



# Reservoir-host amplification of disease impact in an endangered amphibian

Ben C. Scheele,<sup>1,2,\*</sup> David A. Hunter,<sup>3</sup> Laura A. Brannelly,<sup>2</sup> Lee F. Skerratt,<sup>2</sup> and Don A. Driscoll<sup>4</sup>

<sup>1</sup>Fenner School of Environment and Society, Australian National University, Canberra, ACT, 2601, Australia

<sup>2</sup>One Health Research Group, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Queensland, 4811, Australia

<sup>3</sup>New South Wales Office of Environment and Heritage, Albury, NSW, 2640, Australia

<sup>4</sup>Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Burwood, 3125, Australia

**Abstract:** Emerging wildlife pathogens are an increasing threat to biodiversity. One of the most serious wildlife diseases is chytridiomycosis, caused by the fungal pathogen, *Batrachochytrium dendrobatidis* (Bd), which has been documented in over 500 amphibian species. Amphibians vary greatly in their susceptibility to Bd; some species tolerate infection, whereas others experience rapid mortality. Reservoir hosts—species that carry infection while maintaining high abundance but are rarely killed by disease—can increase extinction risk in highly susceptible, sympatric species. However, whether reservoir hosts amplify Bd in declining amphibian species has not been examined. We investigated the role of reservoir hosts in the decline of the threatened northern corroboree frog (*Pseudophryne pengilleyi*) in an amphibian community in southeastern Australia. In the laboratory, we characterized the response of a potential reservoir host, the (nondeclining) common eastern froglet (*Crinia signifera*), to Bd infection. In the field, we conducted frog abundance surveys and Bd sampling for both *P. pengilleyi* and *C. signifera*. We built multinomial logistic regression models to test whether *Crinia signifera* and environmental factors were associated with *P. pengilleyi* decline. *C. signifera* was a reservoir host for Bd. In the laboratory, many individuals maintained intense infections (>1000 zoospore equivalents) over 12 weeks without mortality, and 79% of individuals sampled in the wild also carried infections. The presence of *C. signifera* at a site was strongly associated with increased Bd prevalence in sympatric *P. pengilleyi*. Consistent with disease amplification by a reservoir host, *P. pengilleyi* declined at sites with high *C. signifera* abundance. Our results suggest that when reservoir hosts are present, population declines of susceptible species may continue long after the initial emergence of Bd, highlighting an urgent need to assess extinction risk in remnant populations of other declined amphibian species.

**Keywords:** *Batrachochytrium dendrobatidis*, chytrid fungus, frog decline, pathogen-mediated apparent competition, vector, wildlife disease

Amplificación Reservorio-Hospedero del Impacto de una Enfermedad sobre un Anfibio En Peligro

**Resumen:** Los patógenos emergentes de vida silvestre son una amenaza creciente para la biodiversidad. Una de las enfermedades más serias es la quitridiomycosis, causada por el patógeno micótico *Batrachochytrium dendrobatidis* (Bd), el cual ha sido documentado en más de 500 especies anfibias. Los anfibios varían enormemente en su susceptibilidad al Bd; algunas especies toleran la infección, mientras que otras mueren rápidamente. Los hospederos reservorios – especies que portan la infección mientras mantienen una abundancia alta pero rara vez mueren por la enfermedad – pueden incrementar el riesgo de extinción en especies simpátricas altamente susceptibles. Sin embargo, no se ha examinado si los hospederos reservorios amplifican el Bd en especies anfibias declinantes. Investigamos el papel del hospedero reservorio en la declinación de la rana amenazada *Pseudophryne pengilleyi* en una comunidad de anfibios en el sureste de Australia. En

\*Address for correspondence: Fenner School of Environment and Society, Australian National University, Canberra, ACT 2601, Australia. email [ben.scheele@anu.edu.au](mailto:ben.scheele@anu.edu.au)

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el laboratorio caracterizamos la respuesta de un hospedero reservorio potencial, la rana (sin declinación) *Crinia signifera*, a la infección de *Bd*. En el campo, realizamos censos de abundancia de ranas y muestreos de *Bd* tanto para *P. pengilleyi* como para *C. signifera*. Construimos modelos de regresión logística multinominal para probar si *C. signifera* y los factores ambientales estaban asociados con la declinación de *P. pengilleyi*. *C. signifera* fue un hospedero reservorio para *Bd*. En el laboratorio, muchos individuos mantuvieron infecciones intensas (>1000 equivalentes de zoosporas) durante más de 12 semanas sin mortalidad, y el 79 % de los individuos muestreados en el campo también portaron infecciones. La presencia de *C. signifera* en el sitio estuvo asociada con el incremento en la prevalencia de *Bd* en la simpátrica *P. pengilleyi*. En congruencia con la amplificación de una enfermedad por causa de un hospedero reservorio, *P. pengilleyi* declinó en sitios con una abundancia alta de *C. signifera*. Nuestros resultados sugieren que cuando los hospederos reservorios están presentes, las declinaciones poblacionales de una especie susceptible pueden continuar mucho tiempo después del surgimiento inicial de *Bd*, resaltando una necesidad urgente de valorar el riesgo de extinción en las poblaciones remanentes de otras especies de anfibios que han declinado.

