

Title: After the epidemic: ongoing declines, stabilizations and recoveries in amphibians afflicted by chytridiomycosis

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Abstract

The impacts of pathogen emergence in naïve hosts can be catastrophic, and pathogen spread now ranks as a major threat to biodiversity. However, pathogen impacts can persist for decades after epidemics and produce variable host outcomes. Chytridiomycosis in amphibians (caused by the fungal pathogen *Batrachochytrium dendrobatidis*, Bd) is an exemplar, with impacts ranging from rapid population crashes and extinctions, to population declines and subsequent recoveries. Here, we investigate long-term impacts associated with chytridiomycosis in Australia. We conducted a continent-wide assessment of the disease, reviewing data collected since the arrival of Bd in about 1978, to assess and characterize mechanisms driving past, present and future impacts. We found chytridiomycosis to be implicated in the extinction or decline of 43 of Australia's 238 amphibian species. Population trajectories of declined species are highly variable; six species are experiencing ongoing declines, eight species are apparently stable and 11 species are recovering. Our results highlight that while some species are expanding, Bd continues to threaten species long after its emergence. Australian case-studies and synthesis of the global chytridiomycosis literature suggests that amphibian reservoir hosts are associated with continued declines in endemically infected populations, while population stability is promoted by environmental conditions that restrict Bd impact, and maintenance of high recruitment capacity that can offset mortality. Host genetic adaptation or decreased pathogen virulence may facilitate species recovery, but neither has been empirically demonstrated. Understanding processes that influence Bd-host dynamics and population persistence is crucial for assessing species extinction risk and identifying strategies to conserve disease-threatened species.

Key words: amphibian, chytrid fungus, extinction, recovery, wildlife disease

1. Introduction

Wildlife disease is implicated as a key driver of species extinction (e.g. Daszak et al., 2000; Fisher et al., 2012). In the past decade, disease-driven mass declines have been reported across many groups, including amphibians, reptiles, birds, mammals, fishes and corals (Fisher et al., 2012; Lorch et al., 2015; Tompkins et al., 2015). Immediate effects of outbreaks due to pathogen emergence in naïve wildlife populations can be severe and overt (e.g. Blehert et al., 2009). In contrast, long-term disease impacts are more complex, dynamic and much harder to detect and assess. This is especially true when field studies have been initiated decades after the emergence of the pathogen, and pre-arrival records are lacking, such as for avian malaria (*Plasmodium relictum*) in Hawaiian birds (van Riper III et al., 1986) and big-horn sheep pneumonia (*Mycoplasma ovipneumoniae*) in the Rocky Mountains (Besser et al., 2013). Host-pathogen coevolution theory postulates that a shift from epidemic to endemic states is generally associated with reduced pathogenicity, underpinned by increased host tolerance, resistance and/or reduced pathogen virulence (de Castro and Bolker, 2005). However, whether such shifts apply to generalist pathogens currently impacting biodiversity is uncertain. Generalist pathogens commonly persist in biotic reservoirs, potentially maintaining high disease impact in a sub-set of highly susceptible host species (de Castro and Bolker, 2005). As recently emerged pathogens become endemic in their new hosts and ranges, understanding the threat associated with ongoing pathogen presence is crucial for assessing impacts and informing conservation actions.

One of the most devastating diseases impacting wildlife over recent decades is amphibian chytridiomycosis, caused by the fungal skin pathogen *Batrachochytrium dendrobatidis* ('Bd') (Berger et al., 1998). Since its discovery, chytridiomycosis has been implicated in the decline of > 200 amphibian species, with declines attributed to the pathogen beginning in the 1970s (Skerratt et al., 2007). Bd infection has been reported in > 500 amphibian species (Olson et al., 2013) and although the origin of Bd remains unresolved (Rosenblum et al., 2013), it appears to be present in most climatically suitable regions worldwide (Olson et al., 2013).

The introduction of Bd into susceptible, naïve amphibian populations is characterized by a rapid, simultaneous increase in prevalence and infection intensity, followed by mass mortality and population decline (Lips et al., 2006; Vredenburg et al., 2010). If some individuals survive the initial epidemic, Bd may persist as an endemic pathogen in remnant populations or in resistant species that did not decline (Briggs et al., 2010; Murray et al., 2009; Reeder et al., 2012). Host impacts associated with endemic Bd are variable, with some species experiencing substantial ongoing mortality (Murray et al., 2009; Pilliod et al., 2010; Scheele et al., 2016b), which suppresses population abundance, while other species are stabilizing or beginning to recover (Newell et al., 2013; Scheele et al., 2015b). These variable host responses mean that the long-term threat associated with Bd remains uncertain across many parts of the globe.

Here, we investigate long-term host responses to Bd and mechanisms that influence Bd dynamics in declined species. First, using a continental-scale assessment of Australian amphibians, we review the impacts of chytridiomycosis 37 years post-Bd emergence. We categorize impacted species based on severity of decline, ongoing disease impact and whether population recovery has been observed. Chytridiomycosis was first reported in 1998 in Australia (Berger et al., 1998) and has subsequently been extensively studied across a large number of host species and environmental and climatic conditions (Berger et al., 2016). Recent reviews have focused on identifying actions, research and resources needed to prevent extinctions in threatened Australian frogs (Skerratt et al., 2016) and overviewed the taxonomy, phylogeny, distribution and ecology of Bd (Berger et al., 2016). However, a comprehensive assessment of Bd impacts and long-term population trajectories has not been undertaken, for Australia or other countries. We also review mechanisms and processes that influence endemic Bd dynamics across the globe to better understand factors underlying initial and ongoing disease impacts, incorporating case-studies of Australian species. Our focus on long-term host responses provides new insights into Bd impacts several decades post-emergence, and identifies the processes underlying variable host responses.

