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Short Communication

Amphibian Chytrid Prevalence in an Amphibian Community in Arid Australia

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The amphibian disease chytridiomycosis, caused by the pathogen *Batrachochytrium dendrobatidis* (*Bd*), has dramatically affected amphibians, causing population declines in over 200 species worldwide (Fisher et al. 2009). The disease is widespread, driving amphibian declines in North America (Muths et al. 2003; Briggs et al. 2005), Australia (Berger et al. 1998), Central America (Lips et al. 2006) and South America (Catenazzi et al. 2011). The variation in susceptibility to disease and mortality seen among host species, populations and locations is at least partially driven by interplay between external environmental and internal host-specific factors (Woodhams et al. 2007; Searle et al. 2011; Blaustein et al. 2012). While at a single location some species may be locally extirpated, others may persist (Lips et al. 2006). Amphibian infection prevalence and mortality rates due to chytridiomycosis are correlated with ambient environmental conditions: being highest during cooler months and at higher elevations (Berger et al. 1998; Woodhams and Alford 2005; Kriger and Hero 2008).

Spatial modelling predicts that *Bd* does not occur in arid and semi-arid regions globally (<500 mm mean annual rainfall) (Ron 2005; Rödder et al. 2008; Murray et al. 2011). However, these predictions are biased towards regions with high amphibian densities and amphibian-monitoring progams (Fisher et al. 2009). Recent detections of amphibians infected with *Bd* in wetlands within arid areas of North America (although infection prevalence was low compared to mesic areas, 8.5 ± 11.7% prevalence at arid sites vs. 20.8 ± 8.4% at mesic sites; Lannoo et al. 2011) and from two arid regions of Australia (Murray et al. 2010; Voros et al. 2011), suggest that current distribution models may underestimate *Bd* persistence.

We investigated the prevalence of *Bd* in the Macquarie Marshes, a high conservation value, arid wetland system in the northern Murray-Darling Basin, southeastern Australia (Fig. 1). Annual rainfall in the study area varies considerably (449 mm [±155 SD mm], median 460 mm, minimum 126 mm, maximum 1023 mm; data from 1900 to 2012 at Quambone Station (BoM 2012); Bioclim annual rainfall value = 443 mm (Hijmans et al. 2005)). Flooding of the Marshes is highly dependent on flows from the Macquarie River, usually beginning in early spring. The Macquarie Marshes support a diverse frog fauna (15 species), and was predicted to be unsuitable for *Bd* based on climatic suitability mapping (Murray et al. 2011).

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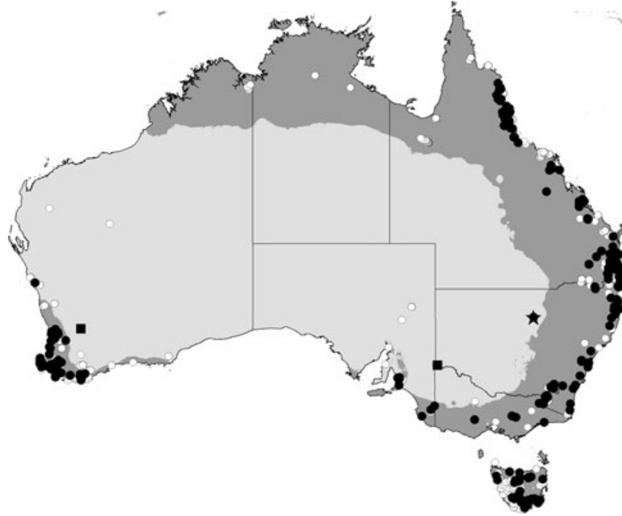

Figure 1. Distribution of *Bd* surveys in Australia showing localities where *Bd* was detected (*black symbols*) and not detected (*white symbols*) (Murray et al. 2010, Aanensen and Fisher 2012). Our sampling site, the Macquarie Marshes, is indicated by a star, and positive records in the arid zone (light grey shading, <500 mm/y based on Worldclim 'BIO12: Annual precipitation' at 1 km^2 resolution (Hijmans et al. 2005) are represented by *squares*.

We sampled for *Bd* in February 2009 and between November 2009 and March 2010, coinciding with a range of flow events into the Macquarie Marshes. These events were rare in recent years due to the prolonged drought across much of western NSW (2000–2010) and resulted in high amphibian abundance and diversity. Sample sites were categorised as permanently or temporarily flooded. Permanent sites were small farm dams or creeks with a constant low flow while temporary water sites were either areas of floodplain marsh and rain-filled depressions that had been dry for at least 6 months before surveys.

