Thermal Physiology, Disease, and Amphibian Declines on the Eastern Slopes of the Andes

ALESSANDRO CATENAZZI,* EDGAR LEHR,† AND VANCE T. VREDENBURG‡

*Department of Zoology, Southern Illinois University, Carbondale, IL 62901, U.S.A., email acatenazzi@siu.edu.
†Department of Biology, Illinois Wesleyan University, 303 East Emerson, Bloomington, IL 61701, U.S.A.
‡Department of Biology, San Francisco State University, San Francisco, CA 94132, U.S.A.

Abstract: *Rising temperatures, a widespread consequence of climate change, have been implicated in enigmatic amphibian declines from habitats with little apparent human impact. The pathogenic fungus Batrachochytrium dendrobatidis (Bd), now widespread in Neotropical mountains, may act in synergy with climate change causing collapse in thermally stressed hosts. We measured the thermal tolerance of frogs along a wide elevational gradient in the Tropical Andes, where frog populations have collapsed. We used the difference between critical thermal maximum and the temperature a frog experiences in nature as a measure of tolerance to high temperatures. Temperature tolerance increased as elevation increased, suggesting that frogs at higher elevations may be less sensitive to rising temperatures. We tested the alternative pathogen optimal growth hypothesis that prevalence of the pathogen should decrease as temperatures fall outside the optimal range of pathogen growth. Our infection-prevalence data supported the pathogen optimal growth hypothesis because we found that prevalence of Bd increased when host temperatures matched its optimal growth range. These findings suggest that rising temperatures may not be the driver of amphibian declines in the eastern slopes of the Andes. Zoonotic outbreaks of Bd are the most parsimonious hypothesis to explain the collapse of montane amphibian faunas; but our results also reveal that lowland tropical amphibians, despite being shielded from Bd by higher temperatures, are vulnerable to climate-warming stress.*

Keywords: critical thermal maximum, elevational gradient, extinction, frogs, montane forest, physiological ecology, threatened species, tropical Andes

Fisiología Termal, Enfermedades y Disminuciones de Anfibios en las Laderas Orientales de los Andes

Resumen: *El incremento en la temperatura, una de las principales consecuencias del cambio climático, se ha implicado en las disminuciones enigmáticas de anfibios en hábitats con poco impacto humano aparente. El hongo patógeno Batrachochytrium dendrobatidis (Bd), actualmente ampliamente distribuido en las montañas neotropicales, puede actuar en conjunto con el cambio climático, causando un colapso en los hospederos termalmente estresados. Medimos la tolerancia termal de las ranas a lo largo de un amplio gradiente de elevación en la zona tropical de los Andes, donde las poblaciones de ranas han colapsado. Usamos la diferencia entre la temperatura crítica máxima y la temperatura que una rana experimenta en la naturaleza como medida de tolerancia a altas temperaturas. La tolerancia a la temperatura incrementó junto con la elevación, sugiriendo que las ranas de elevaciones más altas son menos sensibles al incremento en la temperatura. Probamos la hipótesis alternativa del crecimiento óptimo del patógeno según la cual la prevalencia del patógeno disminuye conforme las temperaturas caen fuera del rango óptimo para su crecimiento. Nuestros datos de prevalencia de infección apoyaron la hipótesis de crecimiento óptimo del patógeno ya que encontramos que la prevalencia de Bd incrementó cuando la temperatura de los hospederos equivalía a su rango de crecimiento óptimo. Estos hallazgos sugieren que el incremento en la temperatura no causa la disminución de anfibios en las laderas orientales de los Andes. Brotes zoonóticos de Bd son la hipótesis más parsimoniosa para explicar el colapso de la batracofauna montañosa; pero nuestros resultados también revelan que los anfibios tropicales de tierras bajas, a pesar de estar protegidos del Bd por temperaturas más altas, son vulnerables al estrés por cambio climático.*

Paper submitted April 29, 2013; revised manuscript accepted July 12, 2013.

Conservation Biology, Volume 00, No. 0, 1–9

DOI: 10.1111/cobi.12194

Palabras Clave: bosque de montaña, ecología fisiológica, especies amenazadas, extinción, gradiente de elevación, máximo termal crítico, ranas, zona tropical de los Andes

Introduction

Amphibians are suffering global extinction at a rate unprecedented in known history (Wake & Vredenburg 2008). About half of the observed declines, which include dramatic decreases in population abundance or the disappearance of 207 species, are enigmatic: they occur in remote habitats with little apparent human influence (Stuart et al. 2004). Neotropical montane amphibians are among the worst affected by enigmatic declines (Lips 1998; La Marca et al. 2005) and have been scrutinized to discover the causes (Lips et al. 2008; Rohr & Raffel 2010). Two hypotheses have been advanced to explain the enigmatic declines (Rachowicz et al. 2005): the novel pathogen hypothesis proposes that the fungus *Batrachochytrium dendrobatidis* (Bd) is a new, highly virulent pathogen that is spreading worldwide and killing amphibians (Berger et al. 1998), whereas the climate-linked epidemic hypothesis proposes that declines might be caused by epizootics triggered by changes in sea surface and air temperatures related to large-scale climate warming (Pounds et al. 2006). Global warming is causing profound ecological changes (Walther et al. 2002), such as shifts in geographic (Parmesan 1996) and elevational distribution (Colwell et al. 2008). In the tropical Andes, climate warming of 0.32–0.34 $^{\circ}$C decade^{-1} between 1975 and 2000 (Vuille & Bradley 2000) is accelerating deglaciation (Vuille et al. 2008). Models based on organismal physiology predict that tropical organisms will be severely affected by climate warming (Deutsch et al. 2008; Dillon et al. 2010).

Rohr and Raffel (2010) reevaluated the link between climate and amphibian declines and propose that increased temperature variability, extreme climatic events, and the strength of Central Pacific El Niño episodes may exacerbate amphibian declines caused by disease. This hypothesis assumes that increased temperature variability driven by climate warming can depress immunity in amphibians, making them more susceptible to Bd or other infections (Raffel et al. 2006). Among vertebrates, the relationship between environmental conditions and host immunity is expected to be especially important in ectotherms (Garcia-Solache & Casadevall 2010; Fisher et al. 2012). Alternatively, shifting temperatures may move pathogens out of their environmental optima (Rowley & Alford 2013) or influence host response by mediating the expression of genotypic variation for susceptibility (Blanford et al. 2003).

