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Title:

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Date:

2017-06-01

Citation:

Scheele, B. C., Hunter, D. A., Brannelly, L. A., Skerratt, L. F. & Driscoll, D. A. (2017).
Reservoir-host amplification of disease impact in an endangered amphibian.
Conservation Biology, 31 (3), pp.592-600. <https://doi.org/10.1111/cobi.12830>.

Persistent Link:

<https://hdl.handle.net/11343/292186>

Reservoir-host amplification of disease impact in an endangered amphibian

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

Running head: Reservoir-host amplification of chytridiomycosis

Abstract

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/cobi.12830](https://doi.org/10.1111/cobi.12830).

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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, with some species tolerating infection, while 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 combine a laboratory study with field surveys, disease sampling, and statistical modeling to investigate the role of reservoir hosts in chytridiomycosis dynamics and species decline in an amphibian community in south-eastern Australia. We show that the non-declining common eastern froglet (*Crinia signifera*) is a reservoir host for Bd, with laboratory animals carrying intense infection burdens over 12 weeks and the majority of wild sampled individuals carrying intense infections. We find that the presence of *C. signifera* is strongly associated with Bd prevalence in the sympatric, IUCN red-listed northern corroboree frog (*Pseudophryne pengilleyi*). Consistent with disease amplification by a reservoir host, we find that *P. pengilleyi* has declined from areas with high *C. signifera* abundance. Our results suggest that when reservoir hosts are present, population declines can continue long after the initial emergence of Bd, highlighting an urgent need to assess extinction risk in remnant populations of other declined amphibian species. Reintroductions and in situ management strategies must focus on identifying reservoir hosts and minimizing exposure of threatened species.

Introduction

Novel pathogens are rapidly emerging as a key threat to wildlife, with 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* (hereafter Bd), which causes the disease chytridiomycosis in amphibians (Berger et al. 1998; Skerratt et al. 2007). *Batrachochytrium dendrobatidis* was first identified in 1998 and has subsequently been implicated in the extinction of over 100 amphibian species and the severe decline of approximately another 100 species (Berger et al. 1998; Lips et al. 2006; Skerratt et al. 2007; Fisher et al. 2009).

The emergence of Bd in naïve amphibian communities can cause rapid species declines and population extinctions (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, as high recruitment may off-set 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 post-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, with some species carrying infection without morbidity, whereas other species are highly susceptible and infection can result in rapid mortality (Fisher et al. 2009; Gahl et al. 2012). To date, both amphibian and non-amphibian 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.

Here, we investigate the role of reservoir hosts in driving ongoing amphibian declines in an endemically infected amphibian community. We study an amphibian community in the highlands of south-eastern Australia, where Bd first emerged in the 1980s (Hunter et al. 2010), resulting in major declines and local extinctions across several species (Osborne et al. 1999; Hunter et al. 2010). Since then, populations of some species have stabilised (Scheele et al. 2015b), while populations of other species are beginning to re-expand (Scheele et al. 2014a). However, worryingly, some species are in a continued state of decline (*Pseudophryne pengilleyi*, *P. corroboree*, *Philoria frosti*) (Hunter et al. 2010; Skerratt et al. 2016). These species experienced an initial period of rapid decline associated with Bd emergence, and are now restricted to remnant populations (Hunter et al. 2010).

In this paper, we focus on processes contributing to the ongoing decline of the endangered, IUCN red-listed northern corroboree frog (*Pseudophryne pengilleyi*). In 2012-2015 we

identified previously undocumented *P. pengilleyi* populations. Preliminary observations indicated higher *P. pengilleyi* abundance at these sites compared to monitoring sites that have been surveyed annually since 1998, and an apparent absence of the non-declining common eastern froglet (*Crinia signifera*) (Gillespie et al. 1995; Osborne et al. 1999; Green & Osborne 2012), which is abundant at monitoring sites. Based on this observation, we hypothesized that *C. signifera* may act as a reservoir host for Bd, increasing pathogen pressure where it is sympatric with *P. pengilleyi*. Specifically, we (1) examine whether *C. signifera* is a reservoir host for Bd using a laboratory study and field sampling; (2) investigate whether the presence of this reservoir host is associated with increased Bd prevalence in sympatric *P. pengilleyi* populations; and (3) test whether reservoir host abundance is associated with spatial variation in the severity of *P. pengilleyi* decline. We investigate these hypotheses using a combination of field surveys, disease sampling, and statistical modeling.

