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Influence of *Batrachochytrium dendrobatidis* isolate and dose on infection outcomes in a critically endangered Australian amphibian

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ABSTRACT

The amphibian chytrid fungus (*Batrachochytrium dendrobatidis* (*Bd*)) is a continuing threat globally, causing widespread declines. Corroboree frogs (*Pseudophryne corroboree*) are critically endangered and no longer have self-sustaining populations in the wild due to the presence of *Bd* in the environment. We aimed to investigate variation in susceptibility to *Bd* in juvenile *P. corroboree* and evaluate the impact of *Bd* isolate and dose on infection outcomes. We experimentally exposed juvenile *P. corroboree* to two *Bd* isolates at five doses, and also characterised the *in vitro* growth of these isolates. Frogs showed high susceptibility to *Bd*, with isolate and dose impacting survival time and infection loads. Additionally, differences in the *in vitro* phenotype of the two *Bd* isolates were linked to their differential virulence. This study highlights the considerable impact that fungal isolate and dose have in shaping disease outcomes in experimental exposures.

1. Introduction

The amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) is continuing to cause severe global amphibian declines, denoting it as one of the worst wildlife diseases in human history (Scheele et al., 2019). Originating in East Asia, *Bd* has since become a global pathogen, adapting and mutating as it spread across all six continents where amphibians occur (O'Hanlon et al., 2018). There are five known distinct genetic lineages of *Bd* (O'Hanlon et al., 2018), however, adaptation within lineages is occurring, with host and environmental pressures giving rise to diverse phenotypes differing in infectivity, virulence, host specificity and *in vitro* fungal growth (Fisher et al., 2021; Berger et al., 2005; Stevenson et al., 2013). In the absence of genetic or phenotypic data, location and host species are often used to distinguish *Bd* isolates, and while this is a crude categorisation, both factors have been linked to differences in isolates (Byrne et al., 2022; Rosenblum et al., 2013).

Australia has likely only had one introduction of *Bd*, in the late 1970s (Skerratt et al., 2007). With no co-evolutionary history with the pathogen, Australian frogs were highly vulnerable, resulting in widespread declines. Currently, one in five Australian amphibians are threatened with extinction, with *Bd* being their main threatening process (Luedtke et al., 2023; Scheele et al., 2017). Of particular concern is the critically endangered southern corroboree frog (*Pseudophryne corroboree*; traditionally known as "Gyack") which is extremely rare in the wild due to chytridiomycosis. The continued survival of this species relies on captive breeding and management, and while captive breeding has been successful (Davidson et al., 2022; McFadden et al., 2013), re-introductions have not produced self-sustaining wild populations due to the continued presence of *Bd* in the environment and ongoing cases of chytridiomycosis (Hunter et al., 2010a, 2018). However, if re-introductions cease, this species will become extinct in the wild. The loss of *P. corroboree* from the environment would not only disrupt the ecological balance of their alpine habitat but also represents a significant loss to Australia's unique biodiversity. Additionally, it is a culturally important species for Aboriginal people, which features prominently in their connection to country (Connolly et al., 2017).

Bd was detected, retrospectively, in the home range of *P. corroboree* in 1984, six years after the initial introduction to Australia (Scheele et al., 2017). Since then, the species has undergone a relatively slow decline due to chytridiomycosis, taking around 30 years to become extinct in the wild, in contrast to many tropical species that disappeared within a few years of *Bd*'s introduction (Laurance et al., 1996). A suggested explanation for their gradual decline was that, given their long lifespan (20–25 years in captivity), some adult frogs survived the initial

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chytridiomycosis outbreak and persisted. However, there was little recruitment to sustain the aging population, and thus their situation became dire in the late 1990s into the early 2000s (Osborne et al., 1999; Osborne, 1989; Hunter et al., 2010b). This suggests that there was variation in susceptibility in adult *P. corroboree*, of which some were able to survive in the presence of *Bd*. The only experimental infection in adult *P. corroboree* resulted in high mortality (93%) but confirmed population differences in susceptibility, with phenotypic and genetic variation detected between four source populations (Kosch et al., 2019). Further work to explore genetic and phenotypic differences in susceptibility is recommended to inform the captive breeding programs used for reintroduction, for example to prioritise breeding from resistant animals (Kosch et al., 2022; Berger et al., 2024). This requires a greater understanding of variation of susceptibility in this species, and standardised experimental infection procedures.