Despite the attention given to the disappearance of Neotropical montane amphibians, little is known about the response of high-elevation species to increases in temperature (Catenazzi 2011; Forero-Medina et al. 2011). In contrast to other terrestrial ectotherms, the moist skin necessary for respiration presumably limits an amphibian's ability to reach and maintain high body temperatures (Tracy & Turner 1992). Previous researchers emphasized distinguishing traits in the thermal physiology of high-elevation amphibians, such as frequent thermoconformity, absence of metabolic acclimation (Navas 1996*b*), lack of conspicuous thermoregulatory behavior (Navas 1997), and avoidance of high body temperatures through thigmothermy (Navas 1996*a*).

We tested 2 hypotheses to explain the recent collapse of frog species richness along the eastern slopes of the Andes in southern Peru (Catenazzi et al. 2011). The host thermal sensitivity hypothesis is that sensitivity to temperature increase drives declines. To better understand the potential effects of temperature increase in frogs, we calculated sensitivity to temperature increase by subtracting the critical thermal maximum (CT_{max}) of individual frogs from the average daily maximum temperature they encounter in their environment (Fig. 1a). A similar metric, warming tolerance (WT) (Deutsch et al. 2008), is the difference between CT_{max} and the mean annual air temperature (T_a). WT should indicate the vulnerability of a species to anticipated climate warming because climate warming will increase the frequency of periods when ambient temperatures exceed the optimal temperature of frogs (Deutsch et al. 2008). We evaluated variation in WTs in frogs along an elevational gradient and compared this variation with the distribution of threatened and declining species.

We also tested the pathogen optimal growth hypothesis that prevalence of Bd is a function of how close the thermal conditions experienced by frogs match the range for optimal growth of Bd in culture (Piotrowski et al. 2004). We found previously that prevalence of Bd is positively correlated with the proportion of frogs that have disappeared along a wide elevational gradient (Catenazzi et al. 2011). We related infection prevalence to the average difference between the temperature of frog microhabitats and the closest limit of the thermal range for Bd growth. Because no data are available on the temperature dependence of montane frog resistance to Bd infection, we assumed that the temperature dependence of Bd infection is directly related to the temperature-dependent growth of Bd in culture. Our measurements of CT_{max} coupled with surveys of frog populations and Bd infection allowed us to simultaneously test hypotheses of a link between climate warming and population declines in a place that is currently experiencing faunal collapse.

