EcoHealth 14, 762–770, 2017
DOI: 10.1007/s10393-017-1269-2

EcoHealth

Original Contribution

The Influence of Temperature on Chytridiomycosis In Vivo

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Abstract: Chytridiomycosis, an amphibian disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), is an ideal system for studying the influence of temperature on host–pathogen relationships because both host and pathogen are ectothermic. Studies of *Bd* in culture suggest that optimal growth occurs between 17 and 23°C, and death of the fungus occurs above 29 or below 0°C. Amphibian immune systems, however, are also temperature dependent and often more effective at higher temperatures. We therefore hypothesized that pathogen load, probability of infection and mortality in *Bd*-exposed frogs would peak at a lower temperature than that at which *Bd* grows best in vitro. To test this, we conducted a study where *Bd*- and sham-exposed Northern cricket frogs (*Acris crepitans*) were incubated at six temperatures between 11 and 26°C. While probability of infection did not differ across temperatures, pathogen load and mortality were inversely related to temperature. Survival of infected hosts was greatest between 20 and 26°C, temperatures where *Bd* grows well in culture. These results demonstrate that the conditions under which a pathogen grows best in culture do not necessarily reflect patterns of pathogenicity, an important consideration for predicting the threat of this and other wildlife pathogens.

Keywords: amphibian, *Batrachochytrium dendrobatidis*, ecophysiology, fungal pathogen, in vitro, thermal

Introduction and Purpose

Fungal pathogens are the greatest infection-related threat to wildlife (Fisher et al. 2012). For example, the honey bee (*Apis mellifera*) is experiencing population declines that have been linked to the fungal pathogen *Nosema ceranae*, as well as other causes (Furst et al. 2014). Triticale, an artificial hybrid of wheat and rye that was developed to be resistant to powdery mildew, is now infected by a hybrid strain of this fungus that was derived from those that infect wheat and rye, respectively (Menardo et al. 2016). The recently emerged *Pseudogymnoascus destructans* fungus causes the disease white-nose syndrome, which is responsible for declines in at least six species of bats (Langwig et al. 2015). In order to mitigate the impact that these and other fungal pathogens have on populations, we need to improve our understanding of the environmental variables that most influence their pathogenicity.

Temperature can be an important limitation to fungal growth (Harvell et al. 2002). For example, most fungi grow best between 25 and 35°C and do not grow well at temperatures above this range (Kwon-Chung and Bennett 1992; Casadevall 2005). In many fungi, reproduction is inhibited

Electronic supplementary material: The online version of this article (doi:10.1007/s10393-017-1269-2) contains supplementary material, which is available to authorized users.

Published online: September 6, 2017

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by light, and this effect is accentuated at high temperatures (Leach 1967). A clearer understanding of these environmental impacts on fungal growth and reproduction may be critical to understanding host–fungal relationships. In vitro growth studies are commonly used as a basis for understanding how temperature influences the growth and reproduction of fungal pathogens (e.g., *P. destructans* in bats: Chaturvedi et al. 2010; *Phytophthora ramorum* in oaks: Grunwald et al. 2008). While these studies can be useful for establishing ranges of thermal tolerance and demonstrating that the growth of a fungal pathogen is temperature dependent, peak infection prevalence, intensity, and mortality in a host may occur at a different temperature than in vitro studies would lead one to predict. For example, in laboratory studies of the fungus *Metarhizium flavoviride,* which was investigated as a potential biocontrol agent for the grasshopper pest *Zonocerus variegatus*, the optimal temperature for infection in the host was found to be 5°C higher than that of optimal growth in culture (Thomas and Jenkins 1997). Strains of this fungus also differed in their sensitivity to temperature such that only those which are more lethal to hosts at lower temperatures would be suitable for use in biocontrol (Thomas and Jenkins 1997).

