Review

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The ecology and impact of chytridiomycosis: an emerging disease of amphibians

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Emerging infectious diseases are increasingly recognized as key threats to wildlife. *Batrachochytrium dendrobatidis* (Bd), the causative agent of chytridiomycosis, has been implicated in widespread amphibian declines and is currently the largest infectious disease threat to biodiversity. Here, we review the causes of Bd emergence, its impact on amphibian populations and the ecology of Bd transmission. We describe studies to answer outstanding issues, including the origin of the pathogen, the effect of Bd relative to other causes of population declines, the modes of Bd dispersal, and factors influencing the intensity of its transmission. Chytridiomycosis is an archetypal emerging disease, with a broad host range and significant impacts on host populations and, as such, poses a crucial challenge for wildlife managers and an urgent conservation concern.

Introduction

Infectious diseases are increasingly recognized as key threats to animal populations, but relatively little is understood about their ecology compared with human pathogens [1]. Several emerging diseases have caused declines in multiple families of plants and animals, including West Nile virus in North American birds [2], avian malaria in Hawaiian birds [3], rinderpest in African ungulates [4], sudden oak death in trees in western North America, Jarrah dieback or rootrot in trees in Australia [5], and chytridiomycosis, first described in 1998 and caused by *Batrachochytrium dendrobatidis* (Bd; Box 1), in amphibians in North and Central America, Europe, and Australia [6–8]. For each of these diseases (many of which were introduced anthropogenically into new regions), the pathogen has a broad host range, with some species showing little pathology (Box 2), whereas others suffer mortality approaching 100%.

The Global Amphibian Assessment (http://www.iucnredlist.org/amphibians) recently argued that the 6000+ species of amphibians are one of the most threatened classes of vertebrate, with 32.5% of species threatened. In addition, 92.5% of the 'critically endangered' group are undergoing 'enigmatic declines' that might be linked to Bd [9,10]. In Latin America, Bd has been linked to possible extinctions in 30 of the 113 species of *Atelopus* harlequin toads [11]. Amphibian declines are currently one of the most compelling conservation issues and Bd, first identified only a decade ago, appears to have had an important role. Here, we focus on outstanding questions in the emergence of this disease, including the origin of the pathogen, its impacts on host populations and the ecology of its transmission.

Introduced or endemic pathogen?

There has been much debate about whether Bd was recently introduced to areas where it is causing population declines (the 'novel pathogen hypothesis'), or whether it has been a long-term endemic pathogen, and population declines result from changes in host susceptibility, pathogen virulence, environmental changes, or a combination of these factors (the 'endemic pathogen hypothesis') [12–17]. This is a crucial question, because conservation actions would differ substantially if it is an introduced pathogen (e.g. implementing trade restrictions to stem additional spread, or attempted eradications), or an endemic pathogen, in which case efforts to determine the environmental changes driving emergence of Bd would become paramount.

Genetic analyses of Bd isolates have been used to attempt to distinguish between the novel and endemic pathogen hypotheses [12,15,17]. Preliminary evidence supports the novel pathogen hypothesis, in that geographically disparate isolates appear genetically similar, and relatively little variation exists globally. However, this evidence is not definitive, because a source population with higher genetic diversity has yet to be identified, and even recently developed markers have few alleles per loci, making it difficult to delineate spatial population structure [12,15,17]. Full sequencing of the Bd genome has been completed (http://www.broad.mit.edu/annotation/genome/batrachochytrium_dendrobatidis.3/Home.html) and should facilitate the development of additional molecular markers and, subsequently, the frequency of recombination [12,17].

Wave-like declines of amphibians have also provided some support for the novel pathogen hypothesis [18] and, in the most well-documented case, the arrival of Bd coincided with declines in amphibian populations [6]. Earlier spatiotemporal patterns of *Atelopus* spp. frog declines from 1979 to 1998 in Central America have also been used

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Box 1. The pathogen: a natural history

Batrachochytrium dendrobatidis (Bd) is a chytrid fungus (Chytridiomycota; Chytridiales), first reported as the cause of chytridiomycosis in wild and captive frogs collected in North and Central America and Australia [7] and described as a species in captive South American frogs [41]. It has been found on six continents [14], and in specimens collected as far back as 1938 [16]. Distributional information for Bd is being compiled by several research groups and is available from two sources: http://www.parcplace.org/bdmap2008update.html and http://www.spatialepidemiology.net/bd.

Bd has two known life stages that are typical of chytrids (Figure I): a sessile, reproductive thallus with a single zoosporangium and motile, uniflagellated zoospores released from the zoosporangium that can swim for up to 24 h covering a distance of 2 cm in still media [41,82,83]. It grows and reproduces in culture under a range of temperatures (4–25 °C) and pH (4–8) and can also withstand freezing to some degree [84]. Optimal growth occurs between 17–25 °C and a pH of 6–7, but tradeoffs exist between the zoosporangium maturation rate (which increases with temperature) and the number of zoospores produced per zoosporangium (which decreases with temperature), such that similar population growth rates can be achieved across a range of temperatures [85]. Nonetheless, several strains show reduced growth and/or mortality above 28 °C, and this appears to influence infection patterns [83].

Survival outside the host is important for the transmission dynamics of Bd and other pathogens, and makes it more likely that the pathogen will drive a host population extinct [64]. Several studies have attempted to determine how long Bd can survive in the environment. In sterile sand or lake water, Bd persisted for up to three months in laboratory conditions [32]. In addition, because Bd can be cultured, it is possible that it can persist indefinitely outside of a host. However, its persistence in non-sterile conditions when faced with competition from other microbes is not known [41] and environmental sampling has not yet demonstrated its presence in the environment following host extinction. It is unknown whether it reproduces sexually but some evidence of recombination exists [12,17]. One study found evidence of spherical unicellular organisms on the surface of frog skin that was PCR-positive for Bd [86]. The identity of these spores is unknown, and they have the same size and morphology as some bacterial spores (Daszak, unpubl. obs.). Demonstrative evidence that these were a resting spore stage would require isolating these spheres, holding them for some time (e.g. weeks to months) under various environmental conditions, and using them to re-infect a susceptible animal.

