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TEMPERATURE AS A DRIVER OF THE PATHOGENICITY AND VIRULENCE OF AMPHIBIAN CHYTRID FUNGUS *BATRACHOCHYTRIUM DENDROBATIDIS*: A SYSTEMATIC REVIEW

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ABSTRACT: Chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), is a leading cause of global amphibian declines. Severe infections with *Bd* can lead to cardiac arrest, and mass deaths during epidemics have been reported. Temperature, pH, salinity, and moisture are important determinants of the survival, growth, reproduction, and pathogenicity of *Bd*, as well as its effect on amphibian populations. Here, we synthesize current knowledge on the role of temperature as a driver of the pathogenicity and virulence of *Bd* to better understand the effects of temperature on amphibian defense mechanisms against infection. This review advises on research direction and management approaches to benefit amphibian populations affected by *Bd*. We conclude by offering guidelines for four levels of temperature monitoring in amphibian field studies to improve consistency between studies: regional climate, habitat, microhabitat, and amphibian host.

Key words: Disease, environmental refuge, freshwater, guideline, intensity, key threatening process, prevalence, thermal, wildlife health.

INTRODUCTION

The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), is a leading cause of amphibian declines globally (Berger et al. 1998; Scheele et al. 2019). Chytridiomycosis, the disease caused by *Bd*, impairs critical skin functions in amphibians, including oxygen regulation, hydration, and electrolyte balance (Voyles et al. 2007). Severe infections can lead to cardiac arrest, and mass deaths can occur during epidemics (Bosch et al. 2001; Lips et al. 2006). Chytridiomycosis has contributed to the decline of more than 500 amphibian species, 18% of which are inferred extinct in the wild, whereas a further 25% have experienced a >90% reduction in abundance (Scheele et al. 2019).

The global impact of *Bd* on amphibian populations has led to considerable research since amphibian declines were first reported because of the fungus in 1997 (Berger et al. 1998, 2016). Temperature, salinity, pH, and moisture availability are now recognized as important drivers of the survival, growth, reproduction, and pathogenicity of *Bd* (Skerratt et al. 2007; Voyles et al. 2011; Berger et al. 2016). The unique immune response and skin microbiota of amphibians can interact with environmental factors to determine their susceptibility to the pathogen (Carey et al. 1999). Temperature has a key role in regulating the growth and survival of *Bd* both in vitro and in vivo and is crucial in regulating the physiology and immunocompetence of amphibian hosts (Carey et al. 1999; Robak and Richards-Zawacki 2018).

Temperature is one of the most important environmental factors influencing the outcome of interactions between *Bd* and amphibian hosts. In the laboratory, *Bd* can survive freezing, displays optimal growth between 17 and 25 C, and fails to grow at 28 C (Johnson et al. 2003; Piotrowski et al. 2004; Kriger and Hero 2008). Sensitivity to temperatures above 28 C is significant, with reports

of 50% mortality of *Bd* cultures when held at 30 C for 8 d and 100% mortality of *Bd* cultures within 4 h when held at 37 C (Johnson et al. 2003; Piotrowski et al. 2004). However, *Bd* is able to survive at 4 C and can, therefore, overwinter in its hosts (Piotrowski et al. 2004; Bosch et al. 2007).

The response of *Bd* to variations in temperature in culture does not fully explain infection outcomes among hosts (Voyles et al. 2017). During in vitro and in vivo experiments, Raffel et al. (2013) found that *Bd* grew slower at 15 C than it did at 25 C but caused greater frog mortality at the lower temperature, a finding consistent with reports of heightened virulence at cooler temperatures (Bustamante et al. 2010; Chatfield and Richards-Zawacki 2011; Raffel et al. 2013). These results align with patterns of infection risk observed in the field: outbreaks of chytridiomycosis occur at cooler, high-elevation sites and when temperatures are at seasonal lows (Berger et al. 2004; Woodhams and Alford 2005; Gillespie et al. 2015). However, some studies have found increased *Bd* prevalence at higher temperatures (Pounds et al. 2006; Bosch et al. 2007), which provides support for the thermal mismatch hypothesis. This hypothesis suggests that host species adapted to warmer temperatures will be more susceptible to disease at relatively cooler temperatures when pathogens are able to outperform their hosts because of their wider thermal breadths (Cohen et al. 2017; Rohr et al. 2018).

Recent reviews have focused on quantifying global effects of *Bd* (Scheele et al. 2019); the actions, research, and resources required to prevent extinctions; and overviews of taxonomy, phylogeny, distribution, and ecology of the pathogen (Berger et al. 2016). Although reviews have documented the role of environmental factors in determining the prevalence and intensity of *Bd* in amphibians (Scheele et al. 2017), there are few peer-reviewed articles that draw together laboratory and field experiments testing the role of temperature on infection risk with *Bd* (Cohen et al. 2017; Rollins-Smith 2020; Sauer et al. 2020). We present a systematic review on temperature as a driver of the pathogenicity and virulence of chytridiomycosis in anurans, with a secondary objective of reviewing methods of temperature measurement during field-based research on *Bd* and provide guidance to researchers on best practices. Increased consistency of temperature measurement in the field will refine our theoretical understanding *Bd*-host dynamics, determining species at risk and informing conservation efforts. This is especially important in the face of a changing climate.