Frogs were swabbed in the field using a standardised protocol (Hyatt et al. 2007). Sterile synthetic swabs (MW112; Medical Wire & Equipment) were used to sample each specimen by swabbing (30 times) the ventral abdomen, thighs and hind feet. Swabs were stored for up to 3 months at 4°C in 1.5 ml tubes until processed. The detection and quantification of *Bd* on swabs was conducted with a TaqMan real-time PCR assay, following standard procedures (Boyle et al. 2004), with the exception of the quantity of PrepMan Ultra (Applied Biosystems) used to extract DNA. The swabs were larger than those recommended in the standard protocol and so 100 µl was required rather than 50 µl.

Each swab was analysed in triplicate. To detect inhibition within the reactions, internal positive controls were included in one replicate of each swab. Where inhibition was detected, a 1/100 dilution of the originally extracted DNA was prepared to dilute inhibitory agents and the reaction repeated. Equivocal results occurred when less than three of the replicates returned positive results, probably from a low density of *Bd* in the original sample, rather than contamination, as negative template controls were included in all assays. Quantitative estimates of *Bd* zoospore loads on positive samples were generated using standard samples of known *Bd* quantity on each plate and summarised as the genomic mean of each positive replicates (Z_{swab}). Samples were defined as "high infection" if the Z_{swab} was >1 and the sample was positive in all three replicates, and "low positive" for $1 < Z_{swab} > 0$ and the sample was positive in less than three replicates (sensu Swei et al. 2011).

We evaluated whether a *Bd*-positive test was independent of site hydroperiod (permanent or temporary water) and species water association category (species were categorised as high, medium, or low association with permanent water bodies based on broad morphological and habitat characteristics, Table 1), using Chi-square tests.

A total of 286 frogs from two families (Hylidae and Myobatrachidae), six genera and 13 species were tested for *Bd*. Fifteen samples of the 286 swabs collected remained inhibited even after dilution and were discarded. Of the remaining 271 swabs, overall infection prevalence (including low and high positives) was 6.36% (95% confidence interval, 3.40–9.85%), with only a single high positive sample (0.37%, CI 0.01–2.04%) and 16 low

Table 1. Total And Number Of Individuals (*N*) of 13 Frog Species Sampled in the Macquarie Marshes, Arid Australia, With Their Association With Water (High, Medium, Or Low) and Infection with *Batrachochytrium dendrobatidis* (*Bd*) Prevalence (High, Low Infection, Respective Percentages And 95% Confidence Limits).

Species	Water association[a]	*n*	High[b]	% High (95% CI)	Low[c]	% Low (95% CI)
Crinia parinsignifera	H	8	0	0 (0–37)	0	No change
Limnodynastes fletcheri	H	49	1	1.69 (0.02–9.08)	3	5.08 (1.06–14.15)
Limnodynastes salmini	H	16	0	0 (0–20.59)	0	No change
Limnodynastes tasmaniensis	H	26	0	0 (0–13.23)	1	3.85 (0.01–19.63)
Litoria latopalmata	H	49	0	0 (0–7.25)	6	12.24 (4.62–24.77)
Litoria caerulea	M	50	0	0 (0–7.11)	1	2.00 (0.05–10.64)
Litoria peroni	M	27	0	0 (0–12.77)	2	7.41 (0.09–24.30)
Litoria rubella	M	16	0	0 (0–20.59)	2	12.5 (1.55–38.35)
Litoria alboguttata	L	10	0	0 (0–31)	0	No change
Litoria platycephala	L	6	0	0 (0–46)	0	No change
Litoria verrucosa	L	8	0	0 (0–37)	0	No change
Neobatrachus sudelli	L	3	0	0 (0–70.80)	1	33.33 (0.84–91.57)
Notaden benneti	L	3	0	0 (0–70.80)	0	No change
Total		271	1	0.37 (0.01–2.04)	16	5.90 (3.41–9.41)

A sample was recorded as high infection if Z_{swab} was >1, or low positive if $1 < Z_{swab} > 0$ and the sample was positive in less than three replicates (sensu Swei et al. 2011)

[a]Species association defined by proximity to water (high—H, medium—M, low—L)

[b]$Z_{swab} > 1$

[c]$0 < Z_{swab} < 1$

positive samples (5.90%, CI 3.41–9.41%) (Table 1). The species with the highest infection prevalence was *Limnodynastes fletcheri*; 1.69% high positive and 5.08% low positive. Overall, infection intensity was low, with a single high positive individual recording a Z_{swab} of 9.48 zoospore equivalents (*L. fletcheri*). There were no significant differences between site hydroperiod (Table 2, Chi-square test $\chi^2 = 2.003$, df = 1, $P = 0.1$) or among species water association categories (Table 2, Chi-square test using Monte Carlo resampling, $\chi^2 = 1.64$, df = NA, $P = 0.5$) in the proportion of frogs with a positive *Bd* result.