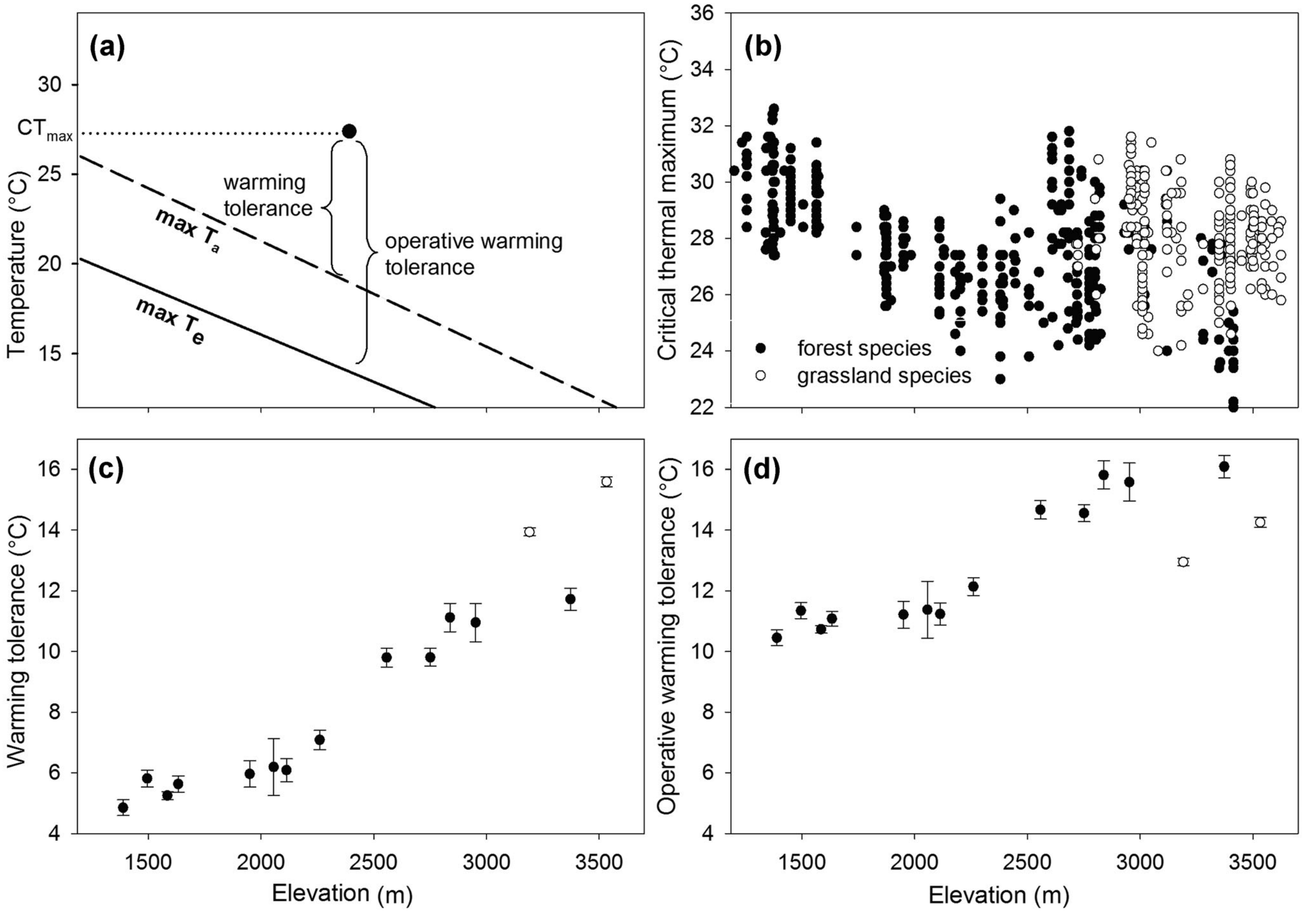

Figure 1. (a) Warming tolerance and operative warming tolerance as a function of average maximum temperature along the elevational gradient in the upper Kosñipata valley (Manu National Park, Peru) (T_a, maximum air temperatures; T_e, maximum operative temperatures; CT_{max}, critical thermal maxima). (b) Critical thermal maxima for 383 forest (solid) and 254 grassland (open) strabomantid frogs ($n = 637$ individuals of 15 species) along the elevational gradient. Average (SE) (c) warming tolerance and (d) operative warming tolerance of 15 species of strabomantid frogs.

Methods

Study Organisms

We sampled frogs of 15 species along the mountain slopes of Manu National Park, southern Peru (Table 1). Frogs were captured from the cloud forest at 1200 m to the high Andean grassland at 3800 m. We reduced phylogenetic effects by examining only species in the family Strabomantidae. These frogs are small, have low vagility, and small geographic distributions on mountaintops (Duellman & Lehr 2009). Stream populations of strabomantid and closely related montane frogs have declined at our site (Catenazzi et al. 2011) and throughout Central America (Ryan et al. 2008).

These frogs inhabit permanently wet microhabitats and likely do not experience evaporative cooling. We recorded T_a and substrate temperatures in microhabitats used by frogs. Although T_a could be a poor surrogate of variation in frogs' body temperatures (Tracy 1976; Huey 1991; Tracy et al. 2007), previous studies in the Colombian Andes have shown that T_a is the best predictor for body temperature in active frogs (Navas 1996*a*), whereas substrate temperature is the best predictor for inactive frogs (Navas 1996*a*). Avenues of heat exchange are assumed to be dominated by heat gains or losses between frogs and the substrate they occupy. Thus, we used substrate temperatures to approximate operative temperatures (T_e) (Navas & Araujo 2000). Operative temperatures represent the predicted equilibrium temperature of nonregulating frogs (Hertz et al. 1993).

Study Site and Regional Climate

We worked in the Kosñipata Valley on the eastern slopes of the Andes, within the Tambopata-Manu wet spot (Killeen et al. 2007). Southern Peru has one of the strongest seasonal variabilities of rainfall throughout the Amazon basin (Villar et al. 2009). At larger timescales,

Table 1. Mean critical thermal maxima ($\mathrm{CT}_{\max}$) (SE) and linear regression between elevation and $\mathrm{CT}_{\max}$ in strabomantid frogs (only species with $n > 10$).

Species	*CT_{max} (SE)*	*df*	*Slope*∗	*R^2*	*p*	*Elevation range (m)^a*
Bryophryne cophites	27.8 (0.2)	36	−0.003	0.01	0.276	3280–3625
Bryophryne hanssaueri	24.9 (0.4)	22	−0.023	0.34	0.002	3280–3414
Bryophryne nubilosus	27.4 (0.4)	19	0.002	0.01	0.343	2610–3120
Noblella pygmaea	26.2 (0.5)	8	0.001	0.01	0.806	2723–3119
Oreobates gemcare	26.7 (0.3)	31	−0.002	0.10	0.03	1422–2950
Pristimantis danae	29.0 (0.1)	83	−0.003	0.40	<0.001	1255–2110
Pr. pharangobates	26.8 (0.2)	60	−0.0001	0.01	0.848	1865–2950
Pristimantis salaputium	29.1 (0.3)	18	−0.004	0.62	<0.001	1255–2300
Pr. toftae	29.8 (0.2)	27	−0.005	0.05	0.138	1255–1742
Psychrophrynella sp. “P”	27.8 (0.3)	65	0.007	0.17	<0.001	2300–2740
Ps. usurpator	28.2 (0.1)	211	−0.001	0.03	0.01	2716–3555
All forest species	28.0 (0.1)	383	−0.001	0.19	<0.001	1200–3414
All grassland species	28.1 (0.1)	254	−0.001	0.03	0.002	2716–3625
All species	28.1 (0.1)	637	−0.0007	0.06	<0.001	1200–3625

∗*Elevations at which experimental animals were captured in the field.*

variability in rainfall is similarly strong or moderate: interannual rainfall variability during the rainy season is related to El Nino-Southern Oscillation (ENSO) and to the sea surface temperature gradient over the tropical Atlantic, whereas interdecadal variability is related to long-term changes in the Pacific Ocean (Villar et al. 2009). During ENSOs, rainfall is below normal (Vuille et al. 2000; Garreaud & Aceituno 2001) and glacier meltdown accelerates (Francou et al. 2003). The opposite pattern occurs in other areas of the Amazon basin: annual rainfall has decreased since the late 1970s in the northern Amazon, but it has increased in the south (Marengo 2004).

Ambient and Operative Temperatures

We inferred maximum air temperatures (T_a) by regressing the corresponding daily averages on elevation between 2001 and 2006 from 4 weather stations operated by the Servicio Nacional de Metereología e Hidrología del Perú from 520 to 3485 m. Correlation coefficients were high ($R^2 = 0.99$, $p = 0.003$), and regression slopes ranged from −0.0059 °C/m for maximum to −0.0050 °C/m for minimum temperatures. Our inferred lapse rates are supported by extensive measurements conducted along the same elevational gradient (Rapp & Silman 2012), which indicate lapse rates from −0.0048 to −0.0063 °C/m.

