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Mitigating Disease Impacts in Amphibian Populations: Capitalizing on the Thermal Optimum Mismatch Between a Pathogen and Its Host

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Understanding how animal behavior can influence the susceptibility of endangered hosts to emerging pathogens and using this knowledge to ameliorate negative effects of infectious wildlife diseases is a promising avenue in conservation biology. Chytridiomycosis, an emerging infectious disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) in amphibians has led to the most spectacular disease-borne loss of vertebrate biodiversity ever recorded in history. Unfortunately, the methods of mitigation that are available today are only practical in captive populations, and an effective method that could be applied in natural habitats without inflicting vast collateral damage is lacking. We suggest here that the thermal tolerance mismatch between *Bd* and its ectothermic hosts coupled with the thermoregulatory behavior of amphibians could be exploited in mitigation interventions combating *Bd* infection *in situ*. If microhabitats with elevated temperatures are made available in their natural environment, individuals taking advantage of the possibility to reach their preferred body temperature could critically lower their infection intensity or even clear the pathogen. We provide a basis for studying this approach by reviewing the evidence that supports the idea, describing how technical difficulties may be overcome, pointing out gaps in our knowledge that need to be filled by future studies, and listing presumable benefits and probable limitations of localized heating. The proposed approach has good potential to become an effective *in situ* mitigation method that can be easily employed in a wide taxonomic range of amphibians, especially in species that are warm-adapted, while causing less collateral damage than any other method that is currently available. If so, it may quickly become a widely applicable tool of biodiversity conservation and may contribute to saving many amphibian populations and species from extinction in the next few decades.

Keywords: *Batrachochytrium dendrobatidis*, *Batrachochytrium salamandrivorans*, emerging infectious disease, heated shelter, mitigation, thermal tolerance

INTRODUCTION

Emerging infectious diseases of wildlife pose a serious threat to biodiversity. They can have large economic costs via spill-over to livestock and, if zoonotic, they may also threaten human health (Daszak et al., 2000). Chytridiomycosis is an emerging infectious disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) in amphibians, where it has already led to the decline or extinction of hundreds of species (Skerratt et al., 2007; Wake and Vredenburg, 2008; Lips, 2016; Scheele et al., 2019). Chytridiomycosis continues to be one of the largest conservation issues because it is still spreading, it is highly virulent, and no widely applicable solution is in sight (Woodhams et al., 2011; Scheele et al., 2014, 2019; Garner et al., 2016). What makes this disease especially worrying is that it affects amphibians, which have been declining for decades and are already one of the most severely threatened vertebrate groups today (Houlahan et al., 2000; Stuart et al., 2004; Wake and Vredenburg, 2008), with 41% of species listed at least as threatened (IUCN, 2016).

Several lineages of *Bd* have been described (O'Hanlon et al., 2018), some of which have existed locally at least for decades without causing mass mortalities, but one hypervirulent lineage, *Bd*GPL, has spread throughout the world in recent years and caused extinctions across several continents (Farrer et al., 2011; James et al., 2015; Lips, 2016; O'Hanlon et al., 2018; Scheele et al., 2019). Spread is attributable to two main factors: human activities (especially transportation and animal husbandry; Weldon et al., 2004; Garner et al., 2006; Scheele et al., 2019) and natural processes (primarily via migrating animals, including non-amphibians carrying the fungus; Vredenburg et al., 2010; Garmyn et al., 2012; McMahon et al., 2013). When *Bd* arrives to a new area, it can spread in a wave-like front, often leading to local extinctions or resulting in sharp declines and leaving just dwindling remnants of amphibian populations (Rachowicz et al., 2005; Lips et al., 2008; Cheng et al., 2011). Moreover, due to climate change, or the appearance of new, more virulent lineages, *Bd* can also suddenly become devastating in locations where it has previously not led to disease outbreaks (Bosch et al., 2007; Rödder et al., 2010; Clare et al., 2016; Jenkinson et al., 2016; Cohen et al., 2018). Furthermore, these two mechanisms leading to mass mortalities in amphibians are not mutually exclusive and may act in concert (Fisher et al., 2009; Rohr and Raffel, 2010; Walker et al., 2010; Lips, 2016; Cohen et al., 2018, 2019).

Bd infects keratinous skin surfaces of amphibians (Berger et al., 1998). In larval amphibians, *Bd* infection usually causes only mild symptoms, including lethargy and poor swimming performance resulting in somewhat lowered body mass, but larval mortality due to chytridiomycosis is rare (Blaustein et al., 2005; Garner et al., 2009; Hanlon et al., 2015). In metamorphs and adults, clinical signs of the disease can include thickening of the outermost skin layer, reddening, ulceration and excessive shedding of the skin, lethargy and anorexia (Berger et al., 1998, 2005). Chytridiomycosis impairs breathing and osmoregulation, facilitates co-infection by other infectious agents, may induce immunopathology, and can ultimately lead to cardiac arrest (Voyles et al., 2009; Campbell et al., 2012; Whitfield et al., 2013).

