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Decline and re-expansion of an amphibian with high prevalence of chytrid fungus

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ABSTRACT

The disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), is a key driver of global amphibian declines. While chytridiomycosis can cause extinction, many susceptible species persist after an initial period of decline, albeit with reduced abundance and distribution. Emerging evidence indicates that amphibian abundance can recover within remnant populations, but to date, the capacity of amphibian populations to re-expand into historically occupied habitat has received limited research attention. We surveyed 145 sites in 2011 and 2012 to determine if populations of the whistling tree frog (*Litoria verreauxii verreauxii*) have re-expanded compared with historical data from 1975–1976, 1990 and 1996. *L. v. verreauxii* underwent a major range contraction likely caused by chytridiomycosis between the first two time periods. Populations have recently re-expanded, with 39 new sites colonised despite high prevalence of Bd. We suspect that changes in disease dynamics have resulted in the increased coexistence of *L. v. verreauxii* and Bd. Habitat attributes at sites that retained frogs for the duration of the study indicate that high quality habitat may contribute to buffering against population level effects of Bd. Colonised sites had more coarse woody debris, suggesting a possible habitat management strategy to encourage range expansion for this species. Given sufficient time and adequate source populations in high quality habitat, it is possible that other amphibian species may re-expand from chytridiomycosis-induced declines. This provides an impetus for the protection of historical, but currently unoccupied amphibian habitats and highlights the importance of maintaining high quality habitat to help species survive novel shocks such as pandemic diseases.

1. Introduction

In an era of rapid biodiversity loss, amphibians are especially vulnerable (Stuart et al., 2004) due to multiple threats, including habitat destruction and emerging infectious disease (Wake and Vredenburg, 2008; Hof et al., 2011). One of the most pressing threats is the pandemic chytridiomycosis (Wake, 2012), an infectious skin disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (hereafter Bd) (Berger et al., 1998) that has caused the decline or extinction of at least 200 species (Skerratt et al., 2007). Chytridiomycosis has resulted in “the most spectacular loss of vertebrate biodiversity due to disease in recorded history” (Skerratt et al., 2007) and provides a devastating example of the threat posed by emerging infectious diseases to biodiversity (Fisher et al., 2012).

Although chytridiomycosis has caused the extinction of many species, the majority of susceptible species experience range contractions but subsequently persist with enzootic Bd infection, albeit with greatly reduced distribution and abundance (Walker et al., 2010; Puschendorf et al., 2011). Disease dynamic models suggest that population recovery is possible in populations with enzootic Bd (Briggs et al., 2010). Recently, Newell et al. (2013) demonstrated that two populations of the endangered *Mixophyes fleayi* have experienced sustained population growth and this combined with observations of increased abundance in several *Litoria serrata* (Syn. *L. genimaculata*) populations (McDonald et al., 2005; Richards and Alford, 2005), indicates that the recovery of at least some remnant populations of these species is occurring. However, despite the extensive Bd literature (Muths et al., 2011), evidence documenting range re-expansion is limited to brief observations from northern Australia described by McDonald (2002) and

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McDonald et al. (2005). Documenting evidence of range re-expansion would greatly aid our understanding of the long-term response of wildlife to novel diseases, and provide an impetus for the protection of historical, but currently unoccupied habitat.

Here, we examine the long-term response of an amphibian species approximately three decades after the emergence of Bd in our study region. *Litoria verreauxii verreauxii* (whistling tree frog) experienced severe declines in upland areas of the Southern Highlands of eastern Australia in the 1980s (Osborne, 1989, 1990, 1992; Osborne and Hunter, 1998). Prior to its decline, *L. v. verreauxii* was considered ubiquitous across the region (Osborne, 1989, 1990, 1992), as demonstrated by non-targeted historical surveys in 1975–1976 that detected the species at 73 of 79 sites (M.J. Littlejohn, University of Melbourne, unpublished results).

Although a direct link between the decline of *L. v. verreauxii* and Bd in the Southern Highlands has not been demonstrated, several lines of evidence indicate that the emergence of Bd provides the most plausible explanation for the decline. First, the decline of *L. v. verreauxii* occurred in the early 1980s and coincided with the local extinction of four co-occurring species (*Litoria aurea*, *Litoria castanea*, *Litoria raniformis* and *Pseudophryne bibronii*) (Osborne, 1990, 1992; Osborne and Hunter, 1998; Hamer et al., 2010). The rapid nature of these declines is consistent with the decline of other species for which chytridiomycosis has been implicated (Berger et al., 1998; Lips et al., 2006; Vredenburg et al., 2010). Disease is a likely cause of mass mortality of adults and the spatiotemporal nature of the decline is consistent with a spreading infectious disease (Laurance et al., 1996; Skerratt et al., 2007). Second, retrospective screening of museum *P. corroboree* and *P. pengilleyi* specimens collected from the Southern Highlands failed to detect Bd prior to 1980, but found Bd was common in specimens collected after populations began to decline (Hunter et al., 2010). Third, experimental work on the closely related subspecies *L. verreauxii alpina* (recent genetic analysis does not support distinguishing this sub-species; L. Price 2012, University of Newcastle, personal communication) has demonstrated very high susceptibility to Bd under laboratory conditions (S. Cashins 2013, James Cook University, personal communication), indicating that *L. v. verreauxii* is susceptible to Bd. Lastly, the species has been killed by chytridiomycosis in the wild (Berger et al., 2004).