2. Chytridiomycosis in Australia: past and present

Some of the earliest reports of enigmatic amphibian declines globally were from eastern Australia, with disappearances of rainforest species first documented in 1979 (Skerratt et al., 2007). Over the following decades dramatic declines were reported in other tropical and temperate species in eastern Australia (Laurance et al., 1996; Osborne, 1989). The unprecedented decline of multiple species across geographic regions and an initial lack of a causal mechanism resulted in considerable debate on the extent and cause(s) of declines (Campbell, 1999). In 1993, frog mortalities were observed in the Australian wet tropics, both in the wild and following an experimental translocation of the sharp-snouted day frog (*Taudactylus acutirostris*) (Dennis and Mahony, 1994; Mahony et al., 1999; Speare, 1995). Due to concern about the potential extinction of *T. acutirostris*, the establishment of a captive insurance colony was attempted in 1993; however, most individuals died soon after being brought into captivity (Banks and McCracken, 2002). Deaths of wild and captive frogs were associated with a skin infection that was later identified as the novel disease, chytridiomycosis (Banks and McCracken, 2002; Berger et al., 1998). The disease hypothesis remained controversial, even after experimental transmission and the description of outbreaks causing mass mortalities of wild frogs in Australia and Central America in 1998 (Berger et al., 1998). Disease spread was not widely supported as the cause of declines until multiple lines of evidence (epidemiological, pathological, theoretical and experimental) were collated and repeated field and laboratory studies were conducted, both in Australia and globally (Berger et al., 2016; Skerratt et al., 2007; Wake and Vredenburg, 2008).

The absence of Bd in amphibians collected prior to the commencement of population declines strongly indicates that Bd is an introduced pathogen to Australia (Australian Government, 2006; Gillespie et al., 2015; Hunter et al., 2010; Skerratt et al., 2007). Furthermore, unlike in other continents where endemic lineages have been detected, only the global pandemic Bd lineage – a hypervirulent lineage associated with amphibian declines across the Americas, the Caribbean and Europe – has been detected in Australia (Farrer et al., 2011). The earliest detection of Bd in Australia is from retrospectively sampled museum specimens collected in 1978 near the major port city of Brisbane (Fig. 1) (Australian Government, 2006). From there, the pathogen appeared to spread north

and south and by 2010 occupied all climatically suitable regions, with several notable exceptions (Fig. 1) (Murray et al., 2011a). The apparent steady spread north in Queensland suggests dispersal via natural methods such as frog-frog contact or water flow. However, dispersal across inhospitable terrain, such as across desert areas and to islands, indicates human actions likely assisted Bd dispersal, probably via the unintentional movement of infected amphibians. This is supported by retrospective evidence of a chytridiomycosis outbreak in a captive amphibian population on the island of Tasmania following contact with a frog accidentally transported from the mainland in vegetable produce in 1993 (Obendorf, 2005).

In the 1970s, 1980s and 1990s many amphibian species experienced declines in Australia. Generally, these declines were only detected in later visits that revealed missing populations, small remnant populations or much reduced distributions and/or abundance (e.g. Hines et al., 1999; Laurance et al., 1996; Osborne, 1989; Osborne et al., 1999; Osborne et al., 1996; Richards et al., 1993). The following sections review all currently available evidence on the impacts of Bd on Australian frogs over the 37 years since its first detection, including immediate population crashes and extinctions through to long-term declines and recoveries.

3. Assessment of Bd impacts in Australia

3.1 Methods

We collated peer-reviewed articles, government technical reports, theses, conference proceedings, and unpublished data on species' status and trends recorded by amphibian ecologists and managers. Our research builds on and underpins the recommendations from the workshop described in Skerratt et al. (2016), which focused on prioritizing management for threatened Australian amphibian species impacted by chytridiomycosis. Literature was searched through Google Scholar and ISI Web of Knowledge, using terms such as; "*Batrachochytrium dendrobatidis*", "chytrid", "chytrid fungus", "chytridiomycosis", "frog decline" coupled with "Australia*". Bibliographies of relevant papers were checked to identify further literature. Once a species was identified as Bd-impacted, it was

individually searched using its scientific name in the above mentioned search engines. Relevant literature for each species is included in Appendix A.

Based on our literature review and expert knowledge, we evaluated the likely extent of species range decline due to Bd. We then categorized species into the following groups: *i*) extinct or probably extinct; *ii*) > 90% decline; *iii*) > 50% and < 90% decline; *iv*) < 50% decline; and *v*) potential to be impacted. We also asked: 1) Has recovery been observed? 2) Is Bd a likely driver of ongoing decline? 3) What key factors are known to influence Bd-host dynamics and population persistence (e.g. reservoir hosts, environmental refugia, high recruitment)? For each species we also recorded evidence on the role of Bd in declines, estimated or exact year of initial decline, elevational range, broad geographic distribution, habitat type, as well as state and federal government and International Union for Conservation of Nature (IUCN) status, and whether species taxonomy remains unresolved. Our assessment is based on current taxonomy; however, it is possible that some species actually form species complexes (i.e., comprising more than one species), while the validity of other species remains uncertain. Resolving taxonomic uncertainty will increase the accuracy of species assessments and assist the allocation of conservation funding (Skerratt et al., 2016).