Several mechanisms of defenses against infectious agents exist in amphibians. Individuals of many species excrete antimicrobial peptides (AMPs) onto their skin and these have been shown to depress *Bd* infection loads *in vitro* (Woodhams et al., 2007a; Rollins-Smith, 2009). However, some species possessing AMPs are highly susceptible to infection (e.g., Rollins-Smith et al., 2006, 2009), and many species lack AMPs completely (Conlon et al., 2009). The adaptive immune system of amphibians may also contribute to the suppression of chytridiomycosis, but results are mixed in this respect as well (Rollins-Smith et al., 2009; Ramsey et al., 2010; Stice and Briggs, 2010; Cashins et al., 2013; McMahon et al., 2014). Individuals may adopt behavioral patterns that prevent infection or lower pathogen burden (i.e., "behavioral fever"; Kluger, 1977; Sherman et al., 1991; Lefcort and Blaustein, 1995; Sherman, 2008; Rakus et al., 2017), but the number of studies reporting such behavioral alterations in response to *Bd* has remained very limited (Murphy et al., 2011; Karavlan and Venesky, 2016; but also see Han et al., 2008; Sauer et al., 2018). Finally, some symbiotic bacteria inhabiting amphibian skin produce antifungal metabolites that can hamper colonization by *Bd* and reduce its growth (Harris et al., 2006, 2009a,b; Lam et al., 2010, 2011). However, symbionts are not ubiquitous (Lam et al., 2010), their performance is environment-dependent (Daskin et al., 2014), and they do not protect against all *Bd* lineages (Antwis et al., 2015). Despite these variable and sometimes powerful defenses, many amphibian populations and species are threatened by chytridiomycosis and are likely to persist only if we implement effective measures against this deadly disease.

Several countermeasures to chytridiomycosis have been proposed as a result of intense research focusing on the biology of *Bd*, its interactions with amphibian hosts, and the factors influencing infection probability and disease progression (for reviews see Woodhams et al., 2011; Scheele et al., 2014; Garner et al., 2016). These include the application of salt and antifungals (Johnson et al., 2003; White, 2006; Pessier, 2008; Heard et al., 2014; Woodward et al., 2014; Bosch et al., 2015; Stockwell et al., 2015; Hudson et al., 2016; Geiger et al., 2017), the addition of probiotics (Woodhams et al., 2007b; Harris et al., 2009a; Muletz et al., 2012), immunization (Ramsey et al., 2010; McMahon et al., 2014), selection for *Bd*-tolerance in captive-bred populations (Garner et al., 2016), enhancement of the density of microscopic aquatic predators of *Bd* zoospores (Buck et al., 2011; Searle et al., 2013; Schmeller et al., 2014), general actions facilitating population persistence and recovery (Muths et al., 2011; Shoo et al., 2011; Heard et al., 2014), and elevating the temperature in the environment of infected individuals (Woodhams et al., 2003; Chatfield and Richards-Zawacki, 2011; Heard et al., 2014; Scheele et al., 2015). Indeed, some of these approaches proved to be highly effective when applied in captive populations. However, because of high costs of maintaining populations in captivity, only a few dozen species may be saved *ex situ* (Zippel et al., 2011), so that mitigation approaches suitable for treating infected amphibian populations in their natural habitats are needed (Scheele et al., 2014; Garner et al., 2016). Unfortunately, the methods listed above are in their current form not yet suitable for *in situ* application. They are either associated with vast collateral damage to the environment, may also harm amphibians, are impractical outside the laboratory, are associated with immense costs, are simply not effective under natural conditions, or their use cannot be permitted because of nature conservation legislation (Scheele et al., 2014; Garner et al., 2016). Consequently, a feasible mitigation strategy for lowering the threat posed by chytridiomycosis in natural populations is still lacking (Scheele et al., 2014, 2019; Garner et al., 2016).

Finding a suitable strategy for mitigation, including those mentioned above, will require further intense research into the basic ecology of the pathogen and of its interactions with amphibian hosts, but may contribute to saving hundreds of amphibian species from extinction. Our aim here is to draw attention to a presumably effective, safely applicable and rather simple method of *in situ* mitigation that conservation managers may readily employ in the fight against chytridiomycosis. We outline the basic idea, list evidence providing support for its potential, describe how it could be realized technically, point out knowledge gaps that need to be filled before its application, list presumable benefits and probable limitations, and thereby provide a basis for researching this promising approach.