To study long-term changes in *L. v. verreauxii* occupancy, we used data collected from three time periods; 1975–1976 before Bd, 1990 and 1996 shortly after Bd arrival, and 2011 and 2012. We were interested in (1) confirming the decline of *L. v. verreauxii* between the first two survey periods and (2) whether *L. v. verreauxii* populations have expanded between surveys from the 1990s compared to surveys from 2011 and 2012, and if so, to what extent. We also investigated whether recent changes in occupancy are affected by breeding habitat variables and quantified the current prevalence of Bd in persistent and recently colonised populations.

2. Methods

2.1. Study area

We conducted our study in the Southern Highlands region of south-eastern Australia (Fig. 1). The region has a temperate climate with an average winter minimum of 0.6 °C and a maximum of 12.2 °C and corresponding summer averages of 12.5 °C and 27 °C (BOM, 2012). Rainfall is consistent throughout the year with an annual average of 616 mm, however, rainfall can be greatly reduced during infrequent El Nino droughts (BOM, 2012). We surveyed 145 sites located in grazing, suburban and protected landscapes. A range of wetland habitat types were surveyed including ponds ($n = 94$), lake shores ($n = 3$) and small streams ($n = 48$). All sites superficially resembled suitable breeding habitat (Anstis, 1976).

2.2. Study species

From late winter through spring, breeding aggregations of *L. v. verreauxii* use a range of habitats including ponds, creeks and swamps (Anstis, 1976). Males call from aquatic vegetation or on adjacent banks and eggs are deposited below the water surface attached to aquatic vegetation (Anstis, 1976).

2.3. Frog surveys

This study is based on frog surveys carried out in 1975–1976 (“historical surveys”) by M.J. Littlejohn (University of Melbourne), 1990 and 1996 (“baseline surveys”) by F.G. and W.O. and 2011 and 2012 (“current surveys”) by B.S. The location of historical surveys was determined by reviewing the field notes of M.J. Littlejohn who undertook extensive surveys throughout the study region in 1975–1976. Using a topographical map, we were able to identify the specific locations of 23 of the sites surveyed by Littlejohn (Fig. 1). To increase the number of sites sampled and provide a robust baseline we selected an additional 122 sites in the immediate vicinity of the historical surveys that form the baseline surveys. These sites were surveyed in 1990 ($n = 20$) and 1996 ($n = 125$). In 1990, surveys were conducted on overcast nights or following rain and in 1996, a reference site was used to check male calling activity prior to survey.

In 2011, all sites were surveyed three times during August and September. The duration of each survey was 5 min. Air temperature, relative humidity, wind speed, time to *L. v. verreauxii* detection and the presence of other amphibian species were recorded. Cognizant of concerns associated with quantifying changes in amphibian site occupancy (see Pechmann et al., 1991; Alford and Richards, 1999), we repeatedly surveyed sites in 2011 to calculate detectability and resurveyed sites once in 2012 to quantify inter-annual variation in site occupancy. Furthermore, to avoid concerns associated with inferring change from presence only records (Skelly et al., 2003) we ensured that our baseline surveys included a large number of presence and absence sites. All surveys were auditory (*L. v. verreauxii* has a clear, loud, easily distinguishable call) and were conducted during the breeding season to maximise likelihood of detection and surveys were not conducted during high winds or heavy rain.

2.4. B. dendrobatidis sampling

Sampling was conducted to investigate if re-expansion had occurred in the ongoing presence of Bd and if Bd was present, to quantify prevalence and intensity of infection. During the 2011 breeding season we used sterile swabs (Medical Wire & Equipment Co., MW 100–100) to sample 65 adult *L. v. verreauxii* at four sites (see Fig. 1). Each sample was collected in a standardised way with three strokes on each side of the abdominal midline, the inner thighs, hands and feet. Samples were analysed by a commercial lab (cesar, Melbourne, Australia) using real-time quantitative PCR following the methodology of Boyle et al. (2004) and Hyatt et al. (2007) with the exception that samples that initially returned equivocal results were re-analysed using a Qiagen master mix instead of the Taqman master mix. We considered a sample positive if all three wells returned a positive reaction. After re-analysis, one sample returned one out of three wells positive and was classified as equivocal.

2.5. Habitat measurements

In October 2011 the following information was collected at all sites: percentage cover of emergent vegetation, percentage of the