While pathogen growth on or in a host may differ from growth in culture for a number of reasons, one important distinction between these two growth environments is that pathogens growing on a host often have to contend with the host's immune system. Host immune systems often have their own thermal performance curves (Butler et al. 2013), which suggests that certain temperatures may be favorable for the pathogen, while others may favor the host. For example, in amphibians, higher body temperatures often enhance the immune response (Maniero and Carey 1997; Carey 2000). Lag effects in spring and seasonal acclimation effects in autumn have also been documented, suggesting that temperature variability can affect susceptibility to infection as well (Raffel et al. 2006). While understanding which temperatures tip the scales in favor of the host versus the pathogen is critical to predicting the potential impact of fungal diseases, at present we know little about the nature of such temperature-dependent shifts. Identifying how the temperature dependence of pathogen growth differs in vitro and in vivo may allow us to better predict where and when epidemics might occur. The present study contributes to our understanding of the influence of temperature on host–fungal dynamics by comparing the in vitro and in vivo effects of temperature on the growth of an amphibian pathogen.

Chytridiomycosis, a disease of amphibians caused by the fungal pathogens *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*) (Longcore et al. 1999; Martel et al. 2013), is ideal for studying the relationship between host, pathogen, and temperature because *Batrachochytrium* pathogens infect amphibians, ectothermic vertebrates whose immune function also depends on temperature (Piotrowski et al. 2004; Fisher et al. 2009; Rollins-Smith and Woodhams 2012). Due to its recent discovery, the full impacts of *Bsal* on host populations likely remain to be seen. *Bd*, however, is well established as one of the most devastating wildlife pathogens as it affects hundreds of amphibian species worldwide and has been linked with declines and even extinctions of amphibian hosts (Wake and Vredenburg 2008).

Temperature is an important life history trait for *Bd* (Voyles et al. 2012) and also one of the most prominent environmental influences on amphibian physiology (Carey and Alexander 2003). Studies of *Bd* in culture have found that optimal growth occurs between 17 and 23°C, and death of the fungus occurs at temperatures above 29°C or below 0°C (Piotrowski et al. 2004; Lips et al. 2008; Woodhams et al. 2008). However, there is evidence to suggest that the pattern of *Bd* growth in vitro does not necessarily predict temperature-dependent patterns of infection prevalence, intensity, or mortality in live hosts. For example, in exposure studies greater numbers of *Bd*-infected mountain yellow-legged frogs (*Rana muscosa,* Andre et al. 2008) and Panamanian golden frogs (*Atelopus zeteki*, Bustamante et al. 2010) survived at 22°C, which is near the thermal optimum for *Bd* growth in vitro, than survived at 17°C. Boreal toads, *Anaxyrus boreas,* also survived longer when infected with *Bd* at 18 than at 15°C (Murphy et al. 2011). The *Bd*-exposure studies in Cohen et al. (2017) also suggest a mismatch in the temperature of peak *Bd* growth in culture versus growth on amphibian hosts, though the direction of this difference appears host-dependent. A clearer understanding of the influence of temperature on this host–pathogen relationship in vivo is needed to predict outbreaks and prevent extinctions.

We conducted a *Bd*-exposure study using Northern cricket frogs (*Acris crepitans*) as a host to investigate: (1) how differences in temperature affect pathogen load, probability of *Bd* infection and survival in vivo, and (2) how this pattern compares to the previously established in vitro temperature-growth curve for *Bd*. *Acris crepitans* was selected because it is widely distributed across the Eastern US and abundant in the southern part of its range,

but is declining in the northern part of its range (Zippel and Tabaka 2008). While the cause of this decline is unknown, chytridiomycosis may have contributed as *Bd* has been detected from many parts of this frog's range and mortality due to chytridiomycosis has been documented (Zippel and Tabaka 2008). To answer our questions, we conducted *Bd*-exposure trials at six temperatures between 11 and 26°C, a temperature range that *A. crepitans* would encounter across its range in nature. We hypothesized that pathogen load, probability of infection (whether a particular frog tested positive for *Bd*) and mortality in *Bd*-exposed frogs would peak at lower temperatures than the peak for *Bd* growth in vitro of 17–23°C. This distinction would have important implications for predicting the vulnerability of wild amphibians threatened by chytridiomycosis and perhaps also for the design of future wildlife disease studies.

