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Running head. Disease reservoirs for chytrid fungus

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Abstract.

Amphibian chytridiomycosis, caused by infection with *Batrachochytrium dendrobatidis* (*Bd*), is the most devastating vertebrate disease on record. Reservoir hosts are likely to be important in the *Bd*-amphibian system because many amphibian species can carry infections without experiencing mortality. However, while a variety of reservoirs have been proposed, few have been empirically demonstrated to act as competent reservoir hosts. In this study we investigate whether the common eastern froglet, *Crinia signifera,* a non-declining species that is widespread in eastern Australia, is a reservoir host for *Bd* infection. We conducted a long-term, large-scale field survey to investigate disease dynamics in *C. signifera* at sites where four sympatric, threatened anuran species have severely declined. We also monitored *Bd* infected *C. signifera* in the laboratory to determine susceptibility and survivorship. Finally, we assessed population age structure to investigate disease impact in the wild. We found that *C. signifera* is a competent reservoir host, maintaining high prevalence and infection intensities in the wild and in the lab, with no signs of sub-lethal effects or clinical disease. In the wild, the modal age is 4 years with individuals living up to 6 years, indicating that adults can survive across multiple years despite high infection prevalence and intensity. The occurrence of *C. signifera* at sites with remnant populations of threatened species likely contributes to ongoing disease impact in declining species decades after the arrival of *Bd*. The presence of *C. signifera* at sites where threatened species have become extinct inhibits effective reintroductions, and we recommend avoiding sites with high reservoir host abundance when planning reintroductions.

Key Words: Australia; Chytridiomycosis; Emerging Infectious Disease; Reservoir Host; Wildlife Disease; Amphibians; *Batrachochytrium dendrobatidis* **Introduction**

Pathogenic fungal diseases have become a major threat to biodiversity over the last three decades, with population declines caused by fungal diseases being reported in bats, corals, bees, snakes and amphibians (Fisher *et al.* 2012; Lorch *et al.* 2016). One particular fungal pathogen, *Batrachochytrium dendrobatidis, Bd,* infects the skin of amphibians and can cause chytridiomycosis, a potentially lethal disease that has resulted in species declines and extinctions around the globe (Berger *et al.* 1998; Skerratt *et al.* 2007). *Bd* infects over 600 species of amphibian and has been implicated as the primary cause of decline in over 200 species (Skerratt *et al.* 2007). *Bd* is found on all continents except Antarctica, which has no amphibians, and the

most dramatic declines of amphibians have occurred in Central and South America, Australia and the west coast of North America (Olson *et al.* 2013). The pathogen was introduced into Australia in the 1970s, and major amphibian declines have occurred since that initial introduction, with six species in Australia currently on the brink of extinction (Skerratt *et al.* 2016; Scheele *et al.* 2017b).

The effects of reservoir hosts on disease risk within a system are complex and context specific. Reservoir hosts are species that can harbour a pathogen by transmitting it among themselves and others, thus maintaining the pathogen within the ecosystem in the absence or with low density of other susceptible hosts (Casadevall & Pirofski 2000; Haydon *et al.* 2002). Competent reservoir hosts can carry and shed high infection loads over time without incurring negative fitness costs of disease; and therefore, they are not considered susceptible to infection (Stockwell *et al.* 2016). Disease carriers are infected individuals that transmit the pathogen, but often do not show signs of infection and are important in transporting pathogens between locations. In contrast, a resistant species is refractory to infection or quickly clears infection, and will have a limited effect on maintaining disease within a system (Keeling & Gilligan 2000).

Bd is known to infect a large number of amphibian species (Skerratt *et al.* 2007) as well as non-amphibian hosts (McMahon *et al.* 2013); however, not all these species are declining or experience mortality from *Bd*. Therefore, when non-declining species are sympatric with susceptible species, they may act as reservoir hosts, leading to increased exposure levels for other hosts and may be important in maintaining the pathogen within a system. Identifying a competent reservoir host requires demonstration of consistently high infection loads in the wild and little impact on host fitness.

Non-declining amphibian species that can become infected with *Bd* are often suggested as reservoir hosts. Some examples of species that are commercially important, or used in medical research and thus heavily traded and have been found to be *Bd* positive are cane/marine toad (*Rhinella marina*), African clawed frog (*Xenopus laevis*) and North American crayfish species (Rollins-Smith *et al.* 2009; McMahon *et al.* 2013). These species are able to quickly clear infection (Rollins-Smith & Conlon 2005; Poorten & Rosenblum 2016) and are found with low infection intensity and prevalence in the wild (Brannelly *et al.* 2015b). The American bullfrog, *Lithobates catesbeiana* is commonly proposed as a reservoir host for *Bd* (Daszak *et al.* 2004; Schloegel *et al.* 2010, 2012) and is a likely candidate for facilitating the global spread of *Bd*

(Schloegel *et al.* 2009, 2010). In the laboratory this species does not appear to successfully maintain an infection (Gervasi *et al.* 2013b); however, within their native range infection prevalence and intensity are high (Garner *et al.* 2006; Schloegel *et al.* 2010). While *L. catesbeiana* may not be a competent reservoir species because it does not retain infection under laboratory conditions, it is likely important in driving disease dynamics in many parts of its native and invasive range. One of the most robust examples of a reservoir host is the Pacific chorus frog, *Pseudacris regilla,* which has high *Bd* prevalence in the wild (Fellers *et al.* 2011), and can maintain a high and stable infection in the lab without signs of disease (Blaustein *et al.* 1994; Reeder, Pessier & Vredenburg 2012; Gervasi *et al.* 2013a). Maintaining a high and stable infection over time is important for a competent reservoir host because it can be the driver of infection within a community of co-occurring susceptible species.

