Abstract

The sublethal effects of infectious disease on reproductive behaviour and mating success are not well understood. Here, we investigate predictors of male mating success in one of Australia's most critically endangered vertebrates: the northern corroboree frog. Using a genomic approach to assign parentage, we explored whether infection with the amphibian chytrid fungus (*Batrachochytrium dendrobatidis, Bd)* a pathogen responsible for amphibian declines globally, influenced male calling behaviour and mating success. We also explored whether male mating success was predicted by phenotypic traits (age, body size, colouration, call characters) that potentially signal genetic quality, and the soil moisture (water potential) of male-constructed terrestrial nests, which may directly impact offspring survival. We found that *Bd* significantly influenced male advertisement; *Bd* infected males produced calls with significantly higher pulse repetition rates than uninfected males. Older males had a higher probability of mating, however variation in the number of eggs in a nest was most strongly explained by an interaction between male *Bd* infection status and call pulse repetition rate. We propose that these relationships may result from either pathogen-mediated changes to host behaviour, or host-mediated changes to behaviour (e.g. terminal investment). Regardless of the mechanism, this is the first evidence that male mating success in an amphibian can be influenced by male *Bd* infection status, highlighting a novel mechanism through which this virulent and now globally distributed pathogen can affect amphibian fitness. More broadly, these findings add to a growing body of evidence that pathogens can alter the reproductive biology of their hosts. From a conservation perspective, increased consideration of how sexual selection operates in altered environments has the potential to assist with the management of threatened amphibians worldwide.

Keywords: amphibian, chytrid fungus, conservation, disease, female mate choice, fitness, male mating success, mating system.

Introduction

Disease can directly impact individual fitness by reducing prospects of survival, but may also have sublethal impacts by modifying reproductive traits that influence host attractiveness and reproductive success (Beltran-Bech & Richard, 2014). In recent years, an increasing number of studies have explored the sublethal effects of disease on reproductive behaviour, and there is mounting evidence that parasites and pathogens can alter male attractiveness (Dass et al., 2011), outcomes of female mate choice (Beltran-Bech & Richard, 2014) and host reproductive success (Pigeault et al., 2018). Such affects have primarily been attributed to pathogen-mediated changes in host physiology, behaviour or secondary sexual traits, or host-mediated changes in reproductive investment (Klein, 2003). Surprisingly, however, we still know very little about the impacts of infection on male mating success in wild populations. Moreover, we also know virtually nothing about interactions between infection and sexual signals across multiple signaling pathways. In most species, sexual signals are composed of multiple components in the same sensory modality and/or concurrent signals across multiple sensory modalities, and females can use a suite of cues to accurately assess mate quality (Candolin, 2003). In principle, because different cues might signal different material or genetic benefits, or act in concert to improve signal efficiency, we should expect a complex interplay between disease and the various cues used in mate choice. Adding to this complexity, females may be under strong selection to use cues to identify male disease status, and to avoid interactions with infected males (Beltran-Bech & Richard, 2014). Studies investigating interrelationships between infection and combinations of male traits expected to influence female mating decisions stand to significantly advance our understanding of how infectious disease alters the operation of sexual selection.

Amphibians provide an excellent model to explore disease-mediated changes in reproductive behaviour, and interrelationships between male traits likely to influence female mate

choice. Since the 1980s, the amphibian chytrid fungus *Batrachochytrium dendrobatidis (Bd)* has emerged worldwide and is now considered the worst wildlife disease in recorded history, having contributed to the decline of over 500 amphibian species globally (Scheele et al., 2019). Chytrid fungus infection can result in the potentially fatal disease chytridiomycosis (Fisher & Garner, 2020). Critically, however, effects of the pathogen are varied, and many species persist with the fungus, or show slow to moderate declines, with the potential for widespread sublethal effects on reproduction (Lips, 2016). Of equal importance, anuran amphibians have been a model group in the study of sexual selection for over 50 years. As such, we have extensive information on sexual signaling and drivers of female mate choice in this taxon, providing a conceptual basis from which to begin exploring effects of *Bd* infection on male attractiveness and mating success.