A SUGGESTION FOR FIGHTING *BD IN SITU*

We suggest exploiting the difference in the thermal optima and maxima between *Bd* and its amphibian hosts in their natural environment simply by providing opportunities for amphibians to reach their preferred body temperature. According to *in vitro* studies, the optimal temperature for *Bd* is around 20°C, it grows well below 24°C, and its critical thermal maximum (CT_{max}) is around 28°C (Johnson et al., 2003; Piotrowski et al., 2004; Stevenson et al., 2013; Cohen et al., 2017; Voyles et al., 2017; also see **Table 1**). However, a global dataset on physiological heat tolerance comprising relevant data on 91 amphibian species suggests that the CT_{max} is higher than 32°C in ca. 80% of amphibian species and lower than 30°C in just 7% (lower than 28°C in just one species; see supplementary dataset in Sunday et al. (2014a); also see Ultsch et al., 1999; Gutiérrez-Pesquera et al., 2016). Importantly, CT_{max} strongly depends on age (Sherman and Levitis, 2003; Turriago et al., 2015) and it is weakly related to altitude and latitude while the phylogenetic footprint is significant (Sunday et al., 2014a,b; Gutiérrez-Pesquera et al., 2016). Nonetheless, exposure to temperatures of 28–30°C for a few days only will not be hazardous to a large number of amphibian species, but caution needs to be applied in case of cold-adapted species (see below). If the CT_{max} of the targeted species, or at least of closely related species is known, it will be possible to set wide enough safety margins on the temperatures to be applied. Elevated temperatures can directly kill *Bd* zoospores and cells encysted in amphibian skin and sporangia. In addition, the immune function of amphibians and the antifungal activity of symbiotic bacteria living on amphibian skin may also increase toward higher temperatures (Raffel et al., 2006; Rollins-Smith et al., 2011; Daskin et al., 2014), especially in warm-adapted-species (Cohen et al., 2017, 2019; Sauer et al., 2018). These mechanisms may act synergistically, lowering the need for exposure of individuals to high temperatures for a prolonged time period (**Table 1**). In cold-adapted species the effectiveness of the immune system may decrease more quickly with increasing temperature than the performance of *Bd*, resulting in relatively low temperature optima for the hosts (Cohen et al., 2017, 2018, 2019; Sauer et al., 2018). Because these cold-adapted species may not endure temperatures that surpass the CT_{max} of *Bd*, applying elevated temperatures may be counterproductive in their case. Consequently, it is important to first assess temperature preferences and critical thermal maxima of the species to be treated and thereafter apply heating, where the applied temperature should ideally surpass the CT_{max} of *Bd*, which is around 28°C (**Table 1**). The use of ambient temperatures elevated to 28–30°C may thereafter be a safe and effective way of treating amphibians infected by *Bd* (Berger et al., 2010; Woodhams et al., 2011; Scheele et al., 2014).

Several lines of evidence provide ample support for the potential of the *in situ* chytridiomycosis-mitigatory use of elevated temperatures. A good number of studies report successful clearing of *Bd*-infection or at least significant lowering of infection prevalence and intensity in captive populations after application of elevated temperatures (**Table 1**). In addition, infection may be prevented in the first place by a warm environment (e.g., Blooi et al., 2015). Also, theoretical models and empirical studies on *Bd*-prevalence and infection load revealed that both increase toward cooler areas and cooler periods of the year in tropical as well as in temperate climate zones (Retallick et al., 2004; Woodhams and Alford, 2005; Kriger et al., 2007; Walker et al., 2010; Forrest and Schlaepfer, 2011; Puschendorf et al., 2011; Fernández-Beaskoetxea et al., 2015; Gabor et al., 2015). These patterns are in accord with reports that in the tropics and subtropics, chytridiomycosis can have devastating effects in cool areas, like highlands, but much less so in warmer lowlands (e.g., Retallick et al., 2004; La Marca et al., 2005; Lips et al., 2006, 2008; Pounds et al., 2006; Walker et al., 2010; Rodríguez-Brenes et al., 2016), and during cool winter months, but much less during warmer parts of the year (Bradley et al., 2002; Berger et al., 2004). It is worth noting, however, that while some species suffer extreme population declines due to chytridiomycosis, others are little affected. This variation in the susceptibility to chytridiomycosis may partly be due to interspecific differences in the effectiveness of the immune system. However, species-, population- and sex-specific thermal profiles may also play a role: individuals that more often experience temperatures higher than 25°C are less likely to carry *Bd* (Rowley and Alford, 2013; Stevenson et al., 2014). Hence, elevating environmental temperatures beyond 25°C or, preferably, to 28–30°C may help amphibians keep *Bd*-infection intensities low or even clear the infection, and, thus, may be an effective strategy of chytridiomycosis-mitigation (Berger et al., 2010; Woodhams et al., 2011; Scheele et al., 2014).

If elevated temperatures are so effective in lowering *Bd*-prevalence and -loads, why has this approach not been applied so far in natural populations? A plausible explanation may be the apparently vast energy demand. Heating up the aquatic environment of larval amphibians to a high enough temperature and also maintaining this temperature would

TABLE 1 | The effectiveness of elevated environmental temperature applied against *Bd* as reported by experiments performed on laboratory cultures of the fungus (*in vitro* studies) and on live and infected amphibians (*in vivo* experiments).