Amphibians in the Australian Alps have been severely impacted by *Bd* (Osborne, Hunter & Hollis 1999; Hunter *et al.* 2010; Scheele *et al.* 2014, 2017b), including the critically endangered southern corroboree frog, *Pseudophryne corroboree*, northern corroboree frog, *P. pengilleyi,* Baw Baw frog, *Philoria frosti,* and the vulnerable alpine tree frog, *Litoria verreauxii alpina* (Skerratt *et al.* 2016; Scheele *et al.* 2017b). The common eastern froglet *Crinia signifera*, is a widespread species found along the east coast of Australia and Tasmania, and is highly abundant throughout the Australian Alps (Hunter *et al.* 2009; Anstis 2013). *Crinia signifera* co-occurs throughout the region with the described declining species and reproduces in the same perennial and ephemeral breeding ponds, and occupies shared terrestrial habitats, and is in close physical proximity to declining species (e.g. nesting burrows of both corroboree frog species) (Green & Osborne 2012). Furthermore, *C. signifera* have not declined despite *Bd* infection (Gillespie, Osborne & McElhinney 1995; Green & Osborne 2012), and populations remain highly abundant and stable in all suitable habitats (Mahony 1996). Tadpoles of this species appear to carry low or no infection (0 of 46 tadpoles positive in November of 2009; N. Clemann and K. Howard unpublished data; Howard et al. 2010). In contrast, adults have been proposed as a reservoir host for *Bd* due to high infection rates (Clemann *et al.* 2009; Hunter *et al.* 2009; Hunter 2012; Brannelly *et al.* 2015a; Scheele *et al.* 2017a). However, survival of infected *C. signifera* or competence as a reservoir host has not been investigated in captivity or the wild.

In this study we investigate the role of *C. signifera* as a reservoir host. We conducted *Bd* sampling in 24 high elevation *C. signifera* populations in New South Wales and Victoria between 2006 and 2013 at locations where *P. corroboree*, *P. pengilleyi*, and *L. v. alpina* have become locally extinct or have experienced severe declines. We then monitored *Bd* infection and health in wild-caught *C. signifera* for 12-weeks in the laboratory to determine the progress and impacts of infection. Finally, we assessed population age structure in wild *C. signifera* populations infected with *Bd* using skeletochronology in order to assess the complexity of population age structure. These various approaches provide strong evidence for an important role of *C. signifera* as a competent reservoir host.

Methods

Study species

The common eastern froglet *Crinia signifera*, is native to eastern Australia and Tasmania, where it occurs in a variety of habitat types. The species has a flexible life-history and can breed in a wide range of permanent and ephemeral water bodies. Males call year round, but the primary breeding period is March-October. Mortality rates of larvae can be high because the water bodies in which this species breeds often dry before the frogs emerge; however, they are frequent breeders, which compensates for failed recruitment (Anstis 2013).

Field survey

Crinia signifera were surveyed for *Bd* at 24 known *Bd*-positive sites in New South Wales and Victoria between 2006 and 2013 (Fig. 1). Surveys occurred between September and January, which incorporates the breeding season for most frog species in the Australian Alps. On a survey night, animal sampling commenced after sun set and concluded several hours later. Animals were captured with a new gloved hand, and either immediately swabbed, or held in individual clean plastic bags until swabbed and then released at the point of capture (see methods "*Testing for Bd presence"*). New gloves and standard hygiene protocols reduce the amount of cross contamination and help to ensure that animals that test positive for *Bd* using qPCR methods are infected and not contaminated with transient *Bd* (Skerratt *et al.* 2011).

The field survey results have been collated from government reports of endangered species monitoring and publications (Clemann *et al.* 2009; Hunter *et al.* 2009; Howard *et al.*

2010; Clemann & Howard 2011; Howard & Clemann 2014; Brannelly *et al.* 2015a, 2016a; Scheele *et al.* 2017a). Here we collate all our studies over this seven-year period (Table 1).

Laboratory Study

Study animals

Fifty mature adult frogs were collected from four sites in the Australian Alps in November 2015 (Fig. 1). These animals were swabbed for *Bd* presence (see methods "*Testing for Bd presence"*) and then transported to James Cook University, Townsville, Queensland. Animals were housed individually in 300 x 195 x 205 mm terraria with gravel and moss substrate, at a room temperature of 14-18°C. They were fed *ad libitum* two times weekly with juvenile crickets (*Acheta domestica*, 8 mm), dusted with amphibian vitamins and gut-loaded. Frogs were misted once daily for 60 seconds with reverse osmosis water. Temperature and humidity were monitored daily. Animals were monitored for 12 weeks after collection for signs of disease and infection.

Disease monitoring

Animals were checked daily for general health. Items monitored each day were mobility, skin sloughing patterns, food consumption and righting reflex. If a frog was unable to right itself within 30 seconds, it was euthanized with an overdose of tricaine methanesulfonate (MS-222). Once per week frogs were swabbed for *Bd* presence (see methods below see methods "*Testing for Bd presence"*), weighed to the nearest 0.01 g, and snout to venter length (SVL) measured to the nearest 0.1 mm.

Histological examination

The ventral skin of euthanized animals was dissected and processed for histological diagnosis of *Bd*. Skin was fixed in 4% phosphate buffered formaldehyde prior to embedding in paraffin wax. Routine histological techniques were used to prepare the tissues for light microscopy. Tissues were dehydrated in a graded series of ethanol, cleared with xylene, and embedded in paraffin. Tissues were serially sectioned at 5 μm, and affixed to hydrophilic glass slides. The slides were stained with hematoxylin followed by eosin counterstaining (H&E), and mounted with coverslips.

At the end of the experiment all remaining animals were euthanised and skeletochronology was performed to determine age.