In anuran amphibians, males of various species are known to use acoustic and/or colour signals to attract mates, and males that invest more heavily in sexual signals typically have higher mating success (Gerhardt & Huber, 2002; Maan & Cummings, 2009; Dreher, Rodríguez, Cummings, & Pröhl, 2017). Females that choose to mate with males that invest more in sexual signals might gain direct material benefits, such as increased fertility (Robertson, 1990; Hettyey, Herczeg & Hoi, 2009), high quality paternal care (Brown, 2013; Pettitt, Bourne, & Bee, 2020) or a decreased risk of disease acquisition (Beltran-Bech & Richard, 2014). Moreover, in resource defense systems, sexual signals may indicate the quality of male-constructed or male-chosen nesting sites (Endler, 1983; Felton, Alford, Felton, & Schwarzkopf, 2006). For terrestrial breeding species, nest moisture is a critical aspect of nest quality because water-holding potential can directly impact egg viability, embryonic development and tadpole size upon hatching (Seymour, Geiser, & Bradford, 1991). In various frog species, it has been demonstrated that aspects of male calling and colouration are intimately linked to hydration state (Withers, 1995; Mitchell, 2001). Therefore, investment in acoustic or colour signals could provide a strong, honest indicator of nest moisture

and nest quality (Felton et al., 2006). Female preferences for males that invest more in sexual signals might also confer indirect genetic benefits through a classic good genes pathway (Forsman & Hagman, 2006; Welch, Semlitsch, & Gerhardt, 1998). Acoustic and colour signals are costly to produce (in terms of energetic expense and increased exposure to predators and parasites) (Kemp, Herberstein, & Grether, 2012; Rojas, 2016), and can reflect male body condition, body size and/or age (Vasquez & Pfennig 2007), all of which have been linked to genetic quality (Welch et al., 1998; Gerhardt & Huber, 2002; Felton et al., 2006; Parris, Velik-Lord, & North, 2009; Rausch, Sztatecsny, Jehle, Ringler, & Hödl, 2014).

Despite enormous potential for *Bd* to have sublethal consequences for amphibian fitness, very few studies have attempted to investigate the influence of this pathogen on amphibian reproductive behaviour. Nevertheless, some recent work draws attention to the potential for widespread evolutionary consequences. Specifically, studies in Japanese and Australian tree frogs (representing two distinct lineages) have revealed that *Bd* can increase male calling effort (e.g. increase probability of calling, and call duration) (An & Waldman, 2016; Roznik, Sapsford, Pike, Schwarzkopf, & Alford, 2015). In regard to colour signaling, impacts of *Bd* infection are yet to be formally investigated, but changes can be expected because chytridiomycosis modifies amphibian skin (through hyperemia, hyperkeratosis and sloughing) (Ohmer, Cramp, White, & Franklin, 2015), with anecdotal reports of skin discolouration. Moving forward, there is a need for field-based studies that use a multivariate approach to explore associations between *Bd* infection and various traits that might influence male attractiveness and mating success (indicative of patterns of female mate choice). Such studies will help direct experimental studies aimed at elucidating causal mechanisms. Furthermore, understanding the impact of *Bd* on reproductive behaviour and mate choice could be integrated into amphibian conservation and management strategies. Globally, numerous threatened amphibian species are the subject of intensive *ex-situ* conservation programs

(Zippel et al., 2011). Knowledge of *Bd*-induced changes to natural reproductive behaviour may be critical to the successful management of declining populations endemically infected with *Bd*.

The aim of this study was to investigate predictors of male mating success in the terrestrial breeding northern corroboree frog, *Pseudophryne pengilleyi*. In this species, male mating success is expected to be a good indicator of female mate choice because females have a high level of control over mating. Females initiate male courtship by entering male-constructed nests, and either accept males by remaining and mating, or reject males by leaving. Females typically move between the nests of multiple males, and may assess individual males' multiple times before mating (displaying high investment in mate assessment). During these interactions, males are not physically aggressive and show no signs of attempting forced copulation (M. McFadden, personal observation). Moreover, there is a distinct absence of alternative male mating tactics common to anurans (such as satellite or sneaking behaviour), or any form of male armament (e.g. enlarged forearms or nuptial spines). Together, these observations indicate that intrasexual selection has not played a major role in the evolution of the *P. pengilleyi* mating system. Over the past thirty years, *P. pengilleyi* has experienced major population declines linked to the spread of *Bd* (Hunter, 2000), and is now one of Australia's most critically endangered vertebrate species. Conservation efforts have focused on large scale captive breeding and reintroduction, but little is known about patterns of mate choice, and whether *Bd* influences mating outcomes in the wild. Using a multivariate approach, and working with the largest remaining wild population, we investigated whether interrelationships between male age, body size, nest moisture, *Bd* infection status and multiple components of male calling and colouration predicted male mating success.

Methods

Field Data Collection

Data collection took place over a two-week period late in the natural breeding season from the 28th February 2018 to the 8th March 2018. The study site was located within Bondo State Forest, approximately 25 kilometres east of Tumut in New South Wales, Australia. Specific location details have been withheld for conservation security reasons. Further details of the study site are provided in the Appendix. During the first week (28th February – 2nd of March) calling males were located by triangulating on their calls. Once located, call sites were marked on the ground using a plastic plant tag with an individual ID number. During the second week (6th March – 7th of March), male calls were recorded. Call recordings were obtained using a Marantz Professional handheld solid state recorder (Model No. PMD661MKII, D&M Holdings Inc. Tokyo, Japan) connected to a directional microphone (Rode Microphones, Sydney, Australia). To minimise disturbance, calls were recorded from a minimum distance of 2 metres and after an acclimation period of 5 minutes from first approaching a nest. Calls were recorded in *.wav* format at a sampling rate of 44.1kHz with a 16-bit resolution. Calls were recorded during periods of peak calling activity between 6.00am and 11.00am and again between 6.00pm and 12.00am, and air temperature was recorded.