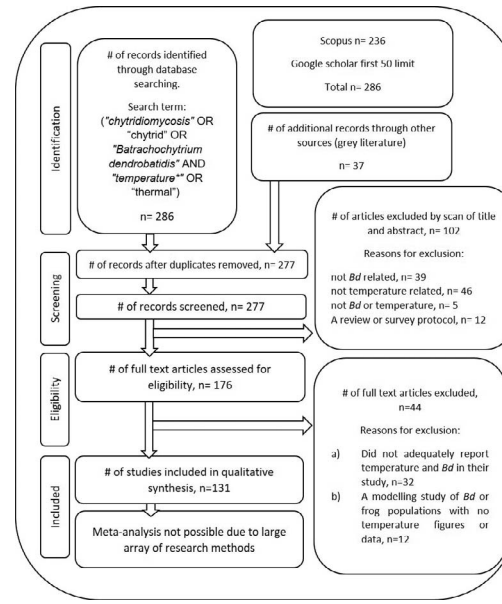

FIGURE 1. PRISMA flow diagram used for inclusion and exclusion of articles for this review on temperature as a driver of the pathogenicity and virulence of amphibian chytrid fungus *Batrachochytrium dendrobatidis*. Adapted from Moher et al. (2009).

MATERIALS AND METHODS

Literature was systematically reviewed using the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) protocol (Fig. 1; Moher et al. 2009). Initial searches were conducted through online databases Scopus (Elsevier, Amsterdam, the Netherlands), Trove (National Library of Australia, Canberra, Australian Capital Territory, Australia), OpenGrey (GreyNet International, Amsterdam, the Netherlands), and Google Scholar (Google, Mountain View, California, USA; from 11 April 2018 to 26

July 2018). The search was repeated using the same terms in December 2020. Exploratory searches in Scopus included search term "chytridiomycosis," which returned 3,496 results; "*Batrachochytrium dendrobatidis*," which returned 3,281 results; and "chytridiomycosis" OR "*Batrachochytrium dendrobatidis*," which returned 4,635 results. We then refined the Scopus search to the effects of temperature on *Bd* using the search modifiers TITLE-ABS-KEY (which searches the title, abstract, and key words) and LIMIT-TO-DOCTYPE "ar" (which limits the results to article document types), and the search terms "chytridiomycosis" OR "chytrid" OR "*Batrachochytrium dendrobatidis*" AND "temperature*" OR "thermal," which resulted in 193 documents. In Trove, the search term "*Batrachochytrium dendrobatidis*" was used and produced 37 articles; of which, four contained temperature data. In OpenGrey, the same search term produced seven papers; of which, none contained temperature data. Google Scholar returned thousands of results. From reading titles, these soon became irrelevant after 50 articles. For that reason, the first 50 were included; of which, only 12 were articles not found through the other search methods. Repeating the refined Scopus search in December 2020 returned an additional 43 articles published since 2018; of those, 35 were included in this review after adhering to selection criteria and are included in the figures.

All articles were first screened by their title and abstract. Articles were rejected if they were 1) not *Bd* related (e.g., other chytrid species), 2) not temperature related, 3) not *Bd* and temperature related, or 4) a review or survey protocol. Reviews were not included in the Results and Discussion of this review; however, they were used in the Introduction to provide background knowledge and identify the knowledge gaps for this current review. The full text of selected articles was examined, and articles were further excluded if they 1) did not adequately report temperature and *Bd* in their study, or 2) were a modeling study of *Bd* or frog populations with no temperature or *Bd* data included in the analysis. Qualitative data analysis software, NVivo12 (2018; version 12, QSR International Pty. Ltd., Doncaster, Victoria, Australia) was used to sort information found in these selected articles using the Query tool.

RESULTS AND DISCUSSION

Literature identified

One hundred and thirty-one articles met all criteria and were included in the review. These were divided into four primary categories: 1) articles including temperature data in a survey of *Bd* prevalence in the field (n=51), 2) articles predicting *Bd* prevalence and intensity based on modeling of temperature regimes and other environmental factors (n=13), 3) in vitro laboratory studies on growth of *Bd* under controlled conditions (n=18), and 4) in vivo laboratory studies testing the effect of temperature on *Bd* (n=60). Seven articles were included in multiple categories. Of the field studies, 25 were conducted in tropical environments and 19 in temperate environments, with two across both tropical and temperate. One field study was in the subarctic, two in arid conditions, two in the Neotropics, and one was subtropical.

Synthesis of the literature

The relationships found between temperature and the prevalence and intensity of *Bd* in frog populations across the globe are complex. Numerous interacting factors drive temperature regimes to which frog populations are exposed. In addition, studies that addressed temperature regimes under laboratory conditions occasionally produced contradictory results to those conducted under field conditions.

Temperature can influence both prevalence and intensity of *Bd*

In epidemiology, "infection prevalence" is defined as the proportion of a population that displays infection at a particular point in time, whereas "infection intensity" refers to the level of infection or pathogen abundance (Porta 2014). Prevalence may be high in species that are able to persist with the pathogen, but immune defenses or behavioral mechanisms may be sufficient to limit pathogen growth; therefore, infection intensity remains low (Daskin et al. 2011; Riley et al. 2013).

Multiple articles reported a significant effect of temperature on either *Bd* prevalence or intensity (Table 1). A reoccurring variable in these articles is the use of lagged temperatures to record the temperature regime experience by an amphibian. Lagged temper-

TABLE 1. Number of articles included in this review with temperature as a driver of the pathogenicity and virulence of amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) that report a significant effect of temperature on either *Bd* prevalence or intensity in the field, laboratory, or a combination of both.

Significant effect detected	Total no. articles included in review	No. field-based studies	No. laboratory-based studies	Studies including field and laboratory components
Effect of temperature on *Bd* infection prevalence and intensity	14	8	5	1
Effect of temperature on *Bd* infection prevalence	44	35	6	0
Effect of temperature on *Bd* infection intensity	19	4	14	0
Effect of temperature on frog mortality from *Bd*	7	0	7	0
No effect of temperature on *Bd* infection prevalence or intensity	7	3	3	1
Effect inferred based on known sensitivity of *Bd* temperature, not specifically measured	5	4	1	0
Simulations of *Bd* prevalence or intensity with temperature variables included	7	0	0	0
Total no. articles	103	54	36	2

ature measurements were strong predictors of *Bd* prevalence or intensity, with average daily air temperature during the 30 d prior being the most commonly used (Kriger and Hero 2007; Murray et al. 2009; Crawford et al. 2017). This aligns with clinical or lethal infections developing after 30 d in infected frogs in laboratory studies (Nichols et al. 2001; Daszak et al. 2004). Lagged measures were typically derived from nearby weather stations or dedicated temperature loggers deployed at survey sites (Kriger and Hero. 2007; Whitfield et al. 2012).