Through identifying a reservoir host and documenting its association with disease impact in an amphibian species of high conservation concern, our study provides important new insights into mechanisms through which Bd may perpetuate amphibian declines decades after its initial emergence. Our work also reveals that reservoir host distribution may drive patterns of within species variation in disease impact. This information is crucial for managing species threatened by chytridiomycosis and informing surveys to locate remnant populations of Bd-declined species.

Methods

Study system and species

Our study was conducted in Kosciuszko, Brindabella and Namadgi National Parks and Micalong State Forest in south-eastern Australia (Fig. 1). The region is undulating to

mountainous, ranging from 750 to 1850 m above sea level. 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 found in isolated frost hollow grasslands, narrow seepages and open bogs and are associated with moist vegetation communities. 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 declines 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 was absent in all specimens collected prior to 1980 but was 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 through-out eastern Australia (Skerratt et al. 2007). Under laboratory conditions, *P. corroboree* (closely related sibling species to *P. pengilleyi*) is highly susceptible to Bd, with 97.5% of exposed adults succumbing to chytridiomycosis (Brannelly et al. 2015a), and chytridiomycosis-induced mortality has been demonstrated in *P. pengilleyi* (Berger et al. 2004). Although available evidence strongly indicates that *P. pengilleyi* is highly susceptible to Bd, controlled exposure experiments to further investigate susceptibility, while not appropriate at this stage due to low abundance, could be undertaken in the future. In contrast, mortality from Bd infection has

not been observed in *C. signifera*, despite persistent and intense infections (see Results).

Drought is also likely to have contributed to the decline of some *P. pengilleyi* populations (Scheele et al. 2012).

Amphibian surveys

Amphibian surveys were undertaken at 75 sites in 2012-2013 and 6 sites in 2015.

Pseudophryne pengilleyi surveys were conducted during the breeding season in February and March using the shout-response technique (Supporting Information). *Crinia signifera* surveys were conducted during the breeding season in September and October (Supporting Information).

Field Batrachochytrium dendrobatidis sampling

We used sterile swabs (Medical Wire & Equipment Co., United Kingdom, MW 100–100) to sample Bd from adult frogs during their breeding seasons, when it is logistically feasible to obtain reasonable sample sizes. We sampled between 20 and 30 adult *C. signifera* at 9 randomly selected sites (total = 204) where the species is sympatric with *P. pengilleyi* in September–October 2012. We sampled 216 adult *P. pengilleyi* at 16 sites in February 2012. We aimed to sample 30 *P. pengilleyi* at each site; however, when <30 individuals were present, we sampled all detected individuals. Although some species exhibit seasonal patterns of Bd prevalence (e.g. Phillott et al. 2013), previous sampling of *C. signifera* in montane and sub-alpine habitats in spring and summer has documented comparable Bd prevalence to what we report (79.4% versus 82.9% and 100%) (Howard et al. 2012; Brannelly et al. 2015b), suggesting that *C. signifera* maintains high Bd loads for at least a

substantial portion of the year. Each sample was collected in a standardised way with 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 analysed in triplicates using real-time quantitative Polymerase Chain Reaction (PCR) following the methodology of Hyatt et al. (2007). We considered a sample positive if all 3 wells returned a positive reaction.