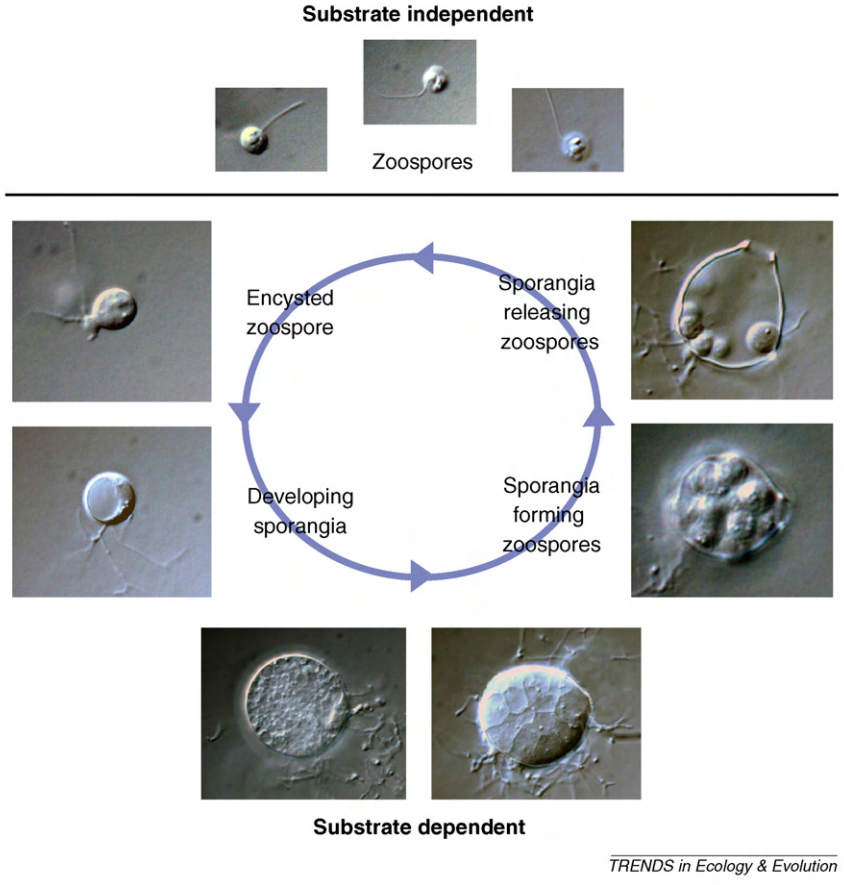

Figure I. The life cycle of Bd. In the substrate-independent part of the life stage, flagellated zoospores are motile and free living. By contrast, in the substrate-dependent part of the life cycle (e.g. after infecting a host), a zoospore encysts and develops into a reproductive thallus (not labeled) with a single sporangium which produce and release new zoospores. Reproduced, with permission, from [79].

to test between the spatial spread expected following the introduction of a novel pathogen and a climate–disease interaction hypothesis termed the 'chytrid-thermal-optimum hypothesis' (CTOH). In this hypothesis increasing temperatures are proposed to have increased Bd growth that then led to the observed population declines [19,20]. Recent work has shown that several of the predictions of the CTOH are not supported. The increased temperatures over the past three decades are more likely to result in decreased, rather than increased, Bd growth (daytime temperatures are too warm; Box 1), the convergence of minimum and maximum daily temperatures did not occur until after most of the *Atelopus* spp. declines, and the temporal patterns of declines were better correlated with

Box 2. Pathogenesis of Bd infections in amphibians

Despite a decade of research on chytridiomcyosis, there is still a lack of clarity on how this pathogen causes sickness and death (its pathogenesis). Understanding pathogenesis of *Batrachochytrium dendrobatidis* (Bd) could be crucial in understanding its ecology because the changes that the infection causes in individual amphibian behavior (e.g. water-seeking behavior, basking [87], reduced movement or increased predation) can alter disease and population dynamics at large scales.

There are two key lesions in Bd infection: First, in larvae, the loss of sections of the keratinized mouthparts; and second, in post-metamorphic individuals, proliferation (hyperplasia) of keratinaceous cells and fusing of the keratin layers (keratosis). In adult amphibians, it is thought that thickening of these impermeable layers might interfere with osmoregulation or ion balance across the skin [7,88], and sporangia size could influence the extent of pathology [45].

In larvae, a growing literature suggests that infection reduces grazing efficiency, food intake, and survival, although impacts in the latter are variable (Online Supplementary Table S1) [89,90]. In a significant recent advance, Garner *et al.* [38] demonstrated that in *Bufo bufo* larvae accrue the costs of infection in a dose-dependent manner during their development, and could die during that stage, at metamorphosis to die without evidence of infection, due to the prior impact of Bd infection on larvae. They further show that adults could be infected and die due to chytridiomycosis, with survival related to body size.

Future research needs

Understanding pathogenesis of Bd infection would be facilitated by development of animal models in which individuals become severely infected (to the point at which they become moribund). This has ethical implications, but is critical to understanding a significant cause of amphibian declines. In these animal models, previously proposed hypotheses (secretion of a toxin, reduced respiration through the skin, osmoregulatory imbalance) could be tested using traditional histopathology, toxicological investigations, and measurements of ion transport, water uptake and electrolyte balance. These studies would need is so long to target not only the very late stages of infection just prior to death, but also demonstrate the time course of pathogenesis, explain why the time course of infection, and how this relates to innate defenses, such as increased skin-shedding rates.

These experiments would be extremely valuable if they could standardize infection protocols within species (infectious dose, passage number, host age, sex and size) with clinical signs (loss of righting reflex, skin-shedding), pathological signs (a standardized lesion score of the size, depth and appearance of lesions at fixed periods post inoculation) and crucially, qPCR counts. This would provide a way for ecologists to correlate qPCR results more accurately with time course of infection and morbidity (i.e. likely outcome). This standardization would need to occur for a range of frog genera, species, age, sex etc. At present there is a lack of an established, suitable model species. Ideally, a model species would be susceptible, common, robust in captivity, and one for which immunological and biochemical reagents are available. It would be a terrestrial, aquatic-breeding species in which Bd induces gradual pathological changes, culminating in severe hyperplasia of the keratinaceous layer, hyperkeratosis and death. White's treefrog (*Litoria caerulea*) fulfils many of these criteria. It is present in the pet trade in many countries, it is easily bred and hardy in captivity, and presents with typical chytridiomycosis when infected experimentally.

Second, further studies of the impact of Bd on larvae could follow through to metamorphosis to test the hypotheses put forward by Garner *et al.* 2009 [38] and others. Scaling up experimental studies in larvae to mesocosms or the wild could be critical in understanding the post-metamorphic mortality events reported anecdotally by many authors. Again, standardization of experimental doses, lesions (number of 'teeth' missing or fraction of tooth rows present or absent), growth rates and other characteristics for each species against field-observable data (qPCR score, missing teeth/tooth rows) is important. Finally, fully understanding pathogenesis could be assisted by the recently analyzed gene expression profiles and use of knockout or other manipulated hosts [91].

local banana and beer production than with temperature, suggesting correlations with temperature were correlative rather than causative [21]. Support was stronger for a spreading pathogen, but in the analyses, the best supported number and location of the center(s) of spread depended on whether the 'year of decline' or the more variable 'last year observed' were analyzed, and resultant locations were spatially inconsistent [13,21]. Overall, these analyses support the novel pathogen hypothesis and refute many aspects of the CTOH. It should be noted that there is strong evidence for climate-caused amphibian declines [22–24] but the best-supported mechanisms are non-disease related (drought and changes in leaf litter owing to warming).