3.2. Results

Batrachochytrium dendrobatidis-associated declines were first observed in Australia in 1979, concomitant with the earliest retrospective detection of Bd (Australian Government, 2006). These declines continue to the present time. Overall, 43 of Australia's 238 amphibian species were assessed as having declined or become extinct due to Bd (Table S1; Fig. 2). A further three species are considered at risk of future Bd-induced declines associated with the continued spread of the pathogen (Tables 1 and S1; Fig. 2). Species declines have been restricted to eastern Australia, across upland tropical, sub-tropical and temperate regions (Fig. 1) and have been most severe in species in high elevation areas where precipitation is high and temperatures are moderate to cold (Table S1). The seven species classified as extinct or probably extinct (Table 1) all occupied high elevation wet forest

stream habitats, while less severe declines occurred in species across diverse habitat associations, ranging from tropical stream breeders to sub-alpine ephemeral pond breeders (Table S1). Direct evidence for the role of Bd in species decline (an outbreak of chytridiomycosis diagnosed during mass declines, emergence of Bd associated with declines and demonstrated high species susceptibility) was more common when species had experienced a >50% decline, compared with <50% decline (Table S1).

Batrachochytrium dendrobatidis remains a driver of ongoing decline for six species (Table 1), while 11 species are reported to be recovering and eight species are apparently stable (species neither experiencing clear ongoing declines nor recoveries) (Table S1; Fig. 3). Population trajectories are unknown for 11 species (including three species experiencing local Bd-associated declines) (Table S1; Fig. 3).

High recruitment (10 species) and environmental conditions that limit Bd growth (eight species) were the most common factors contributing to population persistence, while reservoir hosts were implicated in the ongoing decline of four species (Table S1). High body temperatures (behavioural fever) were reported to moderate Bd infection in three species, and selection for host resistance and/or decreasing pathogen virulence are hypothesized for several species, but have not been demonstrated (Table S1). Factors operating in 17 species were unknown.

4. Perspectives on amphibian conservation and Bd impacts in Australia

Our continent-scale assessment of chytridiomycosis impacts in Australia reveals that the disease is likely to have caused the extinction of seven species and contributed to the decline of a further 36 species since 1970. These declines represent nearly one fifth of the Australian amphibian fauna (238 species). In contrast, no amphibian extinctions were recorded in Australia from the time of European settlement in 1788 until 1970 (Hero et al., 2006; Hero and Morrison, 2004); a period that was associated with mass declines and extinctions in birds and mammals, largely attributed to habitat loss and invasive predators (Garnett et al., 2011; Woinarski et al., 2015). No Australian amphibian

extinctions have been attributed to habitat loss to date, although it is recognized as a key threat for at least 23 species (Gillespie et al., 2011; Hero et al., 2006; Hero and Morrison, 2004). Bd is now endemic throughout most climatically suitable regions of Australia, and is a key feature in the ecology of many declined species. Importantly, hot and dry environmental conditions appear to limit Bd distribution throughout much of Australia, largely restricting Bd impact to the cooler, wetter mountainous areas of eastern Australia (Murray et al., 2011a).

Our results are generally consistent with earlier work predicting decline and disease risk in Australian frogs (Murray et al., 2011a; Murray et al., 2011b; Murray and Skerratt, 2012). In particular, species identified as experiencing severe declines occurred in areas with high predicted environmental suitability for Bd (Murray et al., 2011a) and had life-history characteristics (e.g. stream or moist bog/soak breeding species) associated with increased decline risk (Murray et al., 2011b). However, there were several notable exceptions. For example, we identified evidence that the spotted tree frog (*L. spenceri*) and yellow-spotted bell frog (*L. castanea*) have declined primarily due to Bd; however, environmental suitability for Bd across their distributions was assessed as relatively low by Murray et al. (2011a). Similarly, earlier work predicted *L. spenceri* as stable (Murray et al., 2011b), whereas the species is in fact experiencing ongoing declines associated with Bd (Gillespie et al., 2015).

The long-term population trajectories of Bd-declined species were found to be highly variable across genera, climatic zones and habitat types, with species experiencing a range of outcomes from ongoing decline to apparent stabilization and recovery. Mass population declines due to chytridiomycosis epidemics are well documented internationally (e.g. Berger et al., 1998; Lips et al., 2006; Vredenburg et al., 2010). Our assessment found that the pathogen also remains a major threat to some species decades post emergence, suggesting that even endemic disease can have a dramatic impact on population health and stability. For example, in Australia there are at least six species currently challenged by Bd that are at risk of extinction without conservation intervention. There are also eight declined species that now appear stable with endemic Bd infection (e.g. Murray et al., 2009; Newell et al., 2013; Retallick et al., 2004; Scheele et al., 2014a; Scheele et al., 2015b). However, for

many apparently stable species, the pathogen remains a constraint on abundance and distribution (e.g. Heard et al., 2014; Heard et al., 2015; Murray et al., 2009; Phillott et al., 2013; Scheele et al., 2015b), reducing the resilience of populations and compromising their capacity to withstand other sources of mortality, such as extreme drought (Scheele et al., 2012; Scheele et al., 2016a). Importantly, future shifts in environmental conditions or the emergence of other threatening processes (e.g. predation or habitat loss) may alter this balance and trigger further declines or losses in species that currently appear stable. Furthermore, we caution that apparently stable species may in fact be experiencing gradual, unrecognized declines that have been overlooked due to an absence of robust, targeted monitoring programs. Encouragingly, there is evidence of recovery in 11 species, with increases in population abundance and distribution documented (e.g. McDonald et al., 2005; Newell et al., 2013; Scheele et al., 2014a).