Site classification

To investigate spatial patterns of *P. pengilleyi* decline we used data from 81 sites located 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 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. *Pseudophryne pengilleyi* has high detectability (Supporting Information) and all surveys were undertaken by experienced amphibian ecologists, providing robust results. At *absent* sites we detected no *P. pengilleyi* during surveys in 2012 and 2013. *Pseudophryne pengilleyi* is potentially locally extirpated at these sites, with no individuals recorded in 2014 (author, unpublished data). Sites classified as *declined* have experienced a >80 % decline in *P. pengilleyi* abundance since 2000 (2012 abundance compared to 2000). The third site type was *new* ($n = 25$), and consisted of sites identified during 2012-2015 while surveying new areas for the species. Although the population trajectories of new sites are unknown (due to their recent identification), *P. pengilleyi* densities are generally much higher than at monitoring sites, raising the possibility

that these sites have not experienced major declines in abundance. Most new sites are spatially clustered in areas adjacent to several monitoring sites where the first new sites were discovered, reflecting intensive survey effort in this area (Fig. 1). Notably, two new sites were also found in spatially disjunct locations, reducing the extent of spatial confounding. We also note that new sites are located in the same sub-catchments 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 kilometres 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 if 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, with cooler wetter environments linked with increased Bd prevalence and severe chytridiomycosis outbreaks in other species (Fisher et al. 2009). Similarly, canopy cover influences temperature profiles and high cover has been linked to increased Bd prevalence in temperate environments (Scheele et al. 2015a). Canopy cover was measured by visual estimate, and had either very low or high values. We therefore split the data at 50% cover to create a binary response. To investigate if patterns of decline were related to site drying rather than disease, we measured the number of *Eucalypt* tree seedlings under 5cm diameter at breast height, which has previously been used as a proxy for

site drying in our study region (Scheele et al. 2012). This 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 analysed as described above, except samples were run in singlicate as described in Brannelly et al. (2015b).

Statistical analysis

We used multinomial logistic regression to test whether environmental factors (elevation, canopy cover and site drying) and/or the abundance of *C. signifera* was associated with site type (*absent*, *declined* or *new*) across the 81 sites. Prior to analysis we ensured that there was no collinearity between the explanatory variables. Abundance of *C. signifera* was natural log (n+1) transformed 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 (AICc) using 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 ΔAIC_c values <2 of the best ranked model (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-absence and/or environmental variables.

Pseudophryne pengilleyi was sampled at 16 sites, but only sites with 11 or more samples were used in the analysis (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-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-absence or canopy, as 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

In 2012-2015, *P. pengilleyi* was detected at 42 of 81 sites surveyed. Our surveys documented an ongoing decline of *P. pengilleyi*, with all of the 57 monitoring sites 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. *Crinia signifera* was detected at 55 sites.

Laboratory Crinia signifera study

Infection prevalence in 50 wild caught *C. signifera* was 96% (CI = 82-98%). Infection prevalence remained >96% for the 12 week duration of the study (Supporting Information). No individual returned consecutive negative Bd samples and the small number of negative PCR results from weeks 0 and 1 may represent false negatives (see Supporting Information for further discussion). Mean infection intensity remained above 1000 zoospore equivalents for the duration of the study, with some individuals exceeding 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.

Batrachochytrium dendrobatidis in wild Crinia signifera

Infection prevalence in 204 *C. signifera* adults sampled at 9 sites was 79.4% (CI = 73-84.6%). For infected frogs, the mean infection intensity was 8839 (S.E. = 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, S.E. = 0.01, *T*-value = -2.54, *d.f.* = 7, *P* = 0.04, R^2 = 0.41). No competing models had AICc values within 2 AICc (Supporting Information).

