Evaluating the links between climate, disease spread, and amphibian declines

Jason R. Rohr[a,1], Thomas R. Raffel[a], John M. Romansic[a], Hamish McCallum[b], and Peter J. Hudson[c]

[a]Department of Biology, University of South Florida, Tampa, FL 33620; [b]School of Zoology, University of Tasmania, Private Bag 5, Hobart, Tasmania 7001, Austrailia; and [c]Center for Infectious Disease Dynamics, Penn State University, University Park, PA 16802

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Human alteration of the environment has arguably propelled the Earth into its sixth mass extinction event and amphibians, the most threatened of all vertebrate taxa, are at the forefront. Many of the worldwide amphibian declines have been caused by the chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), and two contrasting hypotheses have been proposed to explain these declines. Positive correlations between global warming and Bd-related declines sparked the chytrid-thermal-optimum hypothesis, which proposes that global warming increased cloud cover in warm years that drove the convergence of daytime and nighttime temperatures toward the thermal optimum for Bd growth. In contrast, the spatiotemporal-spread hypothesis states that Bd-related declines are caused by the introduction and spread of Bd, independent of climate change. We provide a rigorous test of these hypotheses by evaluating (*i*) whether cloud cover, temperature convergence, and predicted temperature-dependent Bd growth are significant positive predictors of amphibian extinctions in the genus *Atelopus* and (*ii*) whether spatial structure in the timing of these extinctions can be detected without making assumptions about the location, timing, or number of Bd emergences. We show that there is spatial structure to the timing of *Atelopus spp.* extinctions but that the cause of this structure remains equivocal, emphasizing the need for further molecular characterization of Bd. We also show that the reported positive multi-decade correlation between *Atelopus spp.* extinctions and mean tropical air temperature in the previous year is indeed robust, but the evidence that it is causal is weak because numerous other variables, including regional banana and beer production, were better predictors of these extinctions. Finally, almost all of our findings were opposite to the predictions of the chytrid-thermal-optimum hypothesis. Although climate change is likely to play an important role in worldwide amphibian declines, more convincing evidence is needed of a causal link.

chytridiomycosis | climate change | emerging infectious disease | extinction | global warming

We are arguably in the midst of a sixth mass extinction event (1–3), and at the forefront are amphibians, the most threatened of all vertebrate taxa (4). More than 32% of amphibian species are threatened and more than 43% are experiencing some form of population decline (4). Unlike past mass extinctions, this one is driven by human activities (1–3). Humans are changing the climate, destroying and polluting habitats, and introducing invasive organisms. These changes threaten thousands and perhaps millions of species worldwide, and certainly impact the "poster child" of this mass extinction episode, amphibians (3–5).

Many of these worldwide amphibian declines have been caused by possibly the most deadly invasive species on the planet (excluding humans), the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (hereafter referred to as Bd) (3, 4, 6, 7). For example, since 1980, the frog genus *Atelopus* has apparently experienced 67 species extinctions, most of which are presumed to be because of Bd (8). The last year observed for each *Atelopus* species (8) represents perhaps the most comprehensive dataset on the timing of a modern day mass extinction within a single genus. Although there is great urgency to understand and curb the drivers of Bd-related declines, there remains unsettled controversy and possibly misconceptions over the causes of these declines that might impede conservation action.

Here, we focus on three important hypotheses that have been proposed for tropical amphibian declines. The "climate-linked-epidemic hypothesis" suggests that tropical declines are caused by an interaction between climate change and an amphibian pathogen (9). In support of this hypothesis, Pounds *et al.* (10) revealed a positive correlation between climatic warming and *Atelopus spp.* declines putatively associated with Bd. Pounds *et al.* (10) then proposed the "chytrid-thermal-optimum hypothesis" to resolve the apparent paradox that Bd, a cold tolerant pathogen that seems to become more pathogenic at low temperatures (11–14), was allegedly causing amphibian declines in warm years. This hypothesis states that increased cloud cover in warm years causes daytime cooling and nighttime warming, converging temperatures toward the thermal optimum for Bd growth [supporting information (SI) Fig. S1]. This study sparked controversy within the scientific community (15–17), most recently receiving criticism by Lips *et al.* (18), who argued that the timing of amphibian extinctions in Latin America is better explained by their "spatiotemporal-spread hypothesis". This hypothesis postulates that Bd-related declines are simply caused by the introduction and spread of Bd from a limited number of introduction sites rather than by any interaction between Bd and climate change (Fig. S1). Lips *et al.* (18) tested the hypothesis by using the variable "year of population decline" rather than "year of last observation", which was used by Pounds *et al.* (10).

These hypotheses for tropical amphibian declines have received substantial media and scientific attention. However, they warrant more thorough investigation given that previous research and media coverage have potentially generated misconceptions about amphibian declines and given their importance to amphibian, and perhaps general biodiversity, losses. For instance, researchers have yet to test whether estimated temperature-dependent Bd growth can predict the timing of *Atelopus spp.* extinctions or whether there is spatial structure to the timing of *Atelopus spp.* extinctions without making assumptions about the location, timing, or number of Bd emergences. By providing innovative and rigorous tests of these hypotheses, we attempt to clarify and advance our understanding of factors involved in widespread amphibian extinctions, which should facilitate conservation action.

