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LETTER

The thermal mismatch hypothesis explains host susceptibility to an emerging infectious disease

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Abstract

Parasites typically have broader thermal limits than hosts, so large performance gaps between pathogens and their cold- and warm-adapted hosts should occur at relatively warm and cold temperatures, respectively. We tested this *thermal mismatch hypothesis* by quantifying the temperature-dependent susceptibility of cold- and warm-adapted amphibian species to the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) using laboratory experiments and field prevalence estimates from 15 410 individuals in 598 populations. In both the laboratory and field, we found that the greatest susceptibility of cold- and warm-adapted hosts occurred at relatively warm and cool temperatures, respectively, providing support for the *thermal mismatch hypothesis*. Our results suggest that as climate change shifts hosts away from their optimal temperatures, the probability of increased host susceptibility to infectious disease might increase, but the effect will depend on the host species and the direction of the climate shift. Our findings help explain the tremendous variation in species responses to *Bd* across climates and spatial, temporal and species-level variation in disease outbreaks associated with extreme weather events that are becoming more common with climate change.

Keywords

Amphibian declines, amphibians, *Atelopus zeteki*, *Batrachochytrium dendrobatidis*, chytrid fungus, climate change, disease, disease ecology, host–parasite interactions, thermal biology.

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INTRODUCTION

One of the most important ecological crises affecting humans and biodiversity is the recent increase in emerging infectious diseases (Daszak 2000; Anderson *et al.* 2004; Jones *et al.* 2008). Since 1950, hundreds of emerging infectious diseases have been recorded (Jones *et al.* 2008), causing widespread declines of individual species and biodiversity in general (Daszak 2000; Skerratt *et al.* 2007; Fisher *et al.* 2012). Many infectious disease outbreaks are associated with extreme weather events, including outbreaks of malaria, dengue, cholera and amphibian and coral diseases (Cazelles *et al.* 2005; Koelle *et al.* 2005; Bruno *et al.* 2007; Pascual *et al.* 2008; Rohr & Raffel 2010). To combat these outbreaks, it is critical that researchers understand the precise environmental conditions that promote them, especially with rapidly changing climates and other sources of human-induced stress on wildlife populations (Rohr *et al.* 2011).

Although it is clear that extreme temperature events cause disease outbreaks, neither warm nor cold spells universally increase outbreaks (Rohr *et al.* 2011). Thus, more nuanced hypotheses regarding the effects of weather and climate on disease are necessary. For example, Nowakowski *et al.* (2016) recently argued that the degree of mismatch between critical thermal tolerances of hosts and parasites might drive disease outbreaks. Similarly, thermal mismatches in temperature optima can occur within host–parasite interactions when cold-adapted hosts and parasites experience warm spells and *vice versa*. Such mismatches may arise because temperatures experienced during extreme weather events fall outside the level of variability to which hosts and their parasites are adapted. If these mismatches are predictive of parasite transmission, they could partially explain spatial, temporal and species-level variation in outbreaks associated with extreme weather events that are becoming more common with climate change (Rosenzweig *et al.* 2001; Anyamba *et al.* 2014).

Here, we propose and test the *thermal mismatch hypothesis*, which posits that hosts should be more susceptible to parasites when environmental conditions shift away from the thermal optima of the host (Fig. 1). This hypothesis is based on a few assumptions. First, we assume that hosts and parasites are locally adapted to their thermal environments (Laine 2008; Sternberg & Thomas 2014). Although we show in Fig. 1 the temperature optima of the host and parasite to be identical because of local adaptation (Fig. 1a,b), we acknowledge that they can be different for several reasons (e.g. different microclimates or breeding times). Even if they are different, the key underlying assumption is that hosts and parasites adapted to similar climates will have more similar temperature optima than hosts and parasites adapted to different climates. As long as this assumption holds, the predictions of the heuristic framework remain the same. Second, we assume that the performances of cold- and warm-adapted hosts and parasites in isolation are approaching their lower and upper thermal limits, respectively. Although this is likely to result in right- and left-skewed thermal performance curves, respectively (Fig. 1a, b), like with the local adaption assumption, the predictions of the heuristic framework remain the same when the skew assumptions are relaxed (Fig. S1). Third, small organisms, such as pathogens, have generally been hypothesised to have

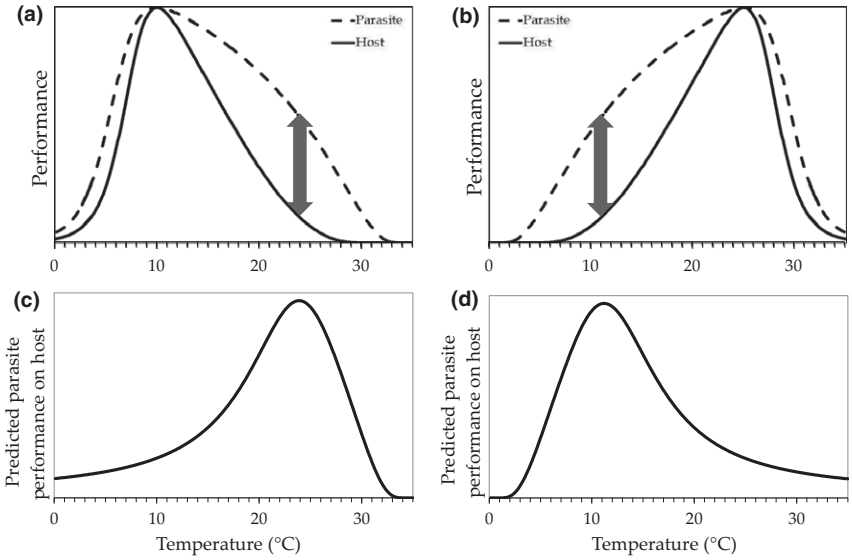

Figure 1 Conceptual figure describing the *thermal mismatch hypothesis*. In isolation, small organisms, such as parasites (dashed lines in panels a and b), generally have broader thermal performance curves than larger organisms, such as hosts (solid lines in panels a and b). Thus, highest parasite growth on hosts is likely to occur at a temperature where a parasite most outperforms its host (bidirectional arrows), and not necessarily at the temperature which a parasite performs best in isolation. Hence, subtracting the thermal performance curves of hosts and parasites reared in isolation (panels a and b) provides a hypothesis for the thermal performance curve of a parasite growing on the host (panels c and d). For interacting cold-adapted hosts and parasites, this subtraction reveals that parasite growth should be maximised at relatively warm temperatures (a and c) and the performance curve should be left skewed (c). In contrast, for interacting warm-adapted hosts and parasites, parasite growth should be maximised at relatively cool temperatures (b and d) and the performance curve should be right skewed (d). Thus, even when hosts and parasites have identical optimum performance temperatures because of local adaptation (although they could differ), small breadth differences in temperature–performance patterns can cause peak growth to occur far from the conditions under which the parasite or host perform best in isolation.

broader thermal breadths than larger organisms, such as hosts (Baas-Becking 1934; Martiny *et al*. 2006). This is because smaller organisms have higher mass-specific metabolic rates (Brown *et al*. 2004; Kingsolver & Huey 2008) and thus might acclimate more quickly to changing conditions than hosts. Faster acclimation should allow them to maintain performance through time over a larger range of temperatures. Indeed, a recent meta-analysis of thermal performance curves revealed that body mass was a significant negative predictor of thermal breadth (Rohr *et al*. in review).