Methods

On each of January 14 and September 2, 2014, we captured 60 wild *A. crepitans* from the F. Edward Hebert Research Center in Belle Chasse, Louisiana and brought them to Tulane University to begin a round of our experiment. The experiment was conducted in two rounds due to limitations on the number of environmental chambers available for housing frogs. Frogs were housed individually in cylindrical plastic tanks (5.5 cm tall, 15 cm diameter) with ventilated lids, containing 300 mL of filtered tap water. Frogs in all treatment groups spent most of their time on the sides of the tanks, descending only occasionally into the water. We heat treated all frogs at 30°C in a temperature controlled environmental chamber (Conviron, Adaptis) for 10 days to ensure that all would be *Bd* negative prior to the start of the experiment (Chatfield and Richards-Zawacki 2011). After this, we lowered the temperature (in 3°C increments every 4 hours) to the inoculation temperature. We then assigned the frogs random numbers, weighed them using a digital scale, measured them (snout vent length, or SVL) using dial calipers, and swabbed them for *Bd* five times on each of the dorsal surface, the sides from groin to armpit, the ventral surface, and the bottom of each foot using a sterile, individually wrapped rayon swab (Medical Wire & Equipment Co., MW-113). We used a random number generator to assign frogs to exposure or control groups in one of three temperatures for each round of the experiment. The positions of the tanks within each environmental chamber were rotated haphazardly each week. Details of the animal husbandry practices used in this study can be found in the electronic supplementary material.

We inoculated all *Bd*-exposure frogs weekly with 40 mL of inoculum at a concentration of 2.5×10^6 of *Bd* zoospores/mL. These frogs were exposed to *Bd* by gentle spraying using a 50 mL syringe onto the frog's dorsal side, over a clean tank, allowing excess inoculum to fall into 300 mL of clean filtered water. We then placed the frog into its tank, containing the 300 mL of water plus excess inoculum. Control frogs were sham inoculated in a similar manner. Additional details regarding the preparation of sham- and *Bd*-exposure inocula are available in the electronic supplementary material. We chose to inoculate weekly because our previous experience with laboratory exposures in *A. crepitans* suggested that these animals can rapidly lose *Bd* infections. We also wanted to replicate the natural scenario for these frogs, which a mark-recapture study in southeastern Louisiana (unpublished data) suggests involves repeated exposure to *Bd* throughout the active season.

The first round of this experiment began in January 2014, and included the 11, 17, and 23°C groups. The second round began September 2014 and included the 14, 20, and 26°C groups. Except as specified below, all other procedures were identical between rounds. On the day of the initial inoculation, we adjusted the environmental chambers to inoculation temperatures of either 17°C (round one) or 20°C (round two), the middle temperatures of what would be the three experimental temperatures for each round. Frogs remained at these temperatures for 14 days, while they received weekly inoculations. All exposure groups had eleven frogs each and control groups contained either eleven frogs (round one) or seven frogs (round two). After 14 days of inoculation, we adjusted the chamber temperatures in 3°C increments every 4 h to one of the following temperatures: 11, 14, 17, 20, 23, or 26°C. To confirm the accuracy and stability of chamber temperatures, we placed a Thermochron iButton (Embedded Data Systems) near the frog tanks in each chamber, which recorded temperature every 10 min.

We swabbed all frogs for *Bd* 2 days prior to their initial inoculation and frogs in *Bd*-exposure groups were also swabbed biweekly for the remainder of the experiment using the protocol described earlier. Swabs were inserted into sterile microcentrifuge tubes and stored at −20°C prior to DNA extraction. To detect and quantify *Bd*

DNA on swabs, we used a Taqman quantitative real-time PCR (qPCR) assay (Boyle et al. 2004) and a series of plasmid standards. All swabs were run in singlicate. Additional methodological details for DNA extraction and the qPCR assay are available in the electronic supplementary material.

We weighed and measured all frogs weekly to assess body condition (calculated using the residuals of the regression between snout vent length and body mass, following Jakob et al. (2011), just prior to the first inoculation). We also monitored frogs weekly for clinical signs of chytridiomycosis (Voyles et al. 2007). We swabbed and recorded the date of death for all frogs that died before the end of the experiment. We terminated rounds one and two 99 and 89 days, respectively, after the initial inoculation. At this point, we swabbed all frogs once more before humanely euthanizing them by bath in a solution of MS-222 (2 g/L).