Our aim was to expand on the previous work in this species, and address recommendations for future efforts by investigating the susceptibility to *Bd* in captive-bred juvenile *P. corroboree*. Specifically, we wanted to assess the impact of different *Bd* isolates and infectious dose on infection outcomes to inform a standardised experimental infection protocol. This protocol aims to increase the likelihood of detecting variation in susceptibility. To achieve this, we experimentally exposed 64 juvenile *P. corroboree* to various doses of two *Bd* isolates, ranging from 1,000 to 500,000 zoospores, across two experiments. Additionally, we compared the *in vitro* growth of *Bd* to disease progression *in vivo*. Overall, our goal was to develop an infection protocol that will facilitate future studies on variation in susceptibility in this species to inform selective breeding programs for reintroduction.

2. Methods

2.1. In vitro evaluation of Bd growth

As growth traits of *Bd* have been documented to undergo adaption to local hosts and environments, we investigated the time to complete lifecycle, zoospore production across one generation and size of zoosporangia between two Australian *Bd* isolates: Wastepoint-L. verreauxii-2013-LB-RW (Wastepoint) and Wongungurra-*L. spenceri*-2021-LB (Wongungurra) (Fig. S1). These two isolates were chosen as they are the closest in location and elevation to the home range of *P. corroboree*, from which no isolate has yet been cultured. Both isolates were collected from similar environments, and then maintained under the same conditions using standard laboratory protocols (Prostak and Fritz-Laylin, 2021; Fisher et al., 2009).

Cryopreserved isolates were defrosted following Boyle et al. (2003), and passed three times before use. *Bd* zoospores were collected from four-day-old tryptone/gelatin hydrolysate/lactose (TGhL) broth cultures, from Wastepoint passage six and Wongungurra passage seven. Zoospores from both isolates were filtered through a 10 μm filter to remove zoosporangia, counted on a haemocytometer and diluted to a concentration of 5×10^4 zoospores/mL. Separate 96 well plates were inoculated with 100 μl of zoospore filtrate from each isolate (n = 60 each), with the perimeter wells of the plate filled with 100 μl TGhL to prevent evaporation and incubated at 20 °C for the duration of the experiment (six days).

Cultures were visually inspected every 24 h, to measure the time to complete lifecycle, which was recorded as the time from zoospore release to their maturation as zoosporangia and release of daughter zoospores. A random number generator was used to select five wells daily for photographing, with five images taken from each using an inverted microscope (Nikon Eclipse TS100). Zoosporangia size was measured by taking the diameter of the two largest zoosporangia per image (Fisher et al., 2009) (n = 50 measurements) for each day, using Fiji software (2.9.0) (Schindelin et al., 2012; Greener et al., 2020). Zoospores were counted daily using a haemocytometer from the onset of zoospore production, using 20 μl of culture from five randomly selected wells. Following sampling, these wells were omitted from the remainder of the experiment.

2.2. Animal exposure experiment

We infected juvenile *P. corroboree* with *Bd* isolates, Wastepoint or Wongungurra, to determine their susceptibility and compare the impacts of isolate and dose on chytridiomycosis outcomes.

2.2.1. Exposure to Bd

Infection trials were performed using 58 captive bred *P. corroboree* embryos, comprised of 11 pedigree families, which we obtained from Melbourne Zoo and raised to six months post metamorphosis. Due to their ongoing conservation value, we were limited in the animals we could obtain for research purposes. *P. corroboree* reach sexual maturity between 2 and 4 years of age, and are classified as juveniles until then (Hunter, 2000). At the start of the experiment, frogs weighed between 0.51 and 1.09 g (mean = 0.79 ± 0.123 g), and their snout-vent lengths were from 16.6 to 23.6 mm (mean = 20.8 ± 1.3 mm). Frogs were moved into individual housing in 230 x 150 × 140 mm enclosures on a damp and crumpled paper towel substrate, where they were maintained on a 12-h light:dark cycle, at 16–18 °C and fed *ad libitum* three times weekly with vitamin and mineral dusted one-day-old crickets (*Acheta domestica*). They were checked and misted with carbon filtered water daily and cleaned weekly by replacing the paper towel substrate. The frogs were acclimated to their new environment for 14 days prior to the start of the experiment.