We recorded operative temperatures (T_e) with 21 iButtons (Maxim Integrated Products, Sunnyvale, California, U.S.A.) every 3 h in microhabitats used by frogs at 5 forest sites from 1525 to 3500 m in the 2 warmest months (January and February 2008), when frogs are most likely to experience temperatures close to their $\mathrm{CT}_{\max}$. We modeled average maximum daily T_e as a function of elevation ($n = 5$ stations). We used a linear function (slope −0.0054 °C/m; $R^2 = 0.99$, $p < 0.001$) to infer T_e at any elevation. For grassland species, we relied on data collected from January to February 2010 at 2800, 2950, and 3450 m (slope −0.0049 °C/m, $R^2 = 1.00$, $p < 0.001$; $n = 3$ stations with 4 iButtons each) because these frogs experience different thermal regimes than forest frogs.

Critical Thermal Maxima and WT

We used the loss of righting response (Navas et al. 2007) to measure $\mathrm{CT}_{\max}$ (Lowe & Vance 1955) in 637 frogs (Table 1). Frogs were acclimated to 16–18 °C for 3–4 d before trials. Frogs were placed in plastic cups with a thin layer of water, and cups were immersed in a water bath. The temperature of the water bath was increased from approximately 18 to 35 °C at a rate of 0.6–0.8 °C/min. Frogs were pushed onto their dorsum first, and then as they righted were forced back on their dorsum. Animals were stimulated to righten by pressing the metal probe of a quick-reading thermometer (accuracy: 0.2 °C) against their flanks. Whenever animals were unable to righten after 5 s, we measured temperature with the probe of the thermometer pressed against the body and immersed in the thin layer of water. We assumed that this temperature was equivalent to the frogs' core temperature (Navas et al. 2007). This assumption is based on the small size of the frogs (range 9.7–44.3 mm), which facilitates heat transfer between the water and the frog's body.

We used 2 approaches to explore the relationship between $\mathrm{CT}_{\max}$ and elevation. First, we averaged $\mathrm{CT}_{\max}$ across individuals of each species and calculated the corresponding midpoint of elevational range. The midpoint of elevational range was defined as the weighted average of elevations at which each frog used in the experiment had been found. In the second approach, we used a randomization procedure to sample 1 $\mathrm{CT}_{\max}$ and its corresponding elevation for each species from the entire data set. We then modeled $\mathrm{CT}_{\max}$ as a function of elevation 1000 times. We subtracted $\mathrm{CT}_{\max}$ from the average maximum T_a to calculate WT, and we subtracted $\mathrm{CT}_{\max}$ from the average maximum T_e to calculate operative WTs (OWTs). We analyzed variation in WT and OWT along elevation with the same statistics used for CT_{max}.

Optimal Growth and Detection of Bd

We used the metric d_e (Hertz et al. 1993) to quantify the suitability of microhabitats occupied by frogs for growth of Bd. This metric is defined as the minimum absolute difference between T_e and the lower (17 °C) or upper (25 °C) limit of optimal growth for Bd in the laboratory (Piotrowski et al. 2004). We used T_e described above, recorded every 3 h, from 4 August 2007 to 7 February 2008, for a total of 1500 measurements for each of 21 data loggers. We modeled d_e as a function of elevation ($d_e = 0.0049 \cdot \text{elevation} - 6.9467$, $R^2 = 0.98, p < 0.001$), and we used this model to calculate d_e for all elevation data associated with frog infection status. We modeled prevalence of Bd as a function of average d_e for 100 m elevational classes.

We collected skin swabs (Hyatt et al. 2007) for 425 frogs in February and March 2008 in order to determine the elevational distribution of Bd. We stroked cotton swabs across the skin of frogs: 5 strokes on each side of the abdominal midline, 5 strokes on the inner thighs, and 5 strokes on the foot webbing of each hind leg. We analyzed swabs with a real-time PCR assay to quantify Bd infection (Boyle et al. 2004). The assay uses genetic markers specific for Bd and compares each sample to a set of standards to calculate a genomic equivalent (Z_{swab}). DNA extracts were analyzed once (Kriger et al. 2006). Swabs were categorized as Bd-positive when $Z_{swab} > 0$ and as Bd-negative when $Z_{swab} = 0$. We calculated prevalence of Bd by dividing the number of infected frogs by the total number of swabbed frogs. We used generalized linear models of logistic regression with binomial errors to relate prevalence with thermal suitability for growth of Bd (d_e) to test the pathogen optimal growth hypothesis. For analyses of infection intensity, we used generalized linear models with log-transformed Z_{swab}.

Results

Critical thermal maxima (CT_{max}) in 15 species of strabomantid frogs ranged from 22.0 to 32.6 °C ($n = 637$; Fig. 1b; Table 1). Considering species as independent data points and averaging across individuals within species (and weighting for elevation), CT_{max} decreased with elevation ($CT_{max} = -0.001 \cdot \text{elevation} + 30.692$; $F_{1,13} = 9.9$, $R^2 = 0.43$, $p < 0.01$), but not as steeply as maximum operative temperature ($T_e = -0.004 \cdot \text{elevation} + 23.636$; $F_{1,13} = 34.79$, $R^2 = 0.73$, $p < 0.001$); estimated difference between slopes was 0.002 (full model ANCOVA, $F_{3,26} = 231.6$, $R^2 = 0.96$, $p < 0.001$). The relationship between CT_{max} and elevation was stronger and steeper when forest species alone were considered in the analysis (i.e., after omitting 2 grassland species: $CT_{max} = -0.002 \cdot \text{elevation} + 32.125$, $F_{1,11} = 32.98$, $R^2 = 0.75$, $p < 0.001$). Consequently, OWT increased with elevation when considering all species (Fig. 1c; $\text{OWT} = 0.002 \cdot \text{elevation} + 7.057$; $F_{1,13} = 33.77$, $R^2 = 0.72$, $p < 0.001$) and when considering forest species alone ($\text{OWT} = 0.003 \cdot \text{elevation} + 5.525$; $F_{1,11} = 87.00$, $R^2 = 0.88$, $p < 0.001$). The randomization procedure confirmed these findings for all species ($p < 0.05$ for 980 regressions; $\text{OWT} = 0.002 \cdot \text{elevation} + 7.005$) and for forest species alone ($p < 0.05$ for 994 regressions; $\text{OWT} = 0.003 \cdot \text{elevation} + 5.608$).