Temperate Australian frog species tended to experience less abrupt declines compared with tropical or sub-tropical species, but were more likely to be experiencing ongoing declines. The slower decline rate may be due to lower Bd exposure associated with many temperate species overwintering in terrestrial environments compared with tropical species that commonly occupy aquatic environments year round (Anstis, 2013). Terrestrial habitat use by temperate species may slow Bd transmission and subsequently the rate of population decline (Skerratt et al., 2010). However, there is considerable uncertainty on initial decline severities in temperate Australia due to limited historical monitoring coinciding with Bd emergence.

5. Mechanisms and processes influencing endemic Bd impacts globally

The ongoing, long-term impact associated with Bd is highly variable among species. Endemic Bd dynamics are influenced by a diverse range of factors operating at the pathogen, individual, and population levels (Fig. 4). Below we focus on mechanisms and processes underlying differential long-term responses to Bd presence. Understanding processes that influence Bd-host dynamics and host

population persistence is crucial for refining our theoretical understanding of generalist host-pathogen dynamics, assessing species risk and informing conservation actions (Scheele et al., 2014b).

5.1. Mechanisms contributing to ongoing declines

5.1.1 Amphibian host reservoirs

Empirical and theoretical investigations indicate that pathogens rarely cause host extinction, with reduced host density limiting transmission as density declines (de Castro and Bolker, 2005; McCallum, 2012). However, when pathogens are capable of persisting in the environment or a biotic reservoir, host extinction is possible (de Castro and Bolker, 2005; McCallum, 2012). The capacity of Bd to persist as a saprobe remains uncertain (Berger et al., 2016). Conversely, Bd is well-known to infect multiple hosts, and the capacity of different amphibian species to acquire, persist with, and transmit Bd is highly variable (Fisher et al., 2009; Reeder et al., 2012; Retallick et al., 2004). Variable host susceptibility can drive pathogen-mediated host competition, whereby the presence of a tolerant species decreases the fitness of sympatric, susceptible hosts through increased pathogen pressure (Holt & Lawton 1994).

Pathogen-mediated host competition is associated with the ongoing decline and population extinctions of the northern corroboree frog (*Pseudophryne pengilleyi*) in temperate Australia (Table 2). The non-declining, highly abundant, common eastern froglet (*Crinia signifera*) acts as reservoir host for Bd; with prevalence generally exceeding 80% and high infection burdens (median zoospore equivalents >1000) (Scheele et al., 2016b). At sites where *C. signifera* is present, average Bd prevalence in *P. pengilleyi* is 41%, while at sites where *C. signifera* is absent, average Bd prevalence among *P. pengilleyi* is only 3%. Long-term monitoring has documented the extirpation or severe decline of *P. pengilleyi* populations that are sympatric with *C. signifera* (Scheele et al., 2016b). Reservoir hosts are also involved in the ongoing decline of at least three other temperate Australian species (*Ph. frosti*, *L. spenceri*, *P. corroboree*) (Clemann et al., 2009; West, 2016). Reservoir hosts

often maintain high Bd prevalence at sites where susceptible species have been extirpated, preventing recolonization and inhibiting reintroduction efforts (Reeder et al., 2012; Scheele et al., 2016b).

5.1.2. Species distribution coincides completely with optimal conditions for Bd

When a species' distribution completely overlaps with conditions favourable for Bd, risk of severe decline or extinction increases (Murray et al., 2011a; Murray et al., 2011b). In Australia, potentially extinct species, and those experiencing ongoing declines, are characterized by relatively small spatial distributions in cool, moist montane and sub-alpine regions. Restricted distributions limit the chance that part of a species' range will fall outside areas optimal for Bd growth, and thus persistence in environmental refugia is unlikely (Puschendorf et al., 2009).

5.2. Mechanisms contributing to population stability and recovery

5.2.1. Environmental conditions unfavourable to Bd

Batrachochytrium dendrobatidis growth and survival is mediated by temperature, desiccation risk, salinity and pH outside the neutral band (Johnson et al., 2003; Piotrowski et al., 2004). As such, environmental suitability for Bd is a key determinant of population-level host-pathogen dynamics (Murray et al., 2011a; Puschendorf et al., 2009). Amphibian habitats with unfavourable conditions for Bd act as disease-refugia, which allows species to persist with infection (Forrest and Schlaepfer, 2011; Heard et al., 2015; Puschendorf et al., 2011).

Unfavourable environmental conditions can inhibit Bd at regional and local scales (Skerratt et al., 2010). Regional refugia occur in areas where climatic conditions are broadly unfavourable for Bd. For example, in tropical regions of Latin America and Australia, Bd-induced declines have been most severe at higher elevations (Berger et al., 1998; La Marca et al., 2005; McDonald et al., 2005), with populations at low elevations (where temperatures may be higher than the thermal optimum of the

pathogen) experiencing reduced host impacts despite Bd presence (Puschendorf et al., 2009). In contrast, site level refugia occur on the scale of a single wetland or stream section. For example, in tropical Australia, all known armoured mist frog (*Litoria lorica*) populations were extirpated from rainforest habitat (McDonald and Alford, 1999). The species was thought to be extinct before being rediscovered on a single stream in open canopy savanna adjacent to rainforest (Puschendorf et al., 2011). The co-existence of this population with Bd is attributed to low canopy cover resulting in warmer rock substrates (and therefore warmer frogs), allowing individuals to moderate infection burden (Puschendorf et al., 2011). Similarly, wetlands occupied by the growling grass frog (*Litoria raniformis*) in south-eastern Australia vary substantially in microclimate due to their elevation, size, depth and vegetation characteristics, producing significant spatial variation in Bd prevalence (Heard et al. 2015). This variation has clear ramifications for the persistence of *L. raniformis* populations, with warm wetlands providing environmental refugia from Bd impacts, which bolsters metapopulation viability. Forrest and Schlaepfer (2011) report a similar situation for the lowland leopard frog (*Lithobates yavapaiensis*) in Arizona, USA, where reduced (or no) Bd impacts are apparent for populations inhabiting geothermal waterbodies in which temperatures regularly exceed 30°C.