We inoculated the frogs with either Wastepoint (passage five) or Wongungurra (passage seven) at three doses: 50,000 zoospores, 200,000 zoospores, or 500,000 zoospores, with N = 8 animals per group. *Bd* zoospores were harvested from four-day-old cultures grown on TGhL agar plates after flooding with 2 mL of sterile Milli-Q water. Zoospores from both isolates were quantified using a haemocytometer, and diluted to the required concentration (50,000, 200,000 or 500,000 zoospores per 3 mL). Frogs were inoculated by 6-h bath exposure in 70 ml containers, exposed by dripping 3 mL of inoculum over their dorsum. Control frogs (n = 10) were inoculated using the same methods, but with *Bd*-negative TGhL agar plates.

Following exposure, frogs were monitored daily for clinical signs of chytridiomycosis (responsiveness, posture and movement changes, reduced appetite, and skin sloughing or erythema). Frogs were euthanised in a buffered 0.2% MS-222 bath if they were deemed in terminal stages of chytridiomycosis (typified by a delay in ability to right themselves).

2.2.2. Bd testing

Frogs were swabbed weekly, using a sterile rayon-tipped swab (MW113, Medical Wire & Equipment), to test for *Bd* infection using a standard approach involving five strokes on their venter, down each ventral side of the midline, and on each thigh and feet (Brannelly et al., 2015a). Swabs were stored at −20 °C until testing.

Genomic DNA was extracted from swabs using Prepman Ultra (Applied Biosystems), with bead beating following Brannelly et al. (2015b). Swab extracts were analysed in singlicate by quantitative PCR (qPCR) (Brannelly et al., 2020b; Boyle et al., 2004), using seven *Bd* plasmid standards (Pisces Molecular).

We also estimated the copy number of the internal transcribed spacer (ITS1) of each isolate (see supplementary 2 for methodology), which allowed us to translate the genomic equivalent values estimated from the qPCR standards to zoospore equivalents (ZE) enabling comparisons across isolates.

2.3. Follow-up exposure experiment

A follow-up *Bd* infection experiment was conducted after our initial experiment (2.2.1) to attempt to identify a dose that produced more

moderate mortality rates and a larger spread of survival times. This follow-up experiment used only the less virulent Wastepoint isolate (as indicated by the initial experiment) at two lower doses of 1,000 zoospores (n = 8) and 10,000 zoospores (n = 8). The controls (n = 10) were maintained under the same conditions and carried over from the initial experiment. All methods used in this follow-up experiment were identical to those used in the initial experiment described above.

2.4. Statistical analysis

All statistical analyses were conducted in R (4.1.2) using the RStudio interface (RSTUDIO TEAM, 2020; R Core Team, 2021).

2.4.1. In vitro evaluation of Bd growth

Zoospore production was $\log_{10}$ transformed, then a two-way ANOVA with Tukey's adjustment was used to compare the zoospore production from each isolate. Zoosporangia size was compared sequentially, using linear mixed models with lme4 (Bates et al., 2015), and emmeans (Lenth, 2022) to obtain and compare model predictions. Isolate and day were included in the model as fixed effects, and sample well was included as a random effect. Two separate models were used to 1) compare the rate of growth, with day treated as a categorical variable; and 2) to compare the rate of increase in growth, with day treated as a continuous variable. For both models, pairwise comparisons were made between isolates with Tukey's adjustment. The size of zoosporangia was also compared between isolates on the day of maturation (Wongungurra = day three and Wastepoint = day four) using a two-sample t-test.