The decrease of average maximum air temperature as elevation increased ($T_a = -0.006 \cdot \text{elevation} + 32.974$; $F_{1,2} = 204.3$, $R^2 = 0.99, p < 0.01$) was much steeper than for CT_{max}; estimated difference was 0.005 (full model ANCOVA, $F_{3,26} = 484.6$, $R^2 = 0.98$, $p < 0.001$). WT calculated from air temperatures increased with elevation (Fig. 1d; for all species: $\text{WT} = 0.005 \cdot \text{elevation} - 2.373$, $F_{1,13} = 134.1$, $R^2 = 0.91$, $p < 0.001$; for forest species alone: $\text{WT} = 0.004 \cdot \text{elevation} - 0.940$, $F_{1,11} = 123.4$, $R^2 = 0.92$, $p < 0.001$). The randomization procedure confirmed the robustness of our results for all species ($p < 0.001$ for 1000 regressions; $\text{WT} = 0.004 \cdot \text{elevation} - 2.091$) and for forest species only ($p = 0.021$ for 1000 regressions; $\text{WT} = 0.004 \cdot \text{elevation} - 0.893$). OWTs were higher than WTs across all individuals (t test for paired samples, $t = -22.15$, df = 632, $p < 0.001$) and species (Wilcoxon Signed-rank test, $W = -114$, $n = 15$, $p = 0.013$).

Overall prevalence of Bd among frogs used for our measurements of CT_{max} was 6.31% (95% confidence interval 4.37–9.02; $n = 425$). In 7 species, no frog was infected, whereas in the other 8 species, prevalence varied between 1.85% and 20.0% (Supporting Information). Thermal physiology of the fungus explained prevalence and infection intensity along the elevational gradient (Fig. 2). Prevalence of Bd was higher in frog microhabitats with temperatures that deviated little from optimal conditions for the growth of Bd (i.e., low d_e) (logistic regression with binomial errors, $p < 0.001$). Moreover, 17 out of 20 frogs with the highest infection intensities were living in microhabitats that average $d_e < 2$. Infection intensity was higher in frog microhabitats with low d_e ($F_{1,22} = 29.2$, $R^2 = 0.55; p < 0.001$).

Discussion

Our study combines for the first time data on amphibian critical thermal maxima and patterns of population declines in a tropical mountain. Montane frogs were less sensitive to temperature increase than those at low elevations, a result supported by analysis of both WTs and OWTs. This finding does not support the idea that climate

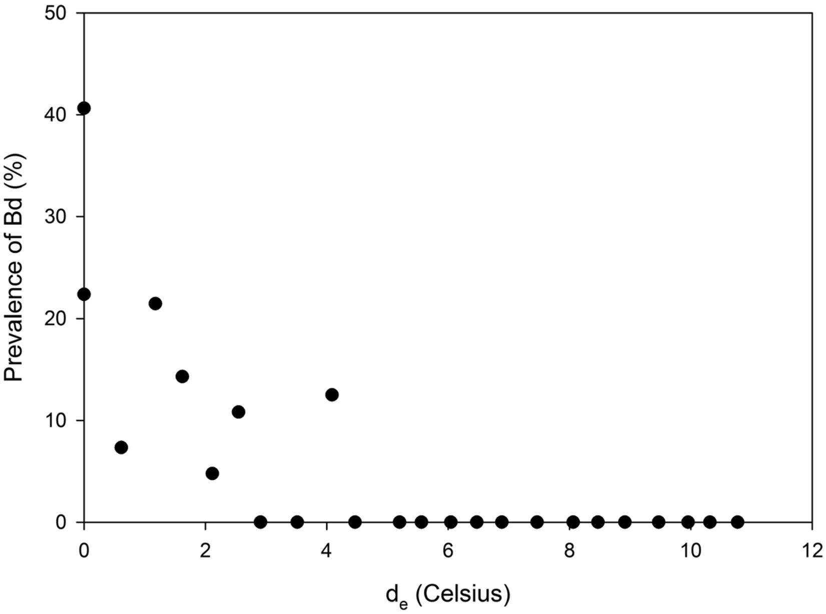

Figure 2. Prevalence of infection by Bd relative to d_e, the difference between temperatures in microhabitats used by frogs and the range of temperatures at which Bd grows best.

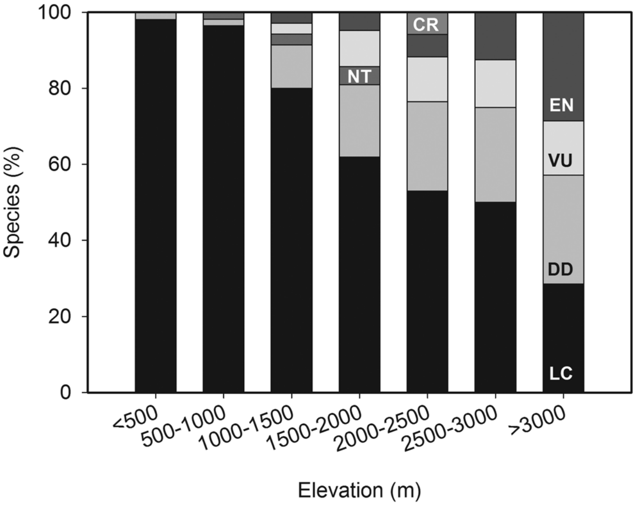

Figure 3. Proportion of amphibians considered threatened in Manu National Park, Peru (IUCN 2013) (Supporting Information) (LC, least concern; DD, data deficient; NT, near threatened; VU, vulnerable; EN, endangered; CR, critically endangered).

warming is causing declines because most threatened frogs in the tropics (IUCN 2013) and in Peru (von May et al. 2008) occur at high elevations (Fig. 3 & Supporting Information). Whereas montane amphibians have disappeared in the Andes over the past 3 decades (Bustamante et al. 2005; Catenazzi et al. 2011), lowland Amazonian amphibians, which live at ambient temperatures that are very close to their CT_{max}, have not declined (von May et al. 2009; Deichmann et al. 2010). Therefore, there is disagreement between our gradient-wide trend of decreased sensitivity to warming as elevation increases and patterns of observed declines centered around midslope and mountaintop regions.