Statistical Analysis

We used a generalized linear mixed model (GLMM) with a binary logistic regression and a logit link function to test whether the probability of infection for individual frogs differed by week and temperature group. To test for differences in pathogen load among temperature groups and across weeks, we fitted a linear mixed model (LMM). To test for effects of *Bd*-exposure and temperature on frog body condition, we used a LMM that included the explanatory (fixed) variables of week, temperature, and exposure group (i.e., whether the individual was in a *Bd*-exposure or sham-exposure group) as well as all two-way and three-way interactions. We attempted to include experimental round as a random effect in mixed models, but in all cases this prevented model convergence. To ascertain whether results differed among rounds, we compared results between models including both rounds and models that considered each round separately. To test for effects of temperature and exposure group on survival, we used a Cox regression with temperature, exposure group and round as covariates. Kaplan–Meier tests and Fisher's LSD tests were used to test for pairwise differences in survival and body condition, respectively, between *Bd*- and sham-exposed animals at each temperature. In both cases, a Bonferroni correction was used to adjust the *P* value for multiple tests. All analyses were performed using SPSS v. 23.

Results

All but five of the frogs exposed to *Bd* (one from 17°C, two from 20°C, and two from 26°C) became infected. Most frogs gained and lost infection throughout the course of the experiment. When considering all six temperatures together, probability of infection (whether a particular frog tested positive for *Bd*) did not differ among weeks or temperature groups and the week by temperature interaction was also non-significant (GLMM, Table S1). The result was the same when round one (Table S2) and round two (Table S3) were analyzed separately.

Pathogen load in *Bd*-exposed frogs did not differ across weeks of the experiment but did differ among temperature groups, with the highest pathogen loads occurring at the lowest temperatures (LMM, Table S4). The 11°C group had the greatest pathogen load (mean ± SE log plasmid equivalents = 3.87 ± 0.61), and the 23°C group had the lowest (0.77 ± 0.33, Fig. 1). The interaction between week and temperature was not significant. The analyses considering each round separately yielded qualitatively similar results (Tables S5, S6).

When both rounds were considered together, body condition differed across weeks, among temperature groups, and between *Bd*- and sham-exposed frogs (LMM, Table S7). The interactions between week and temperature, week and exposure, and temperature and exposure were also significant. Only the three-way interaction between week, temperature, and exposure was not significant. When analyzed separately by round, results for main effects were similar, with the exception of exposure, which was only marginally significant in round one ($P = 0.052$, Table S8).

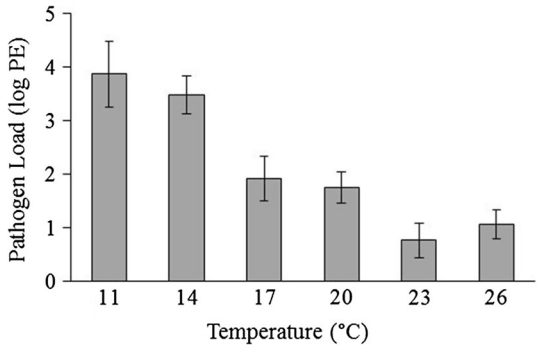

Figure 1. Mean pathogen load, measured in plasmid equivalents (PE) per swab via qPCR, across the experiment for each temperature group. *Error bars* are standard error of the mean.

The interaction between temperature and week was no longer significant when rounds were considered separately, nor were any other interaction effects for round one (Table S8, S9). When round two was considered on its own the three-way interaction between temperature, week and exposure was significant (Table S9). Across the experiment, and considering both exposure groups together, the 26°C group had the lowest body condition. These frogs maintained but did not increase their body condition over the course of the experiment (body condition index ~ 0). Frogs in the sham-exposed groups at all temperatures increased in body condition over the course of the experiment. Frogs in some of the *Bd*-exposure groups increased in body condition as well, but the frogs exposed to *Bd* at 14 and 26°C decreased in body condition (Fig. 2). Body condition was lower in *Bd*-exposed than in sham-exposed frogs at 14 and 23°C (Fisher's LSD test, Bonferroni-corrected $P \leq 0.006$) but not at 11, 17, 20 or 26°C (Fisher's LSD test, Bonferroni-corrected $P \geq 0.198$).