2.4.2. Animal exposure experiment

As all controls remained healthy and uninfected, they were excluded from the analyses. We compared frog survival between isolate and dose groups by Cox regression using the survival package (Therneau and Lumley, 2015). The model was clustered by clutch, and Tukey's adjustment was used for pairwise comparisons. An additional Cox regression model with body condition (mass/snout-venter length) as a covariate was used to assess the effect of body condition on survival.

Infection loads were converted from ITS copy number to zoospore equivalents (ZE) and then $\log_{10}$ transformed before linear mixed model analysis with lme4 (Bates et al., 2015). We then used emmeans (Lenth, 2022) to obtain and compare model predictions. We constructed the model to include week, dose, and isolate as fixed effects, and animal ID as a random effect. Two separate models were used to 1) compare infection loads across the first three weeks, with week treated as a categorical variable, and 2) to compare the rate of increase in infection load, with week treated as a continuous variable. For both models, pairwise comparisons were made between isolates and doses, with Tukey's adjustment. Residual plots were visually inspected to ensure the model was a good fit before proceeding. One frog from the Wastepoint 200,000 dose group, was excluded from the model due to remaining uninfected for the entire experiment.

3. Results

3.1. In vitro evaluation of Bd growth

The time to complete a lifecycle differed between isolates, with Wongungurra completing its lifecycle in three days, and Wastepoint in four. Zoospore encystation in culture also differed between isolates, with all zoospores in the Wongungurra isolate encysted by day two, whereas the Wastepoint isolate still had active zoospores at day three.

In vitro zoospore production was significantly different between the two isolates at days three, four and six (pairwise comparison, $p < 0.05$), but not at day five (pairwise comparison, $p = 0.714$), with the Wongungurra isolate producing more zoospores early, at days three and four, but then appeared to plateau before Wastepoint overtook it on day six (Fig. 1).

Zoosporangia size *in vitro* differed significantly between the two isolates across all time points ($p < 0.05$), except for day three ($p = 0.235$) (Fig. 1). Wongungurra zoosporangia were larger at encystation, but after day three Wastepoint zoosporangia gained greater size. Additionally, at maturation (Wongungurra = day three and Wastepoint = day four) the Wastepoint zoosporangia were 33% larger then Wongungurra zoosporangia (T test, $p < 0.001$, 95% CI: 0.008, 0.012).

3.2. Animal exposure experiment

The overall mortality in the *Bd* exposed frogs across both isolates was 98% (47/48), with a median survival of 30 days (Wastepoint = 35 days, Wongungurra = 24 days; see Fig. S3 for individual treatment groups). One frog in the Wastepoint 200,000 dose group remained negative and survived the 70-day experiment.

Bd isolate significantly impacted frog survival time, with the Wongungurra isolate more virulent when averaged across all doses (Cox regression $p < 0.001$, Hazzard Ratio (HR) = 42, 95% CI: 13, 130), and for each respective dose: 50,000 (Cox regression, $p < 0.001$, HR = 24, 95% CI: 8.3, 71), 200,000 (Cox regression, $p < 0.001$, HR = 64, 95% CI: 20, 201), and 500,000 (Cox regression, $p < 0.001$, HR = 46, 95% CI: 10, 221) (Fig. 2).

Averaged over the isolates, survival time was significantly lower in the 500,000 dose compared to the 50,000 dose treatments (Cox regression, $p = 0.006$, HR = 4.0, 95% CI: 1.4, 12), but it was not significantly different between the 50,000 and 200,000 or the 200,000 and 500,000 doses (Cox regression, $p > 0.05$). Initial body condition did not significantly impact survival (Cox regression, p = 0.44, HR = 14, 95% CI: 0.02, 12741). However, the wide confidence interval suggests that there is little power to assess effect of body condition on survival, likely due to small sample size.