Experimental testing of temperature regimes on *Bd* prevalence and intensity in the laboratory was conducted in 36 studies. In culture, *Bd* grows well between 20 C and 26 C (Piotrowski et al. 2004); however, pathogen growth in vitro does not necessarily reflect patterns of pathogenicity in vivo (Sonn et al. 2017). For that reason, it is important that studies target specific species in different environments to better understand the effect of *Bd* on wild populations. Furthermore, there are many variables (Fig. 2) that influence the temperature that amphibians are subjected to in the wild; these are discussed further soon.

Factors influencing temperature variation and their effect on infection prevalence and intensity

Temperature regimes experienced by amphibian populations vary with elevation, water-body size and depth, and vegetation and canopy cover (Fig. 2). Temperature variation among habitats can influence the prevalence and intensity of *Bd*, as can temperature variation among microhabitats. The presence of microhabitats can create refuges from *Bd*, both among and within amphibian habitats (Heard et al. 2015; Scheele et al. 2017).

Temperature variation because of elevation: Of the 131 articles, 25 mention elevation in their discussion of *Bd* infection prevalence and intensity. However, only six studies included elevation as a variable in their analysis (Drew et al. 2006; Kriger and Hero 2008; Kielgast et al. 2010; Narayan et al. 2014; Heard et al. 2015; LaBumbard et al. 2020). Of those, three found elevation to be an important determinant of *Bd* infection levels (Kielgast et al. 2010; Heard et al. 2015; LaBumbard et al. 2020). LaBumbard et al. (2020) found prevalence decreased with elevation during the wet season but not during the dry season. Heard et al. (2015) found a negative relationship between elevation and nighttime water-surface temperature, which

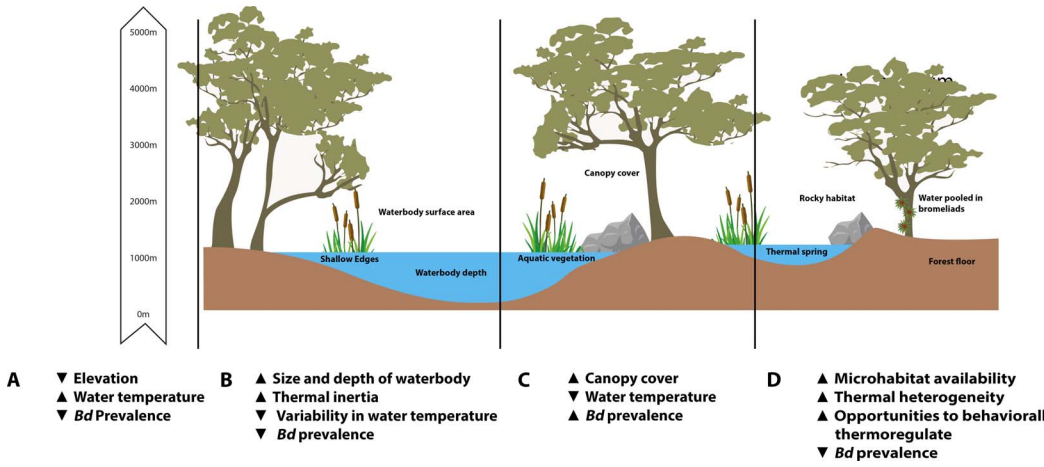

FIGURE 2. Environmental factors which influence prevalence and intensity of *Batrachochytrium dendrobatidis* (*Bd*) in amphibians and their associated relationships. (A) Relationships between elevation and *Bd*. (B) Relationship between waterbody size and depth and *Bd*. (C) Relationship between canopy cover and *Bd*. (D) Relationship between microhabitats and *Bd*.

led us to infer that cooler temperatures at higher elevations affect *Bd* infection status, but elevation alone, did not.

Temperature variation because of water body size and depth: Large, deep water bodies are typically cooler and have temperatures less variable than in small, shallow water bodies, leading to the hypothesis that frog populations that inhabit deeper wetlands show greater *Bd* prevalence and infection intensity (Fig. 2). Five studies used water-body depths as a predictor of *Bd* prevalence or intensity (Woodhams and Alford 2005; Fellers et al. 2011; Beyer et al. 2015; Roznik and Alford 2015; Valencia-Aguilar et al. 2016), but only two found water depth to be significant (Fellers et al. 2011; Valencia-Aguilar et al. 2016). Water-body surface area may also mediate the effects of water depth; for example, there is evidence that the edges of larger, deeper water bodies stay warmer later into the season than do smaller, shallower water bodies (Heard et al. 2015). The effect appears to be driven by thermal inertia of water bodies with larger volume, producing weaker reductions in water temperature through the cooler months of the year and slower increases in water temperature in spring. In turn, larger, deeper wetlands could also be environmental refuges from *Bd* (Heard et al. 2015).