Spread of Bd between countries and continents as suggested by the novel pathogen hypothesis might have been by trade in infected *Xenopus laevis* frogs used for human pregnancy tests [16] and, more recently, by trade in American bullfrogs (*Rana catesbeiana*). Bullfrog trade is widespread, Bd infection is asymptomatic and bullfrogs often establish local populations outside farms, where they can infect native amphibians [25–28]. However, definitive evidence linking distributional patterns of Bd or Bd-caused declines with trade is lacking (but see [29] for an example of ranavirus spread through trade of fish bait).

Local movement of Bd (e.g. over meters to tens of kilometers) is also poorly understood. Although studies have shown apparent waves of Bd spread, and one study found some evidence for local-scale genetic structure in Bd in *R. muscosa*, [6,12,30,31] none of these studies have determined the mode of dispersal. Spread has been hypothesized to occur through frog dispersal, downstream via water and sand, and possibly by bird, human, insect or other animal movement [12,32]. In addition, if Bd is demonstrated to have an environmentally resistant stage, dispersal by wind or other modes would also be possible.

Future research needs

Testing between the novel and endemic pathogen hypotheses will require development of more polymorphic loci for Bd (e.g. genes or spacer regions identified using the full genome sequence) than are currently available to determine population genetic structure [17]. Identification of a single source population with higher genetic diversity (Africa is a hypothesized source [16] but has been poorly sampled), and low genetic variability elsewhere (as is evident so far) would support the novel pathogen hypothesis. By contrast, the identification of significant widespread spatial structure in populations, and a correlation between spatial and genetic distance would support the endemic pathogen hypothesis. It is also possible that Bd was endemic in some areas but the introduction of a novel virulent strain(s) caused the recent declines, as appears to be the case with ranavirus [33]. Alternatively, Bd might have been endemic and widespread in a subset of habitats (e.g. lowlands) but introduced to upland areas by anthropogenic or environmental change

Box 3. Population impacts of Bd on amphibian hosts

Several reviews have implicated Bd in amphibian declines and extinctions of >200 species in six continents [6,7,18,66,91]. Evidence from several well-studied systems has conclusively demonstrated Bd as a cause of declines of many species across large regions [6,8,13,92] (Figure I). In two species exhibiting declines (*Bufo boreas* and *Rana muscosa*), 100% mortality from infection has been demonstrated in the lab (Figure 1, main text; Online Supplementary Table S1). However, in other studies, the link between Bd and observed population declines is less certain [30]. This is important because, in some systems, Bd appears to be present without causing subsequent declines [24], and in other locations, it appears to have been present before widespread declines [93]. Similarly, in some species that declined regionally but persisted in small populations, Bd is still present and infecting frogs without driving these populations to extinction [8,70].

What these studies demonstrate is that linking Bd to declines is a non-trivial exercise [2,21], and host–parasite relationships are often dynamic, with the host and its pathogen coevolving [30,70]. Bd has caused large-scale declines in some species in some locations, but does not cause declines in all species at a site where some species are impacted. This suggests that reservoirs (species in which the reproductive ratio of the pathogen, R_0, is >1) for the pathogen exist, and they are contributing to the declines.

Future research needs

It is still not known what determines whether Bd will cause significant declines in a population, and what determines whether a population will be extirpated or persist with endemic Bd infection. Crucially, it must be determined which species amplify the pathogen and how long Bd can persist in natural environments outside a host. Future studies of the impact of Bd on amphibian populations should use rigorous quantitative analysis [6] and will be more compelling if infection and population data can be gathered before Bd is present and during Bd epidemics, with infection prevalence of dead and live healthy animals [30]. For areas in which Bd is already present and declines have already occurred, studies will be more informative if host susceptibility can be demonstrated by experimental infection, and data on the causal mechanisms for the introduction and/or persistence of Bd can be obtained. Simply documenting a decline and the presence of Bd provides only weak evidence of Bd as a cause of that decline [30].

Figure I. Dead mountain yellow-legged frogs (*Rana muscosa*) at various stages of decay during an outbreak of chytridiomycosis in Sixty Lake Basin, Kings Canyon National Park, California in 2006. Reproduced with permission from Vance Vredenburg.

where it then caused amphibian declines. Testing these hypotheses would require pre-decline sampling for Bd and comparisons of strain genetics and virulence from pre- and post- decline isolates of Bd to demonstrate increased virulence and the presence of novel genotypes of Bd.

Future studies of Bd distribution should include genetic characterization of isolates with highly polymorphic loci, and demonstrate, with appropriate sample sizes, locations where Bd is likely absent from apparently suitable hosts and habitat. These studies would be more useful than finding additional locations where Bd is present because they could be used in demonstrating future spread of the pathogen [6], and in determining environmental factors influencing the presence of Bd.

Analyzing trade flows of Bd hosts (such as *R. catesbeiana* and *X. laevis*) and the spatial genetic structure of Bd (with new markers) could facilitate understanding the regional or between-continent spread of Bd, as has been done for H5N1 avian influenza [34]. At a local scale, it should be possible to test whether birds, insects, or other organisms might transport Bd efficiently over terrain unsuitable for amphibians. As a first step, studies could capture animals from ponds where Bd is known to be present, and place them in water. The water should then be tested for Bd both by PCR and by xenodiagnosis (i.e. by placing a susceptible animal into the water). Controlled lab studies could then be used to determine the details of the transfer process (threshold concentrations of Bd in the water, persistence on different transport hosts, etc.).

Susceptibility to Bd

Individuals from over 250 species of amphibians have been found to be infected with Bd (http://www.spatialepidemiology.net/bd-maps/), but variability exists in prevalence among species and sites, the intensity of infection among individuals, and the impact of Bd on populations (Box 3). A key question concerning generalist pathogens, including Bd, ranavirus [35], West Nile virus [36] and avian mycoplasmal conjunctivitis [37], is what

drives variation in host susceptibility to infection and subsequent infectiousness? Experimental infection studies provide an opportunity to measure susceptibility to infection, mortality from infection and infectiousness to other individuals.

There have been 24 published experimental infection studies with 21 amphibian species, including one salamander, two toads, and 18 species of frogs, with the primary aim being to estimate susceptibility to infection and resulting mortality (Online Supplementary Table S1). Of 90 separate experiments, 20 have been on tadpoles, 66 on post-metamorphic adult amphibians, and four that included both stages. Although methods have differed substantially, some preliminary conclusions can be made.

In eight species (two toads and six frogs), 90–100% mortality was observed after infection with Bd. Other studies found variable survival despite most individuals becoming infected (but see [38] for some decoupling of infection and Bd mortality). Most of the studies demonstrating significant mortality from Bd were performed with post-metamorphic animals, whereas the most consistent effects of Bd infection in tadpoles were slower development to metamorphosis, smaller size at metamorphosis (and increased mortality subsequently), and changes in behavior [38–40]. In one study on tadpoles of the common toad, *Bufo bufo*, significant mortality occurred 20–70 d after Bd infection, and sometimes in animals where no infection could be detected [38]. In another study on tadpoles of *B. boreas*, 33% of the treatment-group animals died in the first 48 h post-exposure (no mortality was observed in control animals). However, this was too early to be due to Bd infection because approximately 4 d is required for one replication cycle of Bd [41]. Instead, it might indicate exposure to a toxin produced by Bd or another unmeasured factor [42]. In all other studies on tadpoles, survival in control and treatment groups were statistically indistinguishable [Online Supplemental Table S1]. In summary, Bd infection frequently caused substantial mortality in post-metamorphs, and mostly sub-lethal, but not-insignificant, effects in tadpoles.