We were unable to compare infection loads past week three, as

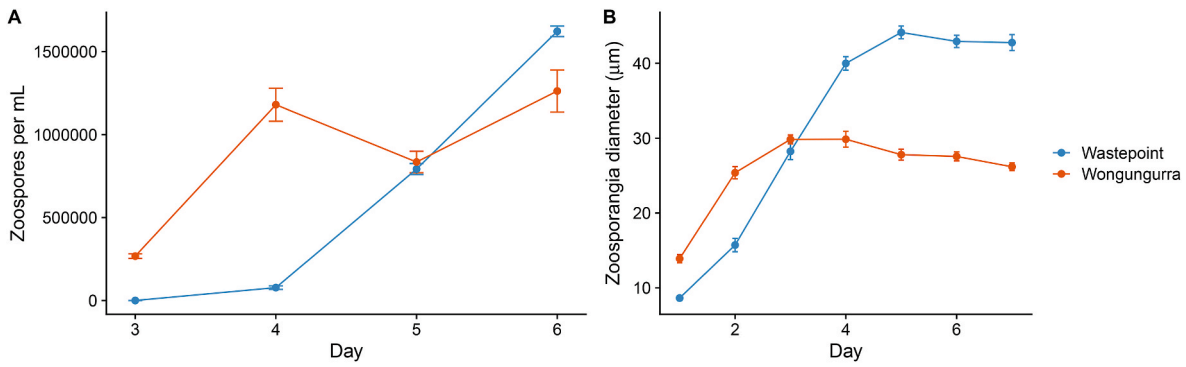

Fig. 1. *In vitro* growth of Wastepoint (blue) and Wongungurra (orange) *Batrachochytrium dendrobatidis* isolates. Error bars indicate the mean (±SE) for **(A)** zoospore production per mL, and **(B)** zoosporangia size (µm).