Results and Discussion

Testing the Spatiotemporal-Spread Hypothesis. Lips *et al.* (18) presented a relationship between the distance from presumed Bd introductions and the timing of *Atelopus spp.* declines, but these findings depended on the number and location of their selected Bd introduction sites (19). Lips *et al.* selected the two earliest amphibian declines in South America as Bd introduction sites, but these two

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[1]To whom correspondence should be addressed. E-mail: jasonrohr@gmail.com.

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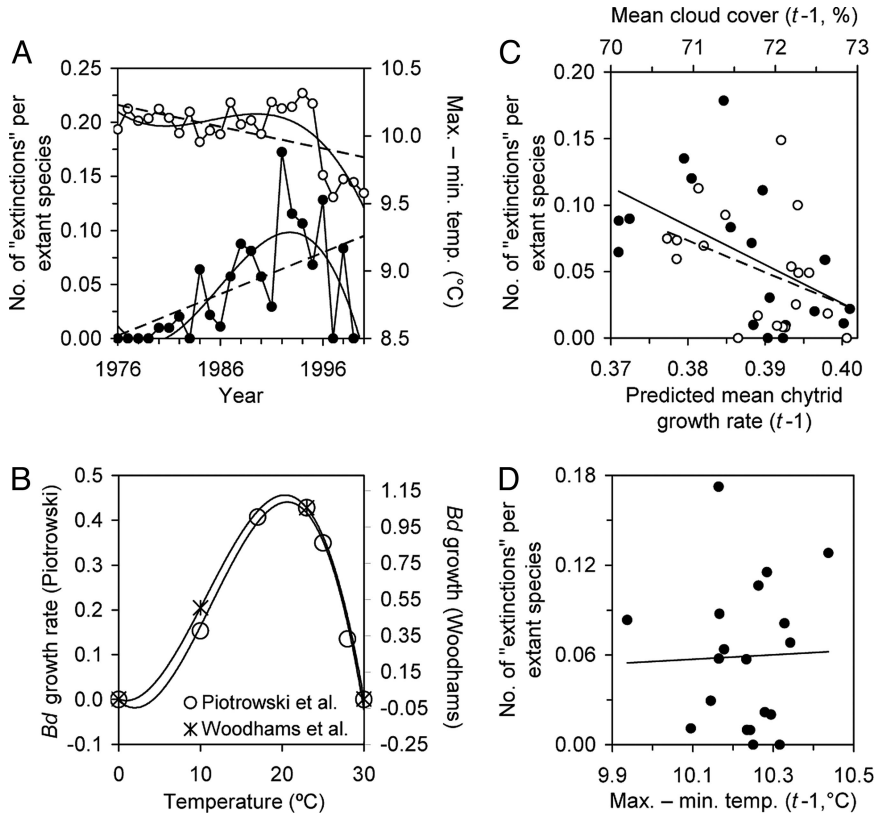

Fig. 1. Evaluation of the chytrid-thermal-optimum hypothesis. (*A*) Proportion of *Atelopus* species extinctions through time (closed circles), and the difference in the mean daily maximum and mean daily minimum temperatures in warmer months (open circles; March–October) for the region where *Atelopus spp.* inhabit. Also shown are the best-fitting third-order polynomial curves selected by using AIC (Table S2 and Table S3). The relationship between temperature difference and time was similar for cooler months (November–February) and, thus, is not displayed. Weighting the analyses by the number of extant species did not affect the statistical results or the model selection. Also shown is the less satisfactory linear fit through time (dashed lines), as suggested by Pounds *et al.* (4), for both *Atelopus spp.* extinctions and the difference in the mean daily maximum and mean daily minimum temperatures. (*B*) Relationship between temperature and exponential growth rate of Bd, per day, in culture based on the data of Piotrowski *et al.* (44) and Woodhams *et al.* (33), and the associated best-fitting third-order polynomial curves: $y = -0.0001414x^3 + 0.0047943x^2 - 0.0172752x - 0.0019076$, $R^2 = 0.9898$; $y = -0.0003107x^3 + 0.0099077x^2 - 0.0176078x + 0.0000000$, respectively). For the Woodhams *et al.* (33) data, we assumed that there was no chytrid growth at zero and 30°C. (*C*) Relationship between the proportion of *Atelopus spp.* extinctions and mean percent cloud cover in the previous year (open symbols, $F_{1,17} = 5.14$, $P = 0.037$, $R^2 = 0.232$) and predicted mean Bd growth rate in the previous year (closed symbols, $F_{1,17} = 5.70$, $P = 0.029$, $R^2 = 0.251$). (*D*) Relationship between the proportion of *Atelopus* species extinctions, and the difference between mean daily maximum and mean daily minimum temperatures in the previous year ($F_{1,17} = 0.020$, $P = 0.889$, $R^2 = 0.001$). Results were consistent for both warmer and cooler months. (See *Methods and Materials* for details.)