Temperatures where the thermal performance of the parasite most exceeds that of the host (arrows in Fig. 1a,b and peak in Fig. 1c,d) might be where host susceptibility is greatest, and thus, subtracting the thermal performance curves of the hosts and parasites in isolation (Fig. 1a,b) offers a hypothesis for their thermal performance when interacting (Fig. 1c,d). The outcome of this subtraction produces two predictions, one about the temperature of maximal parasite growth on hosts and the other about the shape of the thermal performance curve of parasite growth on hosts. First, as a consequence of the broader thermal performance curves of parasites and the physiological upper and lower limits of temperature tolerances of both hosts and parasites (i.e. the right- and left-skewed curves for isolated cold- and warm-adapted organisms, respectively; Fig. 1a,b), hosts should, on average, be susceptible to parasites when temperatures most greatly differ from the temperature to which they are adapted (i.e. long-term average temperature; Raffel *et al*. 2006; Fitt *et al*. 2009). Or, in other words, parasite prevalence and abundance for cold- and warm-adapted hosts should be maximised at warm and cool temperatures, respectively (Fig. 1). We emphasise again that this prediction is robust to the above underlying assumptions regarding local adaptation of hosts and parasites and skew of thermal performance curves (Fig. S1). The second prediction is that, despite cold- and warm-adapted hosts and parasites having right- and left-skewed thermal performance curves in isolation (Fig. 1a,b), the thermal performance curves of parasite growth on cold- and warm-adapted hosts (i.e. when interacting) should be left and right skewed, respectively (Fig. 1c,d). If this thermal mismatch hypothesis is supported, cold-adapted hosts should face considerable risk of parasite transmission during particularly warm periods and thus they should be more at risk from global warming-driven disease outbreaks than warm-adapted hosts.

We set out to test the *thermal mismatch hypothesis* using the amphibian – *Batrachochytrium dendrobatidis* (*Bd*, or chytrid fungus) host–parasite system. Outbreaks of this emerging fungal pathogen are associated with hundreds of amphibian extinctions in the last 50 years (Skerratt *et al*. 2007; Rohr & Raffel 2010) and have occurred under a wide variety of conditions in different hosts (Retallick *et al*. 2004; Bosch *et al*. 2007; Whitfield *et al*. 2012). Thus, *Bd* outbreaks may be predictable based on the *thermal mismatch hypothesis*. *Bd* outbreaks are thought to be directly controlled by climatic conditions because *Bd* has a free-living stage and grows on the external skin of its ectothermic hosts (Kilpatrick *et al*. 2010; Venesky *et al*. 2014) and environmental temperature has been predictive of *Bd* outbreaks in a number of large-scale

analyses (Liu *et al.* 2013; Cohen *et al.* 2016). *Bd* grows best under cool conditions in laboratory culture (18–22 °C) and is generally not as prevalent under warmer field conditions (Retallick *et al.* 2004; Kilpatrick *et al.* 2010), and therefore, it has been assumed that cool conditions typically precede outbreaks. However, retrospective correlations between climate data and previous *Bd* outbreaks have led researchers to conflictingly conclude that cool (Retallick *et al.* 2004; Kriger & Hero 2007b; Whitfield *et al.* 2012), dry (Lampo *et al.* 2006; Laurance 2008), warm (Ron *et al.* 2003; Bosch *et al.* 2007) and wet (Kriger & Hero 2007a; Puschendorf *et al.* 2009) conditions are predictive of high *Bd* prevalence (Venesky *et al.* 2014). Although other factors, such as the availability of reservoirs, can certainly impact *Bd* epidemiology (McMahon *et al.* 2013a), the explanation for such strikingly contradictory patterns in the observed relationships between climate and *Bd* outbreaks may result from contrasting performances of host species or *Bd* isolates across a range of climatic conditions. In fact, this is what would be expected given the immense variation in climatic conditions that hosts and isolates have been exposed to throughout time for this global epizootic (Thomas & Blanford 2003; Rohr *et al.* 2011). Finally, a recent study revealed that *Bd* cultures grew after 24 h exposure to −12 °C and 28 °C (Voyles *et al.* in review). Thus, *Bd* has a much broader breadth, especially at lower temperatures, than many of its amphibian hosts, fulfilling an important assumption of the thermal mismatch hypothesis.

To test the *thermal mismatch hypothesis* experimentally, we quantified (1) host temperature preferences (proxy of host performance in isolation, see Supporting Information), (2) temperature-dependent growth rates of *Bd* isolates in culture (parasite performance in isolation) and (3) temperature-dependent growth rates of *Bd* isolates grown on their local hosts (host and parasite performance when interacting). These experiments were conducted on three phylogenetically, phenotypically and ecologically diverse hosts: Cuban tree frogs (*Osteopilus septentrionalis*), Southern toads (*Anaxyrus terrestris*) and Panamanian golden frogs (*Atelopus zeteki*). *O. septentrionalis* and *An. terrestris* are adapted to warm lowland subtropical habitats, whereas *At. zeteki* are native to high-elevation cloud forests in Central America. Thus, we predicted that the optimal temperature for *Bd* growth on relatively warm-adapted *O. septentrionalis* and *An. terrestris* would be lower than for the relatively cold-adapted *At. zeteki* (see Supporting Information). Finally, to assess the generality of our hypothesis, we searched the literature and collected 598 records of amphibian groups (235 species) previously tested for *Bd* in the field, along with climate data specific to the dates and locations of testing. We predicted that across all of these samples, amphibians in warmer climates would have greater *Bd* prevalence at cooler temperatures than species in cooler climates.

MATERIALS AND METHODS

Animal collection and maintenance

Adult *O. septentrionalis* and *An. terrestris* were collected from Hillsborough County, Florida, and adult *At. zeteki* were obtained from the Maryland Zoo (Baltimore, MD). *Bd* has never been detected on frogs collected from the Tampa, Florida region; thus, these frogs are unlikely to have a history of *Bd* exposure. All animals were maintained individually in vented plastic containers (26 × 16 × 8 cm) on top of two folded paper towels soaked with 15 mL of artificial spring water. Animals were fed vitamin and mineral-dusted crickets *ad libitum* and containers and paper towels were changed twice weekly. Prior to experiments, animals were maintained in a laboratory at 21 °C on a 12 h photoperiod for *c.* 2 months.

Host temperature preference experiment

To ascertain the preferred temperatures of *An. terrestris*, *O. septentrionalis* and *At. zeteki*, we maintained uninfected animals individually in thermal gradient apparatuses (Sauer *et al.* 2016; n = 24, 25 and 9 animals, respectively). Detailed methods describing the construction and application of the apparatuses for temperature preference trials are described in Sauer *et al.* (2016) and thus are only briefly described here. Apparatuses were built out of insulated aluminium downspout gutters cut into dimensions of 137 × 8 × 6 cm^3 and had ice packs (changed every 12 h and frozen at −80 °C) under one end and heat tape under the other. Each apparatus was sealed on top using five 27 × 10 cm^2 plexiglass sheets resting on window weather stripping. Organic sphagnum moss served as a substrate within the apparatuses and kept humidity between 84.1 and 90.7% across the temperature gradient. The apparatuses maintained a consistent temperature gradient across and within gutters (mean ± SD; cold end 9.29 ± 1.33 °C, warm end 33.94 ± 0.46 °C) and the room was kept on a 12 h light cycle. Animals were maintained in the apparatuses for 4 days and fed 10 vitamin-dusted crickets every 2 days. To prevent confounding the temperature preference of the crickets with that of the frogs, we enclosed both in feeding containers (quart-sized zip-top bags with paper clips adhered to the thermal gradient apparatus) at the location where the frog was found before feeding. We took temperature readings of each frog and the substrate occupied by each frog four times a day (10 : 00, 14 : 00, 18 : 00 and 22 : 00) using an Extech® High Temperature Infrared Thermometer (accuracy: ± 2% of rdg < 500°C, emissivity 0.95), which non-invasively measures temperatures accurately (Rowley & Alford 2007). We averaged each individual's mean preferred temperature throughout the trials to determine the overall temperature preference for each species. We excluded data from individuals whose preferences were more than three standard deviations from the mean, which were considered to be extreme outliers (we only removed one *At. zeteki* and one *O. septentrionalis*).