Skeletochronology

All animals that survived through week 12 were analysed using skeletochronology (n = 46). The longest toe on the left hind limb was used for skeletochronological analysis. The whole digit was fixed in 10% neutral buffered formalin, then decalcified in 10% formic acid for 14 h, followed by rinsing in running water for 3 h. Samples were then vertically embedded in paraffin wax and sectioned using a rotary microtome to cut 10 μm sections. The entire third phalange was sectioned to ensure that the mid diaphysis region, which contains the best sections for aging, was identified. Transverse sections were affixed to glass slides and stained for 30 min using Harris's haematoxylin (Sigma-Aldrich) and mounted with a 60 mm cover-slip using D.P.X. mountant. Lines of arrested growth were counted under 400X magnification using a light microscope. Each individual was aged twice without reference to the previous result. When an inconsistent result was obtained, sections were re-examined and if a reliable count could not be obtained, individuals were excluded.

The accuracy of skeletochronology is dependent on the presence of clearly discernible lines of arrested growth, and is based on the assumption that these lines are consistently deposited annually (Smirina 1994). Skeletochronology is a reliable method for aging amphibians in regions that experience strong, consistent seasonal variations in climate, such as sub-alpine environments (Smirina 1994), and has been validated in the study landscape using repeat sampling of individually identified *P. corroboree (*Hunter 2000). Age structure is predicted to be less complex if animals are dying due to disease and has been empirically demonstrated in the amphibian-chytridiomycosis system (Scheele *et al.* 2016).

Testing for Bd presence

We tested for *Bd* infection by using qPCR on skin swabs (Boyle *et al.* 2004). The standard protocol involves running a sterile rayon-tipped swab (MW-113, Medical Wire & Equipment) over the skin. The swabbed regions of the body were the middle of the venter, side of the venter, each thigh, and each foot. The swab was rotated during and between strokes to ensure the greatest amount of DNA was gathered on the swab. Genomic DNA was extracted

from the swabs using the Prepman Ultra kit and 2 minutes of bead beating to break apart the fungal cell walls. The extract was analysed using quantitative PCR following Boyle *et al*. (2004), in singlicate (Kriger, Hero & Ashton 2006; Brannelly *et al.* 2015a) for the laboratory study and 2013 field samples, and triplicate for all other field samples (and the results of the samples run in triplicate were averaged). A positive and negative control, and a series of dilution standards were included in each analysis. Individual frogs were considered positive if all wells returned a positive result. The maximum cycle threshold (CT) value considered positive was 39.5.

Using qPCR for diagnosing infection via skin swab is the standard approach in chytridiomycosis studies. It has been well validated in surveys of wild frogs using three diagnostic methods, and specificity and sensitivity has been estimated (Skerratt *et al.* 2011). While there is small uncertainty regarding the interpretation of results from one lightly infected individual, on a population level the high specificity (94.2%) shows there is high certainty that the PCR is accurate in detecting real infection.

Statistical analysis

For analysis of the field survey data, factors influencing infection intensity were analysed using only *Bd*-positive frogs in a general linear model (GLM). Zoospore equivalents were log base 10 transformed, and was the dependent variable. Date of capture and site were fixed factors and assessed in a full factorial design. In order to determine if infection varied by month or year a second GLM was performed where infection status was the dependent variable and month and year were fixed factors. To determine which factors influenced infection prevalence, a binary logistic regression model was used, where infection status was the dependent variable, and date of capture, altitude and site were covariates. In order to determine if infection varied by month or year a second binary regression was performed where infection status was the dependant variable and month and year were covariates. The test statistic B is represented as e^B, which is equivalent to the odds ratio for a binary logistic regression.

For analysis of the laboratory data, factors influencing infection intensity were assessed using a linear mixed model. Infection intensity was the dependent variable and was log base 10 transformed. Week was a fixed factor, and individual was the subject (random effect). When the model suggested that week influenced infection intensity, an ANOVA test was carried out where infection intensity was the dependent variable and week was the independent variable, and a post

hoc Bonferroni's test was conducted in order to determine how infection intensity varied between each week. In order to determine which factors influenced body condition, a linear mixed model was used. Body condition was the dependent variable and was estimated as the $\log_{10}(\text{mass})/\log_{10}(\text{SVL})$ and week was a fixed factor, and individual was the subject (random effect) . All analyses were carried out in SPSS (v21).

Ethical Approval

Animal ethics was approved by James Cook University in application A2171. Scientific Licence was issued by the Office of Environment and Heritage New South Wales, Licence number SL101584. Work in Victoria was conducted with approval under a research permit (number 10007449) provided by the Victorian Department of Environment, Land, Water and Planning.

Results

Field surveys

From October 2006 to November 2013 at 24 sites across New South Wales and Victoria, 526 individual *C. signifera* were tested for *Bd* (see Table 1). Over all sites and time points 78.67% were *Bd* positive and the infection intensity of positive individuals was 2.66 +/- 1.10 $\log_{10}$(ZE). Date influenced infection intensity (GLM: $F_{df=23} = 1.811$, $p = 0.011$; Fig 2a). The effect of date on infection intensity was both month and year of survey (GLM: year, $F_{df=6} = 22.244$, $p < 0.01$; month, $F_{df=3} = 4.787$, $p < 0.01$; Fig 2a). Infection intensity was not influenced by site or the interaction of date and site (GLM: site, $F_{df=4} = 2.11$, $p = 0.079$; date*site, $F_{df=1} = 2.721$, $p = 0.098$).

Prevalence varied across both dates and sites (binary logistic regression: site, $e^B = 0.96$, $p < 0.01$; date, $e^B = 1.00$, $p = 0.019$), but not altitude (binary regression: altitude, $e^B = 1.0$, $p = 0.492$). However, the effect of date was the year of survey, while the month had no affect on prevalence of infection (binary logistic regression: year, $e^B = 1.225$, $p < 0.01$; month, $e^B = 0.90$, $p = 0.47$) (Fig. 2b).