declines might not have been caused by Bd and, even if they were, they might not have represented the earliest Bd emergences/introductions. Further, there might be either more or fewer Bd emergences/introductions than the three somewhat subjectively selected by Lips *et al.* (one in Central America and two in South America, ref. 18). Consequently, we conducted Mantel tests, which do not to require any assumptions about the location, timing, or number of presumed introductions, to assess whether distances between pairs of *Atelopus* species were correlated with the pairwise differences in the years of their declines or in the years they were last observed. Our Mantel tests revealed significant positive correlations between spatial distance and difference in timing of declines ($r = 0.328$, $P < 0.001$) and last year observed ($r = 0.098$, $P = 0.009$). In addition, the number of species observed for the last time divided by the number of extant species increased exponentially during the 1980s but decreased precipitously during the 1990s (Fig. 1*A*). These results are consistent with the notion of a unique pathogen spreading through the environment and causing fewer extinctions as susceptible species are removed, analogous to the removal of susceptible individuals in classic epidemiological dynamics of host populations. Such spatiotemporal spread of Bd has been supported by Lips *et al.* (7) for frog declines in Panama. However, the temporal pattern of *Atelopus* species extinctions revealed here (Fig. 1*A*) and the spatiotemporal pattern of extinctions revealed by Lips *et al.* (7) in Panama can also be explained by spatiotemporal heterogeneities in either the emergence of a native pathogen or an abiotic stressor that caused localized declines (20). There is evidence from Costa Rica that Bd might have been locally widespread long before any declines were detected (21), but Bd did not appear to be present before it caused declines in Panama (7).

Additional analyses using Bayesian information criteria and Bayesian model averaging (BMA) revealed that two spreading centers, one in Venezuela and one in Ecuador, were the parsimonious explanation for the year of decline data (Table 1 and Table 2), consistent with the number and general locations of introduction sites chosen by Lips *et al.* (18). The precise location of the parsimonious spreading center in Ecuador was equivocal, with four sites explaining the pattern approximately equally well and no strong BMA support for any one of the sites; yet, each site was significant ($P < 0.01$) in a model including only the Venezuelan site

Table 1. Top 7 models for the BMA analysis of the spatiotemporal pattern of declines (DOD) or apparent extinctions (LYO), showing the sites used as spreading centers (predictors) in each analysis and three indicators of parsimony for each model, the Akaike information criterion (AIC), the Bayesian information criterion (BIC) and the adjusted R^2

Analysis	Sites*	AIC	BIC	R^2_{adj}
Date of decline (DOD)	Null	235.7	238.9	
	1 + 2	228.3	234.7	0.227
	1 + 3	228.4	234.8	0.225
	1 + 4	228.6	234.9	0.223
	1 + 5	229.2	235.6	0.208
	1 + 2+5	229.8	237.7	0.260
	1 + 3+5	229.8	237.7	0.215
Last Year Observed (LYO)	Null	262.2	265.8	
	6	258.0	263.4	0.108
	7	258.2	263.6	0.105
	6 + 8	259.6	266.9	0.095
	6 + 7	259.9	267.2	0.089
	7 + 8	260.0	267.2	0.087
	6 + 7+8	261.0	270.0	0.086

Models are considered to explain the data better than the null model if they have lower AIC or BIC values.
*Site numbers correspond to those listed in Table 2.

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Table 2. Predictors remaining in BMA (Bayesian model averaging) analyses of the spatiotemporal pattern of declines (DOD) or apparent extinctions (LYO) after removal of all predictors with negative mean posterior coefficients

Analysis	Site	Species	Country	Longitude; Latitude	DOD/LYO	Posterior Probability	Mean Coefficient	P_{top}‡
Date of decline (DOD)	1*	*A. cruciger*	Venezuela	−67.9; 10.3	1977	68.1	3.09E-01	0.0239
	2	*A. exiguus*	Ecuador	−79.1; −2.9	1988	33.1	2.51E-01	0.0014
	3*	*A. bomolochos*	Ecuador	−78.6; −2.8	1980	32.3	2.05E-01	0.0015
	4	*T. cirrhacelis*†	Ecuador	−79.2; −4.3	1987	29.6	5.06E-02	0.0016
	5	*A. arthuri*	Ecuador	−79.0; −1.8	1988	29.0	4.86E-02	0.0022
Last Year Observed (LYO)	6	*A. chrysocorallus*	Venezuela	−70.4; 9.2	1988	45.4	1.56E-06	0.0156
	7	*A. sanjosei*	Columbia	−74.4; 6.8	1988	41.9	8.66E-07	0.0169
	8	*A. nanay*	Ecuador	−79.2; −2.8	1989	18.9	3.97E-08	0.5553

Each predictor represents distance from the postulated spreading center site, located at the center of the range for the species listed in the table.
*Sites used by Lips et al. (2008) as presumed introduction sites.
†*Telmatobius cirrhacelis*, one of five non-*Atelopus* species listed in the Lips et al. DOD dataset.
‡P-value for significance of each predictor in the best model containing this predictor (Table 1).