Disease refugia also occur in areas where water chemistry inhibits Bd. In particular, water bodies with moderate salinity have been linked to reduced Bd prevalence and disease impact in temperate Australia (Heard et al., 2014; Heard et al., 2015; Stockwell et al., 2015) and the United Kingdom (Bramwell, 2011). For example, the prevalence of Bd infections in populations of *L. raniformis* in south-eastern Australia has been shown to drop by around 40% from fresh to saline wetlands, which in turn leads to a higher probability of persistence for frog populations inhabiting relatively saline wetlands (Heard et al. 2015).

The persistence of Bd-declined species in environments with low Bd suitability highlights the importance of conserving species across their entire distribution (Puschendorf et al., 2011; Scheele et al., 2014a). Persistence across a diverse range of habitat types can help species survive novel, spatially heterogeneous threats, such as disease outbreaks. In the case of Bd, habitats with low perceived value, such as disused quarries or industrial precincts, now act as important disease refugia, supporting

populations of Bd-afflicted species and contributing to metapopulation viability (Heard et al., 2014; Heard et al., 2015; Valdez et al., 2015). The identification and protection of environmental refugia is a conservation priority, whether they be regional zones of low Bd suitability or particular sites in which disease impacts are mitigated by prevailing environmental conditions.

5.2.2. Behavioural fever

Host thermoregulatory behaviour can affect Bd infection. Some amphibian species appear to display behavioural fever during Bd epidemics, modifying their thermoregulatory activities to increase body temperature and inhibit infection development (Richards-Zawacki, 2010). Behavioural fever has also been demonstrated under laboratory conditions, leading to increased survival times (Murphy et al., 2011). In endemically infected populations of several recovering species in tropical Australia, host body temperature has been correlated with individual infection status (Rowley and Alford, 2013). Because individual infection probability was inversely related to time spent above 25°C, natural selection for higher thermal preferences could contribute to population co-existence or recovery from Bd (Rowley and Alford, 2013).

5.2.3. Demographic processes allowing population persistence

Concomitant to environmental refugia that reduce disease impacts, persistence with Bd can also be facilitated by compensatory recruitment, which offsets mortality associated with disease. A number of studies have demonstrated that amphibian populations can remain stable despite high levels of chytridiomycosis-induced adult mortality (e.g. Murray et al., 2009; Muths et al., 2011; Phillott et al., 2013; Scheele et al., 2015b). For example, annual adult survival in the common mist frog (*Litoria rheocola*) (Table 2) in tropical Australia is less than 15% (associated with high Bd impact), but recruitment is also high, resulting in persistent, but seasonally fluctuating populations (Phillott et al., 2013). Importantly, while disease-associated adult mortality may not result in population extinction

due to compensatory recruitment, it may prevent recovery to pre-epidemic abundance if the reserve recruitment capacity is exhausted.

The timing of chytridiomycosis-induced mortality can influence whether mortality from disease affects overall population abundance. For example, in endemically infected yellow-legged frog (*Rana muscosa*) populations, high Bd prevalence in tadpoles is associated with low metamorph survival, with few individuals reaching sexual maturity. However, once mature, adults can survive across multiple years despite Bd infection, although population abundance remains very low because most individuals succumb to disease before reproducing (Briggs et al., 2010). Furthermore, a long-lived tadpole stage acts as an intra-specific reservoir host, maintaining high pathogen-presence (Briggs et al., 2010; Rachowicz and Briggs, 2007). In contrast, in endemically infected alpine tree frog (*Litoria verreauxii alpina*) (Table 2) populations, annual adult mortality approaches 100%. However, Bd prevalence is low in tadpoles and while mortality of recent metamorphs is likely substantial (~20%), recruitment capacity remains sufficient to maintain large population sizes because many individuals reproduce before succumbing to disease (Scheele et al., 2015b). Unfortunately, few studies have simultaneously investigated Bd impacts across all life-stages, limiting our understanding of how variation in the timing of Bd-induced mortality influences population outcome.

Within some endemically infected populations, persistent Bd-induced mortality can drive shifts in host life-history contributing to population persistence. In stable populations of *L. v. alpina* (Table 2), high adult mortality appears to be associated with adaptive shift towards earlier maturation (Scheele et al., 2016a). Early maturation reduces the risk of death before reproduction, selecting for rapidly maturing individuals (Stearns et al., 2000). Elevated rates of mortality in endemically infected populations are also expected to alter selection pressure on other life-history traits, such as fecundity, potentially contributing to population persistence. Consistent with this suggestion, Bd infection has been correlated with increased reproductive effort in infected frogs (Brannelly et al., 2016; Roznik et al., 2015).

Aquatic and terrestrial habitat connectivity can also influence Bd dynamics at the population level (Scheele et al., 2015a). For example, Heard et al. (2015) demonstrated that the density of surrounding populations was an important determinant of metapopulation viability for *L. raniformis* in southern Australia, due to the mitigating effect of migrants on population extinction risk (the so-called ‘rescue effect’) and their capacity to re-establish locally extinct populations. Through simulations, Heard et al. (2015) showed that even in the absence of environmental refugia, large, dense metapopulations could withstand higher overall population-level extinction risk due to Bd by maintaining a balance between population extinctions and recolonizations.