Within a host, three factors have been hypothesized to influence host-Bd interactions: dose, temperature and strain differences (Figure 1). First, increased dose decreased survival in a highly susceptible species (*B. boreas*), but the effect was apparent only at lower doses ($\leq$100 zoospores animal^{-1}); at higher doses survival was 0% (Figure 1a; see also [38]). By contrast, the fraction infected was high at most doses and did not vary in a directional fashion (Figure 1a). Second, temperature had no effect on survival times of *B. boreas* toads held at 12 and 23 °C [40], but in two other studies on different species [43,44], survival or survival times were significantly higher/longer at higher temperatures (Figure 1b). Interestingly, in the study with great barred frogs, *Mixophyes fasciolatus*, animals that died at the warmest temperature (27 °C) did so much earlier than those at the lower temperatures (Figure 1b). This might have been a result of an interaction between the temperature-dependent growth of Bd (Box 1) and the host immune response that might also be temperature dependent. Third, infection with Bd strains isolated from different sites resulted in differences in survival or time until death (Figure 1c and [45]). Intriguing recent evidence suggests that a morphological trait of Bd that differs between strains (sporangia size) and differentially expressed proteins can cause differences in host survival [45].

Immune defenses might also play an important role in Bd infection and amphibians have many of the responses common to other vertebrates: cell-mediated, antibody and innate (naïve) immune defenses [46]. Here, we focus on recent work with skin peptides and anti-fungal bacteria that are present on the skin that might act as defenses against Bd [47]. The best studies have demonstrated direct effects (increased survival or decreased weight loss) of anti-fungal bacteria or skin peptides by adding natural quantities of peptides or bacteria and performing experimental infections [48,49]. A more correlative laboratory infection study of four amphibians found that species that had skin peptides that had higher Bd-inhibiting capacity had higher survival [50] (but see [51]). Less direct studies have included measuring *in vitro* Bd growth with and without peptides obtained from the surface of frog skin [52], and studies comparing population trends and the presence of active peptides against Bd or anti-fungal bacteria [53,54].

Future research needs

Variability in experimental conditions of previous studies hampers comparisons of the susceptibility of species to infection and mortality. A common dosage, temperature regime, strain, water flow rate and study duration are necessary to compare between populations, species or amphibian life-stages. Recommending a common dose to be used with all amphibian species, however, is complicated by the fact that Bd virulence can vary with culture conditions and depending how long Bd has been in culture (C. Briggs, pers. observ.). Additionally, tadpoles are more resistant to infection than post-metamorphic animals (Online Supplementary Table S1), perhaps owing to reduced amounts of keratin, requiring a higher dose to estimate mortality after infecting all animals. Similarly, a common temperature would facilitate comparisons between species, but it would produce unrealistic estimates of the effects of Bd on a species if experimental temperatures are significantly different than the natural environment of the amphibian being studied, and the same logic holds for using local versus foreign strains of Bd. Finally, the flow rate and/or changing frequency of water in animals' chambers and the fraction of time infected animals spent in water can influence re-infection by shed zoospores and thus host mortality [40,55]. It seems that using environmentally relevant temperature profiles, strains, and flow rates is the most appropriate strategy unless an explicit goal is to determine the impact of a novel strain or environment (e.g. owing to introduction or global warming) on susceptibility, mortality or transmission. Furthermore, the use of antibiotics that can interfere with naturally occurring anti-fungal bacteria [55] might alter the outcome of experimental infections and should be avoided. We suggest measuring infection by swabbing at regular intervals (e.g. 10 d), in at least a subset of animals, to facilitate between-study comparisons of infection and to