Type of experiment	Treatment	Effectiveness	References
In vitro	28 or 29°C for 14 days	lowered *Bd*-growth at 28°C, no growth at 29°C	Longcore et al., 1999
In vitro	32°C for 4 days OR 37°C for 4 h	*Bd* wipeout in 100% of cultures	Johnson et al., 2003
In vitro	30°C for 8 days	*Bd* wipeout in 50% of cultures	Piotrowski et al., 2004
In vitro	26,5°C for 8 days	no zoospore release	Woodhams et al., 2008
In vitro	33°C for 1 h daily	lowered *Bd* growth	Daskin et al., 2011
In vitro	23, 25, 26, 27, or 28°C for 14 days	lowered *Bd* growth beyond 26°C	Stevenson et al., 2013
In vitro	26, 27, or 28°C constantly	no *Bd*-growth beyond 28°C	Cohen et al., 2017
In vitro	26°C for 4 h daily	lowered *Bd* growth	Greenspan et al., 2017a
In vitro	26, 27, or 28°C constantly	lowered *Bd*-growth at 27°C, no growth at 28°C	Voyles et al., 2017
In vivo	37°C for 8 h on two consecutive days	clearance of *Bd* from 100% of juvenile frogs	Woodhams et al., 2003
In vivo	27°C for 98 days	clearance of *Bd* from 50% of juvenile frogs	Berger et al., 2004
In vivo	32°C for 5 days	clearance of *Bd* from 100% of adult frogs	Retallick and Miera, 2007
In vivo	17 or 22°C constantly	enhanced survival of juvenile frogs (from 5 to 50%) at 22°C	Andre et al., 2008
In vivo	26°C for 42 days	reduced growth of *Bd* on adult frogs	Ribas et al., 2009
In vivo	17 or 23°C constantly	enhanced survival of adult frogs (from 7 to 81%) at 23°C, at exposure to low zoospore density	Bustamante et al., 2010
In vivo	30°C for 10 days	clearance of *Bd* from 96% of adult frogs	Chatfield and Richards-Zawacki, 2011
In vivo	26°C for 5 days OR 30°C for 8 + 8 + 43 h	clearance of *Bd* from 63 to 88% of tadpoles	Geiger et al., 2011
In vivo	temperature increased from 15 to 18°C	doubling of time to death in juvenile toads	Murphy et al., 2011
In vivo	30°C for 12 h + 35°C for 24 h	ineffective in clearing *Bd* from adult frogs	Woodhams et al., 2012
In vivo	30°C for 11 days	clearance of *Bd* from 100% of adult frogs	McMahon et al., 2014
In vivo	22, 26, or 28°C constantly	growth of *Bd* ceases at around 28°C in adult frogs	Cohen et al., 2017
In vivo	29°C for 4 h/day	clearance of *Bd* after 68 days from adult frogs	Greenspan et al., 2017a
In vivo	20, 23, or 26°C for ca. 90 days	*Bd* load at 23 and 26°C one order of magnitude lower than at 20°C in adult frogs	Sonn et al., 2017
In vivo	26°C for 63 days	lowered *Bd* load and elevated survival in adult frogs	Robak and Richards-Zawacki, 2018

Please note that the effectiveness was usually assessed in in vivo studies using qPCR, which may underestimate the effect of the thermal treatment on Bd cells because DNA extracted from dead cells may still be intact enough to amplify, resulting in false positives.

require a power input that can realistically not be provided in the wild in water bodies exceeding just a few m^3 in size (ca. 12 kWh necessary for each m^3 of water just to increase the temperature by 10°C; calculated as $W(kWh) = \left\{V\left(l\right) \times c(kJ/kg^{\circ}C) \times \Delta T\,(^{\circ}C)\,/3600\right\}$, where V is the water volume, c is the specific heat of water, ΔT is the change in temperature). In the terrestrial environment of adults, on the other hand, animals live dispersed, so that heating up their entire habitat is obviously absolutely impossible. Decreasing canopy cover around ponds and streams may lead to elevated temperatures and thereby provide sufficiently warm thermal refuges in many environments (Raffel et al., 2010; Geiger et al., 2011; Heard et al., 2014), but the approach of felling or pruning trees and shrubs may often not be an acceptable measure due to the collateral damage inflicted, especially so in protected areas, and removal of shading vegetation may simply not elevate temperatures to a sufficient extent in many others.

The obstacle of a vast energy demand can, however, be circumvented in many amphibians, especially in pond-breeding species, by applying localized heating: amphibians may be provided with localities where they can reach their preferred body temperatures also during times and at places where this would otherwise be impossible. It is not necessary to heat up the entire water body, its immediate surroundings or large areas that cover the terrestrial habitat, because if individuals are provided with warm spots, they will use these thanks to their inherent warmth-searching drive (e.g., Heath, 1975; Wilbur, 1980; Dupré and Petranka, 1985). Larvae, juveniles and adults of anuran amphibians are known to select warm areas in their natural habitat (e.g., shallow areas of ponds and sun-lit spots on land) and generally select temperatures between 26 and 30°C (Wells, 2007), while urodelans prefer somewhat lower temperatures ranging from 18 to 26°C, depending on species and on developmental stage (e.g., Licht and Brown, 1967; Heath, 1975; Hutchison and Hill, 1976; Dupré and Petranka, 1985; Fontenot and Lutterschmidt, 2011). Because of their fossorial life history, thermal preferences in caecilians (Gymnophiona) are not well-understood, but Bd has also not been detected in this group yet. Consequently, individuals of many anuran species are likely to readily occupy areas with artificially elevated temperatures.