Temperature variation because of canopy cover and vegetation: Canopy cover increases shading, reduces ambient air and water temperatures, and can reduce temperature variability. Reducing canopy cover may increase temperatures and the likelihood of exceeding the critical thermal maximum of *Bd* spores (Beyer et al. 2015; Heard et al. 2015; Bell et al. 2020). Influence of canopy cover on *Bd* prevalence or intensity was mentioned in 19 articles. Seven of those examined the effect of canopy cover in detail (Becker et al. 2012; Korfel and Hetherington 2014; Beyer et al. 2015; Heard et al. 2015; Roznik et al. 2015; Valencia-Aguilar et al. 2016; Bell et al. 2020). Roznik et al. (2015) reported that a reduction in canopy cover from a cyclone reduced the *Bd* infection risk in an endangered rainforest frog *Litoria rheocola* by 11–28% relative to an unaffected site. Korfel and Hetherington (2014) found the prevalence of *Bd* to be significantly greater in frog populations inhabiting closed canopy streams (52.6% positive, n=247) compared with those in wetlands with emergent vegetation only (15.4%, n=26), but infection intensities, measured in genomic equivalents, tended to be greater in wetlands with emergent vegetation (Korfel and Hether-

ington 2014). Beyer et al. (2015) conducted a 2-yr survey of *Bd* prevalence in wetlands in Illinois, US, and found that canopy cover only had an effect during unusually warm and dry conditions, suggesting that the effect of canopy cover on *Bd* prevalence may be greater in warmer climates. Bell et al. (2020) found a tight inverse correlation between canopy cover and streamside air temperature, with infection probability increasing with canopy cover in the wet tropics of Australia.

Microhabitats act as temperature buffers to facilitate behavioral thermoregulation and reduce Bd infections: When *Bd* is enzootic, microhabitats enable amphibians to behaviorally thermoregulate and the host's infection levels can be determined as a result (Rowley and Alford 2013; Roznik and Alford 2015; Burrowes et al. 2017; Barrile et al. 2020). Microhabitats comprise abiotic factors, such as temperature, wind, and humidity, which can all influence infection risk (Burrowes et al. 2017). Availability of microhabitats, such as a rocks, shallow water, sunlit edges, or vegetation, might provide an opportunity for frogs to raise their body temperature above the *Bd* threshold and clear the infection (Heard et al. 2018). Even in habitats in which average environmental temperatures are suitable for optimum pathogen proliferation, infection risk may be influenced by host behaviors that briefly increase body temperatures to those that are detrimental to *Bd*. Differences in infection prevalence have been observed among species displaying different behaviors that influence their temperature regimes (Daskin et al. 2011; Ruggeri et al. 2015; Burrowes et al. 2017). For example, the common coqui (*Eleutherodactylus coqui*) had twice the likelihood of *Bd* infection on the forest floor, where it was cooler and wetter, compared with those active in vegetation aboveground (Burrowes et al. 2017). At thermal springs in Arizona, water temperatures reach more than 30 C and provided a refuge for the lowland leopard frog (*Rana yavapaiensis*) from *Bd*, which is present in nearby waters (Schlaepfer et al. 2007; Forrest and Schlaepfer 2011). Similarly, water pooled in bromeliads can reach more than 30 C in open patches of the Brazilian Atlantic forest and can provide a microhabitat in which *Phyllodytes edelmoi* (endemic to the northern Brazilian Atlantic Forest) can lower their infection load (Ruano-Fajardo et al. 2016).

Thirty-four articles mentioned microhabitat use by amphibians; however, temperatures within those microhabitats were not always measured. Only nine articles used microhabitat type as a variable in their analysis (Richards-Zawacki 2009; Becker et al. 2012; Korfel and Hetherington 2014; McNab 2015; Roznik and Alford 2015; Roznik et al. 2015; Ruano-Fajardo et al. 2016; Burrowes et al. 2017; Greenspan 2017). Twenty-one articles mentioned behavioral thermoregulation within their discussion. Of those, six were field studies (Richards-Zawacki 2009; Forrest and Schlaepfer 2011; Becker et al. 2012; Rowley and Alford 2013; Ruggeri et al. 2015), and five studies were laboratory-based experiments on frog thermoregulatory behavior and its correlation with body temperature and the level of *Bd* (Woodhams et al. 2003; Murphy et al. 2011; Karavlan and Venesky 2016; Greenspan et al. 2017a, b). Barrile et al. (2020) found that *Bd*-infected boreal toads selected warmer and more-open habitats; that, in turn, was associated with elevated body temperatures and clearing of infection. However, there was no evidence that the boreal toads preemptively avoided microhabitats in which conditions were suitable for high *Bd* growth (Barrile et al. 2020).

Effect of temperature on amphibian defense mechanisms

Amphibians use several mechanisms of defense against *Bd* (Woodhams et al. 2014; Robak and Richards-Zawacki 2018); all of which may be influenced by temperature. Amphibians rely on environmental heat sources to maintain their body temperatures (Richards-Zawacki 2009; Voyles et al. 2017), and amphibian immune function is dependent on temperature (Raffel et al. 2013). As such, capacity to develop acquired immunity through the deployment of lymphocytes and the production of antibodies (McMahon et al. 2014) is temperature dependent. Likewise,

the production of skin secretions that contain antimicrobial peptides (AMPs), which have been shown to inhibit growth of *Bd* in vitro (Rollins-Smith et al. 2002; Woodhams et al. 2012), are affected by temperature, as is the efficacy of the skin microbial community in overcoming infections. In some species, temperature-mediated skin sloughing is an important mechanism for clearing *Bd* infections (Meyer et al. 2012).

Effects of temperature on amphibian AMPs: The AMPs—small amino acid residues in the skin glands of amphibians—serve as initial barriers against *Bd* (Rollins-Smith et al. 2002; Ribas et al. 2009; Márquez et al. 2010). Three articles described the effect of AMPs on *Bd* infection levels (Woodhams et al. 2007, 2012; Robak et al. 2019), and five articles explicitly considered the effect of temperature on AMPs in amphibians (Rollins-Smith et al. 2002; Sheafor et al. 2008; Ribas et al. 2009; Greenspan 2017; Robak et al. 2019).

