Short Communication **Preserving pathogens for wildlife conservation: a case for action on amphibian declines**

JAMIE VOYLES, SCOTT D. CASHINS, ERICA BREE ROSENBLUM

and ROBERT PUSCHENDORF

Abstract Infectious disease is an important driver in biological systems but its importance in conservation has historically been underestimated. Recently, however, researchers have increasingly recognized the impact of diseases on wildlife populations and have grappled with disease-related conservation challenges. For example, the phenomenon of worldwide amphibian declines caused by the fungal disease chytridiomycosis has contributed to the creation of a global Amphibian Conservation Action Plan. The sense of urgency in the protection of amphibians and mitigation of the effects of chytridiomycosis is well-warranted but determining the best way to respond to chytridiomycosis is challenging. Current conservation strategies focus on the preservation of the amphibian hosts, their habitats and their genetic materials. However, we suggest that to confront disease threats fully, particularly in the case of amphibian declines, insight into host-pathogen coevolution may be critical and we must therefore also preserve the pathogen for basic disease research. Here we outline priority targets for virulence research and urge researchers and managers to isolate and archive the pathogen Batrachochytrium dendrobatidis to ensure viable long-term amphibian conservation.

Keywords Amphibian declines, *Batrachochytrium dendrobatidis*, chytridiomycosis, pathogen preservation, wildlife disease

Parasites can regulate host population densities, alter community dynamics and affect entire ecosystems (Scott, 1988; Daszak et al., 2000; Hudson et al., 2002; de Castro & Bolker, 2005; Whiles et al., 2006). Disease has obvious consequences for already-threatened animal populations (e.g. black-footed ferrets, Thorne & Williams, 1988)

SCOTT D. CASHINS and ROBERT PUSCHENDORF School of Marine and Tropical Biology, Amphibian Disease Ecology Group, James Cook University, Townsville, Australia.

ERICA BREE ROSENBLUM Department of Biological Sciences, University of Idaho, Moscow, USA.

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but it can also pose a significant threat to otherwise healthy populations (Scott, 1988; Daszak et al., 2000; de Castro & Bolker, 2005). Many amphibians, for example, are experiencing unprecedented declines because of the disease chytridiomycosis, caused by the fungal pathogen Batrachochytrium dendrobatidis (Berger et al., 1998; Lips et al., 2006; Mendelson et al., 2006; Gascon et al., 2007). The relatively recent discovery of B. dendrobatidis was precipitated by amphibian mass mortality events and disappearances in pristine locations around the world (Berger et al., 1998; Lips et al., 2006; Mendelson et al., 2006; Gascon et al., 2007). Although B. dendrobatidis is confined to the superficial layers of epidermis, infected frogs can die rapidly because of the physiological importance of amphibian skin (Berger et al., 1998; Voyles et al., 2007). Current B. dendrobatidis distribution patterns generated by predictive models are based on the occurrence of the pathogen on the amphibians (Ron, 2005; Puschendorf et al., 2009). It is unclear whether B. dendrobatidis can persist independently of amphibian hosts, in an alternate life stage (Di Rosa et al., 2007), by saprobic growth (Mitchell et al., 2008) or in non-amphibian carriers. In spite of these unresolved possibilities, the loss of biodiversity (Lips et al., 2006; Mendelson et al., 2006), subsequent ecosystem disturbance (Whiles et al., 2006) and the risk for naïve amphibian populations justify immediate conservation efforts.

Management of infectious diseases is not easy; traditional conservation strategies, such as managing reservoir hosts, culling or vaccination, are not practical options where host management is not feasible or where vaccination is unavailable (Hudson et al., 2002). For amphibian conservation, one remediation prescribed by the Amphibian Conservation Action Plan and currently underway is the 'rescue' of amphibians from their natural habitats into ex situ breeding programmes before the disease emerges (Mendelson et al., 2006; Gascon et al., 2007). This strategy is controversial (Pounds et al., 2007) and many scientists recognize the conservation crux: re-establishment of amphibians into habitats where the pathogen is now endemic (Pounds et al., 2007; Schlaepfer, 2007). Understanding host-pathogen interactions and coevolution may be critical for long-term conservation. However, with the current focus on preserving vulnerable amphibian hosts and their genetic material (Gascon et al., 2007), the importance of pathogen collection and preservation is being

JAMIE VOYLES (Corresponding author) School of Public Health, Tropical Medicine and Rehabilitation Sciences, Amphibian Disease Ecology Group, James Cook University, Townsville, Queensland 4811, Australia. E-mail jamie.voyles@gmail.com

overlooked. Chytridiomycosis is an important example of how access to pathogen isolates will be invaluable for disease research and conservation efforts.