Differences in the magnitude of temperature increase at each elevation could affect our conclusion that montane amphibians are less sensitive to warming. Although temperature variability in the tropical Andes is expected to be largest at high elevations (Urrutia & Vuille 2009), the differences in warming tolerances between species at the 2 extremes of the elevational gradient are much larger than the predicted increases in air temperatures. Variation in temperature increases will account for only a small part of the reductions in warming tolerances caused by climate warming. Furthermore, the observed warming trend decreased above 3500 m (Vuille et al. 2003), and locations above 4000 m have been cooling since 1985 (Ohmura 2012). Similarly, cloud frequency at our study site has decreased only in the lowlands, where a trend toward longer and more intense dry seasons is statistically significant (Halladay et al. 2012). Therefore, montane frogs have not been exposed to changes in climate as large as frogs in the lowlands (Table 2).

Differences in habitat structure and the frog's thermal physiology also support the idea that montane species will be less sensitive to temperature increases. Frogs above 3000 m live in a more heterogeneous thermal landscape (Supporting Information) and have access to forest and grassland habitats with different amounts of shade. These frogs will have greater opportunities for thermoregulation than forest species at lower elevations (Huey et al. 2009, 2012; Kearney et al. 2009). Moreover, the thermal breadth of metabolic activities is larger in Andean frogs than in closely related Amazonian lowland frogs (Navas 1996*c*, 1997). This difference in thermal biology reinforces our point that lowland frogs are more vulnerable to warming than montane frogs.

Table 2. Summary of climatic, physiological, and epidemiological data relevant to amphibian declines in southern Peru.

Data relevant to amphibian declines	*Lowland forest (<600 m)*	*Montane forest (forest, 600–3300 m)*	*Grassland (3300–3800 m)*	*Source*
Loss of frog species richness[a]	none	severe (0–57%)	moderate to severe (0–33%)	Duellman 1995; von May et al. 2009; Catenazzi et al. 2011
Sensitivity to temperature increase[b]	high (< 2 °C)	moderate (7.4 °C)	low (13.7 °C)	this study
Thermal performance breadth[c]	narrow	not available	broad	Navas 1996*c*, 1997
Decrease in cloud frequency (1983–2008)	significant	non significant	non significant	Halladay et al. 2012
Increase in dry season length (1983–2008)	significant	non significant	non significant	Halladay et al. 2012
Prevalence of chytrid fungus (Bd)	low (0–0.1%)	very high (0–90%)	high (0–48%)	Catenazzi et al. 2011; Kosch et al. 2012
Deviation from optimal Bd thermal niche[d]	moderate (6 °C)	low (4 °C)	large (10 °C)	this study

[a] *Ranges in parentheses refer to proportion of missing species in 100 m elevational classes (Catenazzi et al. 2011).*
[b] *Values in parentheses are averages.*
[c] *Data from closely related taxa in the Andes and Amazon regions of Colombia.*
[d] *Assuming optimal growth at 17–25 °C (Piotrowski et al. 2004).*

Climatic variability could have affected frogs at our study site, but there was little variability in temperature or rainfall recorded during the period of biodiversity collapse (Appendix S1 in Catenazzi et al. 2011). Moreover, organisms in the southwestern Amazon might be better able to alternate between dry and wet conditions than elsewhere in the basin (Duellman 1995) because the regional climate includes distinct rainy and dry periods that are more intense in montane areas in the eastern Andes such as our study site (Villar et al. 2009). Much of the basin-wide interannual rainfall variability associated with ENSO occurred during the 1970s, in 1983–1986, 1989, 1992–1995, and 1998 (Villar et al. 2009). Recent variability in precipitation has not been large at high elevations in southern Peru (Salzmann et al. 2013). If rainfall variability played a role in amphibian declines, one would expect higher species extirpation rates during these years. However, collections at our study sites in 1972 (specimens at Kansas University), 1991 (National Museum of Natural History), and 1999 (Catenazzi et al. 2011) include species that disappeared only between 2000 and 2007.

Our results support the pathogen optimal growth hypothesis because we found that prevalence of Bd increased when host temperatures matched its optimal growth range. Our data agree with findings from other montane regions (Bosch et al. 2007; Walker et al. 2010), as well as with recent findings that in 3 species of rainforest frogs, individuals' probability of infection by Bd declined rapidly as they spent more time above the pathogen's upper optimum temperature (Rowley & Alford 2013). Because Bd prevalence is correlated with the proportion of missing species at our study site (Catenazzi et al. 2011), a causal link between outbreaks of Bd and population declines could explain the collapse of frog species richness on the eastern slopes of the Andes.

Our results have important implications for amphibian conservation. We found that a simple estimate of frog sensitivity to climate warming yields predictions that are not consistent with patterns of observed declines. Montane frogs, which have declined the most, are less sensitive to warming than lowland species. Declines at our sites mirror those observed at other Neotropical montane sites where Bd epizootics are associated with loss of amphibian biodiversity (Lips et al. 2006; Crawford et al. 2010; Cheng et al. 2011). Therefore, conservation efforts for montane species should be directed toward strategies to mitigate the impact of chytridiomycosis (Woodhams et al. 2011). Our study suggests that the prospects for the lowland Amazonian species are not much better. Although we found that amphibians at low elevations may have been shielded from this fungal pathogen by higher temperatures, they will be vulnerable to predicted warming trends in the future. Many of these lowland species will be restricted in their ability to colonize higher elevations either by their distance from the Andean foothills or by the lack of suitable breeding habitats on montane slopes (Catenazzi 2011). Therefore, future studies should assess the ability of lowland species to move upslope and test the effectiveness of assisted migration and creation of breeding habitats as strategies to conserve Amazonian species.

Acknowledgments

We thank J.C. Jahuanchi and A. Machaca for field assistance and T. Cheng and N. Reeder for laboratory assistance. We thank R.B. Huey and M.E. Power for comments on earlier versions of the manuscript. We thank Manu NP for research permits. Funding was provided by the Amazon Conservation Association, the Rufford Small Grants Foundation, and National Science Foundation Grant 1120283.