Overview of *Bd* experiments

We grew *Bd* in culture and on animals inside of Styrofoam incubators (inner dimensions 37 × 21 × 13 cm^3; Marko Foam Products, Salt Lake City, UT; Fig. S2) that each had a double-pane Plexiglas window in the lid to allow light in and were set to 14, 18, 22, 26 or 28 °C (± 0.5 °C; for more details, see Raffel *et al.* 2013). Incubators were stored in a GR48 environmental chamber (Environmental Growth Chambers,

Chagrin Falls, OH) that maintained 14 °C and a 12-h photoperiod. We also grew *Bd* in culture and on hosts at 10 °C inside of the same incubators in a separate chamber (Hotpack® model #352632, Philadelphia, PA) because the GR48 chamber could not maintain this temperature. Incubator temperatures were monitored throughout the experiments using 15 rotated Hobo pendant temperature/light data loggers (Onset Computer Corporation, Pocasset, MA).

In the *US experiment*, we simultaneously grew *Bd* isolate SRS 812 in culture and on *An. terrestris* and *O. septentrionalis*, and in the *Panama Experiment,* we simultaneously grew isolate JEL 423 in culture and on *At. zeteki*. The *Panama Experiment* was conducted over two temporal blocks (see below). Our goal was to match the host to the isolate in the geographic location where it originated, which is important to ensure ecologically relevant outcomes and to capture any local adaptation between host and parasite (Berger *et al.* 2005; Stevenson *et al.* 2013). JEL 423 was isolated from *Hylomantis lemur* during an epidemic at El Copé, Panamá in 2004, whereas SRS 812 was isolated from a *Lithobates catesbeianus* captured in the south-eastern USA in 2006.

Bd growth in culture

Batrachochytrium dendrobatidis stock cultures (grown in 1% tryptone broth) were maintained at 21 °C for 1 month before use in the experiments. From these, we created six smaller broth cultures and acclimated them to 10, 14, 18, 22, 26 or 28 °C for 12 h. To standardise these acclimated cultures to a common concentration of 3.75×10^5 zoospores/mL, we counted zoospores from 10 μL aliquots of broth using a haemocytometer and Trypan blue (McMahon & Rohr 2014) and diluted them with 1% tryptone. We then filtered cultures using 20-μm nylon filters (Spectrum Laboratories, Inc., Rancho Dominguez, CA) to remove zoosporangia, adding 1 mL of the resulting inoculate to 7 mL of 1% tryptone in 10 mL test tubes. There were 12–16 cultures per temperature depending on the number of incubators used in each experiment.

In the *Panama Experiment* block 1, we measured *Bd* growth in culture after 10 days by manually counting live zoospores from 10 μL samples of each culture using a haemocytometer and Trypan blue. In the *US Experiment* and *Panama Experiment* block 2, we non-destructively transferred 1 mL from each culture at 7 and 14 days into 24-well plates (Falcon®, Corning, NY) and measured their optical densities at 490 nm using a spectrophotometer (Biotech® Epoch Microplate Spectrophotometer, Winooski, VT) (McMahon *et al.* 2013b). We manually counted zoospores in the first block of the *Panama Experiment* because the spectrophotometer was in repair, but we did not observe a strong difference in the temperature-dependent growth curve attained using the two methods (Fig. S3).

Bd growth on hosts

While *Bd* was growing in culture, we simultaneously exposed hosts to *Bd* and maintained them in the same incubators. Hosts were divided into treatments of equal average mass ($n = 6$ for *An. terrestris* and *O. septentrionalis* and $n = 8$ for *At. zeteki*) and were acclimated to their exposure temperature for 14 days prior to *Bd* exposure. Thus, we avoided two issues common to temperature/disease experiments: pseudoreplication within temperature treatments and confounding exposure to a parasite with acclimation to a new temperature (Rohr *et al.* 2011). *An. terrestris* was the only species exposed to 10 °C because we could not obtain permission from the Maryland Zoo to expose *At. zeteki* to 10 °C and all *O. septentrionalis* acclimating to 10 °C died within 2 weeks.

We acclimated *Bd* to each temperature for 12 h and counted and diluted zoospores as described above to standardise cultures to a common concentration. Animals were exposed to 3 mL of 3.75×10^5 zoospores/mL *Bd* pipetted directly on the back of each animal. Runoff remained in the container with the animal for 24 h before a paper towel change. In the *US Experiment* and block 1 of the *Panama Experiment*, all animals were exposed to *Bd* because our focus was on *Bd* growth rates across temperatures and we expected low mortality over the 4-week experiment, as *Bd*-induced amphibian mortality is generally low for at least a month post-exposure. However, we had unexpectedly high mortality of *At. zeteki* and thus, in the second temporal block of the *Panama Experiment* (conducted with new animals), we added control individuals (exposed to 1% tryptone broth not containing *Bd*) at all temperature treatments.

Batrachochytrium dendrobatidis growth on frogs was measured by swabbing the right hind limb of each frog 1, 2 and 4 weeks after *Bd* exposure or on the day of death. The sterile swabs were passed 10 times from hip to toe and then frozen at −80 °C. *Bd* genome equivalents on each swab were determined using quantitative PCR (following methods from Boyle *et al.* 2004) after DNA extraction using Prepman Ultra. We checked each frog for mortality daily, and after 4 weeks, all frogs were weighed and then euthanised with buffered MS-222.

Field study

The experiments described above provided data relevant to our hypothesis on three host species under controlled climatic conditions. However, given the limited number of hosts tested, the experiments do not adequately address the generality of our hypothesis or patterns in the field. To evaluate the hypothesis that cold- and warm-adapted host species are most susceptible to *Bd* in the wild at relatively warm and cold temperatures, respectively, we synthesised field *Bd* prevalence studies. In September 2014, we searched Web of Science for the term "*Batrachochytrium dendrobatidis*", producing 1077 total results. We included swabbed amphibian samples in our analysis if *Bd* prevalence data were reported and ≥ 5 post-metamorphic amphibians were sampled. The resulting dataset consisted of 15 410 animals from 598 samples (groups of amphibians of a common species sampled at a specific location and date) representing 235 species surveyed across 261 sites. We also recorded or calculated the following independent variables directly from content of journal articles: binomial name, developmental stage (adult, metamorph or larva), sample size, dates of collection and geographic coordinates (when not given in the literature, we searched sites on Google Maps). Nomenclature was standardised according to the

IUCN (2015), which generally follows Frost (2015). We obtained mean temperature in each species' geographic range over 50 years from the database compiled by Sodhi *et al.* (2008). Finally, we attached monthly mean temperature and precipitation data from the Hadley Climate Research Unit (Harris *et al.* 2014) specific to the location and month before each amphibian sample was swabbed in the field [*raster* package (Hijmans 2014), extract function; all data compilation and analyses were conducted in R 3.1.0 (R Core Team, 2014)]. We averaged climate data across months if sampling took place over the course of up to three consecutive months and did not use data collected over longer periods of time, requiring us to average climate data over multiple seasons, which we deemed too coarse a climate measurement for our analysis.