**Palabras Clave:** competencia aparente mediada por patógenos, declinación de ranas, enfermedad de vida silvestre, hongo quitridio, vector *Batrachochytrium dendrobatidis*

## Introduction

Novel pathogens are rapidly emerging as a key threat to wildlife; for example, there have been recent high-profile die-offs in bats, bees, and corals (Daszak et al. 2000; Fisher et al. 2012). One of the most devastating wildlife pathogens is *Batrachochytrium dendrobatidis* (*Bd*), which causes the disease chytridiomycosis in amphibians (Berger et al. 1998; Skerratt et al. 2007). First identified in 1998 *Bd* has subsequently been implicated in the extinction of over 100 amphibian species and the severe decline of approximately another 100 species (e.g., Berger et al. 1998; Lips et al. 2006; Skerratt et al. 2007).

The emergence of *Bd* in naïve amphibian communities can cause rapid declines in frog abundance and species extirpations (Berger et al. 1998; Lips et al. 2006; Vredenburg et al. 2010). In populations of susceptible species that survive initial chytridiomycosis epidemics, *Bd* persists (Retallick et al. 2004; Briggs et al. 2010) and has been associated with elevated mortality (Murray et al. 2009). However, there is uncertainty over whether elevated mortality translates into population decline because high recruitment may offset adult mortality in some species (Muths et al. 2011; Phillott et al. 2013; Scheele et al. 2015b). Given the capacity of *Bd* to cause substantial mortality decades after emergence, long-term monitoring and a thorough understanding of *Bd* dynamics are needed to assess the risk of *Bd*-induced extinction in endemically infected amphibian communities.

One mechanism that may drive ongoing amphibian declines in populations with endemic *Bd* is the presence of reservoir hosts, species that carry infection but are rarely killed by disease (Gog et al. 2002; de Castro & Bolker 2005). In contrast to pathogens that primarily infect a single host, which can fade out as host density declines, generalist pathogens, such as *Bd*, can maintain high rates of transmission even as susceptible species become rare, potentially driving species to extinction

(de Castro & Bolker 2005). Amphibians vary greatly in their susceptibility to *Bd*. Some species carry infection without morbidity, whereas other species are highly susceptible, and their infection can result in rapid mortality (Fisher et al. 2009; Gahl et al. 2012). To date, both amphibian and nonamphibian reservoir hosts have been implicated in the spread and persistence of *Bd* (Retallick et al. 2004; Reeder et al. 2012; McMahon et al. 2013). However, despite considerable progress in understanding how species assemblages influence *Bd* dynamics (e.g., Gervasi et al. 2013), it remains unclear whether the presence of reservoir hosts increases the risk of extinction in sympatric species in the wild.

We investigated the role of reservoir hosts in driving ongoing amphibian declines in an endemically infected amphibian community. We studied an amphibian community in the highlands of southeastern Australia, where *Bd* first emerged in the 1980s (Hunter et al. 2010) and resulted in major declines and extirpations across several species (Osborne et al. 1999; Hunter et al. 2010). Since then, populations of some species have stabilized (Scheele et al. 2015b), and populations of other species are beginning to expand (Scheele et al. 2014a). However, some species have continued to decline (*Pseudophryne pengilleyi*, *P. corroboree*, *Philoria frosti*) (Hunter et al. 2010; Skerratt et al. 2016). These species initially declined rapidly when *Bd* emerged and are now restricted to remnant populations (Hunter et al. 2010).

We focused on processes contributing to the ongoing decline of the northern corroboree frog (*Pseudophryne pengilleyi*), which is categorized as endangered by the International Union for Conservation of Nature. From 2012 to 2015, we discovered sites with previously undocumented *P. pengilleyi* populations. Preliminary observations indicated higher *P. pengilleyi* abundance at these sites relative to monitoring sites that have been surveyed annually since 1998. These sites did not contain the nondeclining common eastern froglet (*Crinia*

*signifera*) (Gillespie et al. 1995; Osborne et al. 1999; Green & Osborne 2012), which is abundant at monitoring sites. Based on these observations, we hypothesized that *C. signifera* acts as a reservoir host for Bd; that is, it increases pathogen pressure where it is sympatric with *P. pengilleyi*. Using field surveys, disease sampling, and statistical modeling, we examined whether *C. signifera* is a reservoir host for Bd in a laboratory study and through field sampling; investigated whether the presence of this reservoir host is associated with increased Bd prevalence in sympatric *P. pengilleyi* populations; and determined whether reservoir-host abundance is associated with spatial variation in the severity of *P. pengilleyi* decline.