Determinants of mortality from chytridiomycosis are multiple and complex. Pivotal factors include environmental conditions, population dynamics, host resistance and pathogen virulence. To date, most studies have focused on environmental factors (Woodhams et al., 2003; Berger et al., 2004) and host resistance (Rollins-Smith et al., 2002; Woodhams et al., 2007), whereas differential pathogen virulence has been largely neglected. Preliminary evidence suggests that B. dendrobatidis virulence may differ among isolates (Berger et al., 2005; Retallick & Miera, 2007; Fisher et al., 2009) but the underlying reasons for the variation are unknown. Furthermore, reduced mortality has been observed in populations after surviving initial emergence (Morgan et al., 2007) and different populations, even within species, can experience differential susceptibility to chytridiomycosis (Briggs et al., 2005). This variation in susceptibility to B. dendrobatidis suggests the possibility of increased host resistance, decreased parasite virulence or both.

Determining the factors that influence disease development will lead to a greater understanding of the disease and thus direct more effective conservation action. For example, we may be able to distinguish a novel pathogen from a pathogen that has increased in virulence (Daszak et al., 2000; Rachowicz et al., 2005), assess potential disease risk (Kuiken et al., 2005), track pathogen movements (Kuiken et al., 2005; Kang et al., 2006) or incorporate disease resistance into breeding programmes (Schlaepfer, 2007). Substantial advancements in virulence research are achievable but depend on access to pathogen isolates from diverse spatio-temporal origins. We suggest three priority targets for pathogen collection and cryopreservation: (1) isolates from geographically and taxonomically diverse hosts, (2) isolates preserved during and following pathogen emergence, and (3) isolates collected at finer scales from populations exhibiting differential responses to infection. Such isolates will be a key resource for laboratory experiments and genetic studies aiming to identify pathogenicity factors (Rosenblum et al., 2008) in chytridiomycosis or for any disease that threatens wildlife populations.

In the case of amphibian declines due to chytridiomycosis, the ability to confront the disease threat fully will hinge on coordinating multiple mitigation strategies including careful screening for the pathogen in naïve populations (Skerratt et al., 2008; Cashins et al., in press) and in the international amphibian trade (Australian Government Department of the Environment and Heritage, 2006; Gascon et al., 2007), identifying and preserving vulnerable amphibians (Mendelson et al., 2006; Gascon et al., 2007) and, most importantly, advancing basic disease research (Australian Government Department of the Environment and Heritage, 2006; Mendelson et al., 2006; Gascon et al., 2007). Understanding pathogen virulence is important to this endeavour and we urge managers and researchers to participate in a collaborative effort to archive *B. dendrobatidis*. Established methodologies for isolating (Longcore et al., 1999) and cryo-archiving (Boyle et al., 2003) *B. dendrobatidis* are available in multiple languages at the Global *Bd* Banking Project website (2009). In developing this website as an international forum, we aim to share the necessary resources to preserve *B. dendrobatidis* and to collate information on global isolates into a single database to facilitate virulence research. Confronting disease threats and implementing effective conservation action will require a better understanding of the disease, the host and the pathogen.

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Biographical sketches

JAMIE VOYLES is interested in ecosystem health and the evolution of virulence in host-pathogen systems, focusing on pathogenesis of chytridiomycosis and virulence of *Batrachochytrium dendrobatidis* in amphibians. SCOTT D. CASHINS studies disease ecology, focusing on transmission and seasonal dynamics of *Batrachochytrium dendrobatidis* in tadpoles in rainforest habitats of North Queensland, Australia. ERICA B. ROSENBLUM'S research interests centre on the mechanisms of adaptive evolution, using functional genomics tools to elucidate the genetic basis of pathogenicity of chytridiomycosis. ROBERT PUSCHENDORF is interested in disease ecology and the influence of environmental factors on host-pathogen interactions, especially on dynamics of *Batrachochytrium dendrobatidis* across habitats in Costa Rica and Australia.