Statistical analyses

For the *Host Temperature Preference Experiment*, differences in temperature preferences among the three species were assessed using a one-way ANOVA followed by Tukey's post hoc multiple comparison tests (*stats* package, aov and TukeyHSD functions, assuming normal error distributions). To quantify *Bd* growth rates in culture, we fit a logistic growth model to mean *Bd* optical density or zoospore counts at each time point within each temperature treatment [assuming no growth at t_0; *bbmle* package (Bolker 2014), mle2 function, negative log-likelihood function, assuming a normal error distribution]. We then fit Johnson-Lewin [eqn 1; (Dell *et al.* 2011)] and Weibull [eqn 2; (Angilletta 2006)] growth models to *Bd* growth rates (*r* parameter from logistic growth fits) across temperatures [*bbmle* package (Bolker 2014), mle2 function, assuming a normal error distribution].

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

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

These models are capable of producing asymmetrical temperature–performance curves that do not fall below zero on the *y*-axis. They also produce parameter estimates for temperature of peak growth (T_{opt} in Johnson-Lewin; *b* in Weibull) based on a negative log-likelihood function. To determine peak *Bd* growth of each isolate, we compared the AICs of both models and chose the peak growth parameter of the better performing model. We also fit these models to previously published data describing temperature-dependent *Bd* growth in culture (Piotrowski *et al.* 2004; Woodhams *et al.* 2008) and compared the temperatures of peak growth across the isolates (Fig. S4). For all isolates, we assumed zero growth at 0 and 32 °C. To estimate 95% confidence intervals for our parameters, we profiled all models fit using mle2 (profile function, *stats* package).

To analyse temperature-dependent *Bd* growth patterns on frogs, we fit a logistic growth model to each individual's log *Bd* load over time, after adjusting all *Bd* loads for body size by dividing by mass because larger frogs are swabbed over a larger area [bbmle package (Bolker 2014), mle2 function, normal error distribution]. We then extracted the growth rate parameter, *r*, from each fit and averaged parameters within temperature treatments and species. We fit linear, exponential, Johnson-Lewin and Weibull growth models to *Bd* growth rates (*r*) across temperature and chose the model with the lowest AICc. In addition, we conducted a Mitchell-Olds & Shaw test (Mitchell-Olds & Shaw 1987) to examine whether the temperature-dependent patterns of *Bd* growth on *An. terrestris* and *O. septentrionalis* were unimodal [vegan package (Oksanen *et al.* 2007), MOStest function].

Given that the *thermal mismatch hypothesis* posits that the temperature of greatest prevalence of warm- and cold-adapted species should be at relatively cool and warm temperatures, respectively, it required that we fit thermal performance curves separately to warm- and cold-adapted samples in our *Field Study* and estimate the temperature of maximum prevalence. Hence, this required that we utilise a 'reaction-norm' approach fitting Weibull models to the prevalence data of warm- and cold-adapted samples as a function of the 50-year mean of each sample's environmental temperature [bbmle package (Bolker 2014), mle2 function]. These Weibull models do not assume skew. Rather, the skew emerges from the fit to the data and thus left-skewed, right-skewed or symmetrical curves are possible. The models utilised a binomial error distribution and took into account the number of frogs swabbed in each sample. To assign samples of frogs as either warm or cold adapted, we identified the median environmental temperature of all samples as 17.5 °C and then split the data into cool-adapted host species that had annual 50-year mean environmental temperatures < 15 °C and warm-adapted host species that had annual 50-years mean environmental temperatures of > 20 °C. We also fit models with precipitation as a linear covariate alongside temperature.

The previous analyses dichotomise host samples as either 'cold-' or 'warm-adapted,' but in reality this is a continuous rather than categorical variable. To evaluate the shape of the relationship between the environmental temperature of host populations and the temperature of greatest *Bd* prevalence, we repeatedly fit the same Weibull models as just described but for 4 °C moving windows (e.g. 10–14 °C, 11–15 °C, 12–16 °C, etc.) of 50-year mean environmental temperatures. These windows were fit between mean environmental temperatures of 10–30 °C resulting in a minimum and maximum number of samples in any 4-year bin of 68 and 189, respectively. We then fit a third-order polynomial to the relationship between the temperature of maximal *Bd* prevalence for each of these windows (parameter from the Weibull models) and the midpoint of each mean annual temperature 'window' (i.e. 12 °C for the 10–14 °C window). Hence, a negative relationship would indicate that as mean annual temperature experienced by amphibian populations increases, the temperature where *Bd* prevalence is greatest decreases.

RESULTS

Host temperature preference experiment

Mean temperature preferences of *An. terrestris* (mean ± 1 SE: 24.07 ± 0.19 °C) and *O. septentrionalis* (22.98 ± 0.53 °C) did

not differ significantly (Tukey's HSD, $P = 0.12$), but both species preferred significantly warmer temperatures than *At. zeteki* (17.85 ± 0.14 °C; Tukey's HSD, $P < 0.0001$ for both pairs of species; Fig. 2; Table S1), supporting the hypothesis that *An. terrestris* and *O. septentrionalis* are relatively warm-adapted hosts and *At. zeteki* is a relatively cold-adapted host.

Bd growth experiments

Incubators consistently maintained temperatures within ± 0.5 °C of targets throughout all trials (Fig. S5). We fit non-linear unimodal Johnson-Lewin models to *Bd* growth in culture because these fits had lower AICs than Weibull, linear or exponential fits. In addition, a Mitchell-Olds & Shaw test confirmed that growth patterns for both isolates were unimodal ($P < 0.0001$). In all experiments, the temperature-dependent curve describing *Bd* growth in culture closely followed previously reported patterns (Piotrowski *et al.* 2004; Woodhams *et al.* 2008), slowly rising from 5 °C until peaking around 18–19 °C before quickly crashing around 26 °C (Fig. 3; Fig. S4). Isolate JEL 423 from Panama peaked in growth at 18.0 °C (95% confidence interval 17.1–18.9 °C) and isolate SRS 812 from the south-eastern USA peaked at 18.9 °C (95% CI 18.1–19.6 °C). Thus, the two isolates did not differ significantly in their optimum growth temperatures. In addition, both of the *Bd* isolates we tested were capable of exhibiting reasonably high growth rates (50% of maximum) between 10 and 25 °C, supporting the notion that *Bd* has a reasonably large thermal breadth.