Survival differed across temperature groups in frogs exposed to *Bd* (Cox regression, $\chi^2 = 17.867$, $df = 5$, $P = 0.003$, Fig. 3) but not across rounds of the experiment (Cox regression, $\chi^2 = 1.355$, $df = 1$, $P = 0.245$). Survival was lowest at 11°C, where all frogs had died within 80 days of the first inoculation. As temperature increased, a greater proportion of *Bd*-exposed frogs survived. The only exception was the 23°C exposure group, which saw more mortality than the 20°C group. Survival of sham-exposed frogs also differed among temperature groups (Cox regression, $\chi^2 = 16.536$, $df = 5$, $P = 0.005$, Fig. 3), but not between rounds (Cox regression, $\chi^2 = 0.471$, $df = 1$, $P = 0.493$).

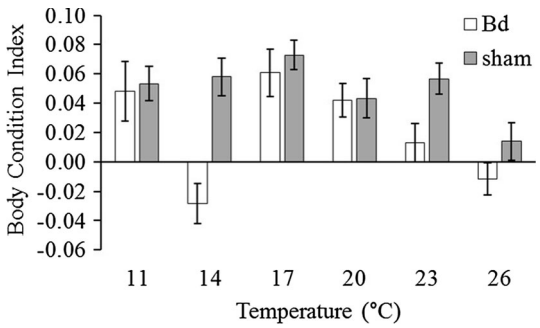

Figure 2. Mean body condition, across the experiment, for *Bd*- and sham-exposed frogs calculated using the residuals of a regression between body mass and snout vent length at day zero. *Error bars* are standard error of the mean. *Bd*-exposed frogs were lower in body condition than sham-exposed frogs only in the 14 and 23°C groups (Fisher's LSD, Bonferroni-corrected $P \leq 0.006$).

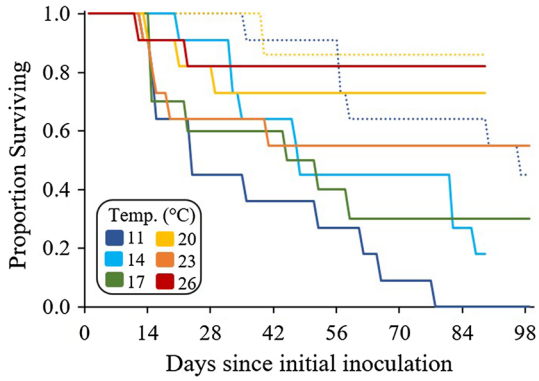

Figure 3. Survival curves for *Bd*-exposed (*solid lines*) and sham-exposed (*dashed lines*) frogs by temperature. Survival in sham-exposure groups at 14, 17, 23 and 26°C (not shown) was 100%. All applicable institutional and/or national guidelines for the care and use of animals were followed.

However, this pattern appears to be driven by the 11°C group, where survival was low (45%). Across all other sham-exposed groups there was only one death, which occurred in the 20°C group. In the 11, 14 and 17°C groups, survival was lower for *Bd*-exposed than for sham-exposed frogs (Kaplan–Meier, Bonferroni-corrected $P \leq 0.012$). In the 20, 23, and 26°C groups, survival did not differ between exposure groups (Kaplan–Meier, Bonferroni-corrected $P \geq 0.106$).

Discussion

In this study, pathogen load and mortality of *Bd*-exposed frogs were inversely related to temperature. The inverse relationship between temperature and mortality, however, appears to be stronger at lower temperatures as survival curves were similar for the three highest temperature groups. Survival was also lower in *Bd*-exposed frogs than in sham-exposed frogs in the 11, 14, and 17°C temperature groups but not in the warmer exposure groups. The fact that we found differences in survival across temperatures in the sham-exposed frogs, with most mortality occurring at 11°C, suggests that this low temperature affected other (non-*Bd*) aspects of the health of these animals as well. While swab results from animals found dead suggest that not all instances of mortality in the *Bd*-exposure groups were attributable to infection with this pathogen, the majority of frogs that died in *Bd*-exposure groups had heavy *Bd* loads at the time of death (Table 1). The frogs that died without a *Bd* infection showed no signs of illness or distress prior to death.

