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An interaction between climate change and infectious disease drove widespread amphibian declines

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Abstract

Climate change might drive species declines by altering species interactions, such as host–parasite interactions. However, few studies have combined experiments, field data, and historical climate records to provide evidence that an interaction between climate change and disease caused any host declines. A recently proposed hypothesis, the *thermal mismatch hypothesis*, could identify host species that are vulnerable to disease under climate change because it predicts that cool- and warm-adapted hosts should be vulnerable to disease at unusually warm and cool temperatures, respectively. Here, we conduct experiments on *Atelopus zeteki*, a critically endangered, captively bred frog that prefers relatively cool temperatures, and show that frogs have high pathogen loads and high mortality rates only when exposed to a combination of the pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*) and high temperatures, as predicted by the *thermal mismatch hypothesis*. Further, we tested various hypotheses to explain recent declines experienced by species in the amphibian genus *Atelopus* that are thought to be associated with *B. dendrobatidis* and reveal that these declines are best explained by the *thermal mismatch hypothesis*. As in our experiments, only the combination of rapid increases in temperature and infectious disease could account for the patterns of declines, especially in species adapted to relatively cool environments. After combining experiments on declining hosts with spatiotemporal patterns in the field, our findings are consistent with the hypothesis that widespread species declines, including possible extinctions, have been driven by an interaction between increasing temperatures and infectious disease. Moreover, our findings suggest that hosts adapted to relatively cool conditions will be most vulnerable to the combination of increases in mean temperature and emerging infectious diseases.

KEYWORDS
amphibians, chytrid fungus, climate change, disease ecology

1 | INTRODUCTION

Global climate change and emerging infectious diseases represent two of the most formidable ecological challenges in modern times, but controversy exists over whether they are causally linked (Harvell et al., 2002; Lafferty, 2009; Rohr et al., 2011). Climatic conditions often directly influence disease outbreaks (Pascual, Chaves, Chaves, Cash, Rodó, & Yunus, 2008), and many predictive models and experiments have revealed that climate change and infectious diseases can

independently drive current and future declines in biodiversity (Liu, Rohr, & Li, 2013; Patz, Campbell-Lendrum, Holloway, & Foley, 2005). However, there is surprisingly little concrete evidence that interactions between climate change and infectious disease are causing widespread population declines (Cahill et al., 2013; Rohr et al., 2011), possibly because of a lack of theoretical frameworks, supported by a combination of experiments and field data, that can relate climatic factors to host–parasite interactions to account for shifts in biodiversity (Rohr et al., 2011). Such frameworks would be valuable in establishing causal links between climate change and declines mediated by disease.

A recently proposed hypothesis, the *thermal mismatch hypothesis* (Cohen et al., 2017), suggests that infectious disease outbreaks are likely to occur at temperatures where the performance gap between pathogens and their hosts is greatest. Because parasites generally have broader thermal performance breadths than hosts (Baas-Becking, 1934; Rohr, et al., 2018), and both hosts and parasites might be locally adapted to climatic conditions in their ranges and limited by extreme conditions, the hypothesis posits that hosts adapted to cooler climates should be especially susceptible to disease under unusually warm conditions, and vice versa (Figure 1a,b). When conditions become too extreme, the hypothesis predicts that any advantage parasites have over hosts will likely cease (Figure 1a,b). Importantly, the predictions of the *thermal mismatch hypothesis* are robust to relaxing several of its assumptions, such as local adaptation of host and parasite and the degree and direction of the skew of the performance curves (Supporting Information Figure S1). Therefore, the *thermal mismatch hypothesis* provides a framework to predict which species might be most likely to experience disease-driven declines under warming and thus might be able to explain patterns in species declines associated with climate-related outbreaks of emerging infectious diseases. Recent experimental and field evidence supports the predictions of the *thermal mismatch hypothesis* (Cohen et al., 2017; Figure 1c,d). For example, the chytrid fungus *Batrachochytrium dendrobatidis*, a pathogen that infects the epidermal layer of adult amphibians and is implicated in worldwide amphibian declines (Collins, 2013), grew best at relatively cool and warm temperatures on species that preferred warm and cool temperatures, respectively (Cohen et al., 2017; Figure 1c). Further, using 598 globally distributed populations from 235 amphibian species, Cohen et al., 2017 revealed that warm- and cool-adapted amphibians had the greatest *B. dendrobatidis* prevalence at cool and warm temperatures, respectively (Figure 1d; possibly because of a temperature-dependent immune response to *B. dendrobatidis*; Ribas et al., 2009), providing broad support for the *thermal mismatch hypothesis*. *Batrachochytrium dendrobatidis* is sensitive to environmental conditions (Kilpatrick, Briggs, & Daszak, 2010), can be locally adapted (Stevenson et al., 2013), and has a broad thermal tolerance (Voyles et al., 2017), fulfilling the assumptions of the *thermal mismatch hypothesis*.

The *thermal mismatch hypothesis*, however, has not yet been applied to predict widespread host declines associated with climate change and infectious disease (but see Clare et al., 2016 for a discussion of climate change impacts on chytridiomycosis). The amphibian genus *Atelopus* has suffered more severe declines attributed to the spread of *B. dendrobatidis* (Lips, Diffendorfer, Mendelson, & Sears, 2008; Pounds et al., 2006; Rohr & Raffel, 2010) than perhaps any other group of amphibians, with approximately 60 species reaching endangered status in the past 50 years. The International Union for Conservation of Nature's (IUCN) Red List Database (IUCN, 2018) is the most up-to-date resource describing whether and when *Atelopus* spp. disappeared for long periods of time (>10 years), and also provides the subsequent level of surveying effort (Table 1). Some *Atelopus* spp. never recovered and have not been observed in decades, while other species have partially recovered in the decades following extreme *B.* dendrobatidis-related declines (Voyles et al., 2018). Still, the IUCN data provide a unique opportunity to examine whether the *thermal mismatch hypothesis* can accurately predict the timing of disease-driven disappearances or declines while competing against alternative climate-related hypotheses for these declines. Previously, several studies explored whether *B. dendrobatidis*-linked declines of *Atelopus* spp. were associated with increases in mean temperature, temperature variability, or are simply explained by the typical spatial spread of the pathogen (Lips et al., 2008; Pounds et al., 2006; Rohr & Raffel, 2010; Rohr, Raffel, Romansic, McCallum, & Hudson, 2008); however, these studies were not backed by experimental data and often correlated extinctions with continental-scale climate data. In addition, these analyses did not address spatial heterogeneity in climate change and geographic variation in host adaptation to climate (See Supporting Information Table S1 and Supporting Information for a comparison and discussion of this literature). Finally, no previous studies have tested hypotheses that consider host-level drivers of declines, such as the *thermal mismatch hypothesis*. Therefore, there is not yet evidence in this system, or any system that we are aware of, that climate change or thermal mismatches interacted with infectious disease to facilitate declines (but see Ben-Horin, Lenihan, & Lafferty, 2013).