By identifying a reservoir host and documenting its association with disease impact in an amphibian species of high conservation concern, we sought to provide important new insights into mechanisms through which Bd may perpetuate amphibian declines decades after its initial emergence. This information can help guide management of species threatened by chytridiomycosis and survey efforts to locate remnant populations of species that have declined due to Bd.

## Methods

### Study System and Species

Our study was conducted in Kosciuszko, Brindabella, and Namadgi National Parks and Micalong State Forest in southeastern Australia (Fig. 1). The region is undulating to mountainous, ranging from 750 to 1850 m asl. The climate is temperate with an average annual rainfall of 1,200 mm and snow at higher elevations during winter. The majority of amphibian breeding sites are in isolated frost-hollow grasslands, narrow seeps, and open bogs and are associated with communities of moist vegetation. In summer, male *P. pengilleyi* construct burrows in vegetation on the edge of small ephemeral ponds in which eggs are laid. Ponds fill in autumn and tadpoles metamorphose in the spring.

Amphibian declines were first observed in the study region in 1984, and since then *P. pengilleyi* has undergone major declines (Osborne et al. 1999; Hunter et al. 2010). In contrast, no changes in distribution or decline have been reported for *C. signifera* (Pengilley 1971, 1992; Gillespie et al. 1995; Osborne et al. 1999; Green & Osborne 2012). Although a direct link between early amphibian declines and Bd in the study region has not been demonstrated, the emergence of Bd provides the most plausible explanation (Hunter 2007; Hunter et al. 2010). Sampling museum *P. corroboree* and *P. pengilleyi* specimens, Hunter et al. (2010) found that Bd is absent in all specimens collected prior to 1980 but is common in specimens collected from declining populations during the 1990s, consistent with the hypothesis that Bd is an introduced pathogen in the region. The

timing of declines are consistent with the spatiotemporal spread of chytridiomycosis throughout eastern Australia (Skerratt et al. 2007). Under laboratory conditions, *P. corroboree* (closely related sibling species to *P. pengilleyi*) is highly susceptible to Bd (97.5% of exposed adults succumb to chytridiomycosis [Brannelly et al. 2015a]), and chytridiomycosis-induced mortality has been observed in *P. pengilleyi* (Berger et al. 2004). Although available evidence strongly indicates *P. pengilleyi* is highly susceptible to Bd, controlled exposure experiments to further investigate susceptibility, although not appropriate at this stage due to low abundance, could be undertaken in the future. Drought is also likely to have contributed to the decline of some *P. pengilleyi* populations (Scheele et al. 2012).