To determine whether temperature inhibits AMPs, Rollins-Smith et al. (2002) tested the effectiveness of 10 peptides derived from North American ranid frogs (*Rana* sp.) against *Bd* at 22 C and 10 C. Inhibition occurred at both temperatures; however, the AMPs appeared more effective against zoospores at lower temperatures. That trial was conducted in vitro and did not account for the effect of temperature on the synthesis and release of the skin peptides (Rollins-Smith et al. 2002). Greenspan (2017) found that cold-acclimated frogs may have primed cellular immune systems that better respond to the challenges of *Bd* infection than frogs in a warmer habitat do, whereas heat pulses simultaneously hindered *Bd* growth and facilitated host immunity. Despite the relatively rapid generation time of *Bd*, *Litoria spenceri* individuals were able to clear infection when subjected to 4-h heat pulses of 29 C (Greenspan et al. 2017b).

Clearance of *Bd* infection is related to the host's innate immune system, rather than to an adaptive immune response (Ribas et al. 2009); either *Bd* does not stimulate or suppress adaptive immunity or trade-offs exist between the two immune systems (Ribas et al. 2009). At cold temperatures, *Silurana* (*Xenopus*) *tropicalis* loses the ability to mount an innate immune response against *Bd*, and instead, an inflammatory reaction occurs. Although there may be differences among species, this is a clear example of temperature dependency in an amphibian response to infection and the importance of the innate immune defenses in resistance to *Bd* (Woodhams et al. 2007; Ribas et al. 2009).

Effects of temperature on amphibian skin microbial community: The mucus on an amphibian's skin is home to a rich community of bacteria, which provides additional immune defense and varies among species (Daskin et al. 2014; Robak and Richards-Zawacki 2018; Assis et al. 2020). That variability could contribute to differences in susceptibility to chytridiomycosis (Woodhams et al. 2014; Robak and Richards-Zawacki 2018). Five articles explicitly studied the temperature-dependent effects of amphibian skin microbial community on the susceptibility to chytridiomycosis (Woodhams et al. 2014; Ackleh et al. 2016; Robak and Richards-Zawacki 2018; Assis et al. 2020; Ruthsatz et al. 2020).

Some skin bacteria have antifungal properties; for example, *Janthinobacterium lividum* produces a fungicide called violacein (Sheafor et al. 2008; Ackleh et al. 2016; Woodhams et al. 2016). The complex role of temperature in *Bd* infection dynamics and control strategies was evident because *J. lividum* was seen to be most inhibitory of *Bd* at 22 C, compared with other cultures of bacterial metabolites that were more inhibitory at lower temperatures (Woodhams et al. 2014). The skin bacterium *Serratia plymuthica* changed from inhibiting to enhancing *Bd* growth with a shift in temperature from 18 C to 25 C. Consistent with that, frogs survived longer at 14 C compared with 26 C after inoculation with antifungal bacterium *Stenotrophomonas maltophilia* (Robak and Richards-Zawacki 2018). Temperature not only affects the activity of *Bd* but also that of beneficial skin microbes; low temperatures reduced anti-*Bd* activity in 11 of 24 bacteria tested in vitro, which could explain why *Bd*-driven declines have occurred in cooler regions (Daskin et al. 2014). Bacterial strains with strong inhibitory effects

are most likely to produce broad-spectrum antimicrobial agents at a range of different temperatures and are, therefore, recommended when using antifungal bacteria as a means to reduce infection burdens (Muletz-Wolz et al. 2017).

Effect of temperature and Bd on amphibian sloughing rates: Sloughing occurs in amphibians on a regular basis, with the outer skin layer being shed in its entirety (Jørgensen and Larsen 1964; Fox 1986). Sloughing is an important first-line component of the innate immune system of amphibians (Meyer et al. 2012; Ohmer et al. 2015) and can determine the levels of beneficial skin microbes and *Bd* zoospores retained on the skin (Meyer et al. 2012). Excessive sloughing has also been described as a sign of infection with *Bd* (Berger et al. 2005; Woodhams et al. 2007; Andre et al. 2008).

Two articles identified in this review considered the effect of temperature on sloughing rates (Murphy et al. 2011; Meyer et al. 2012). Sloughing rate in amphibians was influenced by temperature and by the level of *Bd* infection (Murphy et al. 2011). In controlled laboratory trials, higher ambient temperatures led to increased sloughing in *Bd*-infected cane toads (*Rhinella marina*) and boreal toads (*Anaxyrus (Bufo) boreas boreas*) (Murphy et al. 2011; Meyer et al. 2012). Those trials suggest that increased sloughing rates could be an important defense mechanism to reduce levels of *Bd* infection because sloughing reduced cultivable cutaneous bacteria by up to 100% (Meyer et al. 2012), thus would also be expected to greatly reduce *Bd* loads on the skin.

Methods for measuring temperature

Of the 131 articles included in this systematic review, 54 articles provided detailed information on temperature-measurement methods used during field studies of *Bd*, including measurement of air, water, and host temperature.

Air and water temperate: Thirty-seven articles detailed how air temperature data were obtained, and 17 studies detailed water-temperature measurement methods. Those methods included data collected from nearby weather stations, loggers deployed on-site, handheld instruments, and weather station on site. Hobo loggers (UA-002-64, Onset, Bourne, Massachusetts, USA) or iButtons® (Maxim Integrated Products, Sunnyvale, California, USA) were installed when long-term temperature measurement was required (Becker et al. 2012; Heard et al. 2014; Beyer et al. 2015; Fernández-Beaskoetxea et al. 2015; Heard et al. 2015). If conditions prevented long-term installation, such as high flow rates, manual water-temperature measurements were taken (Kriger and Hero 2007). When sampling frogs in small microclimates, such as within bromeliads, water temperature at the time of capture was measured with a probe thermometer or infrared noncontact thermometer (Forrest and Schlaepfer 2011; Ruano-Fajardo et al. 2016). Those air and water temperature measurement methods are evaluated in Table 2.