We fit linear or exponential models to *Bd* growth on frogs because these fits had lower AICs than the unimodal fits. In addition, a Mitchell-Olds & Shaw test did not support the notion that temperature-dependent growth on frogs was unimodal ($P = 0.91$ for *An. terrestris*, $P = 0.48$ for *O. septentrionalis*). Without extrapolation beyond the tested temperatures, peak *Bd* growth rates were predicted to be at 10 °C on *An. terrestris* and 14 °C on *O. septentrionalis*, which are much lower temperatures than 18.9 °C, the temperature of peak *Bd* growth in culture (Fig. 3a). We

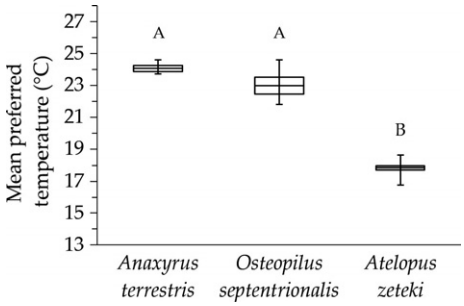

Figure 2 Box plot of temperature preferences of amphibian host species. To ascertain temperature preferences, we maintained uninfected *Anaxyrus terrestris*, *Osteopilus septentrionalis* and *Atelopus zeteki* ($n = 24$, 25 and 9, respectively) in thermal gradient apparatuses containing temperature gradients ranging from 8 to 33 °C. Centre lines represent the mean preferred temperature of each species, boxes are SEs and bars are first and third quartiles. Means with different letters are significantly different from one another based on a Tukey's HSD multiple comparison test.

observed very little mortality among these two species (two and three total deaths, or between 6 and 10% mortality). In contrast to the results for the warm-adapted species, *Bd* growth rates on the cold-adapted *At. zeteki* were positively, not negatively, associated with temperature (Fig. 3b), peaking at warmer temperatures than those where *Bd* grew best in culture. Greatest *Bd* growth for *At. zeteki* occurred at 26 °C, whereas peak *Bd* growth in culture for this isolate occurred at 18.0 °C (Fig. 3b).

Field study

On average, *Bd* prevalence was greatest under conditions only slightly cooler than those that promoted peak growth in culture (17.0 °C, 95% CI 16.4–17.4 °C) (Fig. S6; Table S1). However, *Bd* prevalence was greatest for amphibians from cool climates (averaging < 15 °C) at 20.5 °C (95% CI 19.6–22.1 °C; Fig. 4a). This was significantly higher (i.e. 95% CI did not overlap) than the temperature of greatest *Bd* prevalence for amphibians from warm climates (> 20 °C), which was only 15.9 °C (95% CI 15.4–16.4 °C; Fig. 4b), a result consistent with the predictions of the *thermal mismatch hypothesis*. Furthermore, our 'moving window' approach showed that as the mean annual temperature experienced by amphibians increased, the temperature where prevalence was greatest decreased (third-order polynomial; $R^2 = 0.90$, $P < 0.001$; Fig. 4c). Also consistent with the *thermal mismatch hypothesis* (see Fig. 1c,d), the curve describing *Bd* prevalence of amphibian groups from cool climates was left skewed (Fig. 4a), whereas the curve for samples from warm climates was right skewed (Fig. 4b). Including precipitation in multivariate models with temperature did not improve fits of models predicting *Bd* prevalence.

DISCUSSION

Hosts are likely to experience thermal stress at unusual temperatures (Raffel *et al.* 2006) and microbes and pathogens are known to have broad geographic ranges and thermal breadths (the Baas-Becking Hypothesis, Baas-Becking 1934; Martiny *et al.* 2006; Rohr *et al.* in review). Therefore, we hypothesised that species adapted to warm conditions would experience high *Bd* loads or prevalence at cooler temperatures than host species adapted to cool conditions, which should experience high *Bd* loads or prevalence at relatively warm temperatures. We also hypothesised that parasite prevalence on cold- and warm-adapted hosts as a function of temperature should be left and right skewed, respectively (Fig. 1c,d). As hypothesised, *O. septentrionalis* and *An. terrestris* species from relatively warm climates that preferred higher temperatures (23–24 °C) in our thermal preference trials suffered the highest *Bd* loads at colder temperatures than *Bd*'s optimal growth temperature in culture. Meanwhile, *At. zeteki*, a cool, montane species that preferred cooler temperatures (17.8 °C), experienced rapid *Bd* growth at warmer temperatures than those where *Bd* grew well in culture. Likewise, our analysis of *Bd* prevalence in the field revealed that species adapted to cool conditions experienced greater *Bd* prevalence at warmer temperatures than warm-adapted species. Also as hypothesised,

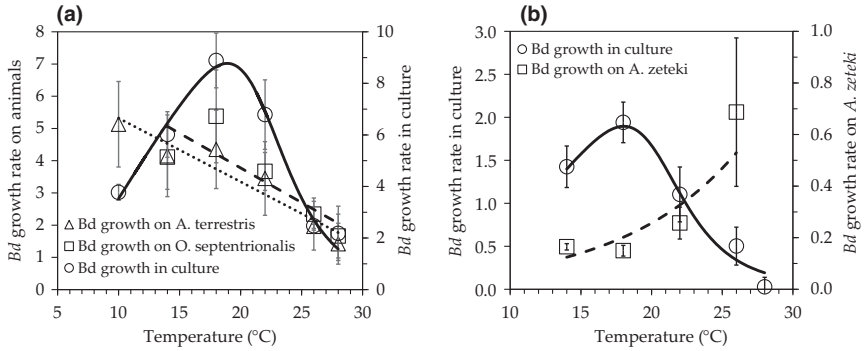

Figure 3 Temperature-dependent growth of *Bd* on hosts vs. in culture is consistent with the *thermal mismatch hypothesis*. In all three species, growth of *Bd* on the host differed from growth in culture. (a) *Bd* growth rates on *Anaxyrus terrestris* (triangles, dotted line) and *Osteopilus septentrionalis* (squares, dashed line) were highest at cold temperatures (10 and 14 °C, respectively, based on linear fits), despite *Bd* growth in culture peaking at 18.9 °C (circles, solid line, Johnson-Lewin fit; 95% confidence interval 18.1–19.6 °C). (b) *Atelopus zeteki* experienced high *Bd* growth (exponential fit) at warm temperatures (squares, dashed line; combined results of two temporal blocks), even though *Bd* growth in culture was poor at these temperatures, peaking at 18.0 °C (circles, solid line, Johnson-Lewin fit; 95% confidence interval 17.1–18.9 °C). We could not measure *Bd* growth rates on *At. zeteki* at 28 °C because too few animals survived long enough to be tested multiple times, which is necessary to fit the logistic growth curves. Shown are means ± 1 SE.