Crinia signifera has both higher infection intensity and prevalence than the sympatric species *P. corroboree, P. pengilleyi* and *L. v. alpina* (see visual comparison in Fig. 3).

Laboratory studies.

Infection intensity increased over time in the laboratory (Mixed models: $F_{df=8, 284.1} = 8.501$, $p < 0.01$) (Fig. 4). Upon capture in the wild, 92.2% of *C. signifera* tested positive for *Bd* infection, and average infection intensity was 2.16 +/- 1.10 $\log_{10}$(ZE). Over the course of the 12 week captive monitoring, infection intensity increased by 48.6% (3.14 +/- 0.75 $\log_{10}$(ZE)) and infection prevalence increased to 100% (d = 0.773). When infection intensities were compared week by week, there was a clear increase after week 3 (Fig. 4a).

Body condition, SVL and mass did not change per week over the course of the experiment (Mixed Models: Mass, $F_{1,331.349} = 0.347$, $p = 0.556$; SVL, $F_{1, 48.171} = 0.123$, $p = 0.727$; Body condition, $F_{1,48.171} = 0.123$, $p = 0.727$) (Fig. 4b). Four animals were euthanized over the course of the study due to lack of a righting response. Upon histological examination of the ventral skin no epidermal damage was observed, and there was low to no *Bd* infection seen. These animals had final infection intensities of 2.53, 2.10, 0.45, 0 $\log_{10}$(ZE), respectively; lower than the average infection intensity over all weeks (2.61 $\log_{10}$(ZE)). The qPCR results coupled with the histological findings suggest that mortality was not due chytridiomycosis.

Population age structure of *Crinia signifera*

The average age of the frogs was 3.53 years +/- 1.01 years. All animals sampled were adults and the youngest was two years old. 37.7% were four years old, and one was six years old (Fig. 5). We excluded one animal from the analysis because consistent lines of arrested growth (annual growth rings in bone) could not be accurately counted (n = 45).

Discussion

In this study we provide four lines of evidence that *C. signifera* is a competent disease reservoir host: 1) high infection prevalence and 2) high infection intensity in the wild, 3) maintenance of infection over time, and 4) no or little mortality associated with pathogen infection. *Crinia signifera* demonstrated high infection intensity and prevalence across all sites surveyed, particularly when compared to other susceptible and declining species across the Australian Alps. In the laboratory, infection was maintained at a high intensity for 12 weeks and was not the suspected cause of death in any individual. Finally, skeletochronology revealed that the age structure of *C. signifera* is complex and that there is a wide range of ages in a disease

endemic region. This empirical evidence suggests that *C. signifera* is a competent and effective reservoir host for *Bd* and maintains high *Bd* prevalence at sites where threatened sympatric species have declined. Our study on *Crinia signifera* is a holistic approach to determining the competency of a reservoir host after multiple reports and studies had suggested their role as reservoir hosts (Clemann *et al.* 2009; Hunter *et al.* 2009; Hunter 2012; Brannelly *et al.* 2015a; Scheele *et al.* 2017a).

Crinia signifera are abundant and widespread across the Australian Alps, and currently inhabit sites where threatened species such as *P. corroboree*, *P. pengilleyi,* and *L. v. alpina* have declined or been extirpated due to chytridiomycosis. The efficacy of *C. signifera* as a reservoir host, coupled with their near ubiquitous presence within the landscape and high infection intensity and prevalence, suggests that they play an important role in exacerbating disease prevalence within co-occurring amphibian species across the Australian Alps. Threatened species are likely to fare better in a landscape where reservoir hosts are absent, as has been demonstrated in the *P. pengilleyi* system (Scheele *et al.* 2017a). Modifying habitat in order to reduce reservoir host abundance has been suggested as an area of research (Skerratt *et al.* 2016), because removing a reservoir host from their preferred habitats is logistically difficult and not currently viable. However, reintroduction of endangered species to sites where *C. signifera* are naturally absent may be feasible and will likely lead to lower exposure to *Bd* and lower mortality. At high elevations, *C. signifera* prefers open habitats with standing water and little shade during the breeding season; therefore, choosing reintroduction sites that are wooded, and have seep water rather than standing water, may improve success for endangered species like *P. pengilleyi* (Scheele *et al.* 2017a).

Many species have been proposed as reservoir hosts for the amphibian chytrid fungus, particularly non-declining but infected species (Daszak, Cunningham & Hyatt 2003; Schloegel *et al.* 2009). However, robust empirical evidence to support these claims is often limited. Evidence to suggest that these putative reservoir hosts are able to maintain infection through time with few consequences is essential to being a competent reservoir host. In our laboratory trial we monitored infection found high infection intensity and increasing infection prevalence over 12 weeks in captivity. Upon capture in the wild, infection was 93%, which rose to 100% over their time in captivity. The animals that tested negative upon capture but became positive during the

study either had a low level infection in the field and the qPCR returned a false negative, or they were exposed to *Bd* during transport (frogs were transported 3-5 per container).

In addition to increasing prevalence, infection intensity also increased during captivity: infection loads increased 48.9%. The reasons for increasing infection intensity in captivity are unclear, however, the temperature environment might be the most parsimonious explanation. The temperature was maintained at a consistent temperature (14-18°C) within the optimal range of the fungal pathogen (Stevenson *et al.* 2013). Infection intensity is an important measure because not only does it indicate the growth rate of the pathogen in the host but also the shed rate of zoospores, and therefore the potential for transmission. Zoospores can be transmitted through both direct contact with a new host and by being expelled into the water (Briggs *et al.* 2005). Skin swabs used for sampling can be used as a proxy of direct contact because the swab is rubbed on the surface of the skin and gathering only available zoospores, not infection deeper in the epidermal tissue (Skerratt *et al.* 2011). Further, high intensity detected by PCR on swabs is highly correlated with rate of zoospore release in other amphibian species, as determined by measuring the number of zoospores released from frogs into water (Reeder *et al.* 2012; Direnzo *et al.* 2014).