Here, we link a theoretical framework, multiple laboratory experiments, and analyses of declines using historic monthly climate data from ranges of individual species (Supporting Information Figure S2; see Methods for more details about climate data), to examine the relationships among amphibian declines, climate change and emerging infectious disease. We tested how mean temperature and temperature shift affects response to *B. dendrobatidis* infection in *Atelopus zeteki*, a species that prefers temperatures between 17°C and 18°C (Cohen et al., 2017) and is known to perform poorly at temperatures exceeding 30°C (see Supporting Information). In addition, we simultaneously tested seven hypotheses for the climate-related declines of *Atelopus*Animals were swabbed for infection at 1, spp.: (a) a null model, (b) spatial spread of the pathogen (see Supporting Information), (c) temperature-dependent growth of *B. dendrobatidis* in culture, (d) temperature variability alone (annual month-to-month variability in temperature), (e) annual mean temperature alone, (f) climate change alone (the 5-year slope of mean temperature), and (g) the *thermal mismatch hypothesis*, measured by the interaction between mean historical climate and climate change in species' ranges. This interaction represents the *thermal mismatch hypothesis* because the hypothesis predicts that the effect of climate change

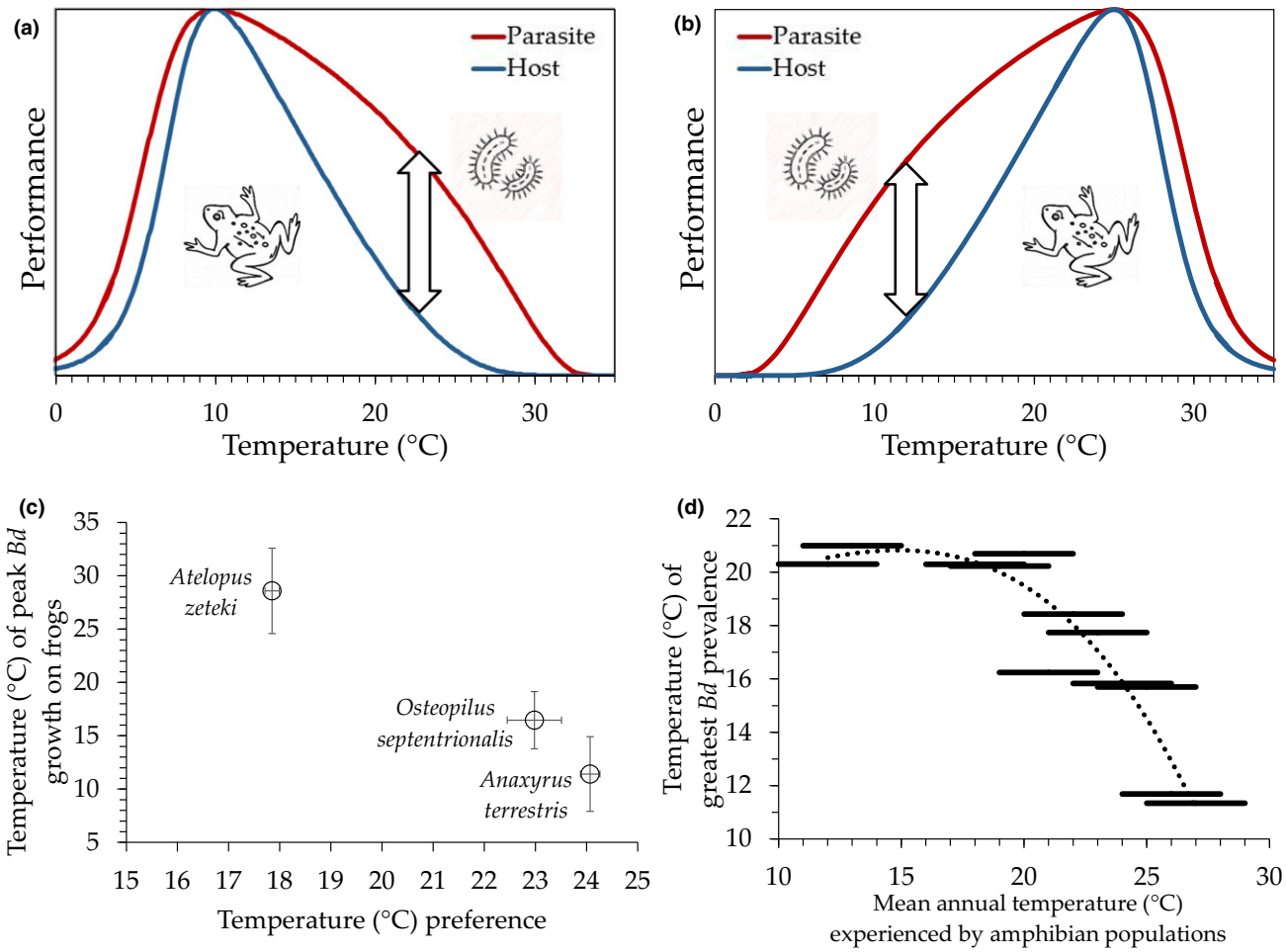

FIGURE 1 The *thermal mismatch hypothesis* In isolation, small organisms, such as parasites (red lines), generally have broader thermal performance curves than larger organisms, such as hosts (blue lines). Parasite growth on hosts is likely to occur at temperatures where a parasite most outperforms its host (bidirectional arrows), and not necessarily at the temperature at which a parasite performs best in isolation. For interacting cool-adapted hosts and parasites (a), parasite growth should be maximized at relatively warm temperatures, whereas for interacting warm-adapted hosts and parasites (b), parasite growth is predicted to be maximized at relatively cool temperatures. Cohen et al., 2017 provided support for this hypothesis by demonstrating that warm- and cool-preferring frogs had the highest abundance of *B. dendrobatidis* at cool and warm temperatures, respectively (see Methods for generation of *B. dendrobatidis* growth peaks and SEMs) (c). Additionally, using 598 globally distributed populations from 235 amphibian species, Cohen et al., 2017 revealed a negative relationship between the 50-year mean annual temperature and the temperature of peak *B. dendrobatidis* prevalence experienced by populations in varying climate windows (bars), indicating that warm- and cool-adapted amphibians tend to have greater disease at cool and warm temperatures, respectively (d). Figures republished with permission from *Ecology Letters*

depends on whether the host is cool- or warm-adapted, which in turn drives the differential performance of host and pathogen. Unlike past investigations, we explicitly competed increasing mean temperatures and increasing temperature variability as explanations for these declines using both laboratory and field data.

2 | MATERIALS AND METHODS

2.1 | Animal care and maintenance

Captive-bred *Atelopus zeteki* were obtained from the Maryland Zoo in Baltimore, MD, as adults and maintained in the laboratory at the University of South Florida at 21°C for approximately one month prior to the start of experiments. Animals were fed vitamin-dusted crickets ad libitum twice per week and were kept in vented plastic containers (26 × 16 × 8 cm) with two folded paper towels that were moistened with artificial spring water and changed twice weekly. Before experiments, all frogs were weighed and assigned to treatments of equal mass. Throughout the experiments, animals were kept in the same plastic containers described above, placed inside of temperature-controlled, insulated Styrofoam incubators (inner dimensions 37 × 21 × 13 cm; Marko Foam Products, Salt Lake City, UT) that had Plexiglas windows to allow in light. Incubators contained adjustable thermostats and were lined on the bottom with heat tape (Raffel et al., 2013). Incubators were stored in a GR48 environmental chamber (Environmental Growth Chambers, Chagrin Falls, OH;

TABLE 1 Year of decline (last observation for a minimum of 10 years) and IUCN conservation status for 66 *Atelopus* species