Male nest sites (Figure 1) were checked on the night of 7th March 2018. For each nest/male ($N = 36$), we recorded mating success (eggs present or absent) and total egg number. When eggs were present, 15% were randomly collected for genetic parentage analysis (see Parentage analysis section). All eggs were carefully collected using a plastic spoon to avoid damaging nest sites and/or causing damage to remaining eggs (*P. pengilleyi* eggs are encased in thick jelly capsules, which afford a high level of protection during handling). If clutches contained embryos at different developmental stages (indicating multiple clutches had been laid), 15% of eggs were sampled from each stage. This level of sampling followed that used to assign paternity in a congener, the brown toadlet *P. bibronii*, and was deemed to be the minimum level required to gain accurate parentage data (Byrne & Keogh, 2009). Overall, a total of 242/1568 eggs were collected from the population.

While eggs were counted, the phenotypic characteristics of the resident male were measured. Males were first digitally photographed to measure aspects of their colouration. Frogs were photographed inside a portable photography light box (60 cm x 60 cm, Puluz Photo Accessories), which contained two 30W 5500K integrated LED strip lights attached to the top inside surface of the box. Power to the photography light box was provided via a 12-volt battery. Photographs were taken with a Canon EOS 70D camera with a standard lens (Canon EFS 18-55mm lens) that was positioned above the light box with a tripod, with the camera lens placed through a 10 cm hole at the top of the light box. The distance between the front of the lens and the bottom of the photo box was 39 cm. Photographs were taken in raw format with the following settings: ISO = 100, f = 10, shutter speed = 1/125. All photographs included a X-rite ColorChecker Passport (X-rite, USA), which consists of 24 coloured and grayscale squares against which the colours in each photo can be standardized (see Appendix Figure A2).

After each male was photographed, body weight (grams) was then measured using a portable balance (On Balance, LS-100 Digital Pocket Scale). Next, to determine male *Bd* infection status, cutaneous swabs were collected from each male using sterile cotton-tip swabs (Medical Wire and Equipment Co. MW 100-100). All males were swabbed in a standardized manner using protocols described previously (Scheele, Hunter, Brannelly, Skerratt, & Driscoll, 2017). In order to prevent the transmission of *Bd* between frogs, sterile gloves were changed and all equipment/instruments wiped clean with ethanol between handling each frog. To further reduce the risk of disease transmission, we placed frogs into individual, single-use plastic weigh trays when weighing them and whilst taking their photograph inside the light box. *Bd* samples were analysed in triplicate with real-time quantitative polymerase chain reaction (PCR) (following the methods of Hyatt et al., 2007) with the exception that Qiagen master mix was used instead of Taqman master mix. We considered a sample positive if one or more wells returned a positive reaction.

176

177 To determine male age, a single toe was removed at the base of the second phalange using
178 surgical scissors for skeletochronology analysis (see details in the Appendix) and preserved in 75%
179 ethanol. This toe tissue sample was also used for parentage analysis to confirm male mating
180 success. Wounds were treated with Bactine (dual antiseptic and analgesic) and Vetbond (a sealant).
181 Frogs were handled for no more than ten minutes in total and placed promptly back on their nest
182 site after all measurements were taken. We observed a number of frogs' resume calling within
183 several of minutes after being returned to their nest. On the 8th of March, nest soil moisture was
184 measured for each male's nest using a soil moisture probe meter (Moisture Probe Meter, MPM-160-
185 B, 12-bit resolution, ICT International Pty Ltd).
186

187 *Male call and colour analysis*

188 For each male, we calculated average call duration, average pulse repetition rate and average call
189 peak frequency using the sound analysis software Raven Pro (see Appendix for detailed methods).
190 To quantify aspects of male colouration, we calculated the chroma (saturation), hue and luminance
191 (brightness) of each male's dorsal yellow markings using an image analysis program written in
192 MATLAB (Cadena, Rankin, Smith, Endler, & Stuart-Fox, 2018) (see Appendix for details).
193

194 *Parentage analysis*

195 To assign parentage to offspring and determine male mating success, we genotyped all sampled
196 males (N = 36) and 15% of offspring from each nest with a large SNP data set. The background and
197 process of the SNP genotype analysis are described in detail elsewhere (see O'Brien et al. 2018). In
198 brief, males and offspring were genotyped from tissue samples using DArTseq$_{TM}$, a widely used
199 genotyping technique. This technique generated a data set of approximately 2548 SNPs, with an
200 average call rate of 98.8% and a reproducibility of 99.8%. From these SNPs, we calculated a