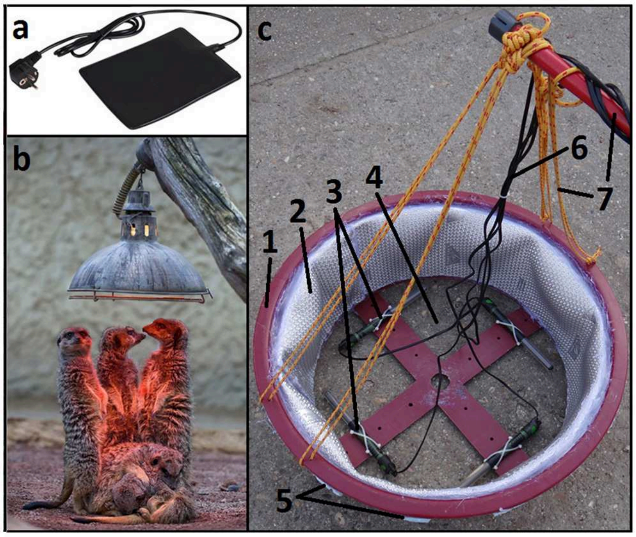

FIGURE 1 | Appliances that may be used for the realization of localized heating: **(a)** heat mat, **(b)** infrared heat lamp, as employed also for mammals in zoos (San Diego Zoo in this case), and **(c)** submersible heater [prototype: (1) skirting preventing heat dissipation via conductance; (2) insulation; (3) aquarium heaters fixed to bottom of appliance; (4) bottom with holes allowing amphibian larvae to enter warm spot; (5) swimmers under the upper edge stabilizing appliance and keeping it afloat; (6) electric cables; and (7) suspension facilitating in-pond placement of appliance].

If individuals can assess their *Bd*-infection status and use warmer locations when necessary (i.e., behavioral fever; Murphy et al., 2011; Karavlan and Venesky, 2016), the effectiveness of localized heating may further be enhanced. Breeding adults, aquatic larvae and newly metamorphosed individuals could be targeted relatively easily in most species, because they occur aggregated both in time and space. Also, amphibians contract *Bd* during these water-dependent events or life stages, and the disease most often leads to mass-mortality shortly after metamorphosis (Van Rooij et al., 2015). Hence, lowering disease transmission rates and infection loads in larvae, metamorphs and reproducing adults using localized heating is likely possible in many species, and this is a plausible objective for any *in situ* chytridiomycosis mitigation method.

The technical realization of localized heating is simple in theory. In the terrestrial environment artificial shelters built around thermostat-controlled heat mats (e.g., 20 × 20 cm, consuming 10 W) or infrared heat lamps (e.g., effectively lit area of 0.1 m^2 at a consumption of 100 W), as applied by terrarists and home gardeners, may be used (**Figure 1**). Heated terrestrial shelters may be dispersed around breeding ponds to target adults and freshly metamorphosed individuals leaving the water. In the aquatic environment, submersible aquarium heaters can be used to locally elevate water temperature. Heat dissipation through convection poses a problem here, but this can be overcome using an insulated skirting around heaters, which helps contain the warm water, but allows amphibians to enter from below (e.g., submersible heaters consuming just 500 W are sufficient to elevate the water temperature by 15°C in a 20 cm deep layer over a semi-isolated area of 0.5 m^2; **Figure 1**). As the power source, a landline is most convenient, but a power generator, a solar power system or their combination may also be suitable for supplying heaters (ca. 100 heated terrestrial shelters or 2 submersible heaters/kW). The requirements these devices have to meet are the ability to heat up their immediate environment and amphibians therein to temperatures between 28 and 30°C, to be portable and usable also in remote areas, to have relatively low power needs, to be cost-effective, be easy and safe to run also by non-academic personnel, and, importantly, to be attractive to amphibians. Solutions for several technical issues will have to be found, including the prevention of dehydration of amphibians using terrestrial warm spots, keeping individuals away from hot surfaces of heaters while still allowing efficient heat uptake, keeping predators away from amphibians aggregating on warm spots, or constructing funnel-trap-like one-way entrances for species or life-stages that would not use the provided warm spots for long enough voluntarily, but would endure these temperatures without damage. Finally, different climates and habitat types may require or allow for the use of different appliances. Consequently, the theoretical and technical expertise of electro technicians and energy engineers is likely to prove helpful during development and optimization, but none of these technical difficulties appear unsolvable.