Species	Year	Status	Species	Year	Status	Species	Year	Status
A. andinus	2004	CE	*A. franciscus*	none	VU	*A. pictiventris*	1996	CE
A. angelito	2000	CE	*A. glyphus*	none	CE	*A. pinangoi*	1997	CE
*A. arsyecue**	1991	CE	*A. guanujo*	1988	CE	*A. planispina*	1985	CE
A. arthuri	1988	CE	*A. guitarraensis**	1990	DD	*A. pulcher*	2004	CE
A. balios	none	CE	*A. halihelos*	1984	CE	*A. quimbaya*	1997	CE
A. bomolochos	2002	CE	*A. ignescens*	1988	EX	*A. sanjosei**	1988	DD
A. boulengeri	1984	CE	*A. laetissimus*	1992	EN	*A. seminiferus*	none	CE
*A. carauta**	1987	DD	*A. limosus*	none	EN	*A. senex*	1986	CE
A. carbonerensis	1998	CE	*A. longirostris*	1989	EX	*A. sernai*	2001	CE
A. carrikeri	1994	EN	*A. lozanoi*	1997	EN	*A. simulatus*	2003	CE
A. certus	2003	EN	*A. lynchi*	1984	CE	*A. siranus**	1988	DD
A. chiriquiensis	1996	CE	*A. mandingues**	1989	DD	*A. sonsonensis*	1996	CE
A. chocoensis	1998	CE	*A. mindoensis*	1989	CE	*A. sorianoi*	1990	CE
*A. chrysocoralis**	1988	CE	*A. minutulus*	1985	CE	*A. spurrelli*	none	NT
A. coynei	1984	CE	*A. mucubajiensis*	1994	CE	*A. subornatus*	1993	CE
A. cruciger	1986	CE	*A. muisca*	1996	EN	*A. tamaense*	1987	CE
*A. dimorphus**	1980	EN	*A. nahumae*	1992	EN	*A. tricolor*	none	VU
A. elegans	1994	CE	*A. nanay*	1989	CE	*A. varius*	none	CE
A. eusebianus	2005	CE	*A. nepiozomus*	1985	CE	*A. walkeri**	1992	DD
A. exiguus	none	CE	*A. oxyrhynchus*	1994	CE	*A. zeteki*	2005	CE
A. famelicus	1994	CE	*A. pachydermus*	1995	CE			
A. farci	1995	CE	*A. peruensis*	1992	CE			
A. flavescens	none	VU	*A. petriruizi**	1998	CE			

Note. Species with asterisks were excluded from our survival model because they lacked the sufficient sampling effort for us to confidently assess a date of decline. Species with "none" below year of decline were surveyed multiple times without failure to appear in multiple consecutive surveys. IUCN classifications for each species are coded as follows: CE, critically endangered, DD, data deficient, EN, endangered, EX, extinct, NT, near threatened, VU, vulnerable.

chamber was set to 14°C ± 0.5°C throughout the experiments; Supporting Information Figure S3). Temperatures inside incubators were monitored using 15 Hobo pendant temperature/light data loggers that were rotated daily (Onset Computer Corporation, Pocasset, MA).

2.2 | Temperature gradient experiment

Atelopus zeteki (n = 8/treatment in each temporal block) were exposed to 3 ml of 1.25×10^5 zoospores/ml of Panamanian *B. dendrobatidis* isolate JEL 423 in 1% tryptone broth at one of five temperature treatments (14°, 18°, 22°, 26°, and 28°C) in two temporal blocks. *Batrachochytrium dendrobatidis* was directly pipetted on the backs of the frogs and the extra inoculate collected in the bottom of their containers until a container change after 24 hr. Control animals (temporal block 2 only) received a sham exposure of 3 ml 1% tryptone. Before exposure, all animals were acclimated to their temperature treatment for 2 weeks to avoid confounding exposure to a pathogen with a simultaneous exposure to a new temperature. *Batrachochytrium dendrobatidis* inoculates were also acclimated to each temperature for 12 hr, were counted using a hemocytometer, and were diluted with 1% tryptone to equal concentrations before exposure. At the time of host exposure, two 8 ml *B. dendrobatidis* cultures containing 1.25×10^5 zoospores were placed in each incubator after acclimating to that temperature for 12 hr and being counted and diluted with 1% tryptone to target concentrations.

Animals were swabbed for infection at 1, 2, and 4 weeks after exposure or on date of death, swabs were frozen at −80°C, and quantitative PCR (qPCR; Boyle, Boyle, Olsen, Morgan, & Hyatt, 2004) was used to determine *B. dendrobatidis* quantities on the swabs. Mortality was monitored daily. After 4 weeks, frogs were swabbed, weighed, and euthanized with buffered MS-222. Cultures were nondestructively sampled (1 ml) and measured at 1 and 2 weeks using manual counts with a hemocytometer (temporal block 1) or a spectrophotometer (block 2). Different methods of quantifying cultures were used because our spectrophotometer was in repair during block 1, but differences in the temperature-dependent growth curves obtained using the two methods were not observed (Supporting Information Figure S4).

The experiment was conducted over two temporal blocks because initially we did not expect rapid mortality and did not have unexposed control frogs present. We had originally intended to

compare *B. dendrobatidis* load intensity across temperature and did not plan to consider mortality. See Supporting Information Figure S4 for a comparison of the results across temporal blocks.

2.3 | Analyses for temperature gradient experiment

To quantify *B. dendrobatidis* growth in culture, we fit a logistic growth model to mean *B. dendrobatidis* optical density or manual counts across each time point within each temperature treatment (assuming no growth at t0; bbmle package; Bolker, 2014; mle2 function). We then fit Johnson–Lewin (Equation (1); a derivative of the Sharpe–Schofield model; Dell, Pawar, & Savage, 2011) and Weibull (Equation (2); Angilletta, 2006) growth models to *B. dendrobatidis* growth rates (*r* parameter from logistic growth fits) across temperatures (bbmle package; Bolker, 2014; mle2 function).

$$h(T) = \frac{c e^{-\frac{E}{kT}}}{1 + e^{-\frac{1}{kT}\left(E_D - \left(\frac{E_D}{T_{opt}} + k \ln\left(\frac{E}{E_D - E}\right)\right)T\right)}} \quad (1)$$

$$P = a\left(\frac{d-1}{d}\right)^{\frac{1-d}{d}}\left[\frac{T-b}{c} + \left(\frac{d-1}{d}\right)^{\frac{1}{d}}\right]^{d-1} e^{-\left(\left(\frac{T-b}{c}\right) + \left(\frac{d-1}{d}\right)^{\frac{1}{d}}\right)^{d}} + \frac{d-1}{d} \quad (2)$$

These models produce asymmetrical temperature performance curves that do not fall below zero on the *y*-axis as well as parameter estimates for temperature of peak growth (T_{opt} for Johnson–Lewin; *b* for Weibull). To determine peak *B. dendrobatidis* growth of each isolate, we compared the AICcs of both models and chose the peak growth parameter of the better performing model. Profiling (stats package, profile function) was used to determine error for the T_{opt} parameter.