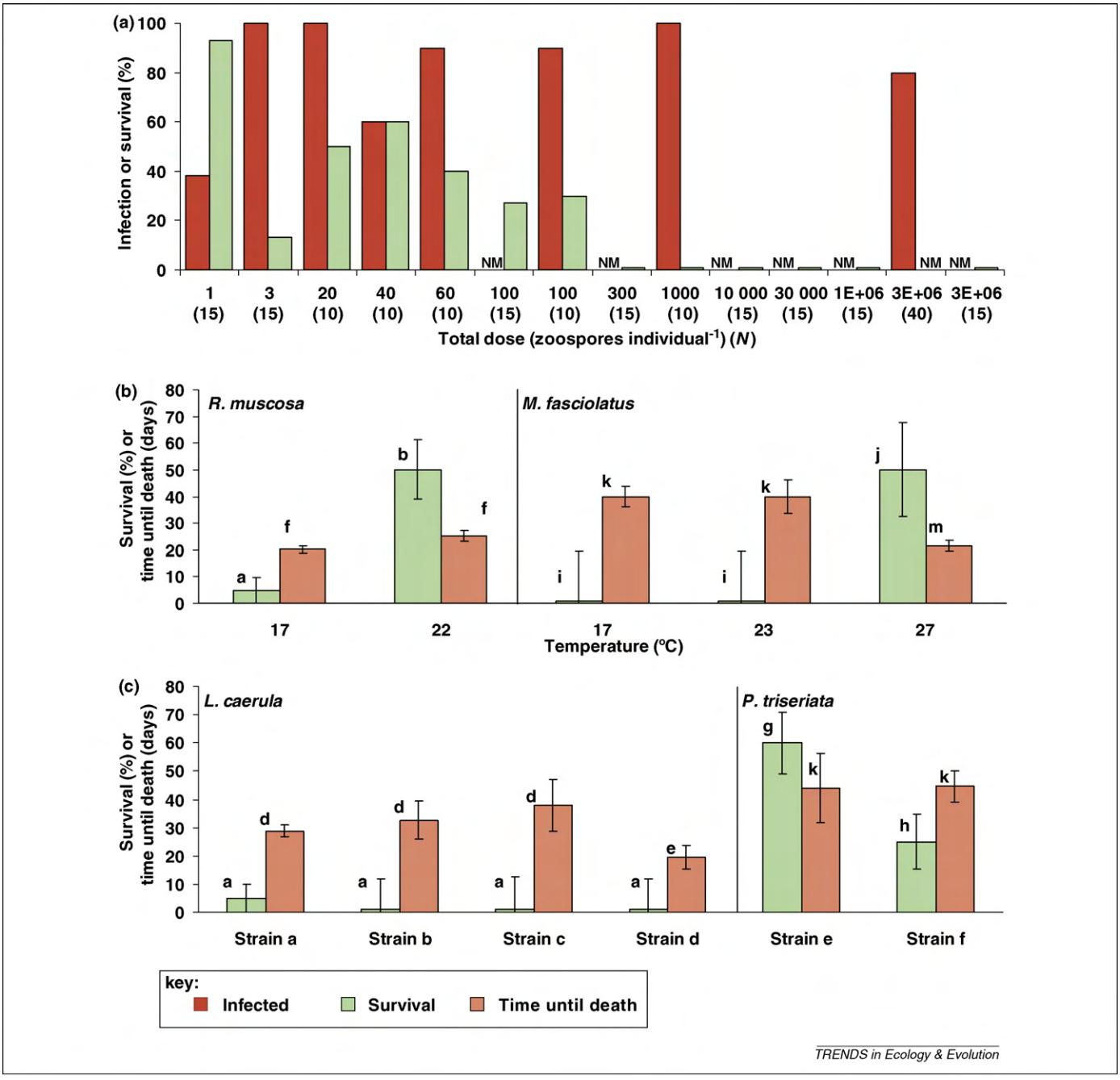

Figure 1. Effects of dose, temperature and strain on fraction infected, survival and time from infection until death following Bd infection. **(a)** Percent infection and survival of *Bufo boreas* (with sample size, *N*, given in parentheses below the dose) infected with different doses of Bd (strain JEL 275) measured 42 d after infection, based on data from [40]. NM indicates that this variable was not measured in the treatment. **(b)** Survival and time until death (± SE; for survival SE is based on the variance for a binomial distribution), for two species (*Rana muscosa* and *Mixophyes fasciolatus*, 85 and 98 d after infection, respectively) at different temperatures based on data from [43,44] and **(c)** for two species (*Litoria caerulea* and *Pseudacris triseriata*) infected with different strains of Bd based on data from [50,69,99]. Strains, dose and other experimental conditions for all experiments are given in Online Supplementary Table S1. Different letters denote significant differences within that portion of the figure, for that measure (survival or time until death). Low values (~1%) indicate a value of 0% and are shown for clarity.

quantify loss of infection that might occur with some regularity [38,55].

With these issues in mind, three types of experimental infection study would provide crucial insight into Bd–host interactions. First, taxonomically focused (e.g. within a genus) or phylogenetic paired sets of species would facilitate geographic or between-habitat comparisons of susceptibility to infection and mortality, especially in investigations of the role of antimicrobial peptides, antifungal bacteria and other host traits. Second, studies in which temperatures cycle, as in natural amphibian microhabitats, and span the full seasonal or geographical range observed will elucidate temperature-caused differences in patterns of infection. Third, differences between strains of Bd or populations of a host species collected from sites according to *a priori* hypotheses (e.g. along an altitudinal gradient, between locations with varying apparent impacts, etc.) would provide more definitive evidence for the causes of Bd emergence and population declines. For example, cross-infection experiments, where pathogens

Box 4. Diagnostic detection of Bd infection in hosts

Early detection of Bd on adult amphibians was done by histological examination of tissue samples from the toes, hindlimbs or pelvic patch of amphibians [94]. Samples were stained with hematoxylin and eosin stain and then examined for 7–15-μm spherical sporangia [7,41]. The advantages of using this technique are that histology can be done in standard laboratories worldwide, and it provides information about the pathology that an infection causes. However, histological surveys underestimate infection prevalence, because even extensive searching of skin cells in sections at low magnifications covers only small areas of the skin.

PCR techniques are the most effective method for identifying the presence of Bd in amphibians [95]. Animals can be assayed for Bd non-destructively through swabbing of the skin in post-metamorphic frogs or of mouthparts of tadpoles, and PCR can also be used to detect Bd in environmental samples [96,97]. Real-time quantitative PCR (qPCR) is now the gold standard for Bd detection and offers substantial advantages over other techniques [95,98] (although it can still miss low-level infections). qPCR is preferable to standard PCR because it provides information about the quantity of DNA present in a specimen. If the collection of the sample is conducted in a uniform way (e.g. same area of skin swabbed on each individual) and if the laboratory testing the specimen validates its qPCR against known standards, this technique could provide a useful estimate of the number of zoospores present in a sample. This provides a proxy measure of the severity of infection, but is not a direct measure of disease severity, which requires histological examination by a pathologist. Care in handling specimens to avoid cross-contamination is also crucial when using PCR techniques for Bd detection.

and hosts (and F_1 crosses of host populations) from sites where declines are and are not occurring could offer insights into the virulence of different strains, susceptibility of host populations and possible co-evolution between host and pathogen [56].

In all three types of study, the infectiousness of experimentally infected animals should be studied by quantifying the number of zoospores animals shed into their containers in a fixed period of time (e.g. daily). Infectiousness has yet to be quantified in any study, let alone how it varies among individuals, life stages, species and under different environmental conditions. This information is crucial for identifying amplification hosts [57] (those in which the pathogen has a reproductive ratio, $R_0 > 1$), and thus quantifying between-individual (and species) transmission. One key challenge, on which progress is being made, is to accurately determine the concentration of Bd in an environmental sample (i.e. water) using quantitative PCR (Box 4).

Finally, the natural concentrations and regulation of skin peptides and anti-fungal bacteria are poorly known and it is unclear which acquired immune responses (e.g. antibodies) are mounted against Bd infection [46,58]. It is unknown if immunity (if any) is lifelong or transient, is effective against multiple strains of Bd, and how this affects Bd transmission dynamics.

Ecology of transmission

Recent work has offered many insights into Bd dynamics. Bd can be transmitted between individuals without direct contact [59], probably as a result of infection by the motile zoospore stage (Box 1). Infected animals can shed zoospores for long periods (24–220 d) before dying [43,60], and Bd can be transmitted between infected tadpoles and from tadpoles to postmetamorphic adults [61]. By contrast, no support was found for crowding increasing susceptibility to infection in *R. muscosa*, or for temperature affecting the probability of transmission [59], although more work is needed, especially with additional species.

In the same study with *R. muscosa*, transmission between individuals in the laboratory appeared to increase asymptotically with the density of infected individuals, which is consistent with several mathematical forms of frequency-dependent transmission [59]. By contrast, in a field experiment in the same study, density-dependent transmission received slightly (but not significantly) higher support than did frequency-dependent transmission. The type of transmission is important because it can influence the impact of pathogens on host populations [62]. Simple models with density-dependent transmission predict that there will be a threshold host population size below which the pathogen cannot persist, whereas models in which the transmission is frequency dependent have no such threshold, and pathogens can drive hosts to extinction.

One early enigma in Bd dynamics was persistent transmission in host populations following declines (Box 3). In many laboratory studies (Online Supplementary Table S1), mortality of post-metamorphic adults approaches 100%, which, in the field, would lead to population collapse of hosts and/or of Bd [8,63]. Models also suggest that host population collapse is likely if Bd can persist in the environment outside the host, saprophytically [64]. By contrast, Bd persistence can occur despite causing 100% mortality in post-metamorphic individuals of a species by: (i) the presence of another reservoir species that can survive infection while still shedding infective zoospores [65]; (ii) persistence of Bd in another life stage of the host (e.g. long-lived tadpoles); or (iii) persistence of Bd in a resting stage or in a stage surviving saprophytically [63,66]. There is now evidence showing that infection of adults is not always fatal in the field even if it is in the laboratory. Infected frogs can sometimes clear the infection, as shown in the laboratory at warmer temperatures [43,67–69], and survival of infected and uninfected post-metamorphic frogs in the field is sometimes similar [68,70].