Throughout the laboratory experiment, no *Bd* related mortality or decreased body condition was observed. *Bd* begins to cause mortality in highly susceptible species (such as *Atelopus zeteki*, *P. corroboree*, *L. v. alpina*) at 20 – 30 days, and 60-90 days in less susceptible species (such as *Litoria caerulea*, *Rhinella marina*) (Voyles *et al.* 2009; Ellison *et al.* 2014; Brannelly *et al.* 2016b). No evidence of disease or decreasing body condition after 84 days at a temperature optimal for *Bd* in a cool adapted region (Stevenson *et al.* 2013), suggests that *C. signifera* is tolerant of heavy *Bd* infection.

In the wild, infection intensity and prevalence varied across date: both month and year. *Bd* growth is temperature dependent, therefore seasonal fluctuations are expected and have been observed in other study systems (Woodhams & Alford 2005; Kriger & Hero 2007; Brannelly, Chatfield & Richards-Zawacki 2012; Phillott *et al.* 2013). Year to year fluctuations are also common with this disease, particularly because amphibian populations are variable even when not declining (Alford & Richards 1999), which affects disease dynamics because *Bd* is density dependent (Briggs, Knapp & Vredenburg 2010; Brannelly *et al.* 2015a). Site also impacted *Bd* prevalence in this system, and *Bd* infection dynamics are affected by microhabitat conditions and

site characteristics (Rohr & Raffel 2010; Puschendorf *et al.* 2011; Becker *et al.* 2012), as well as between different amphibian densities and community composition (Rachowicz & Briggs 2007; Brannelly *et al.* 2015a). However, despite these fluctuations, the infection load and prevalence of *Bd* were consistently high in this species, and were similar or higher than in declining sympatric species (Fig. 3).

We found complex age structure, with some individuals as old as six years of age, despite a high proportion of animals being infected in the wild. This suggests that mortality due to *Bd* is low in *C. signifera* in the wild; consistent with our finding that infected individuals can survive infection under laboratory conditions. In contrast, age structure is highly truncated in infected *L. v. alpina* populations, with near complete turnover of the adult population every year, where prior to *Bd* the animals survived up to seven years old (Scheele *et al.* 2015, 2016). The age structure of *C. signifera* from this study, with the majority of animals 3 – 4 years old and the oldest being six years old, mirrors a Tasmanian population that was sampled between 1997 and 2002 (Lauck 2005), with unknown *Bd* status. The average animal in that study was between three and four years old, but the oldest individuals were seven. The consistency in age structure between these two studies suggests that populations are stable with low or no mortality due to *Bd* infection.

The higher number of four-year-olds than three-year-olds found in this study might reflect higher recruitment four years prior to sampling compared to three years prior, consistent with year-to-year fluctuations in recruitment (Alford & Richards 1999). We found no animals under the age of two years because we only targeted mature adults for sampling. While it is possible that tadpole and juvenile life stages may have different susceptibility to infection than adults, low tadpole infection in the wild suggests mortality from *Bd* immediately post metamorphosis is low in *C. signifera*, as we have observed in the endangered frogs in this region (Scheele *et al.* 2015).

Conclusions

Our multiple lines of empirical evidence suggest that *C. signifera* is an effective and competent reservoir host species for *Bd*. Understanding disease dynamics is important in *C. signifera* because the species is abundant over a large geographical range, and sympatric with four declining species in the Australian Alps. When employing conservation actions and

reintroduction efforts, it is important to consider the ecology of the whole amphibian community so that the risk of disease reservoirs can be mitigated. Disease dynamics within a multi-host system can be complicated, as both disease resistance and general ecology vary among species and habitats. Understanding the nuances of context specific infection dynamics can identify targeted management for threatened species.

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624

Table 1

Sites sampled between 2006 and 2013

Site Number	Site Name	Elevation (m)	Years Sampled	Number of individuals	Percent of individuals infected	Average infection load in ZE
0	Cooelmans	1093	2012	20	80.00%	1,530
1	Brumby Flat	1799	2012	30	56.67%	537
2	Devil Peak	1140	2012	23	95.65%	4,539
3	Peppercorn	1306	2012	20	85.00%	4,230
4	Rings Creek	1241	2012	20	85.00%	7,868
5	Micalong pond	1045	2012	50	100.00%	21,721
6	Big Plain	1375	2012	21	61.90%	364
7	Murphys Swamp	1239	2012	20	50.00%	198
8	Oglivies Ephemeral	1335	2013	21	100.00%	4,617
9	Ogilvies Dam	1404	2013	12	100.00%	5,241
10	Sponars*	1519	2013	26	100.00%	2,664
11	Pipers	1606	2013	41	100.00%	3,250
12	Smiggins	1696	2006	20	95.00%	1,393
13	Kiandra	1417	2006	38	86.84%	423

14	Grey Mare	1593	2006, 2008, 2012	13	92.31%	1,189
15	Ginini Flat	1643	2006	22	68.18%	1,595
16	Bogong	1832	2006, 2007	7	71.43%	10,912
17	Dinner Plain Village	1294	2009, 2010	10	100.00%	140
18	Pond at Dinner Plain	1494	2010	8	100.00%	62
19	Baw Baw Plateau	1567	2006	35	34.29%	17
20	Lake Mountain	1439	2009, 2010	39	51.28%	2,591
21	Airport Road	1130	2010	5	100.00%	613
22	Charlies Creek	1436	2008	5	80.00%	252
23	Lankey Plain	1554	2008, 2009	6	0.00%	
24	Davies Plain	1565	2008	14	57.14%	249
25	Thredbo Diggins*	1154				
26	Blue Lake*	1942				
				Total *C. signifera*	526	78.67%

* sites where *C. signifera* were collected for the laboratory infection experiment in November 2015: 8 animals from site #10, 8 animals from site #25 and 34 animals from site #26.