To determine the temperature-dependent pattern of *B. dendrobatidis* growth on frogs, logistic growth curves were fit to infection loads [log((load +1)/frog mass)] on individual frogs across both temporal blocks via maximum likelihood (All analyses were conducted in R 3.1.0 unless otherwise indicated; mle2 function, bbmle package, Bolker, 2014, assuming a normal error distribution). The mean *r* (-growth rate) parameter was calculated for growth across all temperatures. Growth rates were fit to temperature (mle2 function, bbmle package, Bolker, 2014, same assumptions as above) using an exponential model. To determine temperature of peak parasite growth on frogs, Johnson–Lewin curves were fit to the growth rates across temperature plus an additional point, the CT_{max} for *A. zeteki* (~30°C; pers. comm., Maryland Zoo) where parasite growth is assumed to be zero, so that the pattern would be unimodal and a T_{opt} could be identified. Profiling was again used to determine T_{opt} error (confidence intervals). This method was also applied to determine peak *B. dendrobatidis* growth and error for *Osteopilus septentrionalis* and *Anaxyrus terrestris* data reported previously (Cohen et al., 2017; Figure 1c). For these species, we fit Johnson–Lewin curves to *B. dendrobatidis* growth data on animals from that study as well as the CT_{min} for each species (~10°C for *O. septentrionalis* based on personal experience and ~6.1°C for *A. terrestris* based on CT_{min} in a related species; Johnson, 1972). Although the CT_{min} and CT_{max} values we used are not necessarily accurate, *a* ± 3°C shift in these values only resulted in *a* < 1°C shift in T_{opt} for any species. To examine the relationship between frog mortality, *B. dendrobatidis* exposure, and temperature, Cox proportional hazards models were fit with temperature and *B. dendrobatidis* exposure as either noninteracting or interacting fixed effects, with mortality data censored after day 28 (coxph function, survival package, Therneau, 2014). Data from both temporal blocks were combined for all analyses (controlling for block as a random effect), but see Supporting Information Figure S5 for results from each individual block.

2.4 | Temperature shift experiment

Atelopus zeteki were exposed to 10^5 zoospores of *B. dendrobatidis* (in 1 ml 1% tryptone broth, acclimated to 20°C for 12 hr) at 14°C, 17°C, 23°C, or 26°C after acclimating to either 20°C (shifted group) or their exposure temperature (constant group; *n* = 5 per temperature per group) for 2 weeks. Each incubator contained one animal from the shifted group and one from the constant group. Methods for the production, counting, diluting, and exposure of *B. dendrobatidis* inoculates were identical to those in the previous experiment. Nonexposed frogs were not included in the experiment because not enough frogs were available and because our primary goal was to compare infection loads and mortality between shifted and constant animals at the same after exposure temperatures. Frogs were swabbed 2 and 4 weeks after *B. dendrobatidis* exposure, and infections were quantified as described above. Mortality was monitored for 6 weeks after exposure, after which the experiment was terminated and the animals were weighed and euthanized as described above. Although we acknowledge the lack of ecological realism in this design, we emphasize its elegance in getting at the impacts of a single component of variation. Additionally, it is rare for studies to simultaneously evaluate the effects of both changes to the mean and variance of temperature on responses, which is an advantage of our approach.

2.5 | Analyses for temperature shift experiment

The effects of temperature shifts on mortality were tested by fitting Cox proportional hazards models with temperature and temperature shift (a categorical variable designating whether animals were in the shifted or constant groups) as interacting fixed effects (survival package, coxph function; Therneau, 2014), with mortality censored after day 42. The relative contributions of temperature and temperature shift to mortality risk were evaluated by calculating Cox & Snell pseudo-R^2 for models with all possible combinations of predictors. To test for the effects of temperature shift on mass-adjusted log-*B. dendrobatidis* load, an ANOVA was conducted with temperature and IUCN status as interacting fixed effects, including loads from week 2 and week 4 as response variables in separate models.

2.6 | Survival model predicting atelopus declines

Given the results of our two laboratory experiments and other experiments that suggest that *B. dendrobatidis* infection dynamics are

highly dependent on host species (Figure 1c; Cohen et al., 2017), we hypothesized that *B. dendrobatidis* growth in culture, temperature variability, and mean temperature alone would be relatively poor predictors of *Atelopus* declines in the wild compared to the *thermal mismatch hypothesis*. In this context, the hypothesis posits that as temperature increases, disease and decline risk should be most pronounced among *Atelopus* spp. from cooler environments, which experience a larger performance gap relative to *B. dendrobatidis* than species from warmer environments (Figure 1a). This prediction of the *thermal mismatch hypothesis* would manifest as a statistical interaction between the temperature to which a species is adapted (50-year mean temperature in a species' geographic range) and the level of climate change it has experienced because cool-adapted species should experience disease-associated declines when temperatures increase, whereas warm-adapted species should not.

We used the IUCN red list database (www.iucnredlist.org) to collect date of decline, conservation status, and level of survey effort for 66 *Atelopus* spp. in Latin America (Table 1). Date of decline was defined as the last date a species was observed without any additional observations over a period of ≥10 years from a minimum of two additional attempts at surveying (thus, no dates later than 2008 were considered). We excluded 10 *Atelopus* spp. from our analyses because sampling effort for these species was insufficient (<2 attempts at surveying following the last observation of the species; see Table 1).

We extracted monthly mean temperature and mean precipitation (extract function, raster package, Hijmans, 2014) between 1951 and the present, averaged within the IUCN ranges of each of the remaining 56 *Atelopus* spp (crop function, raster package, Hijmans, 2014). All climate data were sourced from the Climate Research Unit 3.1 rasters (Climate Research Unit 3.1, University of East Anglia, Harris, Jones, Osborn, & Lister, 2014) with a spatial resolution of $0.5°^2$ cells (approx. 55×55 km at the equator). These data are sourced from weather stations and are the highest resolution historic monthly climate data that are currently available. The resolution of these data is also several orders of magnitude finer than any data previously used in analyses of *Atelopus* spp. declines (Pounds et al., 2006; Rohr & Raffel, 2010). Although precipitation levels vary widely within cells of this size across elevational gradients, precipitation was not the focus of our analyses and was only used as a covariate in the models.

To test our hypotheses, we fit a time-dependent Cox proportional hazards model (coxph function, survival package, Therneau, 2014), which nests multiple time intervals per subject (species), to determine how climate variables predicted *Atelopus* spp. dates of decline. The model concurrently evaluated the following predictors of the occurrence and timing of declines: *B. dendrobatidis* growth in culture, temperature variability, mean temperature, climate change, and the *thermal mismatch hypothesis*. For each *Atelopus* spp., we included the following as covariates: geographic range size (based on IUCN range size estimates), long-term (1951–1990) mean temperature in the geographic range, mean altitude in the range, annual mean temperature of each year, slope in temperature over the previous 5 years (recent temperature shift) for each year from 1955 on, mortality probability based on the temperature-dependent *B. dendrobatidis* growth curve in culture (the predicted value of *B. dendrobatidis* growth based on each year's mean temperature and the Johnson–Lewin fit to temperature-dependent *B. dendrobatidis* growth in the *Temperature Gradient Experiment*), annual total precipitation for each year, the log-transformed summed absolute value of the differences in month-to-month temperatures (AVMD) each year (an index of climatic variability; see Rohr & Raffel, 2010), and one of five spatial covariates designed to represent the spatial spread of *B. dendrobatidis* in the region (see below). Our model also included the three-way interaction between log-transformed range size, recent temperature shift, and long-term mean temperature. Mortality probability based on *B. dendrobatidis* growth in culture, AVMD, and annual mean temperature were each included in two-way interactions with range size because they represented competing hypotheses to explain *Atelopus* spp. extinctions: the influence of *B. dendrobatidis* growth in isolation, temperature variability, and simple mean temperature, respectively. Date of decline was included in the model in the form of a binomial variable defining whether a given species declined each year, and the model censored all years after the date of decline. No species were assigned multiple dates of declines.