Regional-scale patterns of infection prevalence demonstrate the influence of climatic and microhabitat conditions. Prevalence and intensity of infection were higher at cooler higher altitudes in more equatorial sites, at sites with more rainfall, at more polar latitudes, and in cooler seasons where conditions are closer to the optimal environment for Bd growth (Box 1) [71–75]. In Australia, ten sampled frog species (with $\geq$ individuals sampled) that bred in permanent ponds and streams had a high prevalence of Bd infection, whereas four species breeding in ephemeral ponds and streams were free from infection [76]. Similarly, more-aquatic amphibian species had a higher prevalence of infection than species that were terrestrial [73].

Finally, two differing size–infection prevalence patterns have been observed in the field. Infected tadpoles of two species were much larger and more developed than uninfected tadpoles [77] whereas in post-metamorphs of another species, smaller frogs were more likely to be

infected [72]. Although the mechanisms for these patterns have yet to be determined, the first (increasing prevalence with size of tadpoles) is consistent with a constant risk of becoming infected and low disease-induced mortality or recovery (i.e. loss of infection). The second pattern (decreasing prevalence with size of post-metamorphs) could be caused by: (i) Bd-related mortality in smaller frogs so that only uninfected frogs reach large sizes [38]; (ii) Bd-related decrease in growth rates [38]; and/or (iii) loss of infection by larger frogs.

Future research needs

Additional studies are needed to quantify population level transmission functions (has been done for *R. muscosa* [59]) across a range of species, life stages, and laboratory and field conditions. To be informative, dosage and exposure duration should be chosen to produce a range of infection prevalences (possibly by measuring infection at regular intervals as outlined above). To be relevant to natural infection, the experiments should be conducted under the most realistic conditions possible, as outlined above.

In a changing climate, it would be prudent to determine more fully the *in vivo* growth and shedding of Bd and the potential for Bd and its hosts to evolve under different temperature regimes, perhaps through an artificial selection experiment. Other pathogens have evolved to be transmitted more efficiently at higher temperatures [78], and selective pressures might exist on Bd to do the same. Experimental infection studies (described above) continued for several transmission cycles and combined with characterization of recently identified host defenses [48,49] and Bd gene expression [45,79] might offer insights, if viewed through the lens of evolution. Finally, at a larger scale, transmission is also likely to be affected by anthropogenic land use, water quality, host community composition and the interaction of these three factors, but research on these topics is only beginning.

Conservation and policy

Ten years after the first published report on chytridiomycosis, actions to combat this threat are now beginning. Agencies have begun to mobilize to mitigate impact, control spread and, ultimately, to attempt to eradicate the pathogen from some areas. In 2008, the Office Internationale des Epizooties (OIE) declared chytridiomycosis and amphibian ranaviral disease as 'notifiable', providing a well-established framework for reporting and control that nation-states registered with the World Trade Organization can use (the same mechanism is used for foot and mouth disease, for example).

Conservation of amphibians in the face of Bd will require addressing other threats to their survival, such as habitat loss, introduced predators and other diseases, so that species will be able to persist in the face of Bd and possibly evolve resistance to it [80]. The urgency of the situation calls for the development of innovative actions [11], such as: (i) captive breeding amphibians for Bd resistance; (ii) development of mobile, low-cost labs for *in situ* Bd-free captive breeding; and (iii) the development of anti-Bd vaccine candidates. Even more radical approaches have been proposed in policy discussions, including the use of anti-fungal bacteria and anti-fungal compounds in the field. These two strategies could be appropriate for species with restricted ranges and that are close to extinction in the wild, such as the Corroborree frog, *Pseudophyrne corroborree*, previously found in only one site in Australia [81] and now in imminent danger of extinction in the wild. For most amphibians, and other wildlife threatened by pathogens, a broader approach that addresses the multiplicity of factors driving their population declines is necessary.

Concluding remarks

Our goal here was to review what is known of the ecology and impact of Bd in amphibian communities and, more importantly, to outline studies that would address outstanding questions. These include the origin of Bd and the factors that drive variation in its transmission and impact. Additional molecular markers, in combination with both focal (especially in Africa) and widespread sampling for the pathogen, should bring us closer to a definitive origin for Bd. Similarly, recently developed diagnostics and refined methods described above in both field and laboratory studies should facilitate: (i) more conclusive determination of the role of Bd as a causative agent in amphibian declines; (ii) the identification of which hosts are amplifying Bd and which are necessary for its persistence; and (iii) how host and environmental factors interact with Bd to determine impacts on species, the persistence of the pathogen, and evolution or coevolution of the pathogen [78] and hosts.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tree.2009.07.011.

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The ecology and impact of chytridiomycosis, an emerging disease of amphibians

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Online Supplementary Table S1. Laboratory experimental infection studies of amphibians with Batrachochytrium dendrobatidis. Stage refers to tadpole (T) or juvenile (J) stage, Te/Tr denotes temperate or tropical species, dose is given in thousands of zoospores (zsp), t_e is the length of the exposure period, in days, Exposure refers to whether animals were exposed individually (I) or in a group (G) of (N) animals in the treatment group, unless otherwise noted, Temp. is the holding temperature following infection, % Infect. is the percent of animals infected, measured at the end of the study by swab, and Mean time to death and standard error (SE) are given in days. Ref gives the reference from the list below this table, not those in the main text. For all measures (e.g. Temp., % infect., etc.), table gives average value if a range was given in the original source. Abbreviations: Unk- Unknown; na – not available.

Species	Stage	Te/ Tr	Strain	Dose (1000s zsp.)	t_e	Expo-sure	N	Temp (ºC)	% Infect.	% Surv. Treat.	% Surv. Cont.	Mean time to death	SE (Mt d)	Ref
Bufo bufo	T,J,A[a]	Te	IA2004 043	9	4	I	105	18	100	2	80	na		[1]
B. bufo	T,J,A[a]	Te	IA2004 043	0.09	4	I	105	18	40	39	80	na		[1]
Bufo boreas	T	Te	JEL215	1.4	2	G	30	14	67	67	95	4.4	6.5	[2]
B. bufo	T	Te	UkTvB	0.19	4	I	40	21	100	46	68	na		[3]
B. bufo	T	Te	TF5a1	0.19	4	I	40	21	100	65	68	na		[3]
B. bufo	T	Te	IA042	0.19	4	I	40	21	100	56	68	na		[3]
B. bufo	T	Te	UkTvB	19	4	I	40	21	100	3	68	na		[3]
B. bufo	T	Te	TF5a1	19	4	I	40	21	100	38	68	na		[3]
B. bufo	T	Te	IA042	19	4	I	40	21	100	8	68	na		[3]
B. fowleri	T	Te	Unk	Unk[c]	10	G	240	27.5	93	50	54	na		[4]
Hyla chrysoscelis	T	Te	Unk	9100	6	I	60	23	85	92	na	na		[5]
H. chrysoscelis	T	Te	Unk	Unk[c]	13	G	480	29.5	86	75	77	na		[6]
H. chrysoscelis	T	Te	Unk	Unk[c]	10	G	240	27.5	93	64	54	na		[4]
H. regilla	T	Te	JEL215	1.4	2	G	30	14	87	73	81	na		[2]
Rana blairi x R. sphenocephala	T	Te	Unk	Unk[c]	9	G	240	27	93	86	83	na		[7]