5.2.4. *Host-pathogen coevolution*

Many recent studies have investigated various aspects of the amphibian immune response to Bd infection, including antimicrobial peptides, symbiotic skin bacteria, immune cells and genes (Ellison et al., 2015; Fites et al., 2013; McMahon et al., 2014; Woodhams et al., 2007; Woodhams et al., 2014). However, evidence for evolved host responses to Bd as the cause of population persistence or recovery is limited. Recent research indicates evolved resistance has facilitated population recovery in the Californian yellow-legged frog (*Rana sierrae*) (Knapp et al., 2016), while improved resistance has been postulated in recovering Fleay’s barred frog (*Mixophyes fleayi*) populations (Newell et al., 2013). Major histocompatibility complex heterozygosity and specific alleles have been associated with survival in laboratory experiments and wild populations (Bataille et al., 2015; Savage and Zamudio, 2011), highlighting potential for evolution of resistance.

Although the complexity of Bd virulence is only beginning to be understood, it may play an important role in determining Bd dynamics in endemically infected populations. In diseases that rely on host survival, pathogen virulence may reduce towards an intermediate level in endemically infected populations, likely due to a trade-off between virulence (host death) and transmission (Lenski and May, 1994). However, as Bd has a broad amphibian host range (Olson et al., 2013), it is possible that reservoir hosts may not only maintain infection at a site but could also enable virulence to remain

high. Rapid changes in Bd virulence are also possible due to frequent genomic recombinations and duplications, genomic heterozygosity, and variations in chromosomal copy number (Farrer et al., 2013; Farrer et al., 2011; Rosenblum et al., 2013). Genome analysis suggests that the most rapidly evolving gene regions in Bd encode putative virulence factors, including proteases and chitin-binding proteins (Farrer et al., 2013; Joneson et al., 2011), suggesting that Bd virulence is evolving in the wild.

Globally, chytridiomycosis-induced amphibian declines have been linked with the hypervirulent global pandemic Bd lineage (Farrer et al., 2011; Jenkinson et al., 2016). Other lineages have been described from Brazil, South Africa and Europe, but have not been associated with amphibian declines (Farrer et al., 2011; Jenkinson et al., 2016). Within the global pandemic lineage, there is evidence for variation in virulence. For example, under laboratory conditions, Bd isolated during an epidemic in California in cascades frogs (*Rana cascadae*) was able to cause higher mortality compared with Bd isolated from stable populations of the same species (Piovia-Scott et al., 2015). This supports the theory that during novel epidemics virulence can become higher when susceptible hosts are plentiful (Hawley et al., 2013) and also suggests Bd virulence may decrease in endemically infected populations. Decreased Bd virulence has been suggested as a mechanism underlying population persistence and recovery with endemic Bd (McDonald et al., 2005; Newell et al., 2013), but is yet to be demonstrated.

6. Conclusions

Batrachochytrium dendrobatidis is now approaching endemism in many regions of the world where it has been associated with declines (Murray et al., 2009; Muths et al., 2011; Olson et al., 2013). Our assessment of the trajectory of Australian amphibians afflicted by Bd illustrates that long-term impacts are highly variable. Nearly four decades post-emergence, some species are apparently stable and others are recovering, while several species are in an ongoing state of decline. We found that rapid impacts on tropical frogs meant at-risk species were lost from these areas soon after the arrival of Bd, but in temperate areas there has been a lag with slower declines, so that despite a zero

extinction record, there are currently more species at high risk of extinction in temperate areas. We expect that similar spatiotemporal patterns are likely in Central and South America where Bd has emerged more recently. We strongly encourage long-term commitment to monitoring and research on Bd impacts in Australia and across the globe, to identify species requiring conservation interventions, and develop management actions to reverse Bd-induced amphibian declines.

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Appendix A. Table S1. Information on 43 Australian frog species impacted by *Batrachochytrium dendrobatidis*.

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Table 1. Some key attributes of Australian frog species 1) likely extinct due to Bd, 2) in an ongoing state of decline due to Bd, and 3) at risk of future declines associated with the continued spread of Bd. Stable or recovering species not listed (see Table S1). *Denotes taxonomic uncertainty.

1. Extinct species	Initial decline date	Elevation range (m)	Adult habitat
<i>Litoria nyakalensis</i> (Mountain Mistfrog)	1989. Last seen: 1990	380-1000	Rainforest stream
<i>Litoria piperata</i> (Peppered Tree Frog)*	Unknown. Last seen 1973	800-1120	Montane wet forest stream
<i>Rheobatrachus silus</i> (Southern Gastric Brooding Frog)	1979. Last seen: 1981	400-800	Rainforest stream
<i>Rheobatrachus vitellinus</i> (Northern Gastric Brooding Frog)	1985. Last seen: 1985	400-1050	Rainforest stream
<i>Taudactylus acutirostris</i> (Sharp Snouted Day Frog)	1984. Last seen: 1997	300-1300	Wet forest stream
<i>Taudactylus diurnus</i> (Mount Glorious Day Frog)	1979. Last seen: 1979	350-800	Rainforest stream
<i>Taudactylus rheophilus</i> (Northern Tinker Frog)	1989. Last seen: 2000	950-1500	Rainforest stream, seepage areas
2. Currently declining species			
<i>Litoria castanea</i> (Yellow-spotted Tree Frog)*	1979	550-1500	Perennial and ephemeral wetlands
<i>Litoria spenceri</i> (Spotted Tree Frog)	Likely commenced in the 1980s	200-1100	Montane forest stream
<i>Philoria frosti</i> (Baw Baw Frog)	Likely commenced in the 1980s	960-1560	Sub-alpine wetlands and woodlands
<i>Pseudophryne corroboree</i> (Southern Corroboree Frog)	1984	1300-1760	Sub-alpine wetlands and woodlands
<i>Pseudophryne pengilleyi</i> (Northern Corroboree Frog)	1984	800-1800	Sub-alpine wetlands and woodlands
<i>Taudactylus pleione</i> (Kroombit Tinker Frog)	Likely commenced in the 1980s	550-900	Rainforest stream, seepage areas
3. Species at risk of future declines			
<i>Litoria andiirmalin</i> (Melville Range Tree Frog)	NA	50-500	Streams in boulder-fields
<i>Litoria burrowsae</i> (Tasmanian Tree Frog)	NA	100-1070	Perennial and ephemeral wetlands
<i>Litoria longirostris</i> (Long-snouted Frog)	NA	400-700	Montane rainforest streams