To test for the effects of spatial spread of *B. dendrobatidis* on *Atelopus* spp. declines in our model, we included one of five possible spatial predictors as fixed effects, calculated for each species and year: (a) the inverse of the minimum Euclidean distance to a decline in the previous year (distances were calculated with species range centroids using the *spDists* function, *sp* package); (b) the inverse of the minimum Euclidean distance to a decline in the previous year multiplied by the range size of the species that declined; (c) the inverse of the minimum Euclidean distance to a decline in the previous 2 years, (d) the inverse of the minimum Euclidean distance to a decline in the previous 2 years multiplied by the range size of the species that declined, and (e), which is the same as (d) but with the declines from 2 years prior down-weighted by 50% relative to those from the previous year. Models 2, 4, and 5 are analogous to gravity models often used to estimate the level of interaction between two points that traditionally incorporate the distance between the points and the mass of each point (in our case, range size). Finally, we fit a sixth model that contained no distance covariate. We acknowledge that sites that are close to one another might have a higher likelihood of being sampled in the same year than sites that are far apart, which could lead to spatial/temporal autocorrelation in declines using this method.

We evaluated the model by calculating Nagelkerke's pseudo-R^2 using likelihood values from (a) the null model, (b) the model containing all variables except those that contribute to the *thermal mismatch hypothesis* (including range size, mortality probability based on growth of *B. dendrobatidis* in culture, annual mean temperature, temperature variability, altitude, total precipitation, and wet day frequency), and (c) the full model. For data visualization, we used the *visreg* package in R (visreg function, Breheny & Burchett, 2012).

3 | RESULTS

3.1 | Temperature gradient experiment

Temperature did not affect *A. zeteki* mortality in the absence of *B. dendrobatidis* (Cox proportional hazards model: χ^2=0.54, p = 0.46), but mortality increased significantly with temperature when *A. zeteki* was exposed to *B. dendrobatidis* (*B. dendrobatidis* × temperature: χ^2 = 4.41, p = 0.036). In fact, within a week of exposure to *B. dendrobatidis*, frogs at 26° and 28°C experienced 69% and 78% mortality, respectively, whereas only one *B. dendrobatidis*-exposed animal died at the two coldest temperatures within a week of exposure (6% mortality) and only four *B. dendrobatidis*-negative animals died throughout the experiment (20% mortality; Figure 2; Supporting Information Figure S4). Similarly, growth rates of *B. dendrobatidis* on frogs increased with temperature (Figure 2a). In contrast, temperature-dependent *B. dendrobatidis* growth in culture closely followed previously reported patterns with growth rates increasing as temperature increased until 18.0°C (optimum) and then decreasing thereafter, with little growth above 26°C (Woodhams, Alford, Briggs, Johnson, & Rollins-Smith, 2008; Figure 2a; Supporting Information Figure S5).

3.2 | Temperature shift experiment

Batrachochytrium dendrobatidis-induced mortality also increased linearly with temperature (Cox proportional hazards model and ANOVA: χ^2 = 4.08, p < 0.05; Supporting Information Table S2). At the same *B. dendrobatidis* exposure temperatures, frogs that experienced temperature shifts had higher *B. dendrobatidis* loads than those that did not experience shifts (ANOVA: $F_{1,34}$ = 8.78, p = 0.005), consistent with the findings of previous studies (Raffel et al., 2013; Raffel, Halstead, McMahon, Davis, & Rohr, 2015). However, we did not observe any significant effect of the temperature shift treatment on mortality (Shift treatment: χ^2 = 0.84, p = 0.36; Shift × temperature: χ^2 = 1.03, p = 0.31), and the temperature gradient (i.e., mean temperature) accounted for >6 times the variance in *B. dendrobatidis*-induced mortality as temperature variability (Supporting Information Table S3).

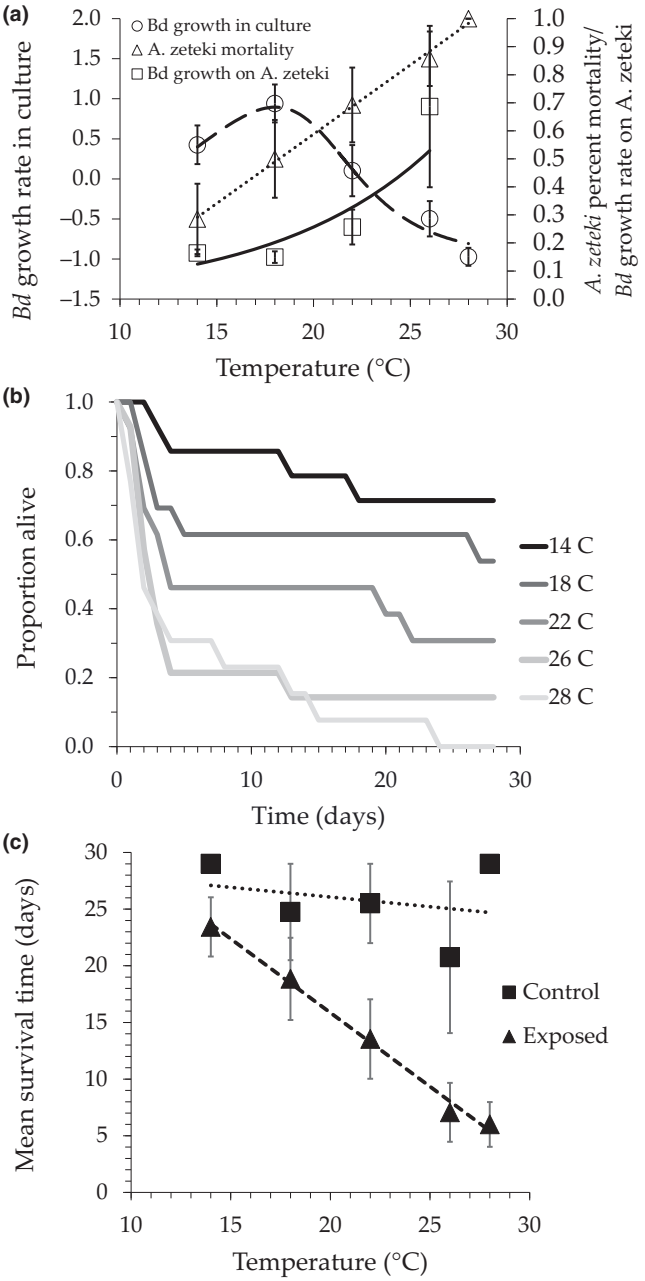

FIGURE 2 Temperature-dependent patterns of *Batrachochytrium dendrobatidis* growth and *Atelopus zeteki* mortality. *A. zeteki* experienced high mortality (triangles, dotted line) and high *B. dendrobatidis* growth (squares, solid line) at warm temperatures after *B. dendrobatidis* exposure (a), even though *B. dendrobatidis* growth rates in culture (circles, dashed line) were low at these temperatures (see Figure S4). We could not measure *B. dendrobatidis* growth rates on *A. zeteki* at 28°C because very few animals survived long enough to be tested multiple times. Mortality over time was highly dependent on temperature for *A. zeteki* exposed to *B. dendrobatidis* across two temporal blocks (b). Mean survival time for *A. zeteki* at each of five temperatures when exposed to *B. dendrobatidis* is given in (c) (triangles; both temporal blocks) or not exposed (squares; 2nd temporal block). Temperature and *B. dendrobatidis* exposure interacted to induce high mortality in *A. zeteki* (χ^2 = 4.41, p = 0.036). Animals surviving the experiment are conservatively assumed to have died on day 29 for these figures only but were censored in the survival analysis. Error bars represent SEMs in all panels