Hamming Distance Matrix for all 220 successfully genotyped individuals to determine paternity, following the methods described in detail in O'Brien et al. (2018). In total 35 nests were sampled, and in the nests that contained eggs (24/35), one resident male was present. The resident male was the genetic sire of all the eggs in his nest in 91.6% (22/24) of cases (based on the 15% of eggs collected from each clutch, see Appendix Table A1). In the remaining two nests with eggs, the resident male was not the genetic sire (indicative of nest takeover) and was therefore excluded from further analysis. The actual genetic sire of one of these nests was not sampled, and the other sire also sired eggs in another nest (he was a resident male of another nest nearby). No nests had single clutches (clutches at the same developmental stage) sired by more than one male (i.e. there were no cases of multiple paternity).

Statistical analyses

In order to determine what factors influence mating success in *P. pengilleyi*, we measured ten explanatory variables: (1) body weight, (2) age, (3) hue, (4) luminance, (5) chroma, (6) average call frequency, (7) average call duration, (8) average pulse repetition rate, (9) *Bd* infection status, and (10) nest soil moisture. In total, 36 males were sampled, however, nine males were excluded during call analysis because their calls temporally overlapped with conspecific calls and were impossible to separate (see call analysis section in the Appendix), and a further two were excluded because parentage analysis showed that they were not the genetic sire of the eggs in their nest (see Parentage analysis section). Thus, the sample size for the analysis was restricted to 25 males. Prior to analysis, all continuous explanatory variables were first tested for collinearity using a Pearson's correlation matrix. Variables with a significant Pearson's correlation coefficient > 0.5 were considered to be highly correlated (Zuur, 2009). Body weight was significantly positively correlated with age (R = 0.55, P = 0.004), and hue and chroma were significantly negatively correlated (R = -0.58, P = < 0.002), so body weight and hue were removed as explanatory variables.

We used linear models to first examine the potential effects of air temperature, age, nest soil moisture and *Bd* infection status on male call characteristics. For each call variable, we separately constructed a set of possible candidate models arising from all combinations of the four explanatory variables. Air temperature, age and soil moisture were entered as continuous variables, and *Bd* status entered as 0 = uninfected, 1 = infected (see Appendix Tables A3-A5). Call duration was log transformed to improve normality. We used an information-theoretic model selection process to rank all possible candidate models based on their Akaike's information criterion value with a correction for small sample size (AIC_c) using the function *dredge* from the package MuMIn version 1.43.15 in R (Bartoń, 2019). The model with the lowest AIC_c score for each call variable was considered to provide the best fit. We ran additional linear models to further examine relationships between air temperature and each call variable, as positive relationships have been found in various frog species (Wells, 2007), and we wanted to ensure that these relationships did not require more detailed examination. However, we found no evidence for significant associations between any of the call variables and temperature (most likely because the vast majority of calls (84%) were recorded between a narrow temperature range (10°C -13°C). To determine whether *Bd*-infected males differed in age, luminance, chroma or nest site soil moisture to uninfected males, we used non-parametric Kruskal Wallis tests.

Next, we investigated factors associated with male mating success. A frequency histogram of the number of eggs in each male's nest (see Figure 3) revealed that the data were zero inflated and that the count data was over dispersed. Therefore, to investigate variables influencing male mating success, we used zero-altered negative binomial (ZANB) generalized linear models, using the function *hurdle* in the R package *pscl* version 1.5.2 (Zeileis et al. 2008). Data were modeled in two parts: 1) a binomial logistic regression with male mating success (yes or no) as the response

variable, and 2) a negative binomial GLM of the count data, with the number of eggs as the response variable, excluding zeros (Zuur, 2009). The two parts of the model explain; 1) the effect of the predictors on whether or not a male mated at all, and 2) the effect of the predictors on mating success for the males that did mate (the total number of eggs). Because our sample size was smaller than anticipated ($N = 25$), limiting the number of explanatory variables that could be incorporated into the same model, we used an information-theoretic model selection process to rank a subset of possible candidate ZANB models based on their AIC values using the AIC function in R (for full details of model selection process, see model selection section in the Appendix and Appendix Table A6 and A7). The model with the lowest AIC value was considered to provide the best fit. Predictions were calculated using the top ranked model using the function *predict.hurdle* from the package *pscl* version 1.5.2 (Zeileis, Kleiber, & Jackman, 2008). We investigated model fit by visually examining diagnostic plots of residual versus fitted values for each predictor variable. All statistical analyses were completed in R version 3.5.1 (The R Foundation for Statistical Computing, 2018).