KNOWLEDGE GAPS THAT NEED TO BE FILLED

Intensive research over the last two decades delivered detailed insights into several aspects of the biology of *Bd* and its interaction with amphibian hosts, but there have remained several important knowledge gaps that would first need to be filled before localized heating can be applied *in situ* routinely. One field where we lack sufficiently detailed information regards the combination of treatment duration and the temperature necessary to lower infection loads or clear *Bd* completely (for basic characteristics of the thermal ecology of *Bd* and of its interaction with amphibian hosts see **Table 1**). For the treatment of captive populations, where elevated temperatures can be provided for long periods without difficulty and immediate surveillance is possible, it may be sufficient to evaluate a combination of temperature and exposure time—which experimentation reveals will clear *Bd* from a treated population—then apply that preventively or in case infection is suspected. However, for the *in situ* application of localized heating, where the energy available for heating is likely limited and the direct observation of treated individuals will often not be possible, we need to know rather exactly the combinations of minimum treatment temperatures and exposure durations that effectively lower infection loads of *Bd*, or, preferably, lead to its complete clearance. In parallel, behavior of amphibians at various life-stages in relation to the use of warm spots will have to be studied to assess if individuals would voluntarily use warm areas for long enough (e.g., Sauer et al., 2018), or if repeated but short stays in warm spots may sufficiently lower *Bd* burdens

(Greenspan et al., 2017a). This is necessary for deciding if heated areas can be designed so that animals can come and go at their will, or if they have to have funnel-trap-like one-way entrances to keep animals inside for long enough. In the latter scenario, conservation officers or scientists would need to manage the process, but this would create the opportunity to mark treated animals and follow up on their future, thereby obtaining reliable estimates on the efficiency of the applied *in situ* treatment.

Temperatures that are effective against *Bd* are relatively low, so that treatment of amphibians with elevated temperatures is considered a safe approach, and has been used in captive populations. Nonetheless, knowing the basics of the thermal ecology of the species to be treated is important because exposure to temperatures around 30°C during the entire larval period can already lower survival and growth rate of tadpoles and metamorphs of some species (Harkey and Semlitsch, 1988; Bellakhal et al., 2014; Goldstein et al., 2017). This knowledge can, however, be obtained by applying simple tests of heat tolerance (e.g., assessment of CT_{max} by observing the temperature at which muscular coordination becomes disorganized; Hutchison, 1961; Huang et al., 2006; McCann et al., 2014; Greenspan et al., 2017b). Elevated temperatures may nonetheless also cause sublethal damage in treated individuals, which have remained largely ignored so far. High temperatures experienced during gametogenesis and gamete maturation have been proposed to impair gamete quality in adult amphibians (Woodhams et al., 2012), but we know of no report documenting such an effect at temperatures around 30°C. Even if remaining below CT_{max}, environmental temperatures maintained at around 28–32°C for several weeks or during the entire larval period can lead to lowered growth and development rates (Angilletta and Dunham, 2003; Bellakhal et al., 2014; Carreira et al., 2016; Goldstein et al., 2017; Phuge, 2017), potentially depressing fitness of treated individuals (Smith, 1987; Semlitsch et al., 1988; Berven, 1990; Altwegg and Reyer, 2003; Schmidt et al., 2012). Also, even brief exposure to 30°C can increase stress hormone levels in adult frogs (Juráni et al., 1973; Narayan and Hero, 2014). However, within pessimal temperature limits, growth rate and overall physiological performance is positively related to temperature in ectotherms (Angilletta and Dunham, 2003; Carreira et al., 2016), and exposure to mildly elevated temperatures applied for a few days only is unlikely to lead to intolerable decreases in fitness even in species with a relatively low CT_{max}. Whether temporally limited exposure to mildly elevated temperatures can have significant negative fitness-effects remains to be assessed.

Elevated temperatures experienced during the sensitive period of larval development can lead to sex reversal in amphibians and only a handful of studies have so far investigated this phenomenon in amphibians (Chardard et al., 2004; Eggert, 2004; Nakamura, 2009). These studies report sex reversal to rarely occur with an effectiveness of 100% and only if environmental temperatures exceeding 28–32°C are maintained for weeks (Hsü et al., 1971; Dournon et al., 1984, 1990; Wallace et al., 1999; Wallace and Wallace, 2000; Chardard et al., 2004; Sakata et al., 2005; Phuge, 2017). Also, sex-reversed individuals may be infertile in some cases but fertile in others (Dournon et al., 1984; Wallace et al., 1999) and the sex-biasing effect may depend strongly on the timing of exposure to elevated temperatures [Muto, 1961 reviewed in Chardard et al. (2004), Wallace et al. (1999) and Sakata et al. (2005)]. Further, temporal variation in temperature may disrupt the sex-reversing effect of heating (Neuwald and Valenzuela, 2011), while the *Bd*-clearing effect may be upheld (Woodhams et al., 2003; Stevenson et al., 2014; Greenspan et al., 2017a). As sex reversal in amphibians (generally masculinization) is likely caused by lowered estrogen synthesis paralleled by elevated androgen and testosterone synthesis (Nakamura, 2009; Kitano et al., 2012), sex reversal could also be avoided by treating individuals with estrogen during heating, as shown in medaka (Kitano et al., 2012). However, the scarcity of reports makes it difficult to draw general conclusions (Chardard et al., 2004). Hence, for a safe application of elevated temperatures, it has to be determined if the temperature necessary to lower *Bd*-burden in infected animals may affect life history traits negatively, lead to lowered fertilization success, to compromised offspring viability, or to high rates of sex reversal. It should also be assessed whether these malign effects may be prevented by allowing temporary fluctuations in body temperature of treated animals, by hormonal balancing or by careful selection of the timing of thermal treatment outside the sensitive developmental window.