Host body temperature: Skin temperature readings can provide a measure of the thermal regime experience by both host and pathogen. Temperature-sensitive radiotransmitters have been used to measure the temperature of individual frogs over longer periods (Rowley and Alford 2013; Stevenson et al. 2014; Greenspan et al. 2017a). Noncontact infrared thermometers have been used in both field and laboratory studies (Rowley and Alford 2007; Richards-Zawacki 2009; Rowley and Alford 2013; Kolby et al. 2015; McNab 2015; Ruano-Fajardo et al. 2016; Longo and Zamudio 2017). Frog body temperature can be measured with a digital pen thermometer against the body and under a folded rear leg (Catenazzi et al. 2014; Spitzen-van der Sluijs et al. 2017). Of the 54 studies that met the criteria for the above review, 14 studies measured frog body temperature in the field and reported the method used in detail (Table 3).

DISCUSSION

Temperature is a significant determinant of the epidemiology of chytridiomycosis. It

TABLE 2. Evaluation of methods used to measure air and water temperature during field-based frog research on chytrid fungus *Batrachochytrium dendrobatidis*.

Method	Source	Evaluation
Nearby weather station accessed via online database	Woodhams and Alford (2005); Drew et al. (2006); Bosch et al. (2007); Kriger and Hero (2007); Murray et al. (2009); Becker et al. (2012); Holmes et al. (2014); Heard et al. (2015); Ruggeri et al. (2015); Whitfield et al. (2017)	Cost: No cost. Weather station installed and managed by government. Reliability: High. Good quality equipment, well maintained. Accuracy: Medium to low. Weather station was often >20 km from the research site, which does not allow for microclimatic effects. Comments: Suitable weather station needs to be chosen and accuracy determined based on proximity to the research site.
Thermochron iButton or Hobo temperature loggers deployed at site	Kriger and Hero (2008); Sapsford et al. (2013); Heard et al. (2014); Korfel and Hetherington (2014); Beyer et al. (2015); Assis et al. (2020); (Bell et al. 2020)	Cost: Low to medium. $50–$100 USD per unit. Plastic Dip required to waterproof iButton. Mounting system, such as posts and cable ties, and a shelter, such as a plastic flowerpot. Multiple loggers required to deploy at individual survey sites. Reliability: Medium. Potential for loggers to fail, water damage, lost or stolen equipment. Accuracy: High. Placed at specific sites at which surveys will take place. Comments: Loggers need to be securely mounted on site to avoid potential damage from livestock, wildlife, or theft. Loggers should be regularly maintained and downloaded to avoid loss of data; once a month advised.
Probe or handheld instrument including infrared thermometer	Kriger and Hero (2007); Richards-Zawacki (2009); Kolby et al. (2015); Forrest et al. (2016); Ruano-Fajardo et al. (2016)	Cost: Low to medium. Regular electronic thermometer (as low as $5 USD) or technical weather meter, which can also measure humidity and wind speed (e.g., Kestrel 3500 Waterproof weather meter $200–$400 USD). Reliability: High. Measured at the site at the time of survey. Not possible to collect regular longer-term data using this method. Accuracy: Medium to high. Depends on quality of equipment and sampling method. Comments: Not possible to collect longer-term data using this method, unless physically visiting the site on a regular schedule. Low cost and minimal equipment required if monitoring many sites.
Weather station deployed at site	Murrieta-Galindo et al. (2014)	Cost: High. $100–$1,000 USD, depends on make and model. Reliability: Medium to high. If good quality equipment and deployed correctly. Requires regular maintenance. Accuracy: High. Located at site of survey. Comments: Suitable for long-term monitoring of sites to provide reliable and accurate air temperature data. Expensive if monitoring multiple sites.

TABLE 3. Evaluation of methods that have been used to measure frog body temperature during field-based frog research on chytrid fungus, *Batrachochytrium dendrobatidis*.

Method	Source	Comments
Contact thermometer against flank	Catenazzi et al. (2014)	Cost: Low. Ranges from $5–$100 USD depending on brand. Reliability: Medium to high. Depends on quality of instrument and methods of use. Accuracy: Medium. Could be influenced more by ambient temperatures depending on how wet the frog is and on the wind-chill factor. Comments: The frog would need to be restrained to take this measurement, which could cause stress. However, swabbing also requires restraint. The temperature needs to be taken immediately upon capture.
Contact thermometer under folded rear leg	Forrest et al. (2016)	Cost: Low. Ranges from $5–$100 USD. Reliability: Medium to high. Depends on quality of instrument and methods of use. Accuracy: High. By placing the thermometer between the abdomen and folded rear leg, a more-accurate reading for body temperature could be taken, which is less influence by wind chill. Comments: Holding the thermometer in this location could be more difficult than simply against the flank.
Noncontact infrared on dorsal surface	Rowley and Alford (2007); Richards-Zawacki (2009); Rowley and Alford (2013); Kolby et al. (2015); Barrile et al. (2020); Sonn et al. (2020)	Cost: High. $400–$600 USD. Reliability: High. Raytek ST-80 has been proven for use in amphibians and Testo-835-T1 has similar specifications. Accuracy: High. Within 0.5 C of cloacal temperature. Comments: Instantaneous and accurate reading of skin temperature, which can be taken before handling the frog. Make sure to take reading from suitable distance depending on instrument specifications.
iButton embedded in agar placed in microhabitat	McNab (2015); Roznik and Alford (2015); Roznik et al. (2015); Burrowes et al. (2017); Stevenson et al. (2020)	Cost: Low–medium. $50–$100 USD per unit. Plastic Dip required to waterproof iButton. Agar and mold required. Reliability: Medium. Potential for loggers to fail, water damage, and lost or stolen equipment. Accuracy: Medium. Placed in specific microhabitats at which target species is found. However, does not encompass full range of temperatures experienced by the frog throughout its daily routine. Comments: Less-invasive way to monitor frog body temperature in specific microclimates. Requires a bit of work in making, deploying, and maintaining the models. Data should be downloaded regularly to avoid loss.

affects not only the viability of the causative agent, *Bd*, but also the behavior, immune response, and microbial community of the amphibian host. Each species has a unique response to *Bd*, and each population and the individuals within it have different capacities to fight the infection load, depending on the biotic and abiotic characteristics of the habitat in which they occur, the microhabitats available therein, and the individual amphibian's microbial community. For species under immediate threat of population declines, research describing the thermal regime at multiple spatial scales is vital. Conservation implications include identification of potential sites for translocations (Puschendorf et al. 2011; Woodhams et al. 2012; Scheele et al. 2014) and manipulating temperature regimes to mitigate *Bd* impacts.