Ethical Note

All procedures conducted during this study were approved by the University of Wollongong Animal Ethics Committee (AE17/30), the Australian National University Animal Experimentation Ethics Committee (A2018/04) and authorized by the New South Wales National Parks and Wildlife Service – Office of Environment and Heritage (SL102049). All sampling was conducted following hygiene protocol guidelines for controlling disease in frogs during field studies (Phillott et al., 2010). Toe clipping was determined to be an appropriate method as it provided substantial information from a single sampling event; a high-quality genetic sample and a sample for skeletochronology to determine age (thereby avoiding the need to repeatedly capture individuals in the wild). Moreover, the sampling detailed in this project also provides data for a comparison to

museum specimens of northern corroboree frogs that were collected in the 1970s and 1980s before the emergence of chytrid fungus, adding to the value of the contemporary sample. Toe clipping was performed with surgical scissors and wounds were treated with Bactine (dual antiseptic and analgesic) and Vetbond (a sealant). At the time of the study, the author that completed the procedure had over 11 years of experience handling and collecting data from threatened frog species, as well as the required animal ethics training. The extent of toe clipping (partial removal of a single toe) used in this study has not been demonstrated to have negative impacts on survival in anuran species (Ginnan et al. 2014; Hudson et al. 2017), including corroboree frogs (Hunter, n.d.). Handling of frogs was kept to a minimum during data collection and frogs were returned promptly to their nest site. We observed no negative effects of the data collection on the frogs. Egg collection was conducted in accordance with the management plans for the study population (as approved by the corroboree frog recovery team) and was deemed highly unlikely to negatively impact population viability given the intrinsically high mortality rate for pre-metamorphic life stages, and because sampling was restricted to a subset of the broader meta-population sampled.

Results

Variation in aspects of male phenotype and nest site

Of the males included in the analyses ($N = 25$), body weight ranged from 0.74 grams – 1.38 grams, with a mean (±SEM) of 0.97 (± 0.03) grams. Male age ranged from 1 year to 3 years of age post metamorphosis, with 60% of males (15/25) aged at two years post metamorphosis. Descriptive statistics for call and colour variables of male *P. pengilleyi* are summarised in Appendix Table A2. Male nest site soil moisture ranged from 33.7% to 83.5% with a mean (± SE) of 56.0% (± 2.8). With regard to *Bd* infection, 40% of males (10/25) were infected with *Bd*. Males infected with *Bd* did not differ in average age (Kruskal-Wallis test: $\chi^2 = 0.5774$, $P = 0.447$), luminance (Kruskal-

300 Wallis test: $\chi^2 = 0.1108$, $P = 0.739$), chroma (Kruskal-Wallis test: $\chi^2 = 0.308$, $P = 0.579$) or nest soil
301 moisture (Kruskal-Wallis test: $\chi^2 = 0.308$, $P = 0.579$) compared to uninfected males.
302
303 *Effects of Bd status, age, nest soil moisture and air temperature on male calling*
304 The best model to explain a male's average pulse repetition rate contained male *Bd* status, age and
305 soil moisture as explanatory variables (see Appendix Table A3). Males infected with *Bd* had a
306 significantly higher pulse repetition rate than uninfected males ($t = 2.318$, $P = 0.031$, Figure 2).
307 There was a significant negative relationship between male age and pulse repetition rate (younger
308 males had a higher pulse repetition rate; $t = -2.297$, $P = 0.032$). There was also a significant
309 negative relationship between soil moisture and pulse repetition rate ($t = -2.728$, $P = 0.013$). The
310 best model to explain a male's average call duration contained male *Bd* status as the explanatory
311 variable (see Appendix Table A4), however this effect was not significant ($t = 1.726$, $P = 0.098$).
312 There were six models within 2 delta AIC of the top ranked model, however the model weights
313 were low (see Appendix Table A4). The best model to explain a male's average call frequency was
314 the intercept only model (see Appendix Table A5). There were four models within 2 delta AIC of
315 the top ranked model, however the model weights were also low and no models were statistically
316 significant (see Appendix Table A5).
317
318 *Variation in male mating success*
319 Of the males included in the analyses ($N = 25$), 68% (17/25) of males received matings (number of
320 eggs > 0). The number of eggs in a single male's nest ranged from 17 to 164 eggs (Figure 3), with
321 an average of 68.5 eggs per nest.
322
323 *Predictors of male mating success*
324 *Probability of mating*

The best model to explain the probability of a male mating contained age as the only predictor variable, with a significant positive effect of increasing age (Table 1, Figure 4).

Total number of eggs

The best model to explain variation in the number of eggs contained the interaction between male *Bd* status and average pulse repetition rate of his call (Table 1). When average pulse repetition rate was low, uninfected males had the highest number of eggs. However, when average pulse repetition rate was high, males infected with *Bd* had higher numbers of eggs than uninfected males (Figure 5).