3.3 | Atelopus Species Declines

In the survival models, neither precipitation, altitude, nor any of our five measures of spatial spread explained significant variation in the risk of *Atelopus* spp. declines (Table 2; Supporting Information Table S4; see Supporting Information for a discussion on why we might not have detected spread). Although the results were highly consistent across all six models (five containing a covariate representing spatial spread, and one without spatial spread), we highlight

Model 2 (the gravity model containing minimum distance to a decline * range size of the declining species) in our results because it had the lowest AIC. Consistent with our experiments, the risk of *Atelopus* spp. declines was not significantly explained by *B. dendrobatidis* growth in culture or temperature variability. However, when interacting with range size, both climate change (range size × temperature shift; $\beta = -17.5$, $df = 16$, $p = 0.0062$; Table 2) and the *thermal mismatch hypothesis* (range size × temperature shift × 40-year mean temperature; $\beta = 0.88$, $df = 16$, $p = 0.0302$; Table 2; Figure 3a) were significant predictors of *Atelopus* spp. decline risk. More specifically, climate change (positive slope of temperature across 5 years) significantly increased the risk of *Atelopus* declines, but only for relatively cool-adapted *Atelopus* spp. and most often in species from relatively small ranges (because species with large range sizes were less likely to experience declines). However, climate change did not predict the occurrence and timing of declines of relatively warm-adapted species (Figure 3b). The model testing the *thermal mismatch hypothesis* explained about 2.5 times more of the variance in declines than a model that did not contain the interaction (Nagelkerke's pseudo-R^2 = 0.406 and 0.151, respectively).

4 | DISCUSSION

Our experiments and analyses of field data are both consistent with the predictions of the *thermal mismatch hypothesis*, which suggests that amphibians from cooler environments are more vulnerable to mortality from chytridiomycosis under warmer conditions than those from warmer environments. Although *A. zeteki*, a species native to intermediate elevations (~150–1,000 m), does not perfectly represent all *Atelopus* spp. and might have different physiology than higher-elevation species, our models suggest that these higher-elevation species are likely to be even more sensitive to disease at warm temperatures than *A. zeteki*, because they should be more cool-adapted. In other words, the pattern we observed in our experiments of high susceptibility after *B. dendrobatidis* exposure at high temperatures we observed in our experiments is likely to be reflected in other *Atelopus* spp, which are almost all native to cooler environments than *A. zeteki*. In support of this assertion, the *thermal mismatch hypothesis* was supported across 598 globally distributed populations from 235 amphibian species (Cohen et al., 2017), suggesting consistency across a wide range of populations and species.

Our experimental results demonstrate that patterns of temperature-dependent *B. dendrobatidis* performance in culture and on hosts differ sharply, a result consistent with the *thermal mismatch hypothesis* and with previous work demonstrating that warm-adapted hosts are most susceptible to *B. dendrobatidis* at cooler temperatures than those at which *B. dendrobatidis* grows best in culture (Cohen et al., 2017; Figure 1c,d). The striking monotonic positive association between temperature and both *B. dendrobatidis* growth on frogs and *B. dendrobatidis*-induced host mortality contradict a common assumption that *B. dendrobatidis* outbreaks only occur at cool or moderate temperatures (Berger et al., 2016; Kilpatrick et al., 2010). We witnessed a strong temperature-dependent "cost of exposure," or cost associated with pathogen exposure rather than infection, among *A. zeteki* in the *Temperature Gradient Experiment* (Figure 2b). Rapid mortality occurred in the two warmest temperature treatments (26° and 28°C) within a week of parasite exposure despite infection protocols that were similar to many previous studies in our laboratory and the literature (i.e., dose of 3.75×10^5 live *B. dendrobatidis* zoospores). However, when we reduced the dose to 10^5 live zoospores during the *Temperature Gradient Experiment*, mortality did not occur rapidly and instead mostly occurred 3–6 weeks after exposure. Strangely, Bustamante, Livo, and Carey (2010) did not report rapid mortality in *A. zeteki* following infections; however, animals were administered with antibiotics during infection, an atypical practice in *Bd* experiments. (antibiotics have been shown to fight Bd infections; Bishop et al., 2009; Bell, Alford, Garland, Padilla, & Thomas, 2013). A cost of exposure to *B. dendrobatidis* has previously been observed in several other host species. For example, *Osteopilus septentrionalis* exposed to *B. dendrobatidis* but not infected had a higher risk of mortality than those never exposed (Rohr et al, 2013) and *B. dendrobatidis* metabolites alone were deadly to crayfish hosts (McMahon et al., 2013).

Our analysis of *Atelopus* spp. declines does not contain data on *B. dendrobatidis* occurrence because no such detailed spatiotemporal infection data exist for many of these remote, poorly understood species with few or no remaining individuals. Instead, we assumed the presence of *B. dendrobatidis* during each of the declines because the pathogen was documented spreading throughout the region during the same period of time (La Marca et al., 2005; Lips et al., 2008). Thus, the results of our survival analysis are consistent with the *thermal mismatch hypothesis*, but we cannot rule out the influence of climate alone as the primary driver of declines. Despite the lack of disease data, it is unlikely that climate change alone could directly cause the 48 *Atelopus* spp. declines observed between 1973 and 2005, as very few disappearances occurred between 1950 and 1973 before chytrid was known to be spreading throughout the area (La Marca et al., 2005).

thermal mismatch hypothesis is unknown, as it has only been evaluated in amphibian systems with directly transmitted pathogens, which might be especially sensitive to environmental conditions. For example, the hypothesis might be less applicable or testable for hosts with body temperatures that do not consistently reflect air temperatures, such as endotherms. Further, terrestrial hosts might be more vulnerable to disease following thermal mismatches than aquatic hosts because they are exposed to air, which fluctuates in temperature more than water. Finally, hosts with larger body sizes might be more sensitive to disease after thermal mismatches than smaller hosts because they have narrower thermal breadths and thus greater performance gaps relative to parasites at nonoptimal temperatures. Additional large-scale analyses of disease datasets are needed to test how well the *thermal mismatch hypothesis* applies across host–parasite systems that vary in host thermal biology and mode of transmission.

As global temperatures and infectious disease outbreaks have increased, these two crises have been repeatedly associated by

TABLE 2 Results of time-dependent Cox proportional hazards model predicting the year of decline of 56 *Atelopus* spp. with a three-way interaction between log-transformed range size, long-term mean temperature (40 year.meantemp), and recent temperature shift (tempchange)

Effect	Coefficient	SE	Robust SE	*z*	*p*
Rangesize	0.79	6.22	2.78	0.28	0.776
Culturemortprob (pathogen only)	0.09	0.42	0.14	0.66	0.506
Log(AVMD; temp. variability)	0.33	0.64	0.58	0.56	0.574
Tempchange (climate change only)	7.73	5.67	4.54	1.70	0.088
40 yr.meantemp (cold- or warm-adapted)	0.01	0.27	0.16	0.09	0.931
Meantemp (mean temp. only)	<0.01	0.27	0.16	0.01	0.995
Logaltitude	0.52	0.77	0.34	1.52	0.128
Total precipitation	<0.01	<0.01	<0.01	0.21	0.834
Distance from nearby extinctions	1.17	0.69	0.83	1.41	0.158
Rangesize:Culturemortprob	1.06	1.58	0.64	1.64	0.101
Rangesize:log(AVMD)	−0.76	1.14	0.68	−1.12	0.261
Rangesize:Tempchange	−17.50	14.20	6.40	−2.74	0.006*
Rangesize:40 yr.meantemp	<0.01	0.44	0.22	0.03	0.977
Tempchange:40 yr.meantemp	−0.31	0.30	0.25	−1.24	0.215
Rangesize:meantemp	0.02	0.47	0.23	0.07	0.942
Rangesize:Tempchange:40 yr.meantemp	0.88	0.84	0.41	2.17	0.030*

Note. Mortality probability based on *B. dendrobatidis* growth in culture (culturemortprob), log-transformed altitude (logaltitude), annual mean temperature (meantemp), a measure of temperature variability (log-transformed AVMD, absolute value of monthly difference in temperature), and a distance covariate representing distance from other declines in the previous year * range size of the declining species were also included. The bolded line represents the test of the *thermal mismatch hypothesis*. Statistical significance is noted with asterisks.