Figure Legends.

Figure 1

Map of the sampling sites in south-eastern Australia, which includes the states of Victoria and New South Wales as well as the Australian Capital Territory. State outlines are in light blue, and land is outlined in yellow. Site information can be found in Table 1.

Figure 2

Infection dynamics of *Crinia signifera* over the survey period from 10/2006-11/2013. Panel (a) is the infection intensity through time, where the y-axis represents the log base 10 transformed zoospore equivalents (ZE). Error bars indicate standard error. To estimate average monthly infection only *Bd* positive animals were included. Panel (b) is the proportion of infected animals at each month of sampling. Error bars indicate 95% confidence interval.

Figure 3

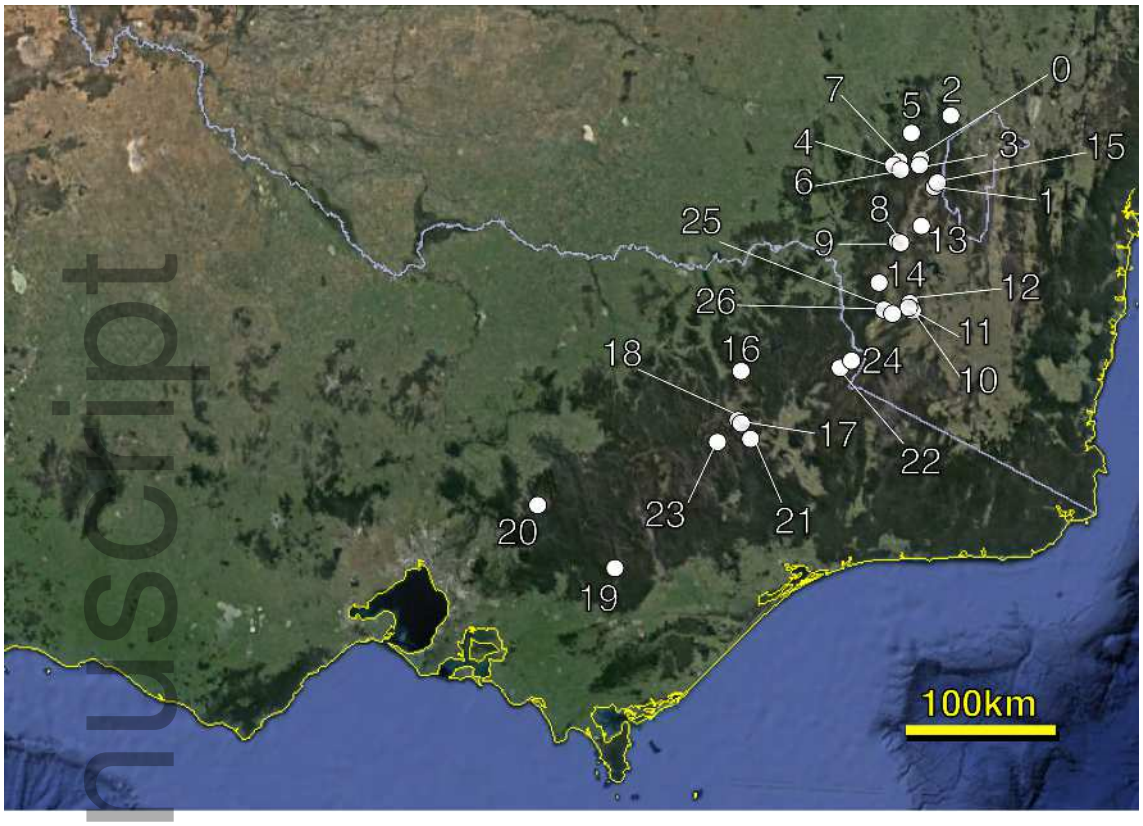
Infection dynamics of *Crinia signifera* compared to sympatric endangered species within their distribution. Data for this graph was collated from different published works: *C. signifera* (this study), *Pseudophryne corroboree* (Hunter *et al.* 2010), *P. pengilleyi* (Scheele *et al.* 2017a) and *Litoria verreauxii alpina* (Hunter *et al.* 2009; Brannelly *et al.* 2015a, 2016a; Scheele *et al.* 2015). Error bars for infection prevalence are 95% confidence intervals, while error bars for infection intensity are standard error.

Figure 4

Infection intensity and body condition of *Crinia signifera* over time in captivity. Panel (a) is infection intensity. Infection intensity is $\log_{10}$(zoospore equivalents), and week 0 was the infection intensity upon capture. *[a] indicates a significant difference from week 12 in infection load, and *[b] indicates a significant difference in infection load from week 0 calculated through a ANOVA Bonferroni post hoc test. Panel (b) is body condition. Week 1 indicates that the animals had been in captivity for one week at the point of first measure. Body condition is calculated as $\log_{10}$(mass)/$\log_{10}$(SVL). In both panels, error bars indicate standard error.

Figure 5

Histogram of the age structure of *Crinia signifera.* The proportion of individuals (of a total of 45 frogs) analysed for age by skeletochronology. The number of bone rings indicates the number of years old.