There is considerable variability in measurement methods among field studies determining the role of temperature in the epidemiology of *Bd*. This variability produces challenges for developing an integrated understanding of the role of temperature in *Bd* dynamics in the field. We, therefore, close with recommendations for field data collection, which have been informed by this literature review process and which seek to facilitate consistent methods of temperature data collection and analysis in research on *Bd* epidemiology.

Recommendations for field measurements of temperature

To quantify the temperature regimes experienced by frogs in the field, we recommend measurement of temperature at four spatial scales: regional climate, habitat, microhabitat, and amphibian host. By including measurements of temperature regimes at those four scales consistently among study species and regions of the globe in which the impact of *Bd* varies, it may be possible to gain an integrative, systematic, synthetic understanding of the role of temperature in the epidemiology of *Bd* in anurans.

Climatic regimes: Climatic data can be accessed from online meteorological resources specific to the region of study. Examples include the Commonwealth of Australia's Bureau of Meteorology (2018) and WorldClim (version 2.1; Fick and Hijmans 2017). Long-term climatic data are important for determining the average seasonal maximum and minimum temperatures for a region, as well as the variability in temperature regimes through time, which may be crucial for understanding *Bd* outbreaks or periods of low prevalence. Observing trends in climate in relation to the prevalence and intensity of *Bd* can enable predictions as to the spread of *Bd* as changes in climate occur (Pounds et al. 2006). Suggested temperature variables to use are monthly mean temperature, mean daily minimum temperature, and mean daily maximum temperature for the focal region. Measures of variability at annual, monthly, and daily scales can also be used (Rohr et al. 2008).

Temperature regimes of the habitat patch: Temperature regimes at a wetland scale can be measured using data loggers, such as Thermochron iButton (accurate to ±0.5 C) or Hobo loggers (0.1 C resolution; Heard et al. 2015). We recommend that at least two temperature loggers be mounted at each site, one to record water temperature and one for air temperature. Hobo loggers are waterproof, whereas iButtons must be sealed with Plasti Dip (Plasti Dip International, Blaine, Minnesota, USA; Roznik and Alford 2012). We recommend that loggers be set to record water surface temperature within 10–15 cm of the water's surface to target the primary active zone, unless aquatic species or tadpoles that experience temperatures at greater depths are the target species.

Water temperature loggers can be mounted inside a slotted polyvinyl chloride pipe, attached vertically on a post within the waterbody being surveyed (Fig. 3A). Attaching a float to the logger enables it to move up and down within the pipe to record water surface temperature (Fig. 3B). Logger placement should target the habitat zones of the target species. For many species, that will be the shallower verges within the littoral zone. However, reductions in water level may leave loggers in that zone exposed to the air,