Table 1. *Bd* Infection Status and Load (Log Plasmid Equivalents) for Animals in *Bd*-Exposure Groups Swabbed on Their Day of Death.

Temperature (°C)	Status at death	
	Bd-positive/ swabbed	Mean (range) load if *Bd*-positive
11	10/10[a]	5.89 (3.18–7.40)
14	6/9	5.00 (3.94–5.46)
17	7/7	6.59 (5.38–7.32)
20	3/3	4.48 (2.82–5.38)
23	3/4[a]	6.11 (5.01–7.62)
26	0/3	

[a] Does not include 1 animal that died but was not swabbed.

Body condition was lower in frogs exposed to *Bd* than in sham-exposed groups at 14 and 23°C. The presence of an effect of *Bd*-exposure on body condition concurs with other studies (e.g., Retallick and Miera 2007), suggesting that exposure to *Bd* can have sub-lethal effects, even when mortality is low. The differences in body condition we saw could have arisen via a number of mechanisms. Inappetence is a well-established clinical sign of chytridiomycosis (Voyles et al. 2007), and our *Bd*-exposed frogs generally had more crickets left over after feedings than sham-exposed frogs. It is also possible that mounting an immune defense against *Bd* is metabolically costly and as a result, frogs fighting infection lose body condition as compared with sham-exposed individuals. Interestingly, no difference in body condition was observed between exposure groups at 11°C, the temperature where the greatest mortality occurred. Perhaps this temperature was so low that *Bd*-exposed frogs were not mounting any immune response, and hence not incurring any additional metabolic costs. It is also possible that because frogs at 11°C died more quickly than other groups there was simply not time for a difference in body condition between exposure groups to manifest itself.

Bd growth in culture peaks around 20°C and the pathogen does not grow as well at 11 or 14°C (Piotrowski et al. 2004). All but one of the disease variables we measured, however, were more severe in our lowest temperature *Bd*-exposure groups (11, 14 and 17°C) than in frogs exposed near the optimal growth temperature for *Bd* in culture. The exception was probability of infection, which showed no relationship with temperature. The fact that we exposed frogs to *Bd* repeatedly may have overwhelmed any potential for resistance and prevented us from seeing a temperature effect for this variable. Mortality was greatest (100%) at 11°C and decreased with each rise in temperature until 20°C, where it was just 27%. This pattern contradicts expectations based on *Bd* growth in culture (Piotrowski et al. 2004), suggesting that the relationship between the amphibian, the fungal pathogen, and temperature is not merely a product of temperature effects on pathogen growth. Our findings are consistent with previous in vivo studies (Andre et al. 2008; Bustamante et al. 2010; Murphy et al. 2011), though the Cohen et al. (2017) study, which included a broader range of temperatures, found *Bd* growth rates on exposed frogs to be inversely related to temperature in two warm-adapted species but not in a third, cold-adapted species. By these authors' definition, Louisiana *A. crepitans* would be considered a warm-adapted species, and so our results appear to be consistent with their "thermal mismatch hypothesis".

Whatever the cause, the difference between the temperature dependence of *Bd* impacts in vivo versus in vitro suggests that some temperatures allow hosts to cope with *Bd* infection better than others. In the wild, *A. crepitans* prefers temperatures from 20.9 to 35.5°C, with a mean body temperature of 27.4°C (Smith et al. 2003). Our findings suggest high mortality and probability of infection in cricket frogs exposed to *Bd* at temperatures below this range. If the cricket frogs' preferred temperature range is an accurate reflection of the temperature range at which its physiological performance is maximal, then it stands to reason that immune defenses against *Bd* would be most effective within this range as well. Perhaps while warmer temperatures are optimal for *Bd* growth, these same temperatures allow the cricket frog's immune system to function optimally making it less susceptible to the pathogen. It is also possible that the temperature dependence of *Bd* growth on hosts is just fundamentally different than in culture. For example, if nutritional environments between culture media and host epidermis differ, this could result in different temperature optima for growth in vivo versus in vitro.