Discussion

We investigated whether infection with the amphibian chytrid fungus *(Bd),* as well as male age, body size, nest moisture and multiple components of male calling and colouration predicted male mating success in the critically endangered northern corroboree frog. Parentage analysis revealed that 68% of males achieved mating success, though there was a skew in the number of eggs males received, even among the males that mated. The most successful males received approximately ten times more eggs than the least successful males. This reproductive skew is common in anuran amphibians, as well as many other animal taxa (e.g. Bray, Pusey, & Gilby, 2016). The probability of a male mating was best predicted by male age (with females preferring older males), and variation in total egg number was most strongly explained by an interaction between male *Bd* infection status and call pulse repetition rate.

Effect of age on male mating success

There may be several reasons why older males were more likely to achieve mating success. In resource-based mating systems, older males may have greater reproductive experience and provide females with higher quality resources, such as nests (Kokko & Lindstrom, 1996; Felton et al. 2006).

In the present study, male age and nest soil moisture were not related, but there may be other nest characteristics, such as nest architecture that influence female preferences (Felton et al. 2006). Another possibility is that females prefer older males because they confer genetic benefits on their offspring (in line with good-genes models of sexual selection) (Beck & Powe, 2000; Brooks & Kemp, 2001). Specifically, male age can act as a viability-indicator; whereby older males demonstrate their high genetic quality by simply surviving (Brooks and Kemp 2001). Increased longevity might also signal that males have accumulated fewer deleterious mutations and are better adapted to current environmental conditions (Brooks and Kemp 2001). In the present study, older males were larger, and also had lower pulse repetition rates, so females might be able to assess male age (and quality) using various phenotypic cues. Assuming this is the case, a critical next step will be to investigate the extent to which male age and genetic quality in *P. pengilleyi* are linked.

Effect of colour and nest soil moisture on male mating success

We found no effect of male colouration (hue, chroma or luminance) on male mating success. The most likely explanation for this finding is that colouration in *P. pengilleyi* has evolved via natural selection and functions as an aposematic warning signal to predators, rather than a sexual signal. This explanation is supported by fact that *P. pengilleyi* does not exhibit obvious sexual dichromatism, and that both males and females possess toxic lipophilic alkaloids in their skin (pumiliotoxins and pseudophrynamines) (Daly, Spande & Garrafo, 2005). Additionally, we found no effect of nest soil moisture on male mating success. This result was surprising because female mate choice in resource-based mating systems is often inextricably linked to the quality of breeding resources provided by a male (Andersson, 1994). It is possible that our single measurement of nest soil moisture did not capture ecologically relevant variation in moisture availability. Egg development is protracted in *P. pengilleyi* (lasting weeks to months), so females might be making strategic nesting decisions based on cues that predict the potential for nests to retain optimal soil

375 moisture levels. Such predictive capacity has been reported in other frog species (Spieler &
376 Linsenmair, 1997; Rudolf & Rödel, 2005). An alternative explanation is that nest soil moisture (and
377 nest quality in general) is not a critically important component of the *P. pengilleyi* mating system.
378 This explanation is supported by our finding that there were extremely low levels of nest takeover
379 (or nest swapping), which suggests that competition for appropriate nest sites is very low.
380 Interestingly however, we found a significant negative relationship between nest soil moisture and
381 male call pulse repetition rate; males in wetter nests had lower pulse repetition rates. The
382 explanation for this pattern is not obvious because high nest moisture has previously been
383 associated with elevated calling investment in the brown toadlet *P. bibronii* (Mitchell, 2001). One
384 possibility is that high soil moisture influences the nest microclimate in a way that leads to reduced
385 investment in calling. For example, wetter nests may be cooler, constraining temperature-dependent
386 advertisement. Ongoing work will be required to ascertain the extent to which nest moisture
387 influences the reproductive behaviour of *P. pengilleyi.*
388
389 *Effect of Bd on male calling and mating success*
390 *Bd* infection status had a significant effect on male calling behaviour; infected males had
391 significantly higher pulse repetition rates than uninfected males. This result aligns with the findings
392 reported in two recent studies in tree frogs, which found that *Bd*-infected males increase their
393 calling effort (Roznik et al., 2015, An & Waldman 2016). Our result also adds to an emerging body
394 of evidence that *Bd* infection can induce various phenotypic changes in male anurans, which likely
395 impact mate attraction and reproductive success (Brannelly, Webb, Skerratt, & Berger, 2016;
396 Chatfield et al., 2013; Roznik et al., 2015). Extending from these findings, we also found that male-
397 mating success (total egg number) was predicted by a male's average pulse repetition rate, however,
398 this relationship differed depending on *Bd* infection status. These results are noteworthy because
399 they suggest that *Bd* can influence both male calling behaviour, and patterns of female mate choice.