The immune function of amphibians and the antibacterial and antifungal activity of their skin microbiome are generally assumed to be highest around 28–30°C (Raffel et al., 2006; Rollins-Smith et al., 2011; Paull et al., 2012; Daskin et al., 2014), while optimal temperatures may depend both on the species of amphibians and skin bacteria. However, elevated temperatures may also promote the replication of microparasites, such as Ranaviruses ((Echaubard et al., 2014; Price et al., 2019); but also see Rojas et al., 2005; Allender et al., 2013) or the growth, reproduction and infection intensity of macroparasites (Mouritsen, 2002; Thieltges and Rick, 2006; Studer et al., 2010; Tinsley et al., 2011; but also see Kluger, 1992; Lafferty, 2009). Relevant tests investigating whether exposure to elevated temperatures—as would be the case at warm spots—enhance the susceptibility of individuals to pathogens and parasites are scarce and contradictory (e.g., Rojas et al., 2005; Allender et al., 2013; Cohen et al., 2017; Price et al., 2019), and do not yet allow general conclusions. Studies on the effects of elevated temperature on disease progression in individuals co-infected with *Bd* and additional pathogens (especially thermophilic Ranaviruses) and parasites are lacking completely, and would be needed urgently. Also, the risk of infection can be positively related to density (Briggs et al., 2010), so that by using warm spots that are attractive to amphibians, individuals may suffer an elevated risk of contracting the disease at the resulting aggregations. However, it appears that disease transmission rates will likely be lowered in heated microhabitats (Blooi et al., 2015). Nonetheless, the net outcome of elevated temperature and high density as resulting from localized heating remains to be investigated.

BENEFITS AND LIMITATIONS

There are multiple benefits of using localized heating *in situ* against chytridiomycosis. It will presumably be effective also under natural conditions and is likely to pose no danger to amphibians because temperatures can be set with a wide enough safety margin (Sunday et al., 2014b). Also, localized heating will cause practically no collateral damage to the environment because it operates by only slightly elevating environmental temperatures in small spatial fractions of the habitat while leaving the microbiome, flora and fauna of the surrounding environment practically unaffected. Most importantly, however, as opposed to the *ex situ* approach, in case of localized heating applied *in situ*, individuals will come into contact with the pathogen and will be cured, often repeatedly, so that they may become immunized (Ramsey et al., 2010; McMahon et al., 2014; but also see Tobler and Schmidt, 2010; Hudson et al., 2016). Populations treated *in situ* may adapt to *Bd*-presence via altered life history traits (Palomar et al., 2016), by the spread of certain MHC class II alleles (Savage and Zamudio, 2011, 2016; Bataille et al., 2015), or by producing more potent skin secretions (Voyles et al., 2018), providing effective defenses against lethal chytridiomycosis. Consequently, via these mechanisms and by preserving enough genetic variation and maintaining infected populations for a sufficiently long time period for microevolutionary changes to occur, *in situ* mitigation using localized heating may often allow for effective adaptation to *Bd* presence, making long-term mitigation interventions obsolete. Based on preliminary studies we estimate that the re-usable equipment (including 25 heated refugia, 5 immersible heaters and a power generator) necessary to provide enough warm spots for treating amphibians in and around a small pond of 1 ha will cost ca. 4,000 *e*. Consumables (mainly petrol for running the power generator) for one season will amount to another 4,000 *e*, but this cost may be significantly lowered if electricity is available. Finally, transportation and personnel costs have to be added. The relatively low total costs of buying and running the equipment and a good transportability will allow for its application basically anywhere.

Nonetheless, there are also limitations to the application of localized heating. Species whose CT_{max} is similar to that of *Bd* may avoid locations of high temperatures and will not profit from this mitigation approach (Nowakowski et al., 2016). Individuals of other species exhibiting a high enough CT_{max}, but a preferred body temperature that is lower than the upper bound of the optimal temperature range of *Bd*, would also not spend enough time voluntarily at areas with elevated temperatures. Funnel-trap-like, one-way entrances of heating appliances, coupled with attentive monitoring by personnel, may provide effective solutions. In very cold habitats, where the temperature stays below what is optimal for *Bd* (i.e., below ca. 12°C; Piotrowski et al., 2004; Stevenson et al., 2013), heating may be impractical because of a high energy demand and because heaters may be unable to create high enough temperatures homogeneously within appliances, thereby increasing the reproductive rate and pathogenicity of the fungus locally (Pounds et al., 2006; Bosch et al., 2007). If environmental temperatures are high enough (higher than ca. 12°C), both heated terrestrial shelters and submersible heaters can provide homogeneous warmth within appliances. However, the heat dissipating from them is not measurable 1 cm away from their walls, leading to essentially no temperature gradient around appliances. This has, however, the consequence that in cold habitats the heated areas may differ too much from the surroundings, so that they may be avoided by cold-habituated amphibians. Importantly, elevated temperatures may enhance replication rates and the spread of Ranaviruses, a group of globally emerging pathogens causing epidemics and mass-mortalities in fishes, amphibians and reptiles (Brunner et al., 2015; Duffus et al., 2015). Although results of the few existing studies are contradictory (Rojas et al., 2005; Allender et al., 2013; Echaubard et al., 2014; Price et al., 2019), a pre-screening for the presence of Ranaviruses is necessary before the application of localized heating, and in populations co-existing with a Ranavirus the application of localized heating can currently not be recommended. Finally, financing the necessary appliances, their transport to the target locations and the personnel running the equipment will be possible in many replicates, but an *en masse* employment of the proposed method will still remain limited by costs and logistics.