Table 2. Examples of Australian amphibian species experiencing different long-term responses following *Batrachochytrium dendrobatidis* emergence.

Ongoing decline	Stable	Recovering
Northern corroboree frog (<i>Pseudophryne pengilleyi</i>)	Alpine tree frog (<i>Litoria verreauxii alpina</i>)	Common mist frog (<i>Litoria rheocola</i>)
The northern corroboree frog (<i>Pseudophryne pengilleyi</i>) occurs in temperate montane forests and sub-alpine woodlands. Population declines were first reported in 1984 (Osborne, 1989), and have continued unabated resulting in the extinction of most populations (Osborne et al., 1999; Scheele et al., 2016b). Bd prevalence in adults is moderate (~30%), likely reflecting their largely terrestrial habitat use and reproduction in ephemeral ponds, with tadpoles rarely infected (D. Hunter unpublished data). The presence of reservoir hosts combined with the slow life-cycle and small clutch size of <i>P. pengilleyi</i> may explain their ongoing decline.	The alpine tree frog (<i>Litoria verreauxii alpina</i>) is an aggregate pond breeder from the sub-alpine zone that experienced major declines from 1984 onwards (Osborne et al., 1999). A small number of stable, but infected populations persist (Hunter et al., 2009). The species remains highly susceptible to Bd infection under laboratory conditions (Bataille et al., 2015), and in the wild annual adult mortality approaches 100% (Brannelly et al., 2015; Scheele et al., 2015b). Low Bd impact in tadpoles and juveniles has enabled populations to survive despite almost complete annual turn-over, but persistence relies on breeding every year, hence they can no longer persist at ephemeral wetland sites (Scheele et al., 2016a).	The common mist frog (<i>Litoria rheocola</i>) is a stream breeding tropical rainforest species. Declines were first observed in 1989, with most populations above 400 m elevation extirpated, coinciding with favourable conditions for Bd (McDonald et al., 2005; Richards et al., 1993). Lowland populations are stable as recruitment is high enough to offset mortality (Grogan et al., 2016; Phillott et al., 2013), and are now re-expanding into upland habitat (McDonald et al., 2005; C. Hoskins unpublished data), which appears consistent with evolution of resistance. In adults, Bd prevalence peaks during winter (~80%), coinciding with optimal climatic conditions for Bd (Phillott et al., 2013).



Figure 1. Dates of some well-documented amphibian declines on a map of the predicted environmental suitability for *Batrachochytrium dendrobatidis* in Australia (modified from Murray et al. (2011a)) Dates from: Richards et al. (1993), Dennis and Mahony (1994), Laurance et al. (1996), Osborne et al. (1996), Osborne et al. (1999), Obendorf (2005) and Murray et al. (2010). Predicted distribution largely coincides with Bd detection, although Bd appears to be absent in south-western Tasmania and northern Cape York (Murray et al., 2010). Amphibian declines have coincided with areas of predicted Bd suitability in eastern Australia, but no Bd-associated declines have been reported in Western Australia (Riley et al., 2013).