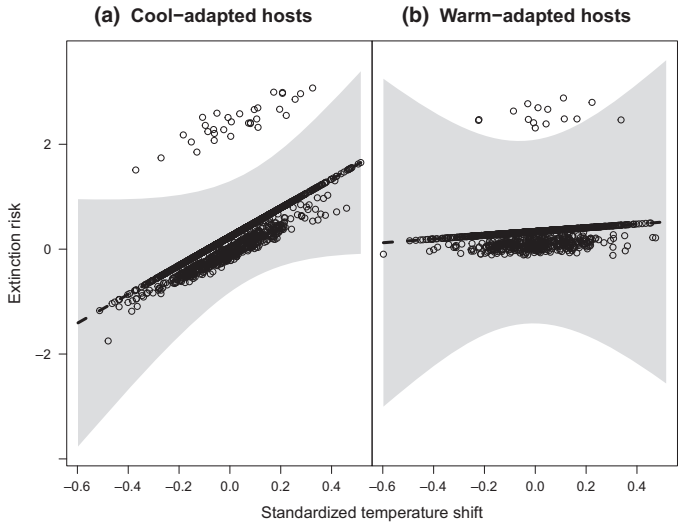

FIGURE 3 Partial residual plot displaying the effects of climate change and annual mean temperature on the decline risk of cool- and warm-adapted *Atelopus* spp. The partial residuals are from the time-dependent Cox proportional hazards model shown in Table 2 and display the significant two-way interaction between 5-year slopes in mean temperature-by-40-year mean temperature. Points represent individual years for each species and gray shading shows associated 95% confidence bands. The model suggests that species from typically cooler climates (a) were at greater risk of a decline (log-odds ratio; *y*-axis) after experiencing climate change (warming, or positive 5-year slope in mean temperature; *x*-axis) than species from warmer climates (b). Breaks are based on 20th and 80th percentiles. Because the response variable in a survival model is essential whether or not the species declined in a given year (coded as 1's and 0's), and there are far more years in which a species did not decline than years in which one did, the mean is close to zero and the residuals cluster slightly below the line (0's) or well above it (1's)

researchers to explain species declines. However, evidence that they interact to cause declines has been elusive, possibly because researchers have tried to merely correlate increases in temperature with infectious disease, rather than looking for more nuanced patterns that depend on the host–parasite interaction (Rohr et al., 2011). Here, we combine experiments with field patterns to examine how mean temperature and temperature variability impact susceptibility to *B. dendrobatidis* in the amphibian genus *Atelopus*, and our findings are consistent with the hypothesis that climate change and infectious disease interacted to drive widespread amphibian declines, especially in cool-adapted species. Recently, some of the *Atelopus* spp. that declined have evolved resistance to *B. dendrobatidis* and now have increasing population growth rates (Voyles et al., 2018). However, it takes time to evolve resistance, and cool-adapted species with restricted ranges might not have enough time to adapt before they are driven extinct by a combination of disease and climate change. Thus, it is important to understand the environmental conditions and traits of hosts that make them particularly susceptible to disease and the rates of both climate change and host adaptations.

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AUTHOR CONTRIBUTIONS

All authors contributed ideas. J.M.C. and M.D.V. wrote proposals to acquire animals. J.M.C., M.D.V., and T.A.M. conducted disease experiments. J.M.C. assembled climate database. J.M.C., D.J.C., and J.R.R. conducted statistical analyses. J.M.C. and J.R.R. wrote the paper, and all authors provided editorial advice.

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REFERENCES

Angilletta, M. J. Jr (2006). Estimating and comparing thermal performance curves. *Journal of Thermal Biology*, *31*(7), 541–545. https://doi.org/10.1016/j.jtherbio.2006.06.002

Baas-Becking, L. G. M. (1934). *Geobiologie; of inleiding tot de milieukunde*. WP Van Stockum &. Zoon NV.

Bell, S. C., Alford, R. A., Garland, S., Padilla, G., & Thomas, A. D. (2013). Screening bacterial metabolites for inhibitory effects against Batrachochytrium dendrobatidis using a spectrophotometric assay. *Diseases of Aquatic Organisms*, *103*(1), 77. https://doi.org/10.3354/dao02560

Ben-Horin, T., Lenihan, H. S., & Lafferty, K. D. (2013). Variable intertidal temperature explains why disease endangers black abalone. *Ecology*, *94*(1), 161–168. https://doi.org/10.1890/11-2257

Berger, L., Roberts, A. A., Voyles, J., Longcore, J. E., Murray, K. A., & Skerratt, L. F. (2016). History and recent progress on chytridiomycosis in amphibians. *Fungal Ecology*, *19*, 89–99. https://doi.org/10.1016/j.funeco.2015.09.007

Bishop, P. J., Speare, R., Poulter, R., Butler, M., Speare, B. J., Hyatt, A., … Haigh, A. (2009). Elimination of the amphibian chytrid fungus Batrachochytrium dendrobatidis by Archey's frog Leiopelma archeyi. *Diseases of Aquatic Organisms*, *84*(1), 9–15. https://doi.org/10.3354/dao02028

Bolker, B. (2014). *Bbmle: Tools for general maximum likelihood estimation*. R package version 1.0.16.

Boyle, D. G., Boyle, D. B., Olsen, V., Morgan, J. A., & Hyatt, A. D. (2004). Rapid quantitative detection of chytridiomycosis (Batrachochytrium dendrobatidis) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms*, *60*(2), 141–148. https://doi.org/10.3354/dao060141

Breheny, P., & Burchett, W. (2012). *Visreg: Visualization of regression models*. R package version 2.