Species	Stage	Te/ Tr	Strain	Dose (1000s zsp.)	t_e	Expo-sure	N	Temp (ºC)	% Infect.	% Surv. Treat.	% Surv. Cont.	Mean time to death	SE (Mt d)	Ref
R. blairi	T	Te	Unk	Unk[c]	9	G	240	27	76	85	82	na		[7]
R. cascadae	T	Te	JEL215	1.4	2	G	11	14	83	100	75	na		[2]
R. catesbeiana	T	Te	JEL215	0.7	2	G	15	14	86	100	100	na		[2]
R. muscosa	T	Te	LJR089	0.107	77	G	94	17	96	100	na	na		[8]
R. muscosa	T	Te	JEL216	900	30	I	26	13	100	0	100	na		[9]
R. muscosa	T	Te	LJR089	1T[h]	28	G(25)	100	17	5	na	na			[8]
R. muscosa	T	Te	LJR089	8T[h]	28	G(25)	100	17	33	na	na			[8]
R. muscosa	T	Te	LJR089	15T[h]	28	G(25)	100	17	17	na	na			[8]
R. muscosa	T	Te	LJR089	22T[h]	28	G(25)	100	17	49	na	na			[8]
R. muscosa	T	Te	LJR089	8T[h]	28	G(25)	100	4	40	na	na			[8]
R. muscosa	T	Te	Unk	0T[i]	49	G	5	9.5	50	na	na			[8]
R. muscosa	T	Te	Unk	5T[i]	49	G	5	9.5	70	na	na			[8]
R. muscosa	T	Te	Unk	15T[i]	49	G	5	9.5	70	na	na			[8]
R. muscosa	T	Te	Unk	0T[i]	49	G	15	9.5	50	na	na			[8]
R. muscosa	T	Te	Unk	5T[i]	49	G	15	9.5	100	na	na			[8]
R. muscosa	T	Te	Unk	15T[i]	49	G	15	9.5	57	na	na			[8]
R. muscosa	T	Te	Unk	0T[i]	49	G	5	9.5	20	na	na			[8]
R. muscosa	T	Te	Unk	5T[i]	49	G	5	9.5	48	na	na			[8]
R. muscosa	T	Te	Unk	15T[i]	49	G	5	9.5	80	na	na			[8]
R. muscosa	T	Te	Unk	0T[i]	49	G	15	9.5	0	na	na			[8]
R. muscosa	T	Te	Unk	5T[i]	49	G	15	9.5	25	na	na			[8]
R. muscosa	T	Te	Unk	15T[i]	49	G	15	9.5	71	na	na			[8]
R. muscosa	T	Te	LJR089	1T[j]	28	G	10	17	16	na	na			[8]
R. muscosa	T	Te	LJR089	1T[k]	28	G	10	17	16	na	na			[8]
R. muscosa	T	Te	LJR089	1T[l]	28	G	10	17	21	na	na			[8]
R. pipiens	T	Te	Unk	2800	7	G	40	22	100	na	na	na		[10]
R. sphenocephala	T	Te	Unk	Unk[c]	9	G	240	27	78	84	84	na		[7]
Ambystoma tigrinum	J	Te	R-230	600	7	I	2	18	50	100	100	na		[11]
A. tigrinum	J	Te	A-277	900	7	I	3	18	100	66	100	na		[11]

Species	Stage	Te/ Tr	Strain	Dose (1000s zsp.)	t_e	Expo-sure	N	Temp (ºC)	% Infect.	% Surv. Treat.	% Surv. Cont.	Mean time to death	SE (Mt d)	Ref
B. boreas[m]	J	Te	JEL275	0.001	1	I	15	23	38	93	100	na		[12]
B. boreas[m]	J	Te	JEL275	0.001	3	I	15	23	100	13	100	na		[12]
B. boreas[m]	J	Te	JEL275	0.02	1	I	10	23	100	50	100	na		[12]
B. boreas[m]	J	Te	JEL275	0.04	1	I	10	23	60	60	100	na		[12]
B. boreas[m]	J	Te	JEL275	0.06	1	I	10	23	90	40	100	na		[12]
B. boreas[m]	J	Te	JEL275	0.1	1	I	15	23	na	27	100	34.5	1.6	[12]
B. boreas[m]	J	Te	JEL275	0.1	3	I	15	23	na	0	100	na		[12]
B. boreas[m]	J	Te	JEL275	0.1	1	I	10	23	90	30	100	33.0	2.3	[12]
B. boreas[m]	J	Te	JEL275	1	1	I	10	23	100	0	100	na		[12]
B. boreas[m]	J	Te	JEL275	10	1	I	15	23	na	0	100	na		[12]
B. boreas[m]	J	Te	JEL275	10	3	I	15	23	na	0	100	na		[12]
B. boreas[m]	J	Te	JEL275	1000	3	I	40	23	96	60	98	13.8	0.8	[12]
B. boreas[m]	J	Te	JEL275	1000	3	I	40	12	96	60	98	14.0	0.8	[12]
B. boreas[m]	J	Te	JEL275	1000	1	I	15	23	na	0	100	16.4	3.9	[12]
B. boreas[m]	J	Te	JEL275	1000	3	I	15	23	na	0	100	16.4	3.9	[12]
B. boreas[n]	J	Te	JEL215	2080	1	G(5)	30	16	na	71	100	8.6	0.6	[13]
B. boreas[n]	J	Te	JEL215	2080	1	G(5)	30	16	na	71	100	8.5	0.7	[13]
B. boreas	J	Te	Unk	505	4	I	18	20	100	0	100	19.9	1.7	[14]
B. boreas	J	Te	Unk	505	4	I	14	20	100	0	93	14.8	2.4	[14]
B. boreas	J	Te	Unk	550	3	I	6	20	100	100	100	na		[14]
B. boreas	J	Te	Unk	550	3	I	6	20	100	100	100	na		[14]
B. boreas	J	Te	Unk	550	3	I	6	20	100	100	100	na		[14]
B. boreas	J	Te	Unk	550	3	I	6	20	100	100	100	na		[14]
B. boreas[m]	J	Te	JEL275	Unk[d]	2	I	11	12	100	9	90	23.8	2.4	[12]
B. boreas[m]	J	Te	JEL275	Unk[d]	2	I	10	23	100	9	90	25.5	2.6	[12]
B. bufo	J	Te	IA2004 043	0.05	0.2	I	10	18	90	40	70	na		[1]
B. bufo	J	Te	IA2004 043	5	0.2	I	10	18	100	10	70	na		[1]
Dendrobates auratus	J	Tr	Unk	Unk[d]	36	I	6	22.5	100	0	84	21.4	1.5	[15]
D. auratus	J	Tr	Unk	Unk[d]	36	I	3	22.5	100	0	na	19.3	3.3	[15]