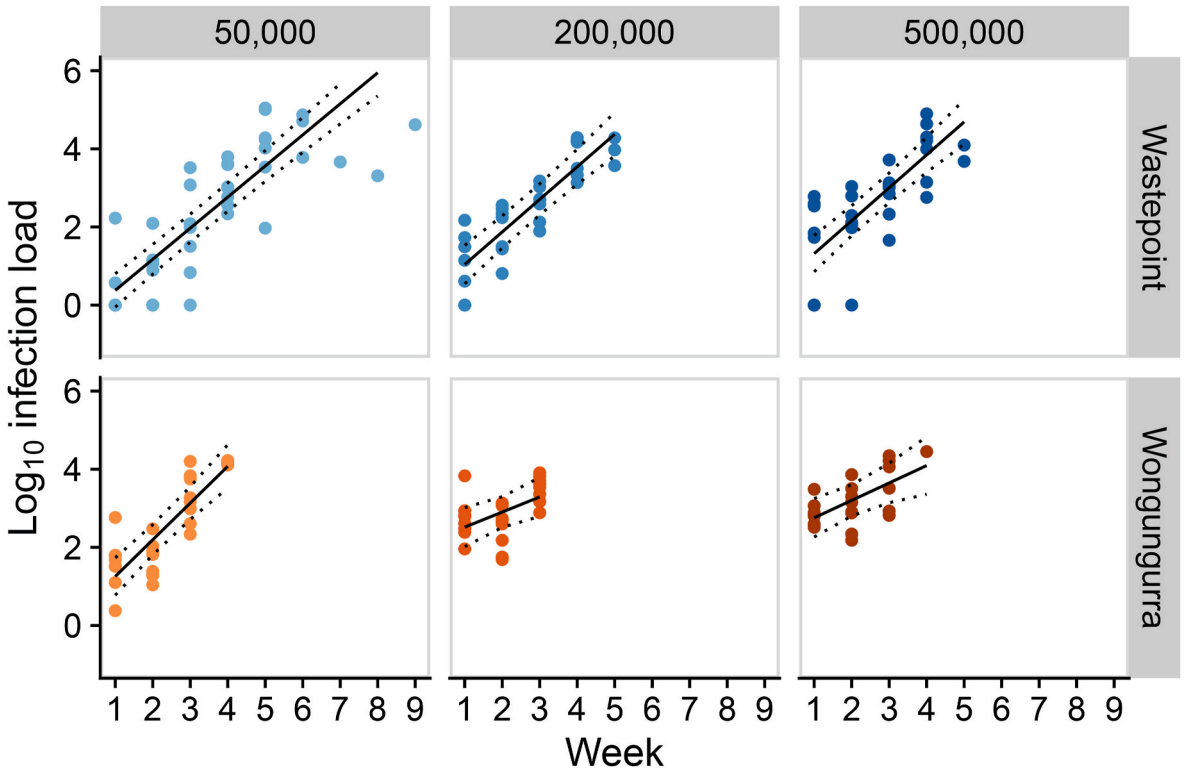

Fig. 2. *Batrachochytrium dendrobatidis* infection loads [$\log_{10}$ (zoospore equivalents +1)] in juvenile *Pseudophryne corroboree*. The predictions from the linear mixed model are indicated by solid lines with 95% CI indicated by dotted lines.

insufficient frogs remained, but over the first three weeks of infection, isolate had a significant impact on infection loads when averaged across doses (pairwise comparison, $p < 0.0001$) (Fig. S4 and Table S4a).

The rate of increase in zoospore load was significantly steeper in the Wastepoint isolates 200,000 and 500,000 doses when compared to Wongungurra (pairwise comparison, $p < 0.05$), but not different in the 50,000 dose treatment (pairwise comparison, $p = 0.214$) (Fig. 3). The rate of increase in zoospore load differed significantly between the Wongungurra 50,000 and 200,000 and the 50,000 and 500,000 dose treatments (Table S4b). The 50,000 dose exhibited a continual increase in infection load, contrasting with the 200,000 and 500,000 doses that had relatively high loads in week one and only experienced a slight increase. The Wastepoint infection loads continuously increased, but no differences in the rate of increase were found between any of the groups (Table S4b).

At one week post infection, the mean *Bd* load was significantly higher in the Wastepoint 500,000 dose group compared to the Wastepoint 50,000 dose group (pairwise comparison, $p = 0.02$, 95% CI: −1.9, −0.13), and the Wongungurra 50,000 dose group was significantly lower than both the 200,000 and 500,000 dose groups (pairwise comparison, $p = 0.007$, 95% CI: −2.1, −0.27, and $p = 0.002$, 95% CI: −2.2, −0.42, respectively).

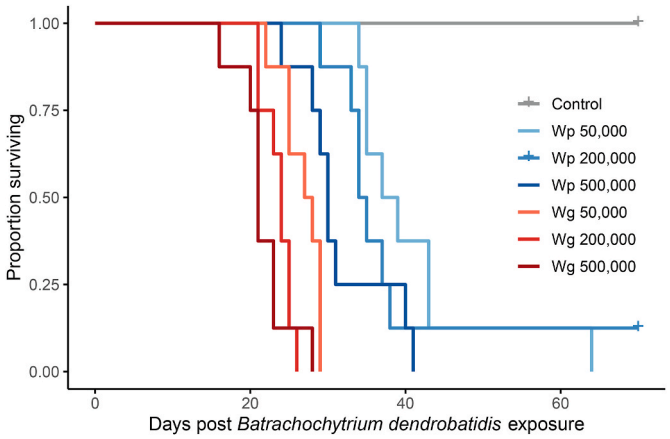

Fig. 3. Survivorship of juvenile *Pseudophryne corroboree* exposed to *Batrachochytrium dendrobatidis* (n = 58 with 8 frogs in each treatment and 10 controls). Two *Bd* isolates, Wastepoint (Wp) (blue) and Wongungurra (Wg) (orange) were used at three doses: 50,000 zoospores, 200,000 zoospores and 500,000 zoospores.

3.3. Follow-up exposure experiment

Overall mortality rate in the *Bd* exposed animals was 100% (16/16), with a median survival time of 39 days (1,000 zoospores = 43.5 days, 10,000 zoospores = 33 days (Fig. S5a). We detected no significant difference in survival time between the 1,000 and 10,000 groups (Cox regression, p = 0.129).