Wetlands in arid Australia are associated with low elevations, high maximum temperatures and low annual rainfall (Rogers and Ralph 2010), factors predicted to be negatively associated with *Bd* (Fisher et al. 2009). However, we detected *Bd* in an arid floodplain wetland, approximately 300 km beyond the predicted area of *Bd* occurrence in Australia (Murray et al. 2011). When combined with the two other *Bd* records from arid wetlands in Australia (Murray et al. 2010, 120 km beyond predicted *Bd* occurence; Voros et al. 2011, 340 km from predicted *Bd* occurence), our data suggest that *Bd* is likely to be widespread in arid area of Australia, contrary to spatial modelling predictions. Although arid regions comprise approximately 47% of the Earth's surface (Thomas and Middleton 1997), only 10% of positive *Bd* records to date (248 of 2401; downloaded from Bd-maps.net 16th January 2013) are located in arid regions (Bioclim annual rainfall, 65–495 mm, Hijmans et al. 2005).

Table 2. Number of Samples and Positive *Bd* Infections (Combined High And Low Positive) for 271 Frogs from 13 Species at Permanent and Temporary Water Bodies, and by Species Water Association Categories in the Macquarie Marshes, Arid Australia.

Category	Type	Positive/total	% Positives (95% CI)
Water permanency	Permanent	12/147	8.16 (4.29–13.82)
	Temporary	5/124	4.03 (1.32–9.16)
Species water association	High	11/148	7.00 (3.80–12.90)
	Medium	5/93	5.38 (1.76–12.10)
	Low	1/30	3.33 (0.08–17.21)

Bd was detected at a number of locations in both temporary and permanent water sites, and on more than half the species surveyed (Table 1). However, infection intensities were all below the critical Z_{swab} threshold above which host mortality causes population declines in other systems (Vredenburg et al. 2010; Kinney et al. 2011). There was also no significant variation in *Bd* infection prevalence among species or with pond hydroperiod (Table 2), though this has often been the case in other studies (Kriger and Hero 2007; Searle et al. 2011). Our failure to detect such patterns may have been due to the small sample size or because our sampling was conducted after flood waters had connected some of the permanent water holes and temporary floodplain sites, potentially blurring hydroperiod categories. Similar to Voros et al. (2011), our sampling period, while coincident with high levels of frog activity, was conducted during warmer months when *Bd* infection prevalence is likely to be lower. Further sampling during colder months will improve our understanding of *Bd* in Australian arid regions.

During subsequent fieldwork (January 2010–February 2012), the seven species we found infected with *Bd* in the Macquarie Marshes remained abundant and all species historically recorded at the site remain present (Shelley 2005). However, long-term, quantitative distribution and abundance data are absent, preventing an accurate assessment of frog population trends. In other arid regions of Australia, amphibian population declines have been observed (Wassens 2008), but the drivers for these declines are poorly understood as there has been limited amphibian ecological research or *Bd* sampling. To date, there is no evidence that the declines have been associated with *Bd* (Wassens 2008). It is possible that habitat modification, associated with regulation of river flows, may act to increase the potential threat of *Bd*, by transforming wetlands from ephemeral to permanent (Wassens and Maher 2011), thereby favouring *Bd* persistence.

Our ability to predict the prevalence of *Bd* remains limited by a poor understanding of its geographic limits, particularly in arid regions. Despite the apparently hostile environment, it appears there are *Bd*-favourable conditions in arid areas that are not recognised by current spatial modelling. While we detected *Bd* at a relatively low prevalence, compared with systematic surveys in other regions, the percentage of species infected was high (Kriger and Hero 2007; Bai et al. 2010; Bell et al. 2011; Lannoo et al. 2011; Hidalgo-Vila et al. 2012; Kaiser and Pollinger 2012). With increasing data from arid regions, spatial models need to be updated so that the potential threat of the pathogen can be considered in regional amphibian conservation decisions.

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