Species	Stage	Te/ Tr	Strain	Dose (1000s zsp.)	t_e	Expo-sure	N	Temp (ºC)	% Infect.	% Surv. Treat.	% Surv. Cont.	Mean time to death	SE (Mt d)	Ref
D. tinctorius	J	Tr	RM-217	10000	31	I	4	21	100	0	100	27.0	1.4	[16]
D. tinctorius	J	Tr	Unk	Unk[d]	30	I	2	22.5	100	0	66	26.5	4.5	[15]
H. regilla	J	Te	JEL215	2080	1	G(5)	30	16	na	100	100	na	na	[13]
H. regilla	J	Te	JEL215	2080	1	G(5)	30	16	na	83	100	7.0	1.3	[13]
Limnodynastes tasmaniensis	J	Tr	GRL-00-LB-1	5	0.6	I	20	18.4	0	100	100	na		[17]
Litoria caerulea	J	Tr	GRL-00-LB-1	5	0.6	I	20	18.4	100	5	100	28.8	2.1	[17]
L. caerulea	J	Tr	98 1469/10	50	1	I	15	18	100	0	100	32.7	6.8	[18]
L. caerulea	J	Tr	99 1385/12	50	1	I	14	18	100	0	100	37.9	9.3	[18]
L. caerulea	J	Tr	00 545	50	1	I	15	18	100	0	100	19.4	4.2	[18]
L. chloris	J	Tr	GRL-00-LB-1	5	0.6	I	20	18.4	100	35	100	65.1	7.4	[17]
Mixophyes fasciolatus	J	Tr	00/545	1	1	I	8	17	100	0	84	40.0	3.8	[19]
M. fasciolatus	J	Tr	00/545	1	1	I	8	23	88	0	50	40.0	6.1	[19]
M. fasciolatus	J	Tr	00/545	1	1	I	8	27	88	50	84	21.5	1.9	[19]
M. fasciolatus	J	Tr	GRL-00-LB-1	5	0.6	I	20	18.4	100	5	100	56.6	3.9	[17]
M. fasciolatus	J	Tr	Unk	3[e]	14	I	6	24	100	0	100	na		[20]
Pseudacris triseriata	J	Te	27 Mile lake	50	2	I	20	20	100	60	100	44.0	12.3	[21]
P. triseriata	J	Te	Lost lake	50	2	I	20	20	95	20	100	44.6	5.6	[21]
R. boyli	J	Te	LRJ119	940	1	I	7	21	0	86	80	na		[22]
R. boyli	J	Te	LRJ119	940	1	I	7	21	14	86	80	na		[22]
R. boylii	J	Te	A-277	127.5	7	I	7	22	14	71	71	10.5	11.7	[11]
R. boylii	J	Te	R-230	127.5	7	I	7	22	0	43	71	22.0	8.3	[11]
R. boylii	J	Te	A-277	1275	7	I	8	22	0	63	71	31.0	9.5	[11]
R. boylii	J	Te	R-230	1275	7	I	8	22	25	63	71	53.3	9.5	[11]
R. cascadae	J	Te	JEL215	2080	1	G(5)	30	16	na	3	26	6.4	0.4	[13]
R. cascadae	J	Te	JEL215	2080	1	G(5)	30	16	na	6	26	5.9	0.4	[13]
R. catesbeiana	J	Te	DA-198	5000	0.5	I	15	15	27	100	100	na		[16]
R. catesbeiana	J	Te	RC-270	5000	2.3	I	4	21	50	100	100	na		[16]
R. catesbeiana	J	Te	RC-260	5000	2.3	I	4	21	25	100	100	na		[16]

Species	Stage	Te/ Tr	Strain	Dose (1000s zsp.)	t_e	Expo-sure	N	Temp (ºC)	% Infect.	% Surv. Treat.	% Surv. Cont.	Mean time to death	SE (Mt d)	Ref
R. catesbeiana	J	Te	RM-216	20000	0.1	I	15	21	27	100	100	na		[16]
R. catesbeiana	J	Te	RY-228	20000	0.1	I	15	21	0	100	100	na		[16]
R. muscosa	J	Te	JEL 215	0.3	1	I	6	17	100[g]	17[g]	100	98.0	11.5	[23]
R. muscosa	J	Te	JEL 215	0.3	1	I	6	17	0[g]	100[g]	100	na		[23]
R. muscosa	J	Te	JEL216	Unk[f]	96	I	4	16	100	0	100	26.8	5.8	[9]
R. muscosa	T,J[b]	Te	LJR089	10000	56	G	20	17	100	5	100	20.1	1.2	[24]
R. muscosa	T,J[b]	Te	LJR089	10000	56	G	20	22	100	50	100	25.1	2.1	[24]
R. yavapaiensis	J	Te	A-277	1275	7	I	3	22	0	66	66	7.0		[11]
R. yavapaiensis	J	Te	R-230	1275	7	I	4	22	50	25	66	26.3	4.7	[11]

Notes:

[a] Animals were infected as tadpoles but monitored through the juvenile stage; mortality occurred during both stages.

[b] Animals were infected as tadpoles but monitored through the juvenile stage; mortality occurred during juvenile stage.

[c] Infection treatment animals were placed with 1 infected adult animal.

[d] Animals were infected with water containing zoospores with an unknown dose.

[e] Dose gives number of sporangia, not zoospores.

[f] Infected treatment animals were placed with 5 infected tadpoles.

[g] Animals in this group were treated with an antifungal bacterial species, *Janthinobacterium lividum* before being exposed to Bd.

[h] Dose gives number of infected tadpoles (T) placed with group of uninfected tadpoles.

[i] Dose gives number of infected tadpoles (T) placed with group of uninfected tadpoles, and all animals were placed in mesh cages in a lake known to be infected with Bd.

[j] first reared for 4 weeks with 26 tadpoles in 12L of H_20 and fed 120 pellets of rabbit chow per week to examine crowding effects (see [k,l]).

[k] first reared for 4 weeks with 47 tadpoles in 12L of H_20 and fed 120 pellets of rabbit chow per week to examine crowding effects.

[l] first reared for 4 weeks with 100 tadpoles in 12L of H_20 and fed 120 pellets of rabbit chow per week to examine crowding effects.

[m] Solution containing Bd zoospores used to infect animals also contained penicillin/streptomycin which may have altered anti-fungal bacteria on animals' skin.

[n] Animals were also exposed to 5 hr UV-B radiation each day (no significant effect on Bd infection was reported).

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