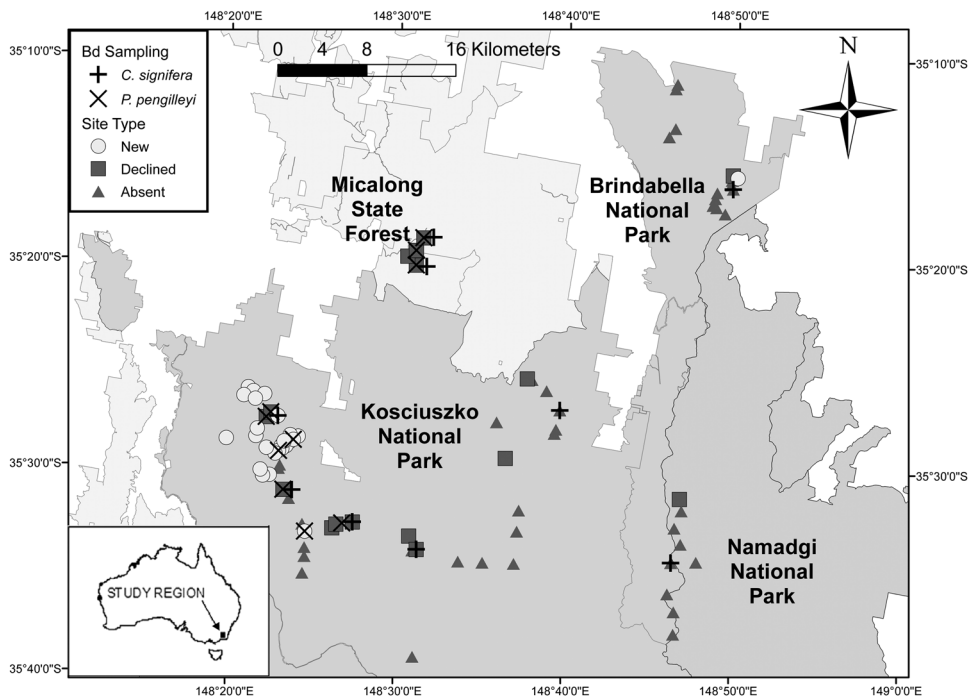
### Amphibian and Bd Field Surveys

Amphibian surveys were undertaken at 75 sites from 2012 to 2013 and 6 sites in 2015. *P. pengilleyi* surveys were conducted during the breeding season in February and March with the shout-response technique (Supporting Information). *C. signifera* surveys were conducted during the breeding season in September and October (Supporting Information).

We used sterile swabs (MW 100-100, Medical Wire & Equipment Co., U.K.) to sample Bd from adult frogs during their breeding seasons, when it is logistically feasible to obtain reasonable sample sizes. We sampled 20–30 adult *C. signifera* at 9 randomly selected sites (total 204) where the species is sympatric with *P. pengilleyi* from September to October 2012. We sampled 216 adult *P. pengilleyi* at 16 sites in February 2012. We aimed to sample 30 *P. pengilleyi* at each site. When <30 individuals were present, we sampled all detected individuals. Although we sampled the 2 species in different seasons, previous research has shown high year-round Bd prevalence in *C. signifera* (Howard et al. 2012; Brannelly et al. 2015b). Each sample was collected in a standardized way; 3 strokes on each side of the abdominal midline, the inner thighs, hands, and feet. A new pair of disposable powder-free nitrile gloves was used for each sample. Samples were analyzed in triplicate with real-time quantitative polymerase chain reaction (PCR) following the method of Hyatt et al. (2007). We considered a sample positive if all 3 wells returned a positive reaction. Field research was completed under New South Wales scientific licenses SL100436 and SL100816 and ethics permits A041025/02 and A2011/19.

### Site Classification

To investigate spatial patterns of *P. pengilleyi* decline, we used data from 81 sites across the species' entire range (Fig. 1). Each site was classified into 1 of 3 categories: absent, declined, or new. All sites were defined as a



**Figure 1.** Location of study sites and *Batrachochytrium dendrobatidis* (Bd) sampling in southeastern Australia (new, sites where new populations of *Pseudophryne pengilleyi* were discovered; declined, monitoring sites where *P. pengilleyi* declined; absent, monitoring sites where *P. pengilleyi* is now absent). Sites where fewer than 11 *P. pengilleyi* were sampled for Bd are not shown.

discrete patch of wetland habitat separated from other sites by >400 m. Sites in the categories absent ( $n = 40$ ) and declined ( $n = 17$ ) had been monitored annually since 1998. Monitoring was undertaken to investigate the trajectories of *P. pengilleyi* populations. All monitoring sites supported *P. pengilleyi* breeding aggregations when established in 1998. *P. pengilleyi* is highly detectable (Supporting Information), and all surveys were undertaken by experienced amphibian ecologists. At absent sites, we detected no *P. pengilleyi* during surveys in 2012 and 2013. *P. pengilleyi* may have been extirpated from these sites; no individuals were recorded in 2014 (B.C.S.). Sites classified as declined had a >80 % decline in *P. pengilleyi* abundance since 2000 (2012 abundance compared to 2000). At new sites ( $n = 25$ ), new populations of *P. pengilleyi* were found from 2012 to 2015 in newly surveyed areas. Although the population trajectories of new sites were unknown (due to their recent identification), *P. pengilleyi* densities were generally much higher than at monitoring sites, raising the possibility that these sites had not experienced major declines in abundance. Most new sites were spatially clustered in areas adjacent to several monitoring sites, where the first new sites were discovered (Fig. 1). Two new sites were also found in spatially disjunct locations, which reduced the extent of spatial confounding. New sites were located in the same subcatchments as monitoring sites where Bd was detected in *C. signifera* and *P. pengilleyi* (Fig. 1), indicating suitable environmental conditions and adequate time for Bd emergence at new sites. New sites are likely to be longstanding rather than recently colonized given their locations, often within several kilometers of sites known to have existed for several decades, and a lack of obvious

dispersal barriers. In contrast to absent and declined sites, which were located in small open areas within the forest, new sites were located within the forest itself.

