	Φ (disease state × KFlast; DC)	2117.89	3.11	0.04	0.21	21	2075.54
10	Φ (disease state × TMAXact; DC)	2118.96	4.18	0.02	0.12	21	2076.61
11	Φ (LT, BR × Bd(0), Bd(+); DC)	2119.06	4.27	0.02	0.12	22	2074.67
12	Φ (disease state × BASKhr; DC)	2119.58	4.79	0.02	0.09	21	2077.22
13	Φ (.)	2135.28	20.50	0.00	0.00	16	2103.08

[a]*The same structure on capture probability* (p) *was used for every model:* p(*site × time). See Supporting Information for descriptions of each model.*

[b]*Key: +, additive model; ×, multiplicative model; disease state Bd(+), positive for Bd; Bd(−), negative for Bd; Bd(0), untested, therefore, unknown; LT, Lost Trail National Wildlife Refuge, Montana; BR, Blackrock, Wyoming; DC, Denny Creek, Colorado; KFdays, annual number of days of killing frost during breeding season; TMINbrd, average daily minimum air temperature during breeding season; KFlast, last day of killing frost during breeding season; TMAXact, average daily maximum air temperature during active season; BASKhr, annual number of basking hours during active season; ΔQAIC$_c$, difference in QAIC$_c$ between the current and the best model; QAIC, quasi Akaike information criterion;* w, *model weight;* K, *number of parameters; Qdeviance, -2 log-likelihood/*ĉ.

The best five models showed no evidence that Φ varied between negative and untested individuals at DC, which lends validity to our supposition that the population was uninfected. The highest ranked model (i.e., lowest QAIC$_c$ value) with different survival probabilities between disease states (untested vs negative for Bd) at DC (model rank 6, Table 2) was approximately 1.9 QAIC$_c$ units higher than the best model of those examined (model rank 1, Table 2) and the associated regression coefficient, although positive, was centered approximately on zero ($\hat{\beta}_{\text{Bd-untested at DC}} = 0.17$; 95% CI −0.73 to 1.07).

Two models that included environmental covariates had strong support from the data (ΔQAIC$_c \leq 2$). Estimates of regression coefficients from these models indicated that individuals that tested negative for Bd were influenced differently by the number of killing frost days and average daily minimum temperature during the breeding season than positive and untested individuals. For example, the estimate from the fifth-ranked model that included the number of killing frost days indicated no relationship between Φ and number of killing frost days for individuals that were negative for Bd ($\hat{\beta} = 0.13$; 95% CI −15.72 to 15.98). The estimated regression coefficients, however, suggested a positive relationship between Φ and number of killing frost days for untested individuals ($\hat{\beta} = 5.60$; 95% CI −17.00 to 28.21) and individuals that were positive for Bd ($\hat{\beta} = 3.35$; 95% CI −26.69 to 33.39).

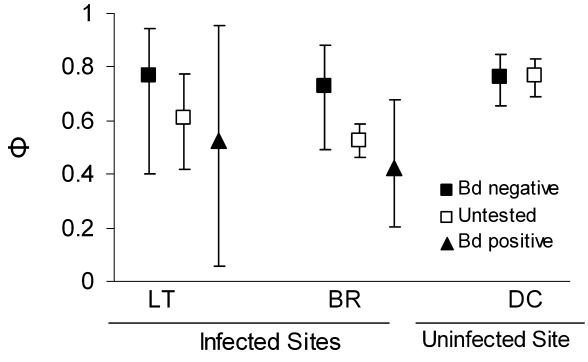

Figure 2. Average annual survival of boreal toads (Φ, 95%CI) at Lost Trail National Wildlife Refuge, Montana (LT), Blackrock, Wyoming (BR), and Denny Creek, Colorado (DC) from 2003 to 2008 relative to the disease state of individuals.

Models of Finite Population Growth

The best model indicated λ was the same for the three sites (Table 3). Due to uncertainty in model selection (ΔQAIC$_c < 2$), however, we derived model-averaged estimates of λ for each population. Over the 6 years of the study, the average population decline at infected sites was 5–7%/year (LT: $\hat{\lambda} = 0.93$, 95% CI 0.49–0.99; BR: $\hat{\lambda} = 0.95$, 95% CI 0.78–0.99), whereas at the uninfected site (DC) the population growth rate was mostly stable ($\hat{\lambda} = 1.00$, 95% CI 0.91–1.09).

Discussion

The pathology of chytridiomycosis in amphibians is complex and variable because host resistance, disease pathogenicity, and environmental conditions vary. Our

Table 3. Results of the analysis of models of finite population growth (λ) of three boreal toad populations from 2003 to 2008.*

Model rank	*Model name*	$QAIC_c$	$\Delta QAIC_c$	w	*Model likelihood*	K	*Qdeviance*
1	λ(.)	6076.48	0	0.42	1.00	22	6032.13
2	λ(LT = BR, DC)	6076.72	0.26	0.37	0.88	23	6030.35
3	λ(LT, BR, DC)	6077.86	1.38	0.21	0.50	24	6029.44

**The reverse-time, capture–recapture models used to estimate the annual rate of population growth followed Pradel (1996). The same structure on capture probability (*p*) and survival probability (Φ) was used for every model:* p*(site × time) and Φ(disease state). Abbreviations: LT, Lost Trail National Wildlife Refuge, Montana; BR, Blackrock, Wyoming; and DC, Denny Creek, Colorado;* $\Delta QAIC_c$*, difference in* $QAIC_c$ *between the current and the best model; QAIC, quasi Akaike information criterion;* w*, model weight;* K*, number of parameters; Qdeviance, −2 log-likelihood/*ĉ.