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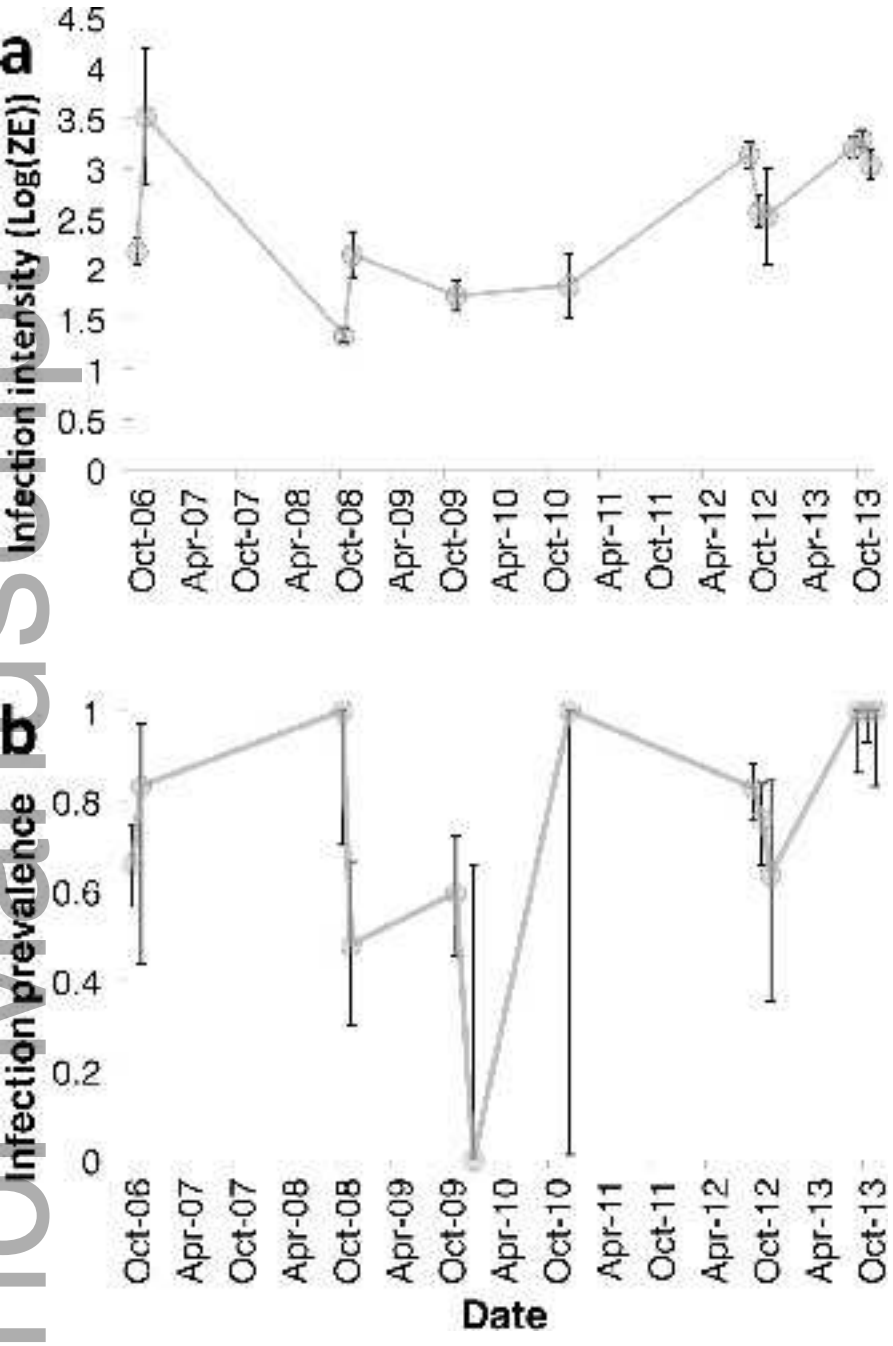

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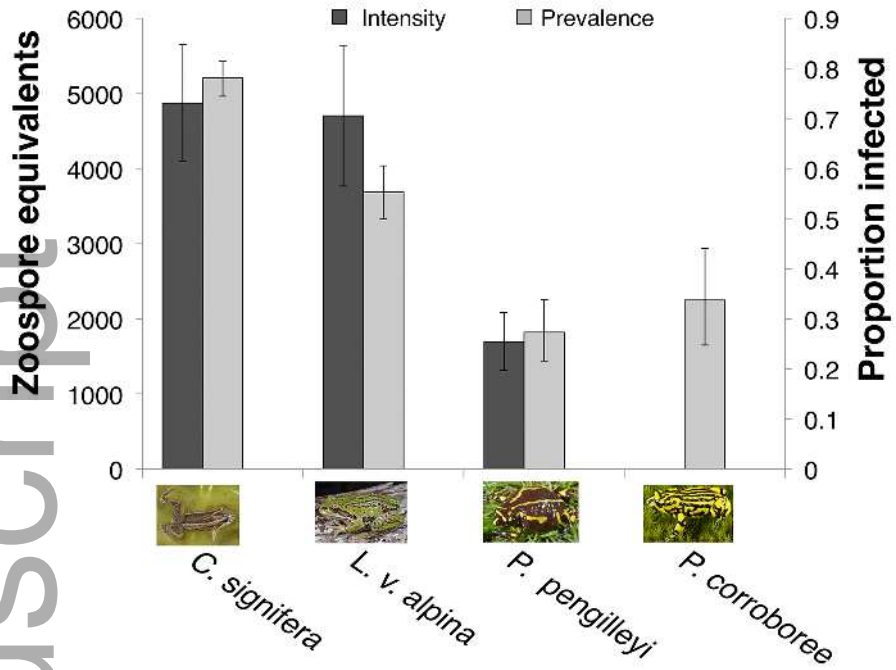