### Environmental Variables

To investigate whether environmental conditions were associated with *P. pengilleyi* decline and Bd prevalence, we collected information on elevation and canopy cover at each site. Elevation was used as a proxy for climatic conditions. Cooler and wetter environments are linked with increased Bd prevalence and severe chytridiomycosis outbreaks in other species (Fisher et al. 2009). Canopy cover influences temperature profiles, and high cover is linked to increased Bd prevalence in temperate environments (Scheele et al. 2015a). Canopy cover was estimated visually, and had either very low or high values. We therefore split the data at 50% cover to create a binary response. To investigate whether patterns of decline were related to site drying rather than disease, we measured the number of *Eucalypt* tree seedlings <5 cm diameter at breast height, which has previously been used as a proxy for site drying in our study region (Scheele et al. 2012). This number was converted to presence-absence data because generally there were either no seedlings or there were many.

### Laboratory *Crinia Signifera* Study

Fifty adult *C. signifera* were collected from three sites (Thredbo Diggins [36°26'55" S, 148°26'06" E], Sponars Lake [36°21'36" S, 148°29'13" E], and Blue Lake [36°23'58" S, 148°18'32" E]) in Kosciuszko National Park



in November 2015. Animals were sampled for Bd on collection and transported to James Cook University, Townsville, Queensland, where they were housed individually for 12 weeks (Supporting Information). Individuals were sampled for Bd in weeks 0 (at collection), 1, and 12. Samples were analyzed as described above, except samples were run in once as described in Brannelly et al. (2015b). Laboratory research was completed under New South Wales scientific licence SL101584 and ethics permit A2171.

### Statistical Analyses

We used multinomial logistic regression to test whether environmental factors (elevation, canopy cover, and site drying) and abundance of *C. signifera* were associated with site type (absent, declined, or new) across the 81 sites. Prior to analyses we ensured there was no collinearity between the explanatory variables. Abundance of *C. signifera* was transformed to the natural log ( $n + 1$ ) because these data were right skewed. We constructed a set of candidate models arising from all combinations of the explanatory variables. We then used an information-theoretic model-selection process to rank models based on their Akaike's information criterion value with a correction for small sample size ( $AIC_c$ ) in the R package AICcmodavg (Mazerolle 2013). We used Wald statistics to determine the relative importance of explanatory variables within a model (Zuur et al. 2007). Multinomial model fit was assessed using likelihood ratio chi-square tests. We considered models with  $\Delta AIC_c$  values  $< 2$  among the best ranked models (Burnham & Anderson 2002).

We used linear models to test whether Bd prevalence in *P. pengilleyi* sampled across 10 sites was influenced by *C. signifera* presence or absence and environmental variables. *P. pengilleyi* was sampled at 16 sites, but only sites with 11 or more samples were used in the analyses (at the remaining 6 sites only 1 or 2 samples were collected due to low frog abundance). We converted *C. signifera* abundance data to binary data because the values exhibited a bimodal distribution. We used the same model selection process described above with *C. signifera* presence or absence, site drying, canopy cover, and elevation as explanatory variables. We did not test for a relationship between *P. pengilleyi* infection intensity and *C. signifera* because only 2 infected individuals were detected at sites where *C. signifera* was absent. We used the same modeling approach and explanatory variables (without *C. signifera* presence or absence or canopy because all sites had  $< 50\%$  canopy cover) to investigate factors influencing Bd prevalence in *C. signifera* sampled across 9 sites. Model fit was assessed using adjusted  $R^2$  values. All analyses were completed in R version 3.1.1 (R Development Core Team 2014).

## Results

### Amphibian Surveys

From 2012 to 2015, *P. pengilleyi* was detected at 42 of the 81 sites. All 57 monitoring sites were classified as either absent (no *P. pengilleyi* in 2012 and 2013) or declined ( $> 80\%$  decline in *P. pengilleyi* abundance between 2000 and 2012), reflecting an ongoing decline of *P. pengilleyi* since monitoring commenced in 1998. *C. signifera* was detected at 55 sites.

### Infection Prevalence

At the commencement of the laboratory study, infection prevalence in *C. signifera* was 96% (CI = 82–98%) and remained  $> 96\%$  throughout the 12-week study (Supporting Information). No individual returned consecutive negative Bd samples, and the small number of negative PCR results from weeks 0 and 1 may have represented false negatives (see Supporting Information for further discussion). Mean infection intensity remained above 1000 zoospore equivalents for the duration of the study; some individuals had over 34,000 zoospore equivalents in week 12 (Supporting Information). Of the 50 animals, four were euthanized due to loss of righting reflex. Histological examination of the epidermis and low infection intensities (0, 1.5, 144, and 1200 zoospore equivalents) indicated loss of righting reflex was not associated with chytridiomycosis.