(Table 1). In contrast, the best models for the last year observed data had only a single spreading center at two possible, disparate locations (Table 1), neither of which were supported by BMA (Table 2). Hence, there was no clear indication of a parsimonious spreading center for this dataset. Additionally, neither of these potential spreading centers were located in Ecuador, the most strongly supported spreading center for the year of decline analysis (Table 1). Thus, although there is evidence of directionality in the timing of *Atelopus spp.* declines and extinctions, there were inconsistencies between the year of decline and the last year observed data in the number and location of introduction/stress events. However, the spatial pattern was stronger in the year of decline analysis than in the last year observed analysis (Tables 1 and 2), consistent with the notion that there is less error in the year of decline dataset.

Testing the Chytrid-Thermal-Optimum Hypothesis. We tested the chytrid-thermal-optimum hypothesis by assessing whether percent cloud cover, convergence of mean daily minimum and mean daily maximum temperatures, and predicted temperature-dependent Bd growth rates (all with a one year lag) (Fig. 1*B*), were significant positive predictors of the number of *Atelopus spp.* extinctions per extant species per year (proportion extinct) and the number of *Atelopus spp.* declines per extant, and data sufficient, species per year (proportion declined). We conducted these analyses by averaging across elevations, but we also examined relationships within the elevation categories selected by Pounds *et al.* (10) and Lips *et al.* (18) because *Atelopus spp.* extinctions increased with elevation, with most extinctions occurring above 1,000 m (10, 18).

Almost all of our findings are contrary to the predictions of the chytrid-thermal-optimum hypothesis. Opposite the general pattern of *Atelopus spp.* extinctions (Fig. 1*A*) was the significant decrease in predicted regional growth of Bd with increasing time (Fig. 2 *A* and *B*) and temperature (Fig. 2*C*) from the 1970s to the 1990s. Moreover, none of the elevation categories had significant increases in estimated Bd growth rates through time during the primary period of *Atelopus spp.* extinctions (Fig. 2*D*). Both growth rates and cloud cover were lowest at the highest elevation category (Fig. 2 *D* and *E*), despite this category having the highest proportion of declines (18) and the second highest proportion of extinctions (10). The relationship between proportion extinct and predicted annual Bd growth rate in the previous year was significantly negative (Fig. 1*C*), regardless of whether we used mean or mean daily maximum temperatures to calculate growth rates, or whether we generated means for either the entire year or just the warm or cool months ($F_{1,17}$>4.63, $P < 0.046$). Qualitatively similar patterns (all negative coefficients) were revealed when we conducted the same analyses on proportion declined ($F_{1,17}$>3.23, $P < 0.090$). Moreover, at elevations >1,000 m, there was no significant relationship between predicted Bd growth rates in the previous year, and either proportion extinct (slope = 1.86, $F_{1,17} = 0.472$, $P = 0.501$) or proportion declined (slope = 2.40, $F_{1,17} = 0.720$, $P = 0.408$).

Percent cloud cover in the previous year (Fig. 1*C*) and temperature convergence in the previous year (Fig. 1*D*, Table S1, and Table S3) were also negative predictors of proportion extinct (slope = −0.089, $F_{1,17} = 5.14$, $P = 0.037$; slope = −0.004, $F_{1,17} = 0.020$, $P = 0.889$, respectively) and proportion declined (slope = −0.079, $F_{1,17} = 4.07$, $P = 0.060$; slope = −0.251, $F_{1,17} = 0.78$, $P = 0.390$, respectively). Additionally, temperature convergence was significantly greater in cool than in warm years, for both cold and warm months (Fig. S2) and despite more extinctions occurring in warm years.

The relationships between minimum-temperature Bd growth rate (based on the mean daily minimum temperature in the previous year) and both proportion extinct and proportion declined were positive but not significant ($F_{1,17} = 3.60$, $P = 0.075$; $F_{1,17} = 3.17$, $P = 0.093$, respectively; Fig. 2*A*). These relationships were positive because nighttime temperatures in the region appeared to be improving for Bd growth (10). However, improved nighttime growth of Bd appears to be offset by reduced daytime growth because growth based on the mean temperature in the previous year is a significant negative predictor of proportion extinct (Fig. 1*C*) and decreases through time (Fig. 2*A*). Additionally, it is possible that frogs either clear or control Bd infections by seeking warm microhabitats that exceed the optimal temperature for Bd growth (11, 14, 22). Since the early 1970s, cloud cover and temperature have decreased and increased, respectively (Fig. 2*C*), at almost all elevations (Fig. 2*E*), which should facilitate daytime thermoregulation to counteract increased nighttime growth of Bd.

Not all of the data presented by Pounds *et al.* (10) are consistent with the chytrid-thermal-optimum hypothesis. Pounds *et al.* (10) argue that the convergence of mean daily minimum and mean daily maximum temperatures through time drives the increase in *Atelopus spp.* extinctions. This conclusion is based on a suggested linear relationship between both variables and time, but the temporal relationships are significantly nonlinear (Fig. 1*A*) and the temporal nonlinearity for temperature convergence is consistent across all elevations (Fig. 2*F*). When these nonlinearities are taken into account, it becomes apparent that there was no regional temperature convergence in the 1980s when extinctions were increasing, and that convergence only occurred in the 1990s when *Atelopus spp.* extinctions were decreasing (Fig. 1*A*), opposite to the conclusions of Pounds *et al.* (10) and the chytrid-thermal-optimum-hypothesis.