Bustamante, H. M., Livo, L. J., & Carey, C. (2010). Effects of temperature and hydric environment on survival of the Panamanian Golden Frog infected with a pathogenic chytrid fungus. *Integrative Zoology*, *5*(2), 143–153. https://doi.org/10.1111/j.1749-4877.2010.00197.x

Cahill, A. E., Aiello-Lammens, M. E., Fisher-Reid, M. C., Hua, X., Karanewsky, C. J., Yeong Ryu, H., … Wiens, J. J. (2013). How does climate change cause extinction? *Proceedings of the Royal Society B-Biological Sciences*, *280*(1750), 20121890. https://doi.org/10.1098/rspb.2012.1890

Clare, F. C., Halder, J. B., Daniel, O., Bielby, J., Semenov, M. A., Jombart, T., … Fisher, M. C. (2016). Climate forcing of an emerging pathogenic fungus across a montane multi-host community. *Philosophical Transactions of the Royal Society B-Biological Sciences*, *371*(1709), 20150454. https://doi.org/10.1098/rstb.2015.0454

Cohen, J. M., Venesky, M. D., Sauer, E. L., Civitello, D. J., McMahon, T. A., Roznik, E. A., & Rohr, J. R. (2017). The thermal mismatch hypothesis explains outbreaks of an emerging infectious disease. *Ecology Letters*, *20*(2), 184–193. https://doi.org/10.1111/ele.12720

Collins, J. P. (2013). History, novelty, and emergence of an infectious amphibian disease. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(23), 9193–9194. https://doi.org/10.1073/pnas.1305730110

Dell, A. I., Pawar, S., & Savage, V. M. (2011). Systematic variation in the temperature dependence of physiological and ecological traits. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(26), 10591–10596. https://doi.org/10.1073/pnas.1015178108

Harris, I., Jones, P. D., Osborn, T. J., & Lister, D. H. (2014). Updated high-resolution grids of monthly climatic observations – the CRU TS3.10 Dataset. *International Journal of Climatology*, *34*(3), 623–642. https://doi.org/10.1002/joc.3711

Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S., & Samuel, M. D. (2002). Ecology - Climate warming and disease risks for terrestrial and marine biota. *Science*, *296*(5576), 2158–2162. https://doi.org/10.1126/science.1063699

Hijmans, R. J. (2014). *Raster: Geographic data analysis and modeling*. R package version, 2.2.31.

IUCN (2018). *The IUCN red list of threatened species*. Version 2018–1.

Johnson, C. R. (1972). Thermal relations and daily variation in the thermal tolerance in Bufo marinus. *Journal of Herpetology*, *31*, 35–38.

Kilpatrick, A. M., Briggs, C. J., & Daszak, P. (2010). The ecology and impact of chytridiomycosis: An emerging disease of amphibians. *Trends in Ecology & Evolution*, *25*(2), 109–118. https://doi.org/10.1016/j.tree.2009.07.011

LaMarca, E., Lips, K. R., Lotters, S., Puschendorf, R., Ibanez, R., Rueda-Almonacid, J. V., … Young, B. E. (2005). Catastrophic population declines and extinctions in neotropical harlequin frogs (Bufonidae : Atelopus). *Biotropica*, *37*(2), 190–201. https://doi.org/10.1111/j.1744-7429.2005.00026.x

Lafferty, K. D. (2009). The ecology of climate change and infectious diseases. *Ecology*, *90*(4), 888–900. https://doi.org/10.1890/08-0079.1

Lips, K. R., Diffendorfer, J., Mendelson, J. R., & Sears, M. W. (2008). Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. *Plos Biology*, *6*(3), 441–454. https://doi.org/10.1371/journal.pbio.0060072

Liu, X., Rohr, J. R., & Li, Y. (2013). Climate, vegetation, introduced hosts and trade shape a global wildlife pandemic. *Proceedings of the Royal Society B-Biological Sciences*, *280*(1753), 20122506. https://doi.org/10.1098/rspb.2012.2506

McMahon, T. A., Brannelly, L. A., Chatfield, M. W., Johnson, P. T., Joseph, M. B., McKenzie, V. J., … Rohr, J. R. (2013). Chytrid fungus Batrachochytrium dendrobatidis has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(1), 210–215. https://doi.org/10.1073/pnas.1200592110

Pascual, M., Chaves, L., Cash, B., Rodó, X., & Yunus, M. (2008). Predicting endemic cholera: The role of climate variability and disease dynamics. *Climate Research*, *36*(2), 131–140. https://doi.org/10.3354/cr00730

Patz, J. A., Campbell-Lendrum, D., Holloway, T., & Foley, J. A. (2005). Impact of regional climate change on human health. *Nature*, *438*(7066), 310–317. https://doi.org/10.1038/nature04188

Pounds, J. A., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P., Foster, P. N., … Young, B. E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, *439*(7073), 161–167. https://doi.org/10.1038/nature04246

Raffel, T. R., Halstead, N. T., McMahon, T. A., Davis, A. K., & Rohr, J. R. (2015). Temperature variability and moisture synergistically interact to exacerbate an epizootic disease. *Proceedings of the Royal Society B-Biological Sciences*, *282*(1801), 20142049. https://doi.org/10.1098/rspb.2014.2039

Raffel, T. R., Romansic, J. M., Halstead, N. T., McMahon, T. A., Venesky, M. D., & Rohr, J. R. (2013). Disease and thermal acclimation in a more variable and unpredictable climate. *Nature Climate Change*, *3*(2), 146–151. https://doi.org/10.1038/nclimate1659

Ribas, L., Li, M.-S., Doddington, B. J., Robert, J., Seidel, J. A., Kroll, J. S., & Fisher, M. C. (2009). Expression profiling the temperature-dependent amphibian response to infection by batrachochytrium dendrobatidis. *PLoS ONE*, *4*(12), e8408. https://doi.org/10.1371/journal.pone.0008408

Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., & Dell, A. I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecology letters*, *21*(9), 1425–1439.

Rohr, J. R., Dobson, A. P., Johnson, P. T. J., Kilpatrick, A. M., Paull, S. H., Raffel, T. R., … Thomas, M. B. (2011). Frontiers in climate change-disease research. *Trends in Ecology & Evolution*, *26*(6), 270–277. https://doi.org/10.1016/j.tree.2011.03.002

Rohr, J. R., & Raffel, T. R. (2010). Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(18), 8269–8274. https://doi.org/10.1073/pnas.0912883107

Rohr, J. R., Raffel, T. R., Halstead, N. T., McMahon, T. A., Johnson, S. A., Boughton, R. K., & Martin, L. B. (2013). Early-life exposure to a herbicide has enduring effects on pathogen-induced mortality. *Proceedings of the Royal Society B-Biological Sciences*, *280*(1772), 20131502. https://doi.org/10.1098/rspb.2013.1502

Rohr, J. R., Raffel, T. R., Romansic, J. M., McCallum, H., & Hudson, P. J. (2008). Evaluating the links between climate, disease spread, and amphibian declines. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(45), 17436–17441. https://doi.org/10.1073/pnas.0806368105

Stevenson, L. A., Alford, R. A., Bell, S. C., Roznik, E. A., Berger, L., & Pike, D. A. (2013). Variation in thermal performance of a widespread pathogen, the amphibian chytrid fungus batrachochytrium dendrobatidis. *PLoS ONE*, *8*(9), e73830. https://doi.org/10.1371/journal.pone.0073830

Therneau, T. (2014). *A package for survival analysis in S*. R package version 2.37.7.

Voyles, J., Johnson, L. R., Rohr, J., Kelly, R., Barron, C., Miller, D., … Rosenblum, E. B. (2017). Diversity in growth patterns among strains of the lethal fungal pathogen Batrachochytrium dendrobatidis across extended thermal optima. *Oecologia*, *184*(2), 363–373.

Voyles, J., Woodhams, D. C., Saenz, V., Byrne, A. Q., Perez, R., Rios-Sotelo, G., … Richards-Zawacki, C. L. (2018). Shifts in disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation. *Science*, *359*(6383), 1517–1519. https://doi.org/10.1126/science.aao4806

Woodhams, D. C., Alford, R. A., Briggs, C. J., Johnson, M., & Rollins-Smith, L. A. (2008). Life-history trade-offs influence disease in changing climates: Strategies of an amphibian pathogen. *Ecology*, *89*(6), 1627–1639. https://doi.org/10.1890/06-1842.1

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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