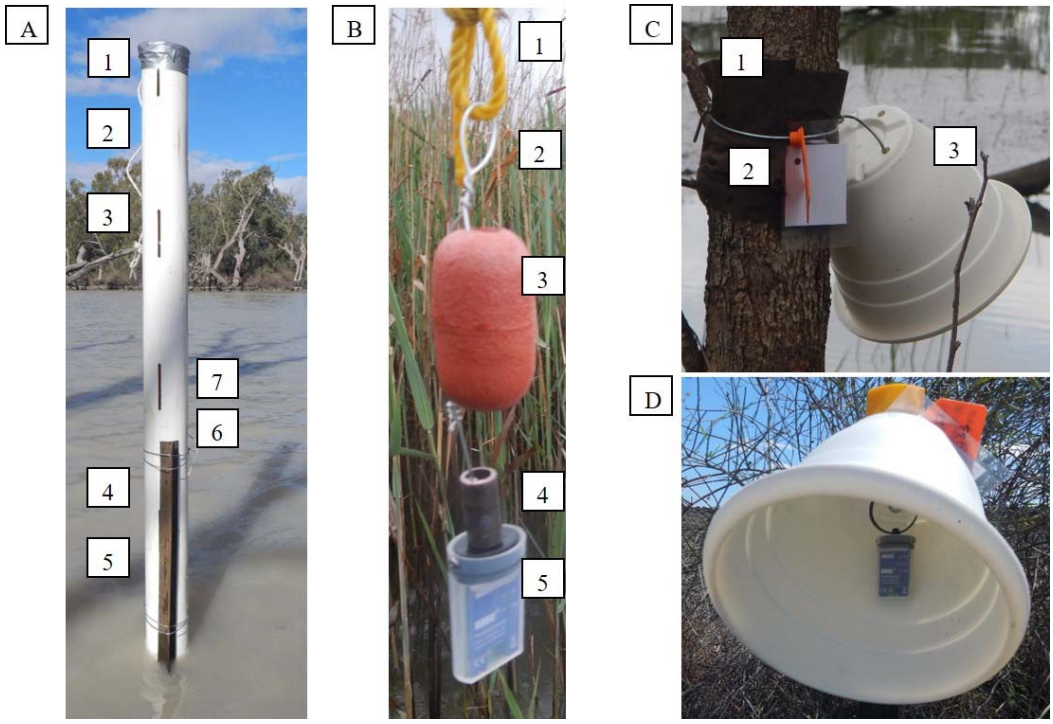

FIGURE 3. (A) Setup for measuring water temperature using data loggers, with logger placement in a protective polyvinyl chloride (PVC) pipe. Components are 1) a 90-mm cap, taped on, screw-top fixture additional cost (plumbing supplies $1.80 AUD each); 2) a 90-mm PVC white, slotted pipe, 2 m long (plumbing supplies $43 AUD for 6 m lengths); 3) rope to retrieve logger; 4) fencing wire to attached pipe to picket; 5) 170-cm steel picket post (fencing supplies approx. $10 AUD each); 6) tag with contact details and wildlife permits (not visible here); and 7) temperature logger inside post (see Fig. 3B). (B) Logger attachment set up for placement within PVC pipe housing shown in Figure S1. Components are 1) rope to retrieve logger from inside pipe; 2) wire to attach pieces together; 3) Wilson Y3 small oval poly float ($20 AUD 12 pack); 4) sinker to keep logger vertical, 10–15 cm below water surface; and 5) temperature logger (UA-001-08 Hobo pendant data logger, $80 AUD per unit). (C) Setup for measuring air temperature using data loggers, with logger placement in a protective white-plastic flower pot. Components are 1) wool tree guard for low-impact attachment to bank-side tree; 2) wire attachment, with ID tag and tag with contact details and wildlife permits; and 3) white ultraviolet-protected, plastic flowerpot. (D) Temperature logger (UA-001-08 Hobo pendant data logger, $80 AUD per unit).

producing inaccurate measurements. Waterline "tracking" may be required in that case (by moving loggers), or a series of loggers could placed perpendicular to the waterline in deeper water to ensure continuous measurement of water temperature as water levels retreat during warmer and drier seasons.

The air temperature logger should be mounted in the shade, on a post, or on a tree at a level that is representative of the zone occupied by the focal species. The use of a Stevenson screen is most appropriate for providing shade from radiant heat while still allowing the air to circulate (Bureau of Meteorology 2018). However, simple alternatives may be constructed by placing the logger within an upturned white plastic flowerpot (or equivalent), giving protection from rain and disturbance while allowing free air circulation to ensure measurements are representative of ambient shade temperature (Fig. 3C). Both water and air temperature loggers should be set to record on at least an hourly basis. Loggers should be deployed at sites 30 d before the first *Bd* sampling. Loggers should remain deployed for the duration of the study, or at least 1 full yr to collect the full annual temperature regime of that site.

Temperature measures that may be derived from the logger data include daily mean temperature, mean maximum temperature, and mean minimum temperatures (Kriger et al. 2007; Spitzen-Van Der Sluijs et al. 2014, 2017). Temperature regimes at this habitat-patch scale can also be estimated using models of water temperature, either those constructed using temperature measurements recorded during surveys (Heard et al. 2018) or those constructed from first principles (Dietze 2017; Kearney and Porter 2017). Building such models can be useful in determining the temperature regimes of wetlands without the time and cost involved in deploying temperature loggers at individual wetlands, especially when there are numerous study sites.

Temperature regimes of microhabitats: Temperature regimes of specific frog microhabitats can be measured using data loggers, such as those mentioned earlier, deployed directly in locations in which frogs are detected within the survey sites (Becker et al. 2012; Roznik et al. 2015). Loggers can be deployed embedded in agar frog models (3% agar) and placed in a range of microhabitats in which frogs are detected within the survey sites (Roznik et al. 2015; Burrowes et al. 2017). Because of data required at this scale, we suggest following the deployment method of Roznik and Alford (2015). Models should be placed in diurnal locations used by frogs to measure temperatures at 30-min intervals from 0700 to 1830 and, then, subsequently moved to nocturnal locations for temperature measurements from 1900 to 0630; these times should be adjusted to coincide with dawn and dusk times during the survey period (Roznik and Alford 2015). This scale of temperature measurement will detect variation in temperature caused by variables such as changes in shading throughout the day or small pockets of more-stable temperatures in which frogs might be sheltered from a cooling breeze (Raffel et al. 2010). Raw temperature measurements can be used in analyses (e.g., mean "body" temperature or maximum diurnal temperature; Richards-Zawacki 2009) or be used to calculate the proportion of time the frog was subject to temperatures below, within, or above the optimal range or above the lethal temperature for *Bd* growth (<17 C, 17–25 C, >25 C, and >28 C, respectively; Johnson et al. 2003; Piotrowski et al. 2004; Rowley and Alford 2013; Roznik and Alford 2015).

Frog body temperature: In some circumstances, thermoregulation by the frog ensures body temperatures are higher or lower than environmental temperatures, on average; in which case, environmental temperatures are not a good reflection of the thermal regime to which *Bd* is being exposed in vivo. In turn, body temperatures may be superior to environmental temperatures for explaining infection dynamics. Frog body temperature is most effectively measured using a noncontact infrared thermometer with a 50:1 distance: spot ratio, emissivity set at 0.95, and an accuracy of ±1.0 C (0.0 to +99.9 C). Raytek ST-80 (Fluke Process Instruments, Everett, Washington, USA) has been proven for use in amphibians (Rowley and Alford 2007) and Testo-835-T1 (Titisee-Neustadt, Germany) has similar specifications. Frog temperature should be taken on the dorsal surface, within 0.5 m, and either immediately before or immediately after capture.

In conclusion, the findings suggest that temperature is a significant driver of *Bd* disease epidemiology. It not only affects the pathogen itself but also the behavior, immune response, and microbial community of the amphibian host. Overall, climatic regimes may determine the infection prevalence of an amphibian population, but microclimate and the host's immune capabilities significantly determine *Bd* infection intensity. It is, therefore, important that the temperature-measurement recommendations made here be considered when conducting field research on amphibian species at risk of decline from chytridiomycosis.

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