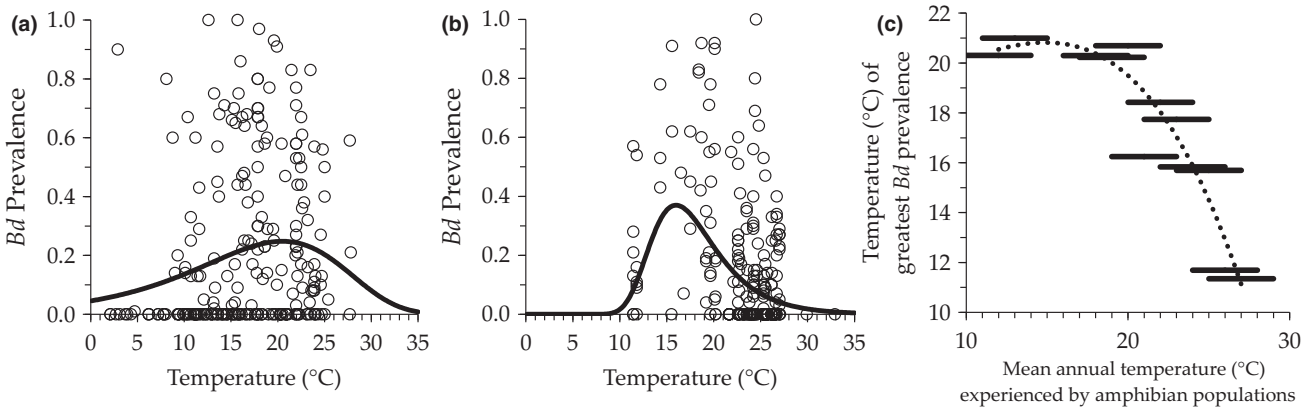

Figure 4 Temperature dependence of peak *Bd* prevalence varies between cold- and warm-tolerant host species in a manner that is consistent with the *thermal mismatch hypothesis*. Amphibian populations were divided into (a) cold (< 15 °C) and (b) warm adapted (> 20 °C) groups based on their 50-year mean annual temperatures. Separately for both the cold- and warm-adapted groups, Weibull models were fit to the relationship between *Bd* prevalence in adult amphibians ($n \geq 5$ animals) and temperature at the specific sampling location during the months of field sampling. Amphibians from cool climates experienced peak *Bd* prevalence at relatively warm temperatures (20.5 °C; 95% confidence interval 19.6–22.1 °C), where groups from warm climates experienced peak prevalence at cooler temperatures (15.9 °C; 95% CI 15.4–16.4 °C), providing support for the *thermal mismatch hypothesis*. (c) Amphibians were divided into subsets based on 50-year mean annual temperatures spanning 4 °C 'windows' (e.g. 10–14 °C, 11–15 °C, etc.) and, for each window, Weibull models were fit to the relationship between temperature during the month of sampling and amphibian *Bd* prevalence. Here, we extracted the Weibull model parameters describing the temperature at which *Bd* prevalence peaked and plotted these against 50-year mean annual temperatures experienced by amphibians in each window (bars). A third-order polynomial model (dotted line) suggests a negative relationship between the temperatures of the climates frogs experience and the temperature at which *Bd* prevalence peaks ($R^2 = 0.90$, $P < 0.001$). Some windows are missing because Weibull models would not fit these data.

the relationship between environmental temperature and parasite prevalence for cold- and warm-adapted hosts in the field was left and right skewed, respectively (Fig. 4).

Our experimental and field results broadly support the *thermal mismatch hypothesis*, suggesting that host species are more susceptible to disease at temperatures far from those to which they are adapted. In a recent article, Nowakowski *et al.* (2016) suggested that another dimension of thermal mismatch, the gap between upper critical thermal tolerances (CT_{max}) of amphibian hosts and *Bd*, is negatively related to disease risk. Nowakowski *et al.* estimated this mismatch in thermal tolerance by calculating the difference between hosts and an assumed constant CT_{max} for *Bd* worldwide. There is evidence, however, for local adaptation of *Bd* isolates to environmental conditions (Berger *et al.* 2005; Stevenson *et al.* 2013; Voyles *et al.* in review). Thus, by subtracting a constant from the CT_{max} of every host, these estimates of thermal mismatch are confounded with host thermal tolerance. In addition, this study did not address the disparity between thermal breadths of hosts and parasites or consider how the host–parasite interaction might differ from host or parasite performance (CT_{max}) in isolation. Our findings address these concepts as well as the fact that *Bd*-related declines often happen in particularly warm years (Rohr *et al.* 2008; Rohr & Raffel 2010).

Although the *thermal mismatch hypothesis* was supported by our findings in a system with pathogens that have free-living

stages and ectothermic hosts, questions remain about the generality of the hypothesis across systems. For example, disease susceptibility is less temperature dependent for endotherms than ectotherms (Harvell *et al*. 2002; Martin *et al*. 2010; Altizer *et al*. 2013). In addition, parasites that lack free-living stages, such as vector-borne pathogens, may also be less directly affected by environmental conditions (Harvell *et al*. 2002; Altizer *et al*. 2013), although traits of some vectors can profoundly depend on temperature (Mordecai *et al*. 2013; Johnson *et al*. 2015). We expect that the *thermal mismatch hypothesis* most likely applies to cases of ectothermic hosts and pathogens with free-living stages, but there is a need to test this hypothesis across systems and pathogens. In addition, in cases where the thermal breadth of the pathogen is narrower than that of the host, extreme temperatures could provide a refuge for hosts (Gsell *et al*. 2013), although such cases may be uncommon (Rohr *et al*. in review). The *thermal mismatch hypothesis* may also be less applicable in cases where hosts behaviourally thermoregulate to control the conditions they experience, although recent evidence suggests that amphibians do not thermoregulate to counter *Bd* infection (Han *et al*. 2008; Rowley & Alford 2013). Even within the amphibian/*Bd* system, the *thermal mismatch hypothesis* cannot fully explain species-level variation in *Bd* prevalence, as there are a number of taxonomic and life-history predictors of outbreaks (Olson *et al*. 2013).

Several of our observations might resolve uncertainties regarding how environmental temperature impacts host susceptibility to *Bd* in the field (Venesky *et al*. 2014). In fact, the *thermal mismatch hypothesis* may help explain some of the apparently contradicting climate–*Bd* patterns in the literature; for example, Retallick *et al*. (2004) found that *Bd* prevalence peaked in the coolest months of the year in tropical northern Queensland, whereas Bosch *et al*. (2007) reported peak *Bd* prevalence during the warmest months in the relatively cool mountaintops of central Spain. In contrast with the considerable variability among hosts in their temperature-dependent susceptibility to *Bd*, we observed much less variation in optimal growth temperatures among *Bd* isolates grown in culture (including two that have been previously reported; Piotrowski *et al*. 2004; Woodhams *et al*. 2008). Importantly, temperature-dependent *Bd* growth in culture would have poorly predicted temperature-dependent host susceptibility for all species we tested (Rohr *et al*. 2008), even though models predicting *Bd* distributions are often parameterised based on growth in culture (Pounds *et al*. 2006; Rohr *et al*. 2008; Woodhams *et al*. 2008; Murray *et al*. 2013). Surprisingly, *Bd* growth patterns on animals were linearly or exponentially related to temperature, implying that poor host resistance can enable pathogen growth even at extreme conditions where pathogens do relatively poorly in culture. We observed large infection loads for *At. zeteki* at high temperatures (26–28 °C), contrasting the observed pattern of *Bd* growth in culture and suggesting that *Bd* has the potential to cause outbreaks well outside of the conditions where it traditionally has been thought to flourish. These results highlight the need to either explicitly consider the host–parasite interaction or evaluate the difference in host and parasite performance in isolation rather than only one of their performances alone.