Evaluation of Evidence for the Climate-Linked Epidemic Hypothesis. Lips *et al.* (18) questioned the multi-decadal correlation between air temperature and extinctions reported by Pounds *et al.* (10), arguing that the last year species were observed is a less accurate measure of the timing of Bd epizootics than the year species declined. Lips *et al.* (18) examined whether the significant relationship between air temperature and last year observed could withstand a "conserva-

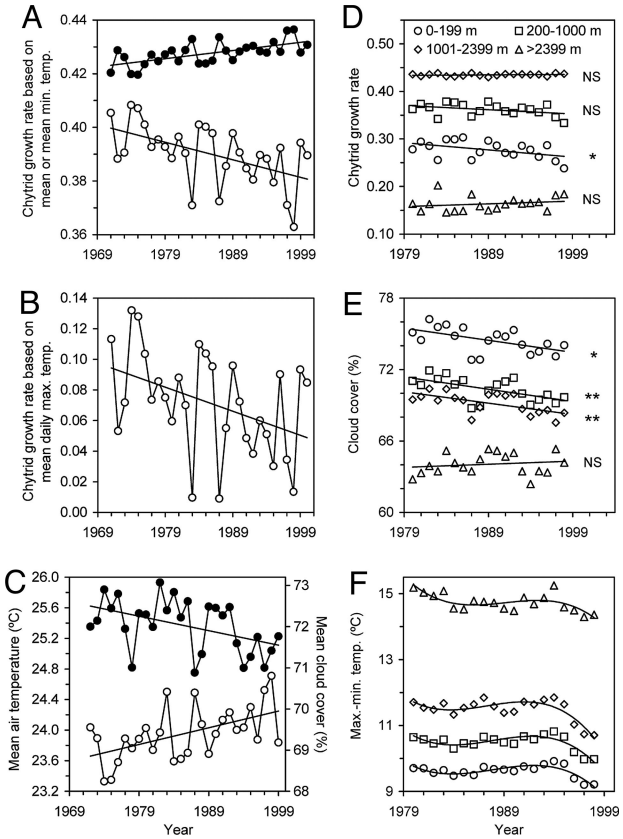

Fig. 2. Bd growth, cloud cover, temperature convergence, and temperature, through time, for the region inhabited by *Atelopus spp*. (*A*) Predicted mean regional Bd growth rate through time, based on mean regional temperatures (open circles; $F_{1,28}$ = 10.80, P = 0.003, R^2 = 0.278) and mean regional daily minimum temperatures (closed circles; $F_{1,28}$ = 5.85, P = 0.022, R^2 = 0.173). (*B*) Predicted mean regional Bd growth rate through time, based on mean regional daily maximum temperatures (open circles, $F_{1,28}$ = 19.60, $P < 0.001$, R^2 = 0.412). (*C*) Mean air temperature (open circles; $F_{1,26}$ = 9.94, P = 0.004, R^2 = 0.276) and cloud cover through time (closed circles; $F_{1,26}$ = 6.79, P = 0.015, R^2 = 0.207; arcsine square root-transformed). (*D*) Mean Bd growth rate through time for four elevation categories. (*E*) Mean cloud cover through time for four elevation categories. (*F*) Nonlinear relationship for mean daily maximum temperature minus mean daily minimum temperature, through time, for four elevation categories. For all images, either best-fit lines or curves are provided. For *D* and *E*, we have provided the results for the relationship between time and the response for each elevation category (*, $P < 0.05$; **, $P < 0.01$; NS = $P > 0.05$).

tive" (their terminology) addition of up to six years of random error to the last year observed data for each species, despite these data already having error in their estimation of the date of decline. Furthermore, Lips *et al.* (18) overestimated the mean difference between the last year observed and year of decline data by excluding the 56% of the species that had identical years of last observation and decline (Fig. 3), resulting in a mean difference of 11.2 years, whereas the mean difference including zeros was 3.7 years (Fig. 3). Hence, the addition of up to 6 years of random error applied to the last year of observed data is arguably not conservative. Additionally, Lips *et al.* (18) randomly applied error from four distributions, all of which were significantly different from the observed error distribution (Fig. 3).

