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Frogs vs fungus: the emergence of amphibian chytridiomycosis

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ABSTRACT

By the late 1980s, widespread dramatic declines in amphibian populations were causing alarm. The culprit was identified as *Batrachochytrium dendrobatidis* (*Bd*), a chytrid fungus that infects the skin of various amphibian hosts, particularly anurans (frogs), and the first example of a chytridiomycete parasitising vertebrates. The disease, chytridiomycosis, has spread globally and is linked to the decline and extinction of many amphibian species. This review summarises the discovery of *Bd*, its emergence as a panzootic pathogen, and some current mitigation strategies to conserve amphibians.

Keywords: amphibian, Australia, *Batrachochytrium dendrobatidis*, chytridiomycosis, conservation, disease, frog, fungal, wildlife.

Introduction

The emergence of chytridiomycosis in amphibians has changed the way we think of wildlife diseases. Never has a fungal disease had such a profound impact on biodiversity.[1] The organism responsible, *Batrachochytrium dendrobatidis* (*Bd*),[2] can infect an unusually broad range of amphibian hosts, with a propensity to cause severe population declines and extinctions.[1] The discovery of chytridiomycosis,[3] and subsequent attempts to control the pathogen, presents unique challenges and highlights the need for cross discipline collaboration.[4]

Discovery of chytridiomycosis

Like many organisms, amphibian populations are declining due to human activities such as deforestation and pollution. When frogs and toads started disappearing from pristine protected areas, however, scientists were puzzled. Throughout the 1980s, reports of amphibian declines were coming from the cloud forests of Costa Rica, the alpine Sierra Nevadas in the USA, and the Atlantic forests of Brazil. Frogs decompose quickly in the forest, hampering efforts to determine the cause of death. In Australia, two species of the unique gastric brooding frog (the northern and southern gastric brooding frogs, *Rheobatrachus vitellinus* and *R. silus*), and two species of day frog (the southern and Eungella day frogs *Taudactylus diurnus* and *T. eungellensis*) went missing from the rainforest of Central and Southern Queensland. Concern was mounting for the North Queensland frogs, and particularly for a related species of day frog,the sharp snouted day frog (*T. acutirostris*). Intense monitoring began at 'Big Tableland', one of the last remaining *T. acutirostris* populations. It was not long before fears were realised and a mass die-off was observed in real time. In addition to *T. acutirostris*, multiple species of stream breeding frogs simultaneously vanished. At the Big Tablelands upland site, the co-occurring common mist frogs (*Litoria rheocola*), Australian lace-lids (*Litoria dayi*), and waterfall frogs (*Litoria nannotis*), disappeared over the space of a few months.[5] Although devastating, this was an opportunity to collect enough freshly dead frogs for pathology to determine the cause of death. A tiny skin parasite in the outer layers of skin, originally dismissed as a secondary infection, was the only common factor. Although such a superficial skin infection seemed unlikely to cause such catastrophic declines, experiments demonstrated that skin from dead frogs could transmit disease to healthy frogs. Electron microscopy and sequencing showed it to be a novel chytrid fungus.[3] When mass die-offs were detected in Panama, an extraordinary international collaborative effort suggested it was the same fungal species causing disease across continents.[3] A parallel investigation into captive frog mortalities in the United States led to the taxonomic description of the culprit, a chytrid fungus: *Batrachochytrium dendrobatidis* (*Bd*).[2] This was the first report of a chytrid parasitising a vertebrate host.

Like other chytrid fungi, *Bd* produces motile zoospores that swim via flagella. But unlike the majority of chytrids, the zoospore is infective, penetrating the hosts skin via germ tube, before developing into a zoosporangium.[6] The zoosporangium asexually produces more

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zoospores, which can infect the same host or spread to a new one. The resulting disease, named chytridiomycosis,[3] presents as lethargy, with characteristic reddened and shedding skin. Death can often occur rapidly after the onset of clinical signs. Such severe outcomes from a seemingly mild, non-systemic skin infection were contrary to conventional wisdom, and it was thought that the fungus must produce a toxin. Thorough investigation revealed that instead, *Bd* infection destroys the integrity of the skin, leading to electrolyte imbalance and cardiac arrest.[7] Supportive supplementation with electrolytes can slow disease progression but cannot keep up with the damage caused by the fungus as it continues to multiply in the skin.[7] Early histological analysis revealed a lack of immune response in infected hosts, with poor infiltration of professional immune cells (e.g. macrophages) to the site of infection and minimal inflammation.[8] The down regulation of immune genes during infection suggested that *Bd* dampens the host immune capabilities.[9] This was further supported by the discovery that *Bd* cells produce a suite of metabolites that can paralyse amphibian immune cells[10,11] and prevent fungal clearance, eventually leading to death of the host. By targeting the skin, an organ universally important for amphibians, the fungus can cause disease in an incredibly broad range of hosts. *Bd* infects all three orders of amphibians, predominantly the frogs and toads, but also salamanders and caecilians, and at least 500 amphibian species have suffered population declines due to chytridiomycosis.[1] In addition, the fungus appears to infect freshwater invertebrates as well, making it especially difficult to eradicate from the environment.