Batrachochytrium dendrobatidis in wild *Pseudophryne pengilleyi*

Infection prevalence in 216 *P. pengilleyi* adults sampled at 16 sites was 27.3% (CI = 21.59-33.85%). For infected frogs, the mean infection intensity was 1697 (S.E. = 385.52) zoospore equivalents and the median was 257. At sites where *C. signifera* was not recorded, Bd prevalence in *P. pengilleyi* was 2.6%, compared with 41.4% at sites where *C. signifera* was present (Fig. 2). The best supported model contained 2 explanatory variables; *C. signifera* presence-absence and elevation (Supporting Information). There was a strong positive relationship between Bd prevalence and *C. signifera* presence (Coefficient = 43.28, S.E. = 6.42, T -value = 6.73, $d.f.$ = 7, P = 0.0002, R^2 = 0.82) and a weak negative relationship with elevation (Coefficient = -0.05, S.E. = 0.02, T -value = -2.54, $d.f.$ = 7, P = 0.04). The second ranked model had a $\Delta AICc$ value of 0.5 (Supporting Information) and contained *C. signifera* presence-absence only (Coefficient = 36.41, S.E. = 7.56, T -value = 4.81, $d.f.$ = 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 (χ^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, Fig. 3). In particular, as *C. signifera* abundance increased the probability of a site being classified as absent versus declined increased, 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, Fig. 3). Three additional models had $\Delta AICc$ values < 2 (Supporting Information). The second ranked model had a $\Delta AICc$ value 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 AICc rankings of models containing solely site drying or elevation, indicated these variables are less important.

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, with high prevalence and heavy infection burdens. We demonstrate a strong positive relationship between Bd prevalence in *P. pengilleyi* and *C. signifera* presence. 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 towards extinction, decades after the pathogen's initial emergence.

Reservoir hosts can amplify disease pressure through increased disease transmission, leading to disease-driven extinctions in sympatric species (Gog et al. 2002; de Castro & Bolker 2005). McCallum (2005) postulated that reservoir hosts could facilitate amphibian declines when; (1) Bd is less pathogenic in reservoir species than in sympatric declining species; (2) Bd prevalence is lower in declining species than in reservoir species; and (3) reservoir species persist at sites where other species have been extirpated. Our findings meet these criteria; (1) under laboratory conditions *C. signifera* maintain infection but do not experience morbidity or mortality, while exposed *P. pengilleyi* experience mortality (Berger et al. 2004; Brannnelly

et al. 2015a), (2) in the wild, Bd prevalence and infection intensity is higher in *C. signifera* (79% and 8839 zse) compared to *P. pengilleyi* (27% and 1697 zse) (despite comparable body sizes); and (3) *C. signifera* persists at sites where *P. pengilleyi* is now absent or has experienced major declines. *Crinia signifera* appears to be both a super-abundant and super-infected host and due to its widespread distribution throughout eastern Australia, it is likely a key determinant of chytridiomycosis dynamics in several amphibian communities. In particular, 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 non-declining species to persist with endemic Bd in areas where susceptible amphibians have declined (e.g. *Pseudacris regilla* in California, USA, Reeder et al. 2012), we anticipate that 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, we found that sites where *P. pengilleyi* appears to have been locally extirpated were associated with higher *C. signifera* abundance; while sites where the species has declined, but still persists have lower *C. signifera* abundance (Fig. 3). 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 appear 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 semi-natural 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 potentially facilitating 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. *Batrachochytrium dendrobatidis* transmission during the non-breeding season may be lower as individuals are generally widely dispersed in terrestrial habitat (Pengilley 1992).

While 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, we note that no changes in *C. signifera* abundance or distribution have been reported across south-eastern Australia following Bd emergence, despite the dramatic decline of several other species (Pengilley 1971, 1992; Gillespie et al. 1995; Green & Osborne 2012). In addition, research conducted prior to Bd arrival at sites used in our study reported that both

species were at high densities in shared habitat, with 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 two 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 unfavourable 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 appears low, were associated with higher canopy cover, which favours 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, as this species appears to favour open, sunny areas in sub-alpine 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. *Batrachochytrium dendrobatidis* growth and survival is temperature dependent, with the pathogen favouring cooler, wetter conditions, commonly associated with higher elevation habitats (Fisher et al. 2009). However, we suggest that the lower Bd prevalence we

documented at high elevation sites may be an artefact of these sites being sampled approximately one month earlier relative to the commencement of breeding compared with low elevation sites. Infection prevalence is likely to be highest towards 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).