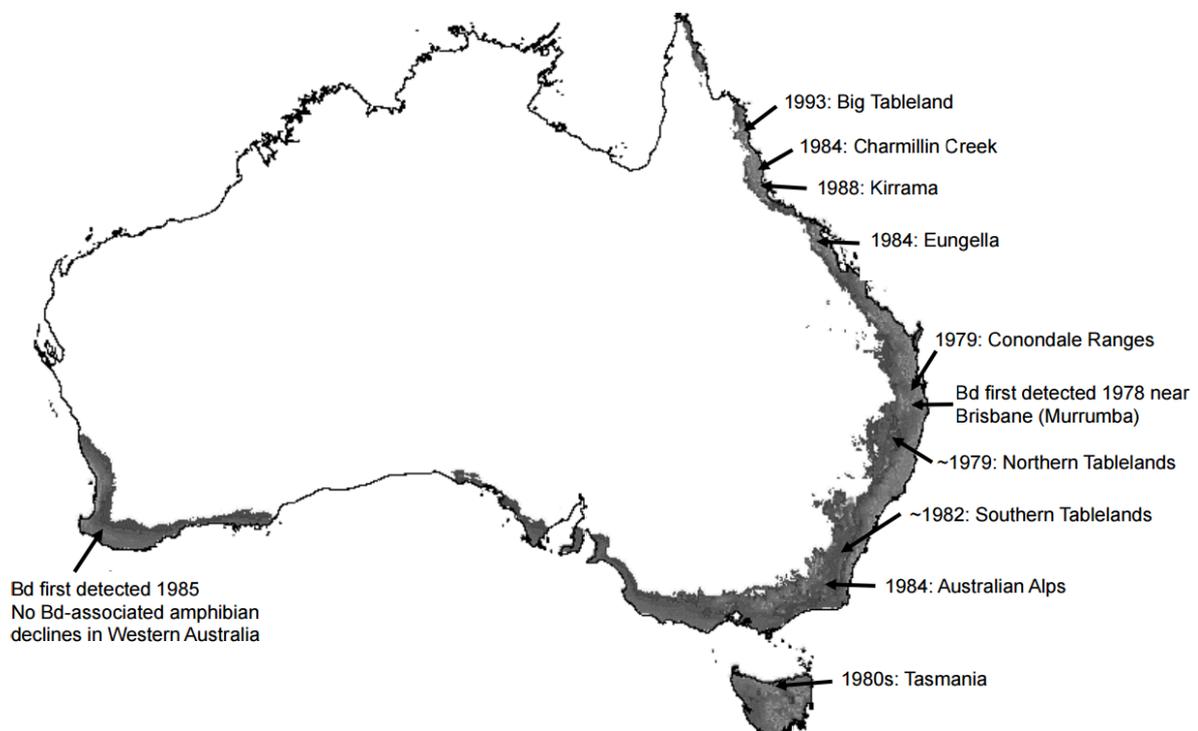


Figure 2. Likely extent of range decline due to population extinctions associated with *Batrachochytrium dendrobatidis* infection among Australian amphibians, classified by geographic distribution.

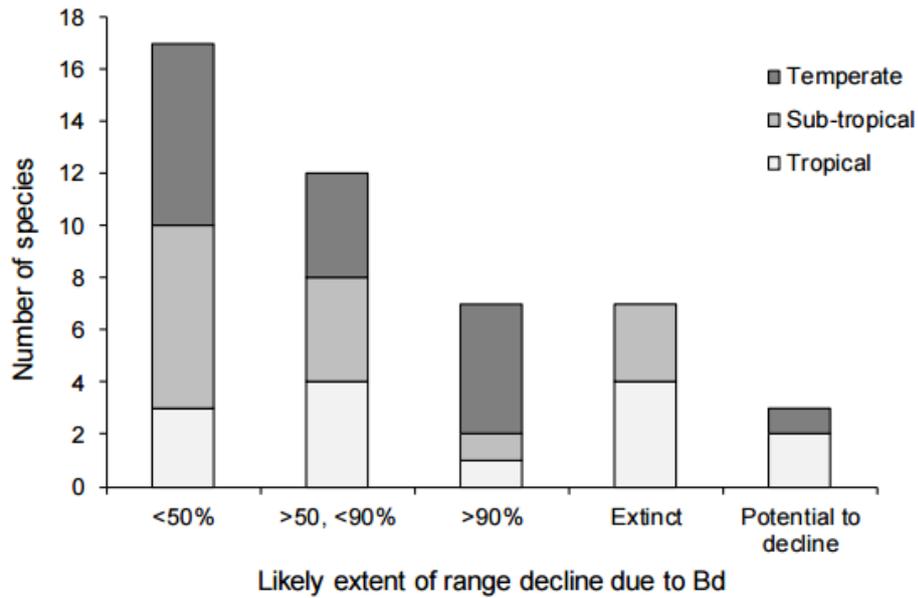


Figure 3. Long-term population trajectories of Australian amphibians that have declined due to *Batrachochytrium dendrobatidis*, classified by geographic distribution.

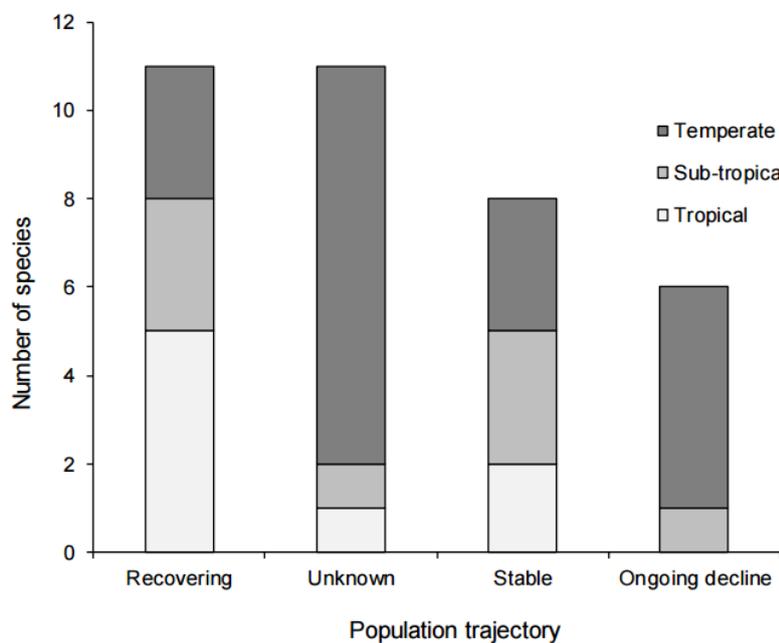


Figure 4. Pathogen, host and environmental factors influencing *Bd*-host dynamics and amphibian population persistence. These factors can also be categorized as affecting either host survival or reproduction. The presence and importance of each factor varies among species, but empirical

evidence from field studies shows these factors are vital to understanding population trajectories in different contexts, except for variation in Bd virulence. Laboratory experiments show Bd virulence varies among strains and over time, and current studies aim to understand the impacts of this variation in the wild.