Supporting Information

Prevalence and intensity of Bd infection (Appendix S1), red-list status (Appendix S2), and variation in operative temperatures (Appendix S3) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

Literature cited

Berger, L., et al. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proceedings of the National Academy of Sciences USA **95**:9031–9036.

Blanford, S., M. B. Thomas, C. Pugh, and J. K. Pell. 2003. Temperature checks the Red Queen? Resistance and virulence in a fluctuating environment. Ecology Letters **6**:2–5.

Bosch, J., L. M. Carrascal, L. Duran, S. Walker, and M. C. Fisher. 2007. Climate change and outbreaks of amphibian chytridiomycosis in a montane area of Central Spain; is there a link? Proceedings of the Royal Society of London, Series B **274**:253–260.

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.

Bustamante, M. R., S. R. Ron, and L. A. Coloma. 2005. Cambios en la diversidad en siete comunidades de anuros en los Andes de Ecuador. Biotropica **37**:180–189.

Catenazzi, A. 2011. Temperature constraint of elevational range of tropical amphibians: response to Forero-Medina et al. Conservation Biology **25**:425–426.

Catenazzi, A., E. Lehr, L. O. Rodriguez, and V. T. Vredenburg. 2011. Batrachochytrium dendrobatidis and the collapse of anuran species richness and abundance in the upper Manu National Park, southeastern Peru. Conservation Biology **25**:382–391.

Cheng, T. L., S. M. Rovito, D. Wake, and V. T. Vredenburg. 2011. Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. Proceedings of the National Academy of Sciences of the United States of America **108**:9502–9507.

Colwell, R. K., G. Brehm, C. L. Cardelús, A. C. Gilman, and J. T. Longino. 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. Science **322**:258–261.

Crawford, A. J., K. R. Lips, and E. Bermingham. 2010. Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. Proceedings of the National Academy of Sciences of the United States of America **107**:13777–13782.

Deichmann, J. L., G. B. Williamson, A. P. Lima, and W. D. Allmon. 2010. A note on amphibian decline in a central Amazonian lowland forest. Biodiversity and Conservation **19**:3619–3627.

Deutsch, C. A., J. J. Tewksbury, R. B. Huey, K. S. Sheldon, C. K. Ghalambor, D. C. Haak, and P. R. Martin. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Sciences USA **105**:6668–6672.

Dillon, M. E., G. Wang, and R. B. Huey. 2010. Global metabolic impacts of recent climate warming. Nature **467**:704–707.

Duellman, W. E. 1995. Temporal fluctuations in abundances of anuran amphibians in a seasonal Amazonian rain-forest. Journal of Herpetology **29**:13–21.

Duellman, W. E., and E. Lehr. 2009. Terrestrial-breeding frogs (Strabomantidae) in Peru. Natur und Tier-Verlag, Münster.

Fisher, M. C., D. A. Henk, C. J. Briggs, J. S. Brownstein, L. C. Madoff, S. L. McCraw, and S. J. Gurr. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature **484**:186–194.

Forero-Medina, G., L. Joppa, and S. L. Pimm. 2011. Constraints to species' elevational range shifts as climate changes. Conservation Biology **25**:163–171.

Francou, B., M. Vuille, P. Wagnon, J. Mendoza, and J. E. Sicart. 2003. Tropical climate change recorded by a glacier in the central Andes during the last decades of the 20th century: Chacaltaya, Bolivia. Journal of Geophysical Research: Atmospheres **108**:4059.

Garcia-Solache, M. A., and A. Casadevall. 2010. Global warming will bring new fungal diseases for mammals. MBio **1**:e00061–00010.

Garreaud, R., and P. Aceituno. 2001. Interannual rainfall variability over the South American Altiplano. Journal of Climate **14**:2779–2789.

Halladay, K., Y. Malhi, and M. New. 2012. Cloud frequency climatology at the Andes/Amazon transition: 2. Trends and variability. Journal of Geophysical Research: Atmospheres **117**:D23103.

Hertz, P. E., R. B. Huey, and R. D. Stevenson. 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. The American Naturalist **142**:796–818.

Huey, R. B. 1991. Physiological consequences of habitat selection. American Naturalist **137**:S91–S115.

Huey, R. B., C. A. Deutsch, J. J. Tewksbury, L. J. Vitt, P. E. Hertz, H. J. Álvarez Pérez, and T. Garland Jr. 2009. Why tropical forest lizards are vulnerable to climate warming. Proceedings of Royal Society B: Biological Sciences **276**:1939–1948.

Huey, R. B., M. R. Kearney, A. Krockenberger, J. A. M. Holtum, M. Jess, and S. E. Williams. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philosophical Transactions of the Royal Society B-Biological Sciences **367**:1665–1679.

Hyatt, A. D., et al. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms **73**:175–192.

IUCN. 2013. IUCN Red List of Threatened Species. Version 2013.1. www.iucnredlist.org.

Kearney, M., R. Shine, and W. P. Porter. 2009. The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. Proceedings of the National Academy of Sciences USA **106**:3835–3840.

Killeen, T. J., M. Douglas, T. Consiglio, P. M. Jorgensen, and J. Mejia. 2007. Dry spots and wet spots in the Andean hotspot. Journal of Biogeography **34**:1357–1373.

Kosch, T. A., V. Morales, and K. Summers. 2012. Batrachochytrium dendrobatidis in Peru. Herpetological Review **43**:288–293.

Kriger, K. M., J. M. Hero, and K. J. Ashton. 2006. Cost efficiency in the detection of chytridiomycosis using PCR assay. Diseases of Aquatic Organisms **71**:149–154.

La Marca, E., et al. 2005. Catastrophic population declines and extinctions in neotropical harlequin frogs (Bufonidae : *Atelopus*). Biotropica **37**:190–201.

Lips, K., J. Diffendorfer, J. I. Mendelson, and M. Sears. 2008. Riding the wave: reconciling the roles of disease and climate change in amphibian declines. PLoS Biology **6**:441–454.