We found that mortality was similarly low in frogs exposed to *Bd* at 20, 23, and 26°C, suggesting that temperatures of $\geq$20°C are likely to give Northern cricket frogs an equal chance of surviving a *Bd* infection, even if repeatedly exposed. At temperatures $\leq$17°C, mortality ranged from 70% (17°C) to 100% (11°C). Given this, Northern cricket frogs may be particularly susceptible to *Bd* at cooler temperatures, such as those that occur in winter,

spring and fall. At higher latitudes, these temperatures occur for a greater portion of the year and a greater portion of each day, and as a result, this species may be more susceptible to chytridiomycosis related declines in the northern part of its range. This is consistent with the geographic pattern of declines in this species, which was once common in Michigan but has since all but disappeared from that state and also from much of Ohio (Lehtinen and Skinner 2006).

Because this experiment was conducted in two rounds, it is possible that the frogs in each round, which were collected at different times of year, differed in their responses to *Bd*. The fact that inoculation occurred at 17°C in round one and at 20°C in round two may also have contributed to differences between rounds as thermal acclimation is known to influence frogs' responses to *Bd* infection (Raffel et al. 2013). Differences between rounds also could have resulted from stochastic variation in inocula. However, the only results that differed when rounds were analyzed separately versus together were for body condition, and these differences did not involve the main effect of exposure, which was significant in all cases. In the future, studies like one, where limitations on space in environmental chambers necessitates multiple experimental rounds, may be improved by including one common temperature (e.g., 20°C) in both rounds. This would aid in assessing how well experimental conditions (e.g., inocula) were replicated across rounds.

Conclusion

A clearer understanding of the relationship between temperature, infection, and ectotherm physiology will improve our ability to predict where and when hosts will be most susceptible to disease. For chytridiomycosis, species distribution models (SDMs) have been useful in predicting the potential range of *Bd* (e.g., Ron 2005; Puschendorf et al. 2009; Rödder et al. 2009; Murray et al. 2010), but these have no way of discriminating where, when or in which hosts *Bd* infections may cause declines. For example, SDMs suggest that most of North America, including the eastern US, is suitable for *Bd* (Ron 2005) and field surveys have confirmed that *Bd* is widespread in the US (Ouellet et al. 2005; Longcore et al. 2007; Pearl et al. 2007; Rothermel et al. 2008), yet it has only been associated with declines in certain areas (e.g., Arizona: Bradley et al. 2002; Colorado: Muths et al. 2003; California: Rachowicz et al. 2006). Incorporating our understanding of host and pathogen ecophysiology should improve our ability to predict outbreaks (e.g., Murray and Skerratt 2012), but our findings underscore the need for caution when considering the physiology of the pathogen in isolation from the host.

Our results highlight the importance of in vivo studies in understanding the often complex relationships between hosts, their pathogens, and the environment. There is a striking mismatch between the temperature at which *Bd* grows best in culture and that at which it grows best on (and causes the greatest mortality in) *A.crepitans*. This type of mismatch between in vitro and in vivo patterns of pathogen growth may be common, though it remains unclear whether the pattern seen here is the most common one. In at least one frog (*A. zeteki*), Cohen et al. (2017) found the opposite that pathogen growth on hosts peaked at higher temperatures than did *Bd* growth in vitro. These authors attributed this to *A. zeteki* being cold-adapted. Additional studies of this nature on hosts adapted to different climates are needed to determine the extent to which this pattern holds true.

Acknowledgements

The authors thank David Heins, Sunshine Van Bael and Warren Porter for feedback on earlier drafts. Thanks also to Gina Zwicky, Megan Exnicios, Tammy Vo, Xander Rose, Megan McWilliams, and Ian Buchta who assisted with animal husbandry and data collection and Mary Neligh who assisted with database design. This work was funded by grants from the National Science Foundation (Award No. 1649443) and Louisiana Board of Regents (Award No. LEQSF (2011-14)-RD-A-26) to CLRZ. Permission to collect *A. crepitans* was provided by the Louisiana Department of Wildlife and Fisheries (Permit Nos. WL-Research-2012-06 and LNHP-14-060). This study and its methods were approved by the Institutional Animal Care and Use Committees (IACUC) at Tulane University (Protocol Nos. 0391 – 0391R2).

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