Much of the recent debate over whether global climate change is likely to increase the spread of disease focuses on how changing climates will impact pathogen ranges (Epstein 2001; Lafferty 2009; Murray *et al*. 2011), with fewer studies exploring the effect of climatic shifts on host immunity or resistance (Martin *et al*. 2010; Rohr *et al*. 2011). In general, parasites might be more adaptable to climate change than their hosts because of their shorter generation times and faster acclimation rates (Raffel *et al*. 2013; Rohr *et al*. in review), although the adaptability of host microbiomes to climate change remains unknown. Warmer mean temperatures are likely to push hosts away from their optimal temperatures and result in greater thermal mismatches between hosts and parasites, particularly for cold-adapted hosts. Meanwhile, sudden temperature drops that occur following warm periods (Rohr & Raffel 2010) may cause thermal mismatches between warm-adapted hosts and their parasites. In addition, extreme weather events are likely to increase with climate change (Rosenzweig *et al*. 2001), exposing hosts to unusual temperatures and lending pathogens an advantage in host–parasite interactions. To manage disease, it will be critical that researchers account for thermal mismatches between the performance of hosts and parasites when predicting temperature-dependent disease outcomes across systems.

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AUTHORSHIP

All authors agreed to submission of the manuscript and accept the responsibility for the accuracy and integrity of the manuscript.

AUTHOR CONTRIBUTIONS

All authors contributed ideas, JMC and MDV wrote proposals to acquire animals, JMC, MDV and TAM conducted disease experiments, ELS conducted host preference experiments, JMC and DJC conducted statistical analyses, all authors assembled the field database, JMC and JRR wrote the article and all authors provided editorial advice.

REFERENCES

Altizer, S., Ostfeld, R.S., Johnson, P.T.J., Kutz, S. & Harvell, C.D. (2013). Climate change and infectious diseases: from evidence to a predictive framework. *Science*, 341, 514–519.

Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R. & Daszak, P. (2004). Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends Ecol. Evol.*, 19, 535–544.

Angilletta Jr, M.J. (2006). Estimating and comparing thermal performance curves. *J. Therm. Biol.*, 31, 541–545.

Anyamba, A., Small, J.L., Britch, S.C., Tucker, C.J., Pak, E.W., Reynolds, C.A. *et al.* (2014). Recent weather extremes and impacts on agricultural production and vector-borne disease outbreak patterns. *PLoS ONE*, 9, e92538.

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

Berger, L., Marantelli, G., Skerratt, L.L. & Speare, R. (2005). Virulence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* varies with the strain. *Dis. Aquat. Organ.*, 68, 47–50.

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

Bosch, J., Carrascal, L.M., Duran, L., Walker, S. & Fisher, M.C. (2007). Climate change and outbreaks of amphibian chytridiomycosis in a montane area of Central Spain; is there a link? *Proc. R. Soc. B Biol. Sci.*, 274, 253–260.

Boyle, D.G., Boyle, D.B., Olsen, V., Morgan, J.A.T. & Hyatt, A.D. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Dis. Aquat. Organ.*, 60, 141–148.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004). Toward a metabolic theory of ecology. *Ecology*, 85, 1771–1789.

Bruno, J.F., Selig, E.R., Casey, K.S., Page, C.A., Willis, B.L., Harvell, C.D. *et al.* (2007). Thermal stress and coral cover as drivers of coral disease outbreaks. *PLoS Biol.*, 5, 1220–1227.

Cazelles, B., Chavez, M., McMichael, A.J. & Hales, S. (2005). Nonstationary influence of El Nino on the synchronous dengue epidemics in Thailand. *PLoS Med.*, 2, 313–318.

Cohen, J.M., Civitello, D.J., Brace, A.J., Feichtinger, E.M., Ortega, C.N., Richardson, J.C. *et al.* (2016). Spatial scale modulates the strength of ecological processes driving disease distributions. *Proc. Natl Acad. Sci. USA*, 113, E3359–E3364.

Daszak, P. (2000). Emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science*, 287, 1756.

Dell, A.I., Pawar, S. & Savage, V.M. (2011). Systematic variation in the temperature dependence of physiological and ecological traits. *Proc. Natl Acad. Sci. USA*, 108, 10591–10596.

Epstein, P.R. (2001). Climate change and emerging infectious diseases. *Microbes Infect.*, 3, 747–754.

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

Fitt, W.K., Gates, R.D., Hoegh-Guldberg, O., Bythell, J.C., Jatkar, A., Grottoli, A.G. *et al.* (2009). Response of two species of Indo-Pacific corals, Porites cylindrica and Stylophora pistillata, to short-term thermal stress: the host does matter in determining the tolerance of corals to bleaching. *J. Exp. Mar. Biol. Ecol.*, 373, 102–110.

Frost, D.R. (2015). Amphibian Species of the World: An Online Reference. Version 6.0. American Museum of Natural History, New York, USA. Accessible at: http://research.amnh.org/herpetology/amphibia/index.html. Last accessed September 10 2015.

Gsell, A.S., Domis, L.N.D., van Donk, E. & Ibelings, B.W. (2013). Temperature alters host genotype-specific susceptibility to chytrid infection. *PLoS ONE*, 8, e71737.

Han, B.A., Bradley, P.W. & Blaustein, A.R. (2008). Ancient behaviors of larval amphibians in response to an emerging fungal pathogen, *Batrachochytrium dendrobatidis*. *Behav. Ecol. Sociobiol.*, 63, 241–250.

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. *Int. J. Climatol.*, 34, 623–642.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S. *et al.* (2002). Ecology – climate warming and disease risks for terrestrial and marine biota. *Science*, 296, 2158–2162.

Hijmans, R.J. (2014). raster: geographic data analysis and modeling, R package version 2.2.31.

IUCN. (2015). The IUCN red list of threatened species. Version 2015.2. Available at: www.iucnredlist.org. Last accessed June 13 2015.

Johnson, L.R., Ben-Horin, T., Lafferty, K.D., McNally, A., Mordecai, E., Paaijmans, K.P. *et al.* (2015). Understanding uncertainty in temperature effects on vector-borne disease: a Bayesian approach. *Ecology*, 96, 203–213.

Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L. *et al.* (2008). Global trends in emerging infectious diseases. *Nature*, 451, 990–U994.

Kilpatrick, A.M., Briggs, C.J. & Daszak, P. (2010). The ecology and impact of chytridiomycosis: an emerging disease of amphibians. *Trends Ecol. Evol.*, 25, 109–118.

Kingsolver, J.G. & Huey, R.B. (2008). Size, temperature, and fitness: three rules. *Evol. Ecol. Res.*, 10, 251–268.

Koelle, K., Rodo, X., Pascual, M., Yunus, M. & Mostafa, G. (2005). Refractory periods and climate forcing in cholera dynamics. *Nature*, 436, 696–700.

Kriger, K.M. & Hero, J.M. (2007a). The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. *Divers. Distrib.*, 13, 781–788.

Kriger, K.M. & Hero, J.M. (2007b). Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *J. Zool.*, 271, 352–359.

Lafferty, K.D. (2009). The ecology of climate change and infectious diseases. *Ecology*, 90, 888–900.

Laine, A.L. (2008). Temperature-mediated patterns of local adaptation in a natural plant-pathogen metapopulation. *Ecol. Lett.*, 11, 327–337.

Lampo, M., Rodriguez-Contreras, A., La Marca, E. & Daszak, P. (2006). A chytridiomycosis epidemic and a severe dry season precede the disappearance of Atelopus species from the Venezuelan Andes. *Herpetol. J.*, 16, 395–402.

Laurance, W.F. (2008). Global warming and amphibian extinctions in eastern Australia. *Austral Ecol.*, 33, 1–9.

Liu, X., Rohr, J.R. & Li, Y. (2013). Climate, vegetation, introduced hosts and trade shape a global wildlife pandemic. *Proc. R. Soc. B Biol. Sci.*, 280, 20122506.