More critically, this is the first evidence that male mating success in an amphibian can be influenced by male *Bd* infection status, drawing attention to the potential for this virulent, globally distributed, pathogen to have major indirect impacts on host fitness.

The finding that uninfected males with low pulse repetition rates received the highest numbers of eggs was unexpected because female anurans typically prefer males who invest more in calling (Gerhardt & Huber, 2002). However, in our system, males with lower pulse repetition rates were also larger and older, and older males had a higher probability of mating. Therefore, age may be the primary determinant of male attractiveness. An alternative explanation is that uninfected males with highly pulsatile calls are more aggressive, and that females avoid these males to reduce mating costs, as reported in other vertebrates (Ophir, Persaud, & Galef Jr, 2005). However, this seems unlikely, as there is no evidence for forced copulation or sexual coercion in *P. pengilleyi.* Adding to the complexity of our findings was the result that infected males had higher mating success than uninfected males when pulse repetition rate was high. While the reason for this relationship is unclear, one explanation may be that pulse repetition rate is an uninformative threshold cue (Candolin, 2003). Females may ignore pulse repetition rate at lower values, with mating decisions based on other age-related cues, but, once pulse repetition rate surpasses a threshold, pay greater attention to this cue. High pulse repetition rate may not signal mate viability or any direct benefits, but may exploit a pre-existing sensory bias (Candolin, 2003). Indeed, there is some evidence that female frogs are intrinsically more sensitive to call traits that deviate from the population mean (Ryan & Rand, 1990). Another possibility is that female preference for high pulse repetition rate is a remnant from past selection. This could occur, for example, if high pulse rate in ancestral populations reduced costs of mate assessment by facilitating signal detection and/or reception (Candolin, 2003). Clearly, until further research is conducted, explanations for our complex findings will remain speculative. Nevertheless, assuming that male-male competition plays

425 a limited role in male mating success in *P. pengilleyi*, our findings imply that female preferences
426 can be altered by male disease status. Pathogen and parasite-mediated models of sexual selection
427 propose that disease infection may facilitate sexual selection by mediating changes in aspects of a
428 host's phenotype (Klein, 2003; Beltran-Bech & Richard, 2014). Most commonly, these changes are
429 reported to inhibit courtship behaviour or diminish secondary sexual traits (Beltran-Bech &
430 Richard, 2014). However, empirical evidence in numerous vertebrate species indicates that
431 following infection, pathogens can directly manipulate aspects of host physiology and behaviour to
432 increase transmission (Klein, 2003). In particular, pathogens can mediate changes in courtship
433 behaviour or secondary sexual traits that result in an increase in contact between infected and
434 uninfected individuals (Klein, 2003). Alternatively, pathogen-induced phenotypic changes may be
435 host-mediated (as opposed to pathogen mediated). This may occur when individuals threatened with
436 disease allocate more resources to current reproductive effort due to a heightened risk of diseased-
437 induced mortality (referred to as the terminal-investment hypothesis) (Clutton-Brock, 1984).
438
439 More broadly, our findings add to a growing body of evidence that pathogens can alter the
440 reproductive biology of their hosts (Klein, 2003). Specifically, our findings add to extensive
441 observational and empirical evidence across multiple animal groups that pathogens can modify
442 various aspects of animal behaviour, spanning foraging, movement, anti-predatory and social
443 behaviour (Hawley, Davis, & Dhondt, 2007; Lefcort, Lefcort, Eiger, & Eiger, 1993). The major
444 advance made by our study, however, is that it was conducted in a wild population, and considered
445 interactions between infection and sexual signals across multiple signaling pathways. Through this
446 approach we draw attention to the likelihood that interactions between disease and behaviour are far
447 more complex that currently realised. Regardless of the mechanisms causing behavioural change,
448 resultant impacts on mating success can have profound effects on population dynamics and
449 population viability (McKnight, Schwarzkopf, Alford, Bower, & Zenger, 2017). By altering male

traits that likely signal either direct or indirect benefits, infectious disease could have pervasive, though currently underappreciated, indirect impacts on individual fitness. In turn, such impacts could alter the strengths and targets of sexual selection and contribute to mating system variation, both within and across populations (Ashby & Boots, 2015; Kokko, Ranta, Ruxton, & Lundberg, 2002). Both factors could affect the direction and tempo of evolution (Ashby & Boots, 2015; Lockhart, Thrall, & Antonovics, 1996). Continuing to explore indirect effects of disease on mating behaviour and individual reproductive success will broaden our understanding of the dynamic interplay between disease and sexual selection.