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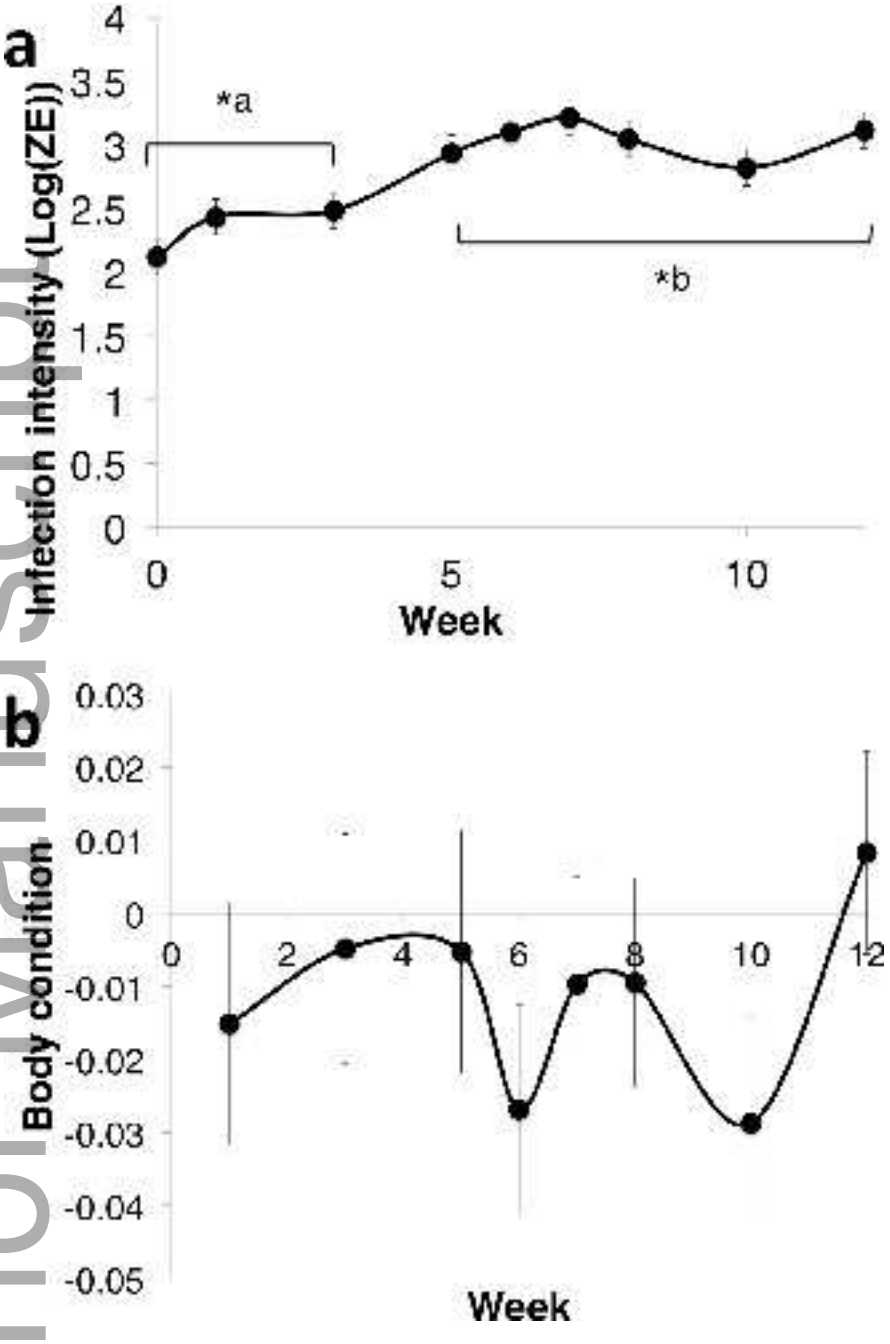

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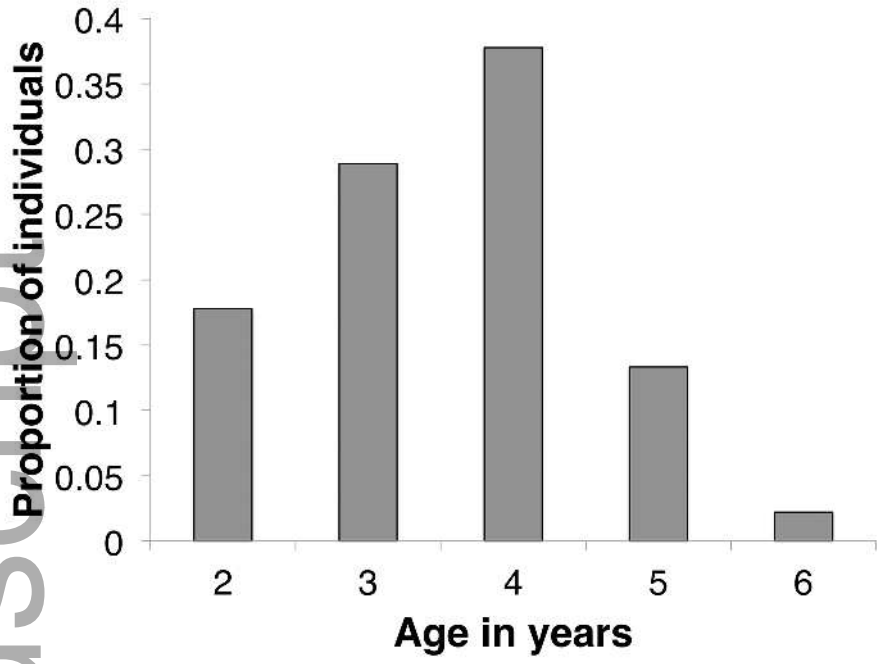

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