To address these issues, we tested whether the relationship between last year observed and air temperature in the previous year could withstand the addition of randomly selected error from the actual last year observed minus the year of decline distribution. The mean slope of this relationship was significantly positive (1.16, 95% CI: 0.36–1.86). Moreover, air temperature in the previous year was a significant positive predictor of the number of declines per year

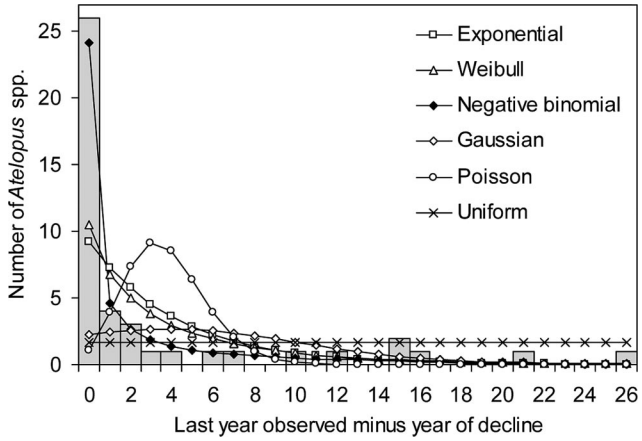

Fig. 3. Frequency distribution for the last year observed minus the year of decline data from Lips *et al.* (18), and normal (mean = 4.23, SD = 6.56), uniform (mean = 1.63), Poisson (lambda = 3.73), exponential (rate = 0.0237), Weibull (shape = 0.833, scale = 4.20), and negative binomial (size = 0.203, μ = 3.73) fits. All four of the distributions (normal, uniform, Poisson, and exponential) used by Lips *et al.* (12), and the Weibull, were significantly different from the observed distribution ($P < 0.000003$). The observed distribution was not significantly different from a negative binomial distribution (P = 0.700). There was a total of 44 species used in this analysis. For species with ranges of years for their year of decline, we used the mean of the range.

(X^2 = 8.92, P = 0.003). Thus, all evidence indicates that the relationships between air temperature in the previous year and both *Atelopus spp.* extinctions and declines are positive and robust, contrary to the conclusions of Lips *et al.* (18).

Nevertheless, these correlations should not be interpreted as causal because they are driven by multi-decadal increases in each variable. For this reason, almost anything that increased between 1970 and 1990 will be positively related to *Atelopus spp.* declines (Fig. 1*B*). For example, mean annual log-transformed gross domestic product, human population size, banana production, and even beer production, for countries harboring *Atelopus spp.*, were all better positive predictors of the number of *Atelopus spp.* extinctions per year (all X^2>19.48, all $P < 0.000011$) than was air temperature across the tropics (X^2 = 14.33, P = 0.000153). We intentionally selected these variables to demonstrate that strong correlations can occur between amphibian extinctions and both plausible and implausible drivers of declines. At this point, although there is evidence that "disease is the bullet killing frogs", we still need further research before we can conclude that "climate change is pulling the trigger" (23).

Conclusions

There are many convincing examples of the consequences of modern climate change on biological systems (24–27), many of which signify an urgency to curb global warming; therefore, it seems probable that climate is a factor in amphibian declines (10, 15, 17, 28–31). However, at the current time, the evidence that climate change is causing amphibian declines and extinctions is incomplete (20, 32). Our analysis identified that temperature-dependent Bd growth alone cannot explain the patterns of *Atelopus spp.* extinctions. Some possible explanations for the lack of a positive relationship between predicted Bd growth and *Atelopus spp.* declines are (*i*) Bd growth in the laboratory might not accurately reflect growth in the field, (*ii*) *Atelopus spp.* might be well defended against Bd at Bd's optimal temperature for growth, (*iii*) Bd might have been coincidentally introduced and spread during a time when predicted Bd growth conditions were worsening, or (*iv*) many *Atelopus spp.* extinctions might not be directly caused by Bd (20). Alternatively, the lack of a positive relationship might reflect how little we know about Bd biology. For instance, recent evidence suggests that Bd

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exhibits substantial life-stage variation in response to temperature (33), which could represent a beneficial bet-hedging strategy in nature, where temperatures can be highly variable. We suspect that, to thoroughly understand climatic effects on amphibian declines, investigators will need to integrate climate-dependent parasite growth with climate-dependent amphibian susceptibility (34), as well as consider other factors altering host-parasite dynamics (5, 35–37).

Our analyses also reinforce the weakness of multi-decadal correlations as evidence for a causal relationship between climatic variables and biodiversity losses. We need to move beyond simply identifying multi-decadal correlations and begin examining whether fluctuations around these long-term temporal trends are also predictive of the timing of declines. As shown, many factors can increase or decrease across decades, but many fewer factors will correlate with fluctuations around long-term trends, vastly reducing the probability of spurious correlations. This should provide more convincing evidence for a causal relationship between climate and declines, considering that retrospective correlation analyses might be our best method for understanding the causes of any mass extinction given that the species are unavailable for experimentation. Nevertheless, using extant species to experimentally test hypotheses derived from retrospective correlation analyses will also be important to assess mechanistic links between climate and declines. Regardless of the methodological approach used to identify factors in biodiversity declines, both scientists and the media should take great care in distinguishing hypotheses from findings and in inferring causation from correlation.