Emergence of chytridiomycosis

Histological analysis of museum specimens and a newly developed PCR diagnostic assay provided evidence linking *Bd* to many more past and current amphibian declines globally.[12,13] But where had *Bd* come from and what was behind the sudden emergence at so many sites globally? The pathogen is in fact an ancient, early diverging fungal species thought to have originated in Asia. Genetic analysis revealed that *Bd* is comprised of several distinct lineages, some of which appear to be endemic and not associated with amphibian declines.[14–16] However, the hypervirulent global panzootic lineage '*Bd*GPL' emerged in the early 20th century, and spread most likely via the amphibian trade.[15] The *Bd*GPL lineage appears responsible for the dramatic amphibian declines observed in Australia, the Americas and Europe, which are estimated to have caused the extinction of at least 90 amphibian species.[1] Chytridiomycosis has had flow on effects extending beyond amphibians, as seen in the loss of neotropical snakes once their prey disappeared.[17] The fungus thrives in cool, wet environments, therefore patterns of chytridiomycosis emergence can be explained predominately by temperature and rainfall.[18] However, in some cases, co-occurring species can have vastly different responses despite a shared niche. For example, the iconic corroboree frog (*Pseudophryne corroboree*) is incredibly susceptible to *Bd* and has declined to near extinction,[19] whereas the co-occurring common froglet (*Crinia signifera*) appears tolerant[20] (Fig. 1). Uncovering the mechanism behind differential susceptibility is an important area of future research.

Combatting chytridiomycosis

Antifungal drugs borrowed from veterinary medicine are sufficient for the treatment of captive animals.[21] Targeted antifungal treatment of wild amphibians can reduce infection and increase survival.[22,23] However, as the fungus appears impossible to eradicate from the environment, antifungal treatment of wild amphibians only provides a short-term solution. Feasible, long-term solutions to combat chytridiomycosis are urgently required to prevent further loss of biodiversity.[4] Potential mitigation strategies can be broadly divided into those that target the pathogen and those that aim to increase host resistance. Anti-pathogen strategies include modifying natural areas and creating unsuitable microhabitats, for example by increasing salt content[24] or providing basking opportunities to help hosts overcome infection (Fig. 2). There has also been interest in developing viral biocontrol to reduce fungal virulence, as this technique has been successful in curing fungal diseases in wild plants.[25] By reducing the virulence of *Bd* without

Pseudophryne corroboree

A myobatrachid frog restricted to high elevation bogs and woodland in Kosciuszko National Park. Wild populations have declined dramatically. Exposure to low dose of *Bd* can result in 100% mortality in a few weeks.

Crinia signifera

A myobatrachid frog occupying a range of habitats along the east coast, including co-occurring with *P. corroboree*. No evidence of *Bd* related population declines and infected individuals can resolve infection.

Fig. 1. Two Australian species exhibiting different responses to chytridiomycosis. Photos by R. Webb.