results show that in the wild Bd reduces survival of infected adult boreal toads, a species that has declined in parts of its range and is considered "near threatened" by the International Union for Conservation of Nature (IUCN 2009). Nevertheless, contrary to patterns of rapid decline observed elsewhere following outbreaks of chytridiomycosis in amphibian populations (Skerratt et al. 2007; Ryan et al. 2008), we found that infected populations were declining relatively slowly even when over 40% of tested individuals in a population were infected with Bd. Our findings contribute to a growing body of evidence that some amphibian species and populations may coexist with Bd or that Bd is not an invariably lethal pathogen for all amphibians (Carey et al. 2006; Rachowicz et al. 2006). Given the widespread distribution of Bd and evidence that Bd has been present in many regions for ≥50 years (Ouellet et al. 2005), amphibian coexistence with Bd may now be the prevailing situation in some regions, particularly North America (Briggs et al. 2005; Longcore et al. 2007; Pearl et al. 2007; Muths et al. 2008; Rothermel et al. 2008; Padgett-Flohr & Hopkins 2009). Although there are few long-term population data on which to base this claim, a 35-year study in South Carolina (U.S.A.) showed multiple amphibian species have persisted at a site where Bd was first detected in 1978 (Daszak et al. 2005).

Understanding the effects of disease on population persistence requires estimates of survival, information that rarely is available for wild host populations (Jolles et al. 2005). We found that infected toads were 31% (LT) and 42% (BR) less likely to survive compared with uninfected toads at the infected sites. Furthermore, survival probabilities for uninfected toads at both infected and uninfected sites were high and similar. These findings are consistent with those of the only other analysis of survival probability of infected anurans in the wild. In a population of *L. pearsoniana* in Australia, monthly survival was consistently 38% lower in infected (Φ = 0.1–0.6) than in uninfected male frogs (Φ = 0.35–0.95; Murray et al. 2009). These findings contrast with two other Australian studies in which there were no statistical differences between return rates of infected and uninfected frogs, even in populations where 32% of tested frogs were infected with Bd (Retallick et al. 2004; Kriger & Hero 2006).

Our study provides additional evidence that some amphibian species and populations around the world are able to persist in the presence of Bd. The annual survival probabilities for infected toads were lower than for uninfected toads, but survival probability was still often >0.50 for those that were infected. This suggests that infected toads are more likely to die than uninfected toads, but the infected toads still have a fairly high probability of surviving to the next year. Two of our toads that tested positive for Bd in 1 year, tested negative in the next year (D.S.P. and E.M., unpublished data), which indicates individuals can rid themselves of the fungus over time. Clearing Bd infection has been reported in amphibians in Australia (Kriger & Hero 2006; Murray et al. 2009) and the United States (Corn 2007).

The influence of the environment on the dynamics of chytridiomycosis has not been well studied in the field because of the complexity of natural environments (e.g., microhabitat conditions), microhabitat selection by hosts, and the physiological responses of host and pathogen to the environment. Our results suggested that the direct effect of Bd on survival of adult boreal toads was larger than the indirect effect of the environmental covariates (i.e., temperature) on disease and toad survival. Results of model selection from among the models that included environmental covariates suggested that colder temperatures during the breeding season (i.e., more killing frost days) may have created less favorable conditions for Bd and thus increased survival of infected toads. These findings are consistent with Piotrowski et al. (2004), who suggest that infections at temperatures below 10 °C may not be fatal because growth of Bd is not favored. Nevertheless, the CIs around the regression coefficients for the number of killing frost days were approximately centered on zero; therefore, the potential for cold temperatures to suppress Bd activity needs further exploration.

Over the 6 years of our study, infected populations declined by about 5–7%/year, whereas the uninfected population remained relatively stable. Assuming mortality related to Bd is additive relative to other sources of mortality, it is possible that chytridiomycosis is removing some individuals from the population each year, but not causing mass mortality. This suggests that chytridiomycos can function as an enzootic disease in which host

and pathogen coexist. Although a 5–7% annual decline could lead to extinction within a few years, models of amphibian extinction risk suggest that population persistence is possible if some infected individuals survive (Briggs et al. 2005), which appears to be the case in our infected populations. Rates of population decline, however, may also be influenced by population size; smaller populations may be affected disproportionately by demographic stochasticity or loss of alleles that afford disease resistance.

A complete understanding of this host–pathogen dynamic at the sites we examined depends on information on first arrival of Bd at infected sites and the time that elapsed to first infection. We have, however, only a general understanding of the history of Bd in the region. From museum records, it appears Bd first appeared in the western United States in the 1960s (Ouellet et al. 2005; Padgett-Flohr & Hopkins 2009). It is now widespread (Pearl et al. 2007; Muths et al. 2008; Padgett-Flohr & Hopkins 2009), and sufficient time has passed for some populations to have developed resistance. Therefore, we speculate our toad populations have experienced one of the following scenarios: (1) Bd arrived years or decades prior to initial surveys of these populations as an epizootic pathogen, and its presence resulted in low survivorship and a declining population. Initial population declines were followed by recovery of the population by resistant individuals, but the disease continues to reduce survival of some infected toads; (2) Bd arrived (possibly even recently) as a novel pathogen and has had low-level effects on survival since its arrival; (3) Bd arrived (possibly even recently) as a novel pathogen that caused no mortality until its interaction with other stressors associated with changing climatic conditions (e.g., warmer or drier winters, earlier springs).

The future of these populations is uncertain. Our data indicate chytridiomycosis is decreasing survival of adult male toads and causing slow population declines. But given the short duration of our study relative to the long lifespan of boreal toads, we are not yet convinced that these infected populations are threatened with extirpation.

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Supporting Information

Details of the hypotheses and descriptions of the Cormack-Jolly-Seber models used to evaluate the effect of disease state and environmental variables on survival are available as part of the online article (Appendix S1). The authors are responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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