Finally, our spatial analyses provide clear evidence of spatial structure in the timing of *Atelopus spp.* declines, but the causes of this structure remain equivocal, underscoring the need for more molecular data on Bd to discriminate between the hypotheses that Bd is an endemic pathogen that emerged versus a novel pathogen that has recently spread (38, 39). Although there is evidence that the last year observed data occasionally, but significantly, misrepresents the actual start of a decline (i.e., year of population decline), the difference between the two variables was usually small ($\approx$70% of the time the difference was less than 3 years with a median difference of zero) (Fig. 3). Further, we have no reason to believe that this error was biased with respect to the hypotheses of interest (19). The addition of random error makes patterns more difficult to detect; thus, there is more danger of missing important correlations than of falsely concluding that correlations exist (19). This suggests that any correlations detected, by using the last year observed or year of decline datasets, are unlikely to be caused by chance (but they still might not represent causal relationships). Despite their flaws, the last year observed and year of decline datasets represent possibly the most comprehensive spatiotemporal data on a modern day mass extinction, and will undoubtedly have much to offer science for years to come.

Ultimately, we have much to learn about amphibian declines and their contribution to the sixth mass extinction event. Hence, we must ensure that diligent, careful, and concerted efforts are made to further advance our understanding and protection of this critically imperiled taxon (40–42). We hope that these analyses have clarified factors involved in losses of amphibians and, most importantly, facilitate their protection.

Materials and Methods

Spatiotemporal-Spread Hypothesis. We conducted Mantel tests to assess whether distances between pairs of *Atelopus* species were correlated with either the pairwise differences in the years of their declines or the years they were last observed. These tests were based on the center of gravity of species' ranges (range maps attained from the Global Amphibian Assessment) and the Euclidean distance of both spatial and temporal dissimilarity (10,000 randomizations). These tests excluded extant species, undescribed species, and species with last years observed either before 1980 or after 1998.

We assessed whether the number (two) and location (Ecuador and Venezuela) of Bd introduction sites (which we refer to as ''spreading centers'' to avoid implying a particular cause for spatial structure) selected by Lips *et al.* (18) were warranted based on available data. Like Lips *et al.* (18), we predicted that, if there was spatiotemporal spread, there should be a positive correlation between distance of a species from a spreading center and either its year of decline or its last year observed. Unlike Lips *et al.* (18), we conducted a single analysis for South America rather than dividing the analysis into regional sections, reasoning that, if there were multiple spreading centers, the distance from each of these spreading centers should be a positive predictor within a multiple regression analysis. We used a multiple regression analysis, with distance from the center of a species's range to each postulated spreading center as a separate predictor, and either year of decline or last year observed, for that species, as the response. To avoid bias in the choice of number and location of potential spreading centers, we assumed that the center of any species's range could be a potential spreading center. We constructed separate multiple regression models for all possible combinations of these predictors, with the number of predictors in a single model ranging from one to all 36 species in the year of decline analysis and from one to all 45 species in the last year observed analysis. We calculated Bayesian information criterion values for all possible combinations of these predictors and used Bayesian model averaging to determine which potential predictors were parsimonious locations of spreading centers. Only those predictors that correlate positively with declines are likely to be spreading centers; therefore, we iteratively removed the most negative predictor and reconducted the Bayesian model averaging analysis until all remaining predictors had positive mean posterior coefficients. We assessed the importance of each remaining predictor by using a combination of Bayesian model averaging, Bayesian information criteria, and traditional F-ratio statistics. Mantel tests and Bayesian model averaging analyses were run by using ''vegan'' and ''BMA'' packages in R statistical software (www.r-project.org).

Chytrid-Thermal-Optimum Hypothesis. For tests of the chytrid-thermal-optimum hypothesis, we focused on last year observed and year of decline data from 1980 to 1998 for the following reasons. Most *Atelopus spp.* extinctions occurred after 1980 (Fig. 1*A*), there is no convincing evidence that Bd was widely present in this region before the late 1970s, and Pounds *et al.* (10) stopped their analyses in 1998. Unlike Pounds *et al.* (10), who used air temperature data for all of the tropics, our climate data are strictly for the land masses in the region where *Atelopus spp.* either did or does inhabit. Hence, our temperature data were gathered from a polygon with coordinates 13.5 and −20.5° lat. and −44.5 and −82.5° long, but excluding a subpolygon with coordinates −4.5 to −20. 5° lat. and −45.5 to −65. 5° long., where no *Atelopus spp.* are known to have inhabited. All of the climate data, including cloud data, were obtained from the Climate Research Unit (CRU TS 2.1), University of East Anglia (43). These data were interpolated to 0.5° resolution by using all quality climate stations in the region, passed extensive quality control measures, and met the quality standards of the Intergovernmental Panel on Climate Change (43). This dataset arguably represents the most comprehensive and precise historical estimates of local climate across the region inhabited by *Atelopus spp.* In this dataset, each 0.5 × 0.5 degree grid cell has a mean elevation that was used for our elevation analyses.

Bd growth in culture is temperature dependent (44), and, thus, we used temperature to estimate Bd growth rate (Fig. 1*B*). However, the growth curve of Bd as a function of temperature is not symmetric (44). Thus, equal negative and positive deviations away from the optimum will not produce equal reductions in Bd growth. Consequently, to more accurately estimate Bd growth based on temperature, we generated a temperature-dependent growth curve to estimate growth from temperature.