Lips, K. R. 1998. Decline of a tropical montane amphibian fauna. Conservation Biology **12**:106–117.

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 USA **103**:3165–3170.

Lowe, C. H. J., and V. J. Vance. 1955. Acclimation of the critical thermal maximum of the reptile *Urosaurus ornatus*. Science **122**:73–74.

Marengo, J. A. 2004. Interdecadal variability and trends of rainfall across the Amazon basin. Theoretical and Applied Climatology **78**:79–96.

Navas, C. A. 1996*a*. Implications of microhabitat selection and patterns of activity on the thermal ecology of high elevation neotropical anurans. Oecologia **108**:617–626.

Navas, C. A. 1996*b*. Metabolic physiology, locomotor performance, and thermal niche breadth in neotropical anurans. Physiological Zoology **69**:1481–1501.

Navas, C. A. 1996*c*. Thermal dependency of field locomotor and vocal performance of high-elevation anurans in the tropical Andes. Journal of Herpetology **30**:478–487.

Navas, C. A. 1997. Thermal extremes at high elevations in the Andes: physiological ecology of frogs. Journal of Thermal Biology **22**:467–477.

Navas, C. A., M. M. Antoniazzi, J. E. Carvalho, H. Suzuki, and C. Jared. 2007. Physiological basis for diurnal activity in dispersing juvenile *Bufo granulosus* in the Caatinga, a Brazilian semi-arid environment. Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology **147**:647–657.

Navas, C. A., and C. Araujo. 2000. The use of agar models to study amphibian thermal ecology. Journal of Herpetology **34**:330–334.

Ohmura, A. 2012. Enhanced temperature variability in high-altitude climate change. Theoretical and Applied Climatology **110**:499–508.

Parmesan, C. 1996. Climate and species' range. Nature **382**:756–766.

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

Pounds, J. A., et al. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature **439**:161–167.

Rachowicz, L. J., J. M. Hero, R. A. Alford, J. W. Taylor, J. A. T. Morgan, V. T. Vredenburg, J. P. Collins, and C. J. Briggs. 2005. The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife. Conservation Biology **19**:1441–1448.

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.

Rapp, J. M., and M. R. Silman. 2012. Diurnal, seasonal, and altitudinal trends in microclimate across a tropical montane cloud forest. Climate Research **55**:17–32.

Rohr, J. R., and T. R. Raffel. 2010. Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. Proceedings of the National Academy of Sciences USA **107**:8269–8274.

Rowley, J. J. L., and R. A. Alford. 2013. Hot bodies protect amphibians against chytrid infection in nature. Scientific Reports **3**:1515.

Ryan, M. J., K. R. Lips, and M. W. Eichholz. 2008. Decline and extirpation of an endangered Panamanian stream frog population (*Craugastor punctariolus*) due to an outbreak of chytridiomycosis. Biological Conservation **141**:1636–1647.

Salzmann, N., C. Huggel, M. Rohrer, W. Silverio, B. G. Mark, P. Burns, and C. Portocarrero. 2013. Glacier changes and climate trends derived from multiple sources in the data scarce Cordillera Vilcanota region, southern Peruvian Andes. Cryosphere **7**:103–118.

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 extinction worldwide. Science **306**:1783–1786.

Tracy, C. R. 1976. A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment. Ecological Monographs **46**:293–326.

Tracy, C. R., G. Betts, C. R. Tracy, and K. A. Christian. 2007. Plaster models to measure operative temperatures and evaporative water loss of amphibians. Journal of Herpetology **41**:597–603.

Tracy, C. R., and J. S. Turner. 1992. Contrasting physiological abilities for heating and cooling in an amphibian (*Rana pipiens*) and a reptile (*Sauromalus obesus*). Herpetologica **48**:57–60.

Urrutia, R., and M. Vuille. 2009. Climate change projections for the tropical Andes using a regional climate model: Temperature and precipitation simulations for the end of the 21st century. Journal of Geophysical Research: Atmospheres **114**:D02108.

Villar, J. C. E., J. Ronchail, J. L. Guyot, G. Cochonneau, F. Naziano, W. Lavado, E. De Oliveira, R. Pombosa, and P. Vauchel. 2009. Spatio-temporal rainfall variability in the Amazon basin countries (Brazil, Peru, Bolivia, Colombia, and Ecuador). International Journal of Climatology **29**:1574–1594.

von May, R., et al. 2008. Current state of conservation knowledge on threatened amphibian species in Peru. Tropical Conservation Science **1**:376–396.

von May, R., K. Siu-Ting, J. M. Jacobs, M. Medina-Müller, G. Gagliardi, L. O. Rodríguez, and M. A. Donnelly. 2009. Species diversity and conservation status of amphibians in Madre de Dios, Perú. Herpetological Conservation and Biology **4**:14–29.

Vuille, M., and R. S. Bradley. 2000. Mean annual temperature trends and their vertical structure in the tropical Andes. Geophysical Research Letters **27**:3885–3888.

Vuille, M., R. S. Bradley, and F. Keiming. 2000. Interannual climate variability in the clentral Andes and its relation to tropical Pacific and Atlantic forcing. Journal of Geophysical Research **105**:12447–12460.

Vuille, M., R. S. Bradley, M. Werner, and F. Keimig. 2003. 20th century climate change in the tropical Andes: observations and model results. Climatic Change **59**:75–99.

Vuille, M., B. Francou, P. Wagnon, I. Juen, G. Kaser, B. G. Mark, and G. A. Bradley. 2008. Climate change and tropical Andean glaciers: past, present and future. Earth-Science Reviews **89**:79–96.

Wake, D., and V. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Sciences USA **105**:11466–11473.

Walker, S. F., et al. 2010. Factors driving pathogenicity vs. prevalence of amphibian panzootic chytridiomycosis in Iberia. Ecology Letters **13**:372–382.

Walther, G.-R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J.-M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. Nature **416**:389–395.

Woodhams, D. C., et al. 2011. Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. Frontiers in Zoology **8**:8.