Martin, L.B., Hopkins, W.A., Mydlarz, L.D. & Rohr, J.R. (2010). The effects of anthropogenic global changes on immune functions and disease resistance. *Year Ecol. Conserv. Biol. 2010*, 1195, 129–148.

Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L. *et al.* (2006). Microbial biogeography: putting microorganisms on the map. *Nat. Rev. Microbiol.*, 4, 102–112.

McMahon, T.A. & Rohr, J.R. (2014). Trypan blue dye is an effective and inexpensive way to determine the viability of *Batrachochytrium dendrobatidis* Zoospores. *EcoHealth*, 11, 164–167.

McMahon, T.A., Brannelly, L.A., Chatfield, M.W.H., Johnson, P.T.J., Joseph, M.B., McKenzie, V.J. *et al.* (2013a). Chytrid fungus *Batrachochytrium dendrobatidis* has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. *Proc. Natl Acad. Sci. USA*, 110, 210–215.

McMahon, T.A., Romansic, J.M. & Rohr, J.R. (2013b). Nonmonotonic and monotonic effects of pesticides on the pathogenic fungus *Batrachochytrium dendrobatidis* in culture and on tadpoles. *Environ. Sci. Technol.*, 47, 7958–7964.

Mitchell-Olds, T. & Shaw, R.G. (1987). Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution*, 41, 1149–1161.

Mordecai, E.A., Paaijmans, K.P., Johnson, L.R., Balzer, C., Ben-Horin, T., Moor, E. *et al.* (2013). Optimal temperature for malaria

transmission is dramatically lower than previously predicted. *Ecol. Lett.*, 16, 22–30.
Murray, K.A., Retallick, R.W.R., Puschendorf, R., Skerratt, L.F., Rosauer, D., McCallum, H.I. *et al.* (2011). Assessing spatial patterns of disease risk to biodiversity: implications for the management of the amphibian pathogen, *Batrachochytrium dendrobatidis*. *J. Appl. Ecol.*, 48, 163–173.
Murray, K.A., Skerratt, L.F., Garland, S., Kriticos, D. & McCallum, H. (2013). Whether the weather drives patterns of endemic amphibian chytridiomycosis: a pathogen proliferation approach. *PLoS ONE*, 8, e61061.
Nowakowski, A.J., Whitfield, S.M., Eskew, E.A., Thompson, M.E., Rose, J.P., Caraballo, B.L. *et al.* (2016). Infection risk decreases with increasing mismatch in host and pathogen environmental tolerances. *Ecol. Lett.*, 19, 1051–1061.
Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J. *et al.* (2007). The vegan package. Community ecology package 10.
Olson, D.H., Aanensen, D.M., Ronnenberg, K.L., Powell, C.I., Walker, S.F., Bielby, J. *et al.* (2013). Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. *PLoS ONE*, 8, e56802.
Pascual, M., Cazelles, B., Bouma, M.J., Chaves, L.F. & Koelle, K. (2008). Shifting patterns: malaria dynamics and rainfall variability in an African highland. *Proc. R. Soc. B Biol. Sci.*, 275, 123–132.
Piotrowski, J.S., Annis, S.L. & Longcore, J.E. (2004). Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia*, 96, 9–15.
Pounds, J.A., Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.N. *et al.* (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, 439, 161–167.
Puschendorf, R., Carnaval, A.C., VanDerWal, J., Zumbado-Ulate, H., Chaves, G., Bolanos, F. *et al.* (2009). Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. *Divers. Distrib.*, 15, 401–408.
R Core Team, (2014). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
Raffel, T.R., Rohr, J.R., Kiesecker, J.M. & Hudson, P.J. (2006). Negative effects of changing temperature on amphibian immunity under field conditions. *Funct. Ecol.*, 20, 819–828.
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. *Nat. Clim. Chang.*, 3, 146–151.
Retallick, R.W.R., McCallum, H. & Speare, R. (2004). Endemic infection of the amphibian chytrid fungus in a frog community post-decline. *PLoS Biol.*, 2, 1965–1971.
Rohr, J.R. & Raffel, T.R. (2010). Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proc. Natl Acad. Sci. USA*, 107, 8269–8274.
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. *Proc. Natl. Acad. Sci. USA*, 105, 17436–17441.
Rohr, J.R., Dobson, A.P., Johnson, P.T.J., Kilpatrick, A.M., Paull, S.H., Raffel, T.R. *et al.* (2011). Frontiers in climate change-disease research. *Trends Ecol. Evol.*, 26, 270–277.
Rohr, J., Civitello, D.J., Cohen, J., Roznik, E.A., Sinervo, B. & Dell, A. (in review). A global framework for estimating acclimation and thermal breadth predicts risk from climate change. *Proc. Natl. Acad. Sci. USA*.
Ron, S.R., Duellman, W.E., Coloma, L.A. & Bustamante, M.R. (2003). Population decline of the Jambato Toad Atelopus ignescens (Anura: Bufonidae) in the Andes of Ecuador. *J. Herpetol.*, 37, 116–126.
Rosenzweig, C., Iglesias, A., Yang, X., Epstein, P.R. & Chivian, E. (2001). Climate change and extreme weather events; implications for food production, plant diseases, and pests. *Global Change Hum. Health*, 2, 90–104.
Rowley, J.J. & Alford, R.A. (2007). Non-contact infrared thermometers can accurately measure amphibian body temperatures. *Herpetol. Rev.*, 38, 308–316.
Rowley, J.J.L. & Alford, R.A. (2013). Hot bodies protect amphibians against chytrid infection in nature. *Sci. Rep.*, 3, Scientific Reports, 3, Article Number 1515.
Sauer, E., Sperry, J. & Rohr, J. (2016). An efficient and inexpensive method for measuring long-term thermoreulatory behavior of terrestrial and semi-terrestrial organisms. *Therm. Biol.*, 60, 231–236.
Skerratt, L.F., Berger, L., Speare, R., Cashins, S., McDonald, K.R., Phillott, A.D. *et al.* (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth*, 4, 125–134.
Sodhi, N.S., Bickford, D., Diesmos, A.C., Lee, T.M., Koh, L.P., Brook, B.W. *et al.* (2008). Measuring the meltdown: drivers of global amphibian extinction and decline. *PLoS ONE*, 3, e1636.
Sternberg, E.D. & Thomas, M.B. (2014). Local adaptation to temperature and the implications for vector-borne diseases. *Trends Parasitol.*, 30, 115–122.
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, e73830.
Thomas, M.B. & Blanford, S. (2003). Thermal biology in insect-parasite interactions. *Trends Ecol. Evol.*, 18, 344–350.
Venesky, M.D., Raffel, T.R., McMahon, T.A. & Rohr, J.R. (2014). Confronting inconsistencies in the amphibian-chytridiomycosis system: implications for disease management. *Biol. Rev.*, 89, 477–483.
Voyles, J., Johnson, L.R., Kelly, R., Barron, C., Miller, D., Minster, J. *et al.* (in review). Living on the edge: growth of the lethal fungal pathogen *Batrachochytrium dendrobatidis* at its thermal extremes. *Oecologia*.
Whitfield, S.M., Kerby, J., Gentry, L.R. & Donnelly, M.A. (2012). Temporal variation in infection prevalence by the amphibian chytrid fungus in three species of frogs at La Selva, Costa Rica. *Biotropica*, 44, 779–784.
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, 1627–1639.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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