Infection prevalence in the wild for 204 *C. signifera* adults sampled at 9 sites was 79.4% (CI = 73–84.6%). For infected frogs, the mean infection intensity was 8839 (SE 1664.16) zoospore equivalents, and the median was 1094. The best supported model demonstrated a negative association between Bd prevalence and elevation (coefficient =  $-0.049$  [SE 0.01],  $t = -2.54$ ,  $df = 7$ ,  $p = 0.04$ ,  $R^2 = 0.41$ ). No competing models had  $AIC_c$  values within 2  $AIC_c$  (Supporting Information).

Infection prevalence in 216 *P. pengilleyi* adults sampled at 16 sites was 27.3% (95% CI = 21.59–33.85). For infected frogs, the mean infection intensity was 1697 (SE 385.52) zoospore equivalents, and the median was 257. At sites where *C. signifera* was not recorded, mean Bd prevalence in *P. pengilleyi* was 2.6% (range 0–10%). At sites where *C. signifera* was present, mean Bd prevalence was 41.4% (range 27–58%). The best supported model contained 2 explanatory variables: *C. signifera* presence or absence and elevation (Supporting Information). There was a strong positive relationship between Bd prevalence and *C. signifera* presence (coefficient = 43.28 [SE 6.42],  $t = 6.73$ ,  $df = 7$ ,  $p = 0.0002$ ,  $R^2 = 0.82$ ) and a weak negative relationship with elevation (coefficient =  $-0.05$  [SE 0.02],  $t = -2.54$ ,  $df = 7$ ,  $p = 0.04$ ). The second ranked model had a  $\Delta AIC_c$  value of 0.5 (Supporting Information) and contained *C. signifera*

**Table 1.** Model coefficients (SE) of variables from the best supported model of site classification.<sup>a</sup>

Site type	Intercept	Wald	<i>Crinia signifera</i> abundance	Wald	Canopy cover	Wald
Declined <sup>b</sup>	-1.64 (0.82)	-2.00	3.60 (1.07)	3.33	-2.66 (1.37)	-1.94
Absent <sup>c</sup>	-1.36 (0.76)	-1.77	3.78 (1.06)	3.56	-1.22 (1.04)	-1.04

<sup>a</sup>Sites where new populations of *Pseudophryne pengilleyi* were discovered were used as the baseline in the analysis, so coefficients represent differences relative to new sites.

<sup>b</sup>Monitoring sites where *P. pengilleyi* declined.

<sup>c</sup>Monitoring sites where *P. pengilleyi* is now absent.

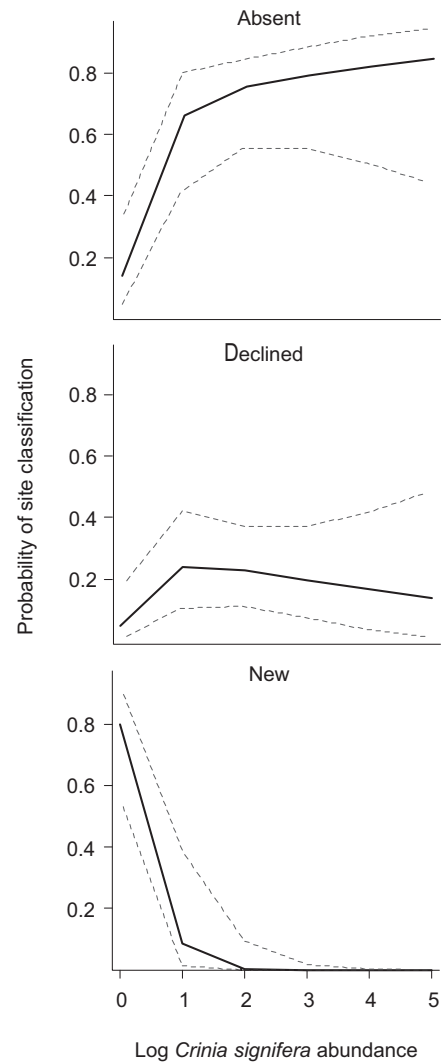
presence or absence only (coefficient = 36.41 [SE 7.56],  $t = 4.81$ ,  $df = 8$ ,  $p = 0.001$ ,  $R^2 = 0.71$ ).