Woodhams *et al.* (33) and Piotrowski *et al.* (44) offer data on the growth of Bd as a function of temperature. Using the Piotrowski *et al.* (44) data, we calculated the population growth rate parameter for the exponential portion of the Bd growth curve at each selected temperature. Woodhams *et al.* (33) only had data on overall Bd growth for two temperatures (10 and 23°C). It is well established that Bd growth ceases at temperatures ≤0°C, and ≥30°C (14, 45, 46); thus, we added these two data points to the Woodhams *et al.* (33) data, fit third-order polynomial functions to each dataset, and then compared the two temperature-dependent growth curves (Fig. 1*B*). The relative temperature-dependent growth curves of Woodhams *et al.* (33) and Piotrowski *et al.* (44) were nearly identical. We use the Piotrowski *et al.* (44) dataset from hereon to calculate estimated Bd growth because it offers greater precision (i.e., seven temperatures tested) than the Woodhams *et al.* (33) dataset (two temperatures tested). We entered monthly regional mean temperature, mean daily minimum temperature, or mean daily maximum temperature into the polynomial fitting equation of temperature versus exponential Bd growth rate (Fig. 1*B*) to calculate the predicted regional monthly growth rate of Bd. We then calculated the geometric mean for the 12 months of each year because Bd growth is multiplicative. In addition, we calculated regional geometric mean growth rates for the warm (March–October) and cold months (November–February), separately for each year, because many Bd-related declines appear to have occurred in cold months (11, 12, 47).

We conducted simple linear regressions to evaluate various predictors of the number of extinctions, or declines, per extant species per year (arcsine square root-transformed), weighted by the number of extant species each year. These response variables controlled for the reduction in the probability of extinctions or declines, as the number of extant species decreased, whereas weighting by the number of extant species controlled for the change in precision of estimates, associated with the reduced sample size through time. For the year of decline analyses, the denominator for the proportion of declines per extant species included species with a year of decline between 1980 and 1998 and species that we knew were extant during this period (i.e., species with last year observed after 1998). Both the denominator and numerator excluded species with ranges of years for their year of decline.

Many predictors might have nonlinear relationships with these response variables. Thus, to determine which relationships were significantly nonlinear, we selected among first–fourth order polynomial models based on AIC values. Before analyses, the variable ''year'' was centered to minimize multicolinearity. The goodness-of-fit of the selected model was also verified by comparing its residuals to those estimated from the generalized additive model (e.g., Tables S2 and S3 and Figs. S3 and S4).

Climate-Linked-Epidemic Hypothesis. Last year observed and year of decline data were collected from Lips *et al.* (18) for species where both types of data were available. A frequency distribution was generated for the last year observed minus the year of decline data and we tested the fit of normal, uniform, Poisson, exponential, Weibull, and negative binomial distributions to these data.

The positive relationship between tropical air temperature in the previous year and *Atelopus spp.* extinctions revealed by Pounds *et al.* (10) was placed into question by Lips *et al.* (18). However, there were some issues with the analyses used by Lips *et al.* (18) to cast doubt on this relationship (see *Evaluation of evidence for the climate-linked epidemic hypothesis* above); thus, we set out to evaluate the relationship between tropical air temperatures in the previous years and *Atelopus spp.* extinctions and declines by using analyses with fewer inherent assumptions than those used by Lips *et al.* (18). We used the number of extinctions (last year observed) and declines each year, between 1970 and 1998, as our response variables, and air temperature anomalies for all of the tropics as our predictor. We used this time period and these response variables, rather than the number of extinctions or declines per extant species, because they were used by Pounds *et al.* (10) and Lips *et al.* (18) in their analyses. We randomly selected data from the actual-last-year-observed-minus-year-of-decline distribution (with replacement) and subtracted this value from each species's year of last observation because every year of last observation occurred in a year greater than or equal to the corresponding year of decline, despite the Lips *et al.* (18) suggestion that a species could decline after it was observed for the last time (figure 1 of ref. 18). We then tallied these new last year observed estimates for each year between 1980 and 1999, and generated a slope parameter for the relationship between last year observed plus error and air temperature for the tropics in the previous year by using Poisson regression. We did this 1,000 times and calculated the mean slope and associated 95% confidence interval. The confidence interval was determined by ranking the 1,000 slope parameters, removing the top and bottom 2.5%, and using the remaining largest and smallest parameters as the upper and lower bounds of the interval.

We assessed the quality of tropical air temperature in the previous year as a predictor of *Atelopus spp.* extinctions by comparing the amount of variation in *Atelopus spp.* extinctions accounted for by this variable to the amount accounted for by annual gross domestic product, human population size, banana production, and beer production for countries harboring *Atelopus spp*. We intentionally selected variables that were arguably plausible (e.g. gross domestic product and population size) and implausible (banana production and beer production) predictors of *Atelopus spp.* extinctions. Data on annual gross domestic product, population size, banana production, and beer production were obtained from the Oxford Latin American Economic History Database (http://oxlad.qeh.ox.ac.uk/search.php).

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