Infection loads were significantly higher in the 10,000 zoospore group, compared to the 1,000 zoospore treated frogs at week 1, 2, 3, and 4 ($p < 0.05$) but not for week 5 (p = 0.54) (Fig. S5b and Table S5). The mean infection load at week one was significantly higher in the 10,000 zoospore group (pairwise comparison, p = 0.001, 95% CI: −1.9, −0.53), and thus the rate of increase in zoospore load was also significantly different between the two doses (pairwise comparison, p = 0.04, 95% CI: 0.01, 0.35), with the 1,000 zoospore group having a lower load at week one and experienced a steeper increase in loads. The 10 control animals remained free of infection for the duration of the experiment.

4. Discussion

In this study we have developed an infection method that will enable future studies that rely on variation in disease progression for identifying resistant phenotypes in juvenile *P. corroboree*. Our results

demonstrate that juvenile southern corroboree frogs are highly susceptible to the amphibian chytrid fungus, and that both *Bd* isolate and dose can have a significant impact on survival time and infection loads. We compared two *Bd* isolates and found phenotypic differences *in vitro*, as well as virulence differences *in vivo*. The more virulent isolate, Wongungurra, had a shorter generation time, and initially grew faster *in vitro*, but zoospore concentrations plateaued at lower levels than the less virulent isolate, Wastepoint.

Although infected frogs had 100% mortality, there were significant differences in survival time between the two *Bd* isolates and across doses. Frogs infected with the Wastepoint isolate survived on average for eight days longer than those infected with the Wongungurra isolate. Within isolates, lower *Bd* doses resulted in longer survival times, as has been reported in other species (Carey et al., 2006; Carvalho et al., 2023b). The follow-up experiment confirmed this inverse relationship between initial infectious dose and survival in this species, with lower doses resulting in prolonged survival times (Fig. S5a).

Infection loads were also significantly impacted by isolate and dose, with a marked decrease in rate of load increase for the lowest dose, possibly due to host response. In the initial experiment, significant differences were seen between the 50,000 and 200,000 and the 50,000 and 500,000 doses in the first two weeks of infection; however, by three weeks post infection, all treatment groups reached a similar infection load. This suggests a correlation between the initial infectious dose and infection load, with lower doses requiring additional time to build comparable levels of infection. For the Wastepoint isolate, the rate of load increase was the same for all doses, so while the dose impacted the level of infection load it did not affect the progression of infection. However, in our follow-up experiment using lower doses of the Wastepoint isolate, the rate of load increase was lower in the 1,000 group compared to the 10,000 dose group (Fig. S5b), and infection load was also lower until five weeks post infection when it then reached a similar level (Fig. S5b and Table S5). Thus, frogs exposed to very low doses had both a lower initial infection load and a lower rate of increase, possibly because innate immunity can better resist low initial infection loads. This may allow time for the host immune response to influence the rate of increase in infection loads, altering disease progression and, in turn, provide greater opportunity to detect variation in individual responses.

Experimental factors that may have artificially influenced these results include the use of captive-bred animals and fungal isolates obtained from alternative host species. For example, the microbial diversity and richness in captive frogs differs compared to their wild counterparts, potentially making them more susceptible to *Bd* (Becker et al., 2014; Woodhams et al., 2023). The *Bd* isolates used in this experiment were cultured from hylid (tree frog) species from nearby habitats and may have adapted to these different hosts. *Bd* evolution is driven by selective pressures from the host (Byrne et al., 2022), and the environment (Sheets et al., 2021). Evidence from laboratory experiments suggest that host mediated adaptation in *Bd* can result in more severe disease when frogs are exposed to isolates collected from a different host species (Langhammer et al., 2013; Berger et al., 2005; Retallick and Miera, 2007; Carvalho et al., 2023a). Additionally, *Bd* growth *in vitro* is artificially influenced by factors such as resource limitations (e.g., space and nutrients) and handling practices (e.g., passage number and cryopreservation) (Langhammer et al., 2013; Vredenburg et al., 2010), therefore, caution is advised when comparing disease outcomes across species, isolates, or experiments.