### Spatial Pattern of *Pseudophryne Pengilleyi* Decline

In the multinomial analysis the best ranked model contained 2 explanatory variables: *C. signifera* abundance and canopy cover ( $\chi^2 = 72.81$ ,  $p < 0.0001$ ). The probability of a site being classified as either absent or declined had a strong positive association with *C. signifera* abundance (Table 1 and Fig. 2). In particular, as *C. signifera* abundance increased the probability of a site being classified as absent versus declined increased, which is consistent with the hypothesis that high densities of *C. signifera* drive severe disease impact. New sites with relatively high *P. pengilleyi* abundance were associated with few or no *C. signifera* (Table 1 and Fig. 2). Three additional models had  $\Delta AIC_c$  values  $< 2$  (Supporting Information). The second ranked model had a  $\Delta AIC_c$  of 0.04 and contained only *C. signifera* abundance. The third and fourth ranked models included *C. signifera* abundance plus site drying, and canopy cover and elevation, respectively. Strong support for the model containing solely *C. signifera* abundance combined with the low  $AIC_c$  rankings of models containing only site drying or elevation indicated these variables were less important than *C. signifera* abundance.

### Discussion

Mechanisms that may drive ongoing amphibian declines decades after the initial emergence of Bd are poorly understood. We found that *C. signifera* acts as a reservoir host for Bd; Bd prevalence was high and infection burdens heavy. The relationship between Bd prevalence in *P. pengilleyi* and *C. signifera* presence was strong and positive. Based on annual monitoring throughout *P. pengilleyi*'s range, the species has experienced severe declines or is now likely extirpated in areas where *C. signifera* is abundant. Combined, our results suggest that when reservoir hosts are present, endemic Bd may drive highly susceptible, sympatric amphibian species toward extinction decades after the pathogen's initial emergence.



**Figure 2.** Probability of a site being classified as either absent (not containing *Pseudophryne pengilleyi*), declined (*P. pengilleyi* declined), or new (sites where new populations of *P. pengilleyi* were found) based on *Crinia signifera* abundance (solid lines, model predictions; dashed lines, 95% CI).

Reservoir hosts can amplify disease pressure by increasing disease transmission, which can then lead to disease-driven extinctions in sympatric species (Gog et al. 2002;

de Castro & Bolker 2005). McCallum (2005) postulates that reservoir hosts could facilitate amphibian declines when Bd is less pathogenic in reservoir species than in sympatric declining species; Bd prevalence is lower in declining species than in reservoir species; and reservoir species persist at sites where other species have been extirpated. Our findings are consistent with these postulates: under laboratory conditions *C. signifera* maintained infection but did not die, whereas *P. pengilleyi* infected with Bd died (Berger et al. 2004; Brannelly et al. 2015a); in the wild, Bd prevalence and infection intensity was higher in *C. signifera* (79% and 8839 zse) than in *P. pengilleyi* (27% and 1697 zoospore equivalents) (despite comparable body sizes); and *C. signifera* persisted at sites where *P. pengilleyi* was absent or had experienced major declines. *C. signifera* appeared to be both a superabundant and superinfected host, and due to its widespread distribution throughout eastern Australia, it is likely a key determinant of chytridiomycosis dynamics in several amphibian communities. Our results indicate that remnant populations of the critically endangered *P. corroboree* and *Ph. frosti* are at high risk of extinction when sympatric with *C. signifera*. More broadly, given the capacity for nondeclining species to persist with endemic Bd in areas where susceptible amphibians have declined (e.g., *Pseudacris regilla* in California [Reeder et al. 2012]), we anticipate disease amplification by reservoir hosts could drive ongoing declines in other regions.

The higher prevalence of Bd in *P. pengilleyi*, where it is sympatric with *C. signifera*, and the spatial pattern of *P. pengilleyi* decline, is consistent with the idea that pathogen-mediated apparent competition underpins the continued decline of *P. pengilleyi*. Pathogen-mediated apparent competition, whereby the presence of one species decreases the fitness of another through a shared enemy, has been documented in both animals and plants (Holt 1977; Bonsall & Hassell 1997) but has not been investigated in the amphibian Bd system. Consistent with apparent competition, sites where *P. pengilleyi* appeared to have been locally extirpated were associated with higher *C. signifera* abundance, whereas sites where the species had declined but still persisted had lower *C. signifera* abundance (Fig. 2). Furthermore, no declines were recorded in 2013 at the new site where Bd was detected in 2012, indicating that in the absence of *C. signifera* Bd impacts appeared to be low. To further investigate the role of apparent competition in *P. pengilleyi*'s decline, manipulative experiments could be undertaken to quantify rates of Bd transmission and mortality for the 2 species in seminatural mesocosm enclosures.