Eradicating *Bd* from entire geographic regions using localized heating, or, indeed, any mitigation method will often not be possible (Garner et al., 2016). It may, however, be suitable for the preservation of the most valuable populations. Also, it is an encouraging conclusion of previous studies that we do not have to eliminate *Bd* to prevent mass mortalities and extinctions due to chytridiomycosis (Briggs et al., 2010; Tobler et al., 2012; Schmeller et al., 2014; Hudson et al., 2016). For example, Vredenburg et al. (2010) observed in three North-American frog metapopulations that mass mortality only commenced once infection intensities passed a threshold. Experimental laboratory-based studies confirm that many amphibians do not show clinical signs and mortalities as long as infection intensities remain low (e.g., Carey et al., 2006; Cheng et al., 2011). Also, amphibian populations where the local climate allows individuals to elevate their body temperatures at least temporarily above the CT_{max} of *Bd*, and can thereby lower infection intensities, have been shown to maintain their population sizes even at high *Bd* prevalence (Riley et al., 2013; Rowley and Alford, 2013). Consequently, suppressing infection intensities and thereby ensuring the survival of amphibian populations in the presence of *Bd* may represent an effective, and usually the only viable *in situ* mitigation strategy (Garner et al., 2016).

Besides fighting *Bd*, localized heating may also be applied against other emerging infectious diseases caused by agents whose CT_{max} is lower than that of their hosts. For example, *Batrachochytrium salamandrivorans* (*Bsal*), a sister species of *Bd* that causes disease in urodelan amphibians (Martel et al., 2014) and has recently led to severe population declines in European newts and salamanders (Spitzen-van der Sluijs et al., 2013, 2016; Stegen et al., 2017), grows best at temperatures between 10 and 15°C and its CT_{max} is around 25–26°C (Martel et al., 2013; Blooi et al., 2015; Laking et al., 2017; Beukema et al., 2018). Although these decisive temperatures are lower in *Bsal* than in *Bd*, the preferred temperatures and CT_{max} of urodeles are in general also lower than those of most anurans, so that localized heating may not work for clearing *Bsal* from many cold-preferring urodeles just by the effect of elevated temperatures. Nonetheless, it may very well be effective as a supportive mitigation action in many salamander and newt species, because even mildly elevated temperatures (i.e., 20°C) may decisively limit the growth of *Bsal* (Martel et al., 2013; Blooi et al., 2015). Also, exogenous heat introduced into water bodies used by urodelans for reproduction accumulates via convection at the water-air interface, where *Bsal* cysts float out of reach for most aquatic predators (Stegen et al., 2017). If *Bsal* cysts exhibit similarly low CT_{max} as non-encysted cells, the application of localized heating may, as a beneficial side-effect, also critically reduce densities of these otherwise resistant and infective life-stages of *Bsal*.

SUMMARY

Widely deployable and effective measures of *Bd*-mitigation are urgently needed for averting the ongoing biodiversity crisis caused by chytridiomycosis. After an epizootic caused by *Bd* lead to sharp declines in previously stable amphibian metapopulations, a slow recovery may occur in some species (Newell et al., 2013; Knapp et al., 2016; Scheele et al., 2017; Voyles et al., 2018), but many others will be extinct. Also, new *Bd* strains, other infections or invasive alien predators may arrive, anthropogenic pollution, or simply chance events may cause the weakened amphibian populations to disappear (Murray et al., 2009; Puschendorf et al., 2011). We suggest a method that could be used far more easily and more widely while causing less collateral damage than any method which has been suggested so far for *in situ Bd*-mitigation. It is unlikely that one single method will solve the conservation problem posed by *Bd* (Garner et al., 2016), and research into other methods that have proven to be successful in captive populations and that are being tested and optimized for application in the field are urgently needed. Nonetheless, localized heating will likely prove to be a highly valuable approach, especially in the case of disease outbreaks and when the most threatened species or populations are to be saved. If mortality rates due to chytridiomycosis are suppressed by the application of localized heating, genetic variability may be retained in populations that selection can act upon: alleles promoting resistance or tolerance to *Bd* may not disappear during severe genetic bottlenecks and may spread so that prolonged mitigation may become obsolete. We do not propose that by researching and applying localized heating it will become possible to eradicate *Bd* from entire regions or save all extant amphibian species from extinction due to chytridiomycosis, but suggest that this method has a good potential for significantly contributing to the preservation of hundreds of populations and dozens of species that are likely to go extinct unless we find and employ an effective mitigation strategy against this deadly disease.

DATA AVAILABILITY

No datasets were generated or analyzed for this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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