In conclusion, we provide 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 experienced severe decline, or is likely extirpated from areas where *C. signifera* is abundant. We suggest that 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.

Acknowledgments

Funding was provided by the Australian Academy of Science and a Taronga Zoo Field Conservation Grant. B. Scheele was supported by Australian Research Council grant LP110200240 during the revisions of this manuscript. In kind field support and monitoring data was provided by the NSW Office of Environment and Heritage and NSW National Parks. Field assistance was provided by C. Scheele, R. Pietsch and C. Portway. W. Blanchard provided valuable statistical advice. C. Foster provided valuable comments on a draft. Field research was completed under NSW National Parks and Wildlife (NPW) Scientific Licences SL100436 and SL100816 and ethics permits A041025/02 and A2011/19. Laboratory research was completed under NSW NPW Scientific Licence SL101584 and ethics permits A2171.

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Table 1. Model coefficients (and standard errors) of variables from the best supported model; new sites were used as the baseline in the analysis so coefficients represent differences relative to new sites.

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

Figure 1. Location of study sites and *Batrachochytrium dendrobatidis* (Bd) sampling in south-eastern Australia. Site where fewer than 11 *Pseudophryne pengilleyi* were sampled for Bd are not shown.

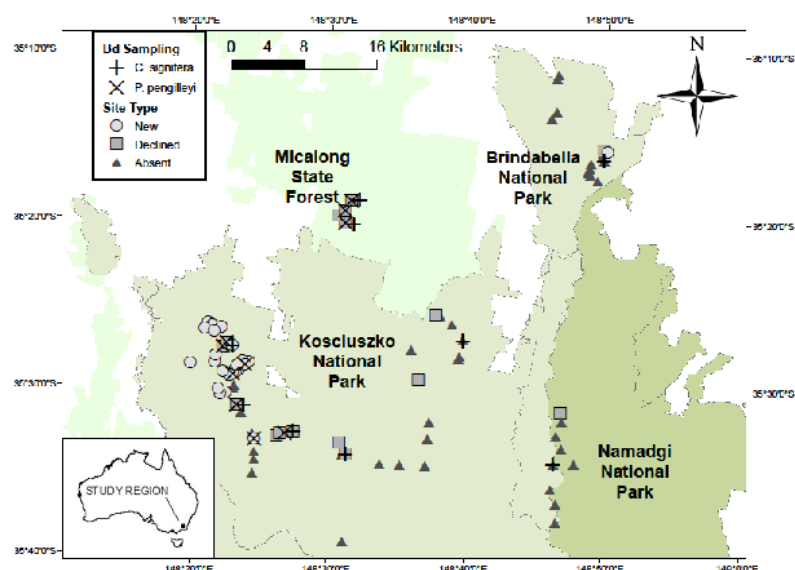


Figure 2. *Batrachochytrium dendrobatidis* prevalence in 10 *Pseudophryne pengilleyi* sites where *Crinia signifera* was either absent or present, showing the median, 25th and 75th percentiles and minimum and maximum values.

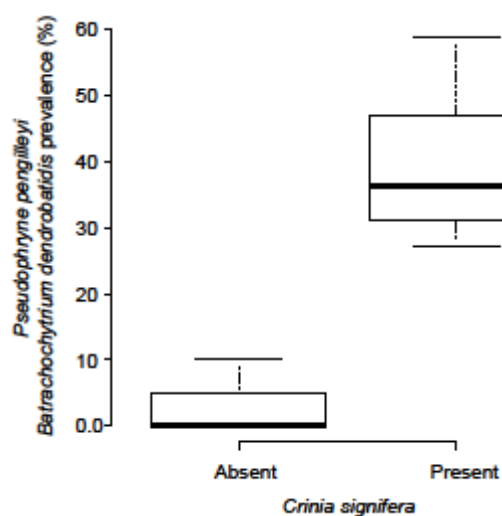


Figure 3. The predicted probability of a site being classified as either absent, declined or new based on *Crinia signifera* abundance with 95% CI.