Direct frog-to-frog contact provides a plausible mechanism for Bd transmission (Rowley & Alford 2007). While sampling *P. pengilleyi*, we frequently observed *C. signifera* within or close to *P. pengilleyi* burrows. We also observed several instances of attempted amplexus between individuals of the 2 species. These observations are

supported by research conducted prior to population declines at several sites used in this study that documented a high level of interaction between adult *P. pengilleyi* and *C. signifera*, including prolonged physical contact between the 2 species in *P. pengilleyi* burrows (Pengilley 1971, 1973, 1992). Pitfall trapping has demonstrated that both species occupy shared breeding habitat for at least 2 months annually (Pengilley 1973, 1992). Furthermore, *P. pengilleyi* and *C. signifera* tadpoles inhabit shared ponds, which may facilitate waterborne Bd transmission (Berger et al. 1998), and metamorphs from both species occupy moist vegetation around ponds for several months prior to dispersal from breeding habitat. The transmission of Bd during the nonbreeding season may be lower because individuals are generally widely dispersed in terrestrial habitat (Pengilley 1992).

Although a reservoir-host explanation is consistent with the spatial pattern of *P. pengilleyi* decline and Bd infection, we cannot rule out the role of competitive release of *C. signifera*. However, no changes in *C. signifera* abundance or distribution have been reported across southeastern Australia following Bd emergence, despite the dramatic decline of several other species (Pengilley 1971, 1992; Gillespie et al. 1995; Green & Osborne 2012). Research conducted prior to Bd arrival at sites used in our study showed that both species were at high densities in shared habitat and had approximately equal relative abundances (Pengilley 1992). Furthermore, given substantial differences in breeding phenology (Pengilley 1992; Lemckert & Shine 1993), life-history traits (Bull & Williamson 1996; Hunter 2007), and resource use (Pengilley 1971), it seems unlikely the 2 species are major competitors. Combined, the available evidence suggests competitive release provides a less likely explanation for the spatial pattern of *P. pengilleyi* decline.

Environmental conditions can influence Bd prevalence and infection intensity (Puschendorf et al. 2011; Stockwell et al. 2015). However, we found no evidence that unfavorable environmental conditions explain low Bd prevalence at new sites or the spatial pattern of *P. pengilleyi* decline, reinforcing the likely role of *C. signifera* as a disease amplifier. In fact, new sites, where disease burden appeared low, were associated with higher canopy cover, which favors Bd growth and persistence in other systems (e.g. Puschendorf et al. 2011; Scheele et al. 2015a). Rather than directly influencing Bd, we suggest that shading at new sites may limit *C. signifera* occupancy because this species appears to favour open, sunny areas in subalpine environments. Finally, differential tolerance to drought could explain persistence of *C. signifera* and not *P. pengilleyi* in some sites (Scheele et al. 2012). However, a drought explanation is not consistent with the strong relationship between *P. pengilleyi* infection rates and *C. signifera* presence.

We found that Bd prevalence in *C. signifera* and *P. pengilleyi* was negatively associated with elevation.

Growth and survival of Bd is temperature dependent; the pathogen favors cool, wet conditions commonly associated with higher elevation habitats (Fisher et al. 2009). We suggest the lower Bd prevalence we documented at high-elevation sites may be an artifact of these sites being sampled approximately 1 month earlier relative to the commencement of breeding compared with low-elevation sites. Infection prevalence is likely to be highest toward the end of the breeding season because individuals congregate for breeding under conditions that promote pathogen transmission (e.g., Scheele et al. 2015b).

Our results have important implications for the management of species threatened by Bd. Areas where reservoir hosts are absent or at low abundance may provide important refugia where risk of contracting Bd infection is low. As such, identifying reservoir hosts and mapping their distribution could help locate remnant populations of declining amphibians. Similarly, our results indicate that when developing reintroduction programs, potential reservoir hosts need to be identified and their likely impact on the success of the reintroduction assessed. In cases where reservoir hosts are likely to have a major negative impact, introductions should be into new areas where reservoir hosts are absent (Scheele et al. 2014b).

We found evidence that the presence of a reservoir host, *C. signifera*, is associated with increased disease prevalence in a declining species, *P. pengilleyi*. This finding is congruent with the pattern of decline that has been observed for this species across its distribution – it has declined severely or is likely extirpated from areas where *C. signifera* is abundant. We suggest reservoir hosts may play a previously underappreciated role in causing amphibian declines in other regions where Bd is now endemic. This highlights an urgent need to assess extinction risk in remnant amphibian populations where reservoir hosts are present.

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## Supporting Information

Detailed information on frog surveys (Appendix S1) and on the laboratory *C. signifera* study (Appendix S2) and

AICc model-ranking tables (Appendix S3) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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