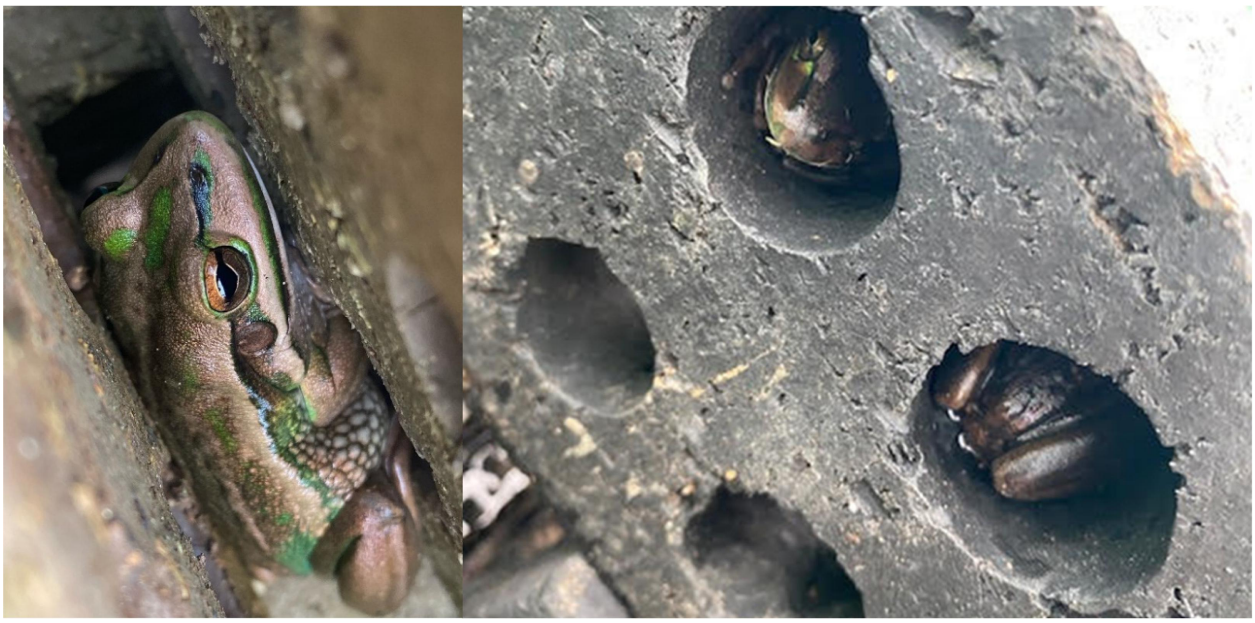

Fig. 2. Adult *Litoria aurea* using a recently developed 'thermal refugia' during an experimental trial at Macquarie University, Sydney, NSW. Photos by A. Waddle.

eradicating it, these approaches could allow wild amphibians the opportunity to slowly develop natural resistance.

Alternatively, artificially increasing resistance of captive bred and released animals could create self-sustaining wild populations.[4] Early attempts at vaccination were unsuccessful,[26] which is not surprising given the suppressed host immune system. However, recent research suggests that vaccination might be protective in some species. Inoculations with low virulence strains or infection with highly virulent strains followed by antifungal treatment can bolster host defences to future infections.[27,28] However, a vaccination strategy might still require ongoing intervention. Research into selective breeding of resistant individuals is underway, with the aim of using whole genome sequencing to identify alleles associated with survival (discussed in Kosch *et al.*[4]). Release of resistant animals could reduce the need for constant intervention. However, this is an expensive and long-term approach, which will likely only be feasible for a few select species. Therefore, a 'silver bullet' is unlikely, and a combination of approaches will be required for amphibian conservation.

Continued threat of chytridiomycosis

Although *Bd* has now spread around the globe, restricting pathogen movement remains a priority. Hybrid strains can display increased virulence,[29] highlighting the importance of minimising opportunities for contact between divergent *Bd* lineages. In Australia, strict biosecurity and field hygiene protocols are necessary to prevent further *Bd* introductions, and to slow pathogen spread into uninfected areas such as parts of the Tasmanian Wilderness World Heritage Area and Cape York Peninsula, Queensland. These protocols are also essential to prevent the spread of other amphibian diseases. For example, another species of parasitic chytrid fungus; *Batrachochytrium salamandrivorans* or '*Bsal*', was described in 2013 after causing fire salamander (*Salamandra salamandra*) mortality in Belgium.[30] It is now reported in various locations around Europe, having likely emerged and spread from Asia in much the same way as *Bd*.[31] There is mounting concern that the pathogen will reach North America and have devastating effects on the diverse salamander communities there.[32] A collaborative taskforce is currently assessing the risk of *Bsal* invasion to North America using species susceptibility and environmental suitability modelling, as well as monitoring wild and imported salamanders. Although there are no native salamanders in Australia, *Bsal* may still pose a risk to our native amphibians. Preliminary laboratory experiments indicate that *Bsal* can infect frog species, highlighting the need for targeted monitoring and preventative measures.[33] Australia has already lost six amphibian species due to chytridiomycosis.[34] The good news is that some species, such as Fleay's barred frog (*Mixophyes fleayi*), are showing signs of recovery and coexistence with *Bd*, but at least seven species are still in dire risk of extinction without intervention. Continued research and multidisciplinary collaboration are required to mitigate the current *Bd* epidemic, understand the mechanisms by which some species are developing resistance, and to prevent the introduction of a second amphibian chytrid species.

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Biographies

Rebecca Webb is a Research Fellow with the One Health Research Group, at the University of Melbourne. Rebecca is passionate about amphibian conservation, especially in regards to their biggest threat; the chytrid fungus. Her research aims to increase understanding of this fungal pathogen and identify its weaknesses, and use this information to inform potential control mechanisms. Currently her research focusses on using gene silencing techniques to characterise important fungal genes, and as a possible tool to reduce fungal virulence.

Anthony Waddle is a Schmidt Science Fellow and postdoctoral scholar working at Macquarie University in Applied BioSciences. His research focuses on developing conservation interventions for amphibian species that have been impacted by chytrid fungus. Prior to his current position, Anthony worked on developing vaccines against chytrid, determining the mechanisms by which frog populations can persist despite the presence of chytrid, and developing habitat manipulations which can buffer against chytrid. He is now developing genome resources and synthetic biology techniques, which can be used to understand and promote host resistance to disease.