Comparing *in vitro* fungal growth of two *Bd* isolates revealed distinct differences, with the more virulent isolate exhibiting faster initial growth and turnover but had lower overall zoospore productivity. These isolates (Wastepoint and Wongungurra) likely belong to the highly pathogenic global panzootic lineage (*Bd*-GPL), as all Australian isolates sequenced to date are in this lineage, and epidemiological and genetic analyses suggest that there has only been one introduction of *Bd* to Australia (Murray et al., 2010; O'Hanlon et al., 2018; Scheele et al., 2017). Both isolates were collected from similar geographic areas (Figure S1), however, *in vitro* growth of the isolates differed substantially, so we anticipated that growth and virulence *in vivo* would also differ. As the Wastepoint isolate grew larger and produced more zoospores *in vitro*, and these traits have been linked to virulence in *Bd* infections (Voyles, 2011), we expected that Wastepoint would be more virulent *in vivo*, however, survival was prolonged for this isolate compared to Wongungurra. This highlights the complexity of intrinsic and extrinsic factors that can influence disease outcomes, and that *in vitro* growth may not necessarily be a reliable method to predict virulence *in vivo*, as results are highly contingent on the specific isolate and host.

For laboratory exposures, optimising the choice of *Bd* isolate, dose and experimental conditions can be challenging and depends on the aims of the study. Increasingly, isolates cultured from the host species are used if available (Sauer et al., 2020). Exposure doses are often selected with the aim of producing infection or mortality rates or incubation times to suit the experimental aim, and not necessarily to reflect natural exposures. As the primary goal of many infection experiments is to assess susceptibility to *Bd*, high experimental doses may not be realistic as fast disease progression can overcome host resistance factors, and mask variation in susceptibility. Here we found that juvenile *P. corroboree* frogs exposed to lower doses expressed more variation in disease outcomes, in agreement with other experiments comparing infectious doses (Carey et al., 2006). Despite the lack of information on the typical *Bd* dose in natural exposures, studies using eDNA methods (Brannelly et al., 2020a) show levels in water bodies are often low. Therefore, aside from direct contact with infected frogs (e.g., at breeding; Stegen et al., 2017), frogs may be unlikely to come into contact with a high doses of zoospores. Similarly, light infection loads in susceptible wild frogs are common, perhaps suggesting low levels of exposure (Phillott et al., 2013; Grogan et al., 2016). Therefore, exposing frogs to lower doses of *Bd* could more accurately simulate typical natural exposures, facilitating a more realistic assessment of host susceptibility.

The complexity of the *Bd* disease system, with its variable infection outcomes, stems from its broad host range across diverse environments. Recent efforts have established a model amphibian species to study chytridiomycosis, allowing the mechanisms of disease to be studied under standardised methods (Carvalho et al., 2023b). However, as chytridiomycosis impacts amphibians with a diverse range of physiological and biological traits, results from disease studies cannot be extrapolated across species with confidence. In addition, the choice of isolate used can result in varying outcomes. Hence, validating methods for use in non-model species remains essential.

Despite the challenges of conducting experimental exposures in endangered species, given constraints such as limited animal availability and ethical considerations, they are necessary for applied conservation research and management. Assessing the variation of susceptibility to chytridiomycosis in threatened species such as *P. corroboree* is key to understanding factors influencing their survival. In this study we developed an infection protocol in juvenile *P. corroboree* that challenged the frogs at a low enough disease burden, allowing them to express measurable variation in susceptibility. This will enable further investigation into the genetic basis of immunity, which will inform selective breeding approaches to enhance host resistance and ultimately improve conservation outcomes in this species.

CRediT authorship contribution statement

Mikaeylah J. Davidson: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Tiffany A. Kosch:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Amy Aquilina:** Writing – review & editing, Investigation. **Rebecca J. Webb:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Lee F. Skerratt:** Writing – review & editing, Supervision, Methodology, Funding acquisition,

Conceptualization. **Lee Berger:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Data availability statement

The data that supports this study is available in the accompanying online supplementary material.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics

All work was conducted under approval of the University of Melbourne's Animal Ethics Committee (Application, 2021-22144-24454-5), and Wildlife Act 1975 Research Authorisation permit number 10010261.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2024.101397.

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