Conservation implications for P. pengilleyi

Our findings may help direct *P. pengilleyi* captive breeding and reintroduction. Under existing captive breeding protocols females are offered a choice of multiple males (typically 4-6) based on a maximum avoidance of inbreeding (MAI) genetic breeding scheme (OEH, 2012). Based on our findings, we conclude that maintaining this approach is appropriate. However, there may be scope for manipulating the group composition of males offered to females (in line with the overarching genetic management plan) to maximize reproductive output. For instance, conservation managers may consider maintaining a broad age demographic in captive populations and manipulating the age cohort of males presented to females to maximize mating success. Although, it is important to recognise that patterns of mate choice may differ between wild and captive populations due to the presence of *Bd*. As such, we recommend that patterns of female mate choice also be investigated in captivity. In regard to reintroduction, current *P. pengilleyi* release strategies generally involve releasing 1-year-old frogs (McFadden et al., 2016). However, there may be value in releasing older frogs (2-3 years post-metamorphosis), if these individuals have a higher probability of achieving reproductive success. Supplementing wild populations with older individuals may help maintain a broad age structure and substantially increase recruitment.

475

476 *Global conservation implications*

477 More broadly, the results of this study have significant implications for the conservation and
478 management of threatened amphibians globally. Our findings emphasise the potential for *Bd* to alter
479 reproductive processes in declining populations of endangered amphibians, spanning many
480 hundreds of species worldwide. Given this potential, we encourage conservation managers to
481 explore whether disease-mediated changes in reproductive behaviour need to be incorporated into
482 conservation practices. Knowledge of how *Bd*-induced changes in sexual selection processes impact
483 an individual's reproductive success and probability of infection (with subsequent flow on effects
484 for population growth, viability, genetic diversity and mating system variation) will likely be
485 critical to the design and operation of conservation breeding programs, as well as the management
486 of reintroduced and wild populations (Herrera & Nunn, 2019; Hoverman & Searle, 2016). As the
487 amphibian chytrid fungus is the most destructive wildlife disease ever recorded (Scheele et al.
488 2019), we emphasise the urgent need to account for the interplay between *Bd* infection and
489 behaviour in amphibian conservation actions.

490

491 *Conclusion*

492 The results of our study suggest that disease influences male calling behaviour and mating
493 success (likely reflecting female mate preferences), in *P. pengilleyi*. *Bd* infected males produced
494 calls with significantly higher pulse repetition rates than uninfected males, and variation in total egg
495 number was strongly explained by an interaction between male *Bd* infection status and call pulse
496 repetition rate. This is the first evidence that male mating success in an amphibian can be influenced
497 by *Bd*, drawing attention to a novel mechanism through which this potentially fatal, globally-
498 distributed pathogen can indirectly influence amphibian fitness. From a conservation perspective,
499 our findings highlight the potential for a pervasive disease to alter reproductive outcomes in

500 threatened amphibian populations, and emphasise the urgent need to integrate knowledge of
501 reproductive ecology into conservation and management plans.
502
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522
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Appendix

Study site

This study was conducted on one of the largest remaining wild populations of *P. pengilleyi*, during the species' breeding season (February to March). The study site was located within Bondo State Forest, approximately 25 kilometres east of Tumut in New South Wales, Australia. Specific location details have been withheld for conservation security reasons. The study site was at an elevation of approximately 1000 metres above sea level, and was a discrete patch, approximately two hectares in size, containing aquatic herb field and montane bog heathland vegetation communities (vegetation communities A9 and E59 as described in the NSW Office of Environment and Heritage Report, 2011). The area adjacent to the study site was montane woodland dominated by the tree *Eucalyptus stellulata*. The study site contained numerous seasonally inundated pools ranging in size from 1 m^2 to 15 m^2, which provide breeding habitat for *P. pengilleyi*. The dominant vegetation fringing these pools consisted of the sedge *Carex gaudichaudiana*, the grass tussock *Poa labillardierei*, and rope grass *Empodisma minus*. The majority of male *P. pengilleyi* nest sites were at the base of *P. labillardierei* tussock grass, but some nest sites were also found within the other plant species fringing the ephemeral breeding pools.

Call analysis

P. pengilleyi produces two main call types while advertising to females; a two-part call and a one-part call (Pengilley 1971; Pengilley 1973) (Appendix Figure A1). For the present study, we focused our call analysis on the one-part call only, as this call type was recorded for nearly all individuals and was the predominant call type being made by males during the sampling period. The two-part call was only recorded for a smaller subset of the sampled males (36%, and was therefore not used

751 in this analysis). Oscillograms and spectrograms of the different call types are presented in
752 Appendix Figure A1. Calls were excluded for nine males, as their individual call traits could not be
753 accurately analysed; two of the nine males shared the same nest, so we were unable to determine
754 which male was calling on the recording whilst the other seven males had significant call overlap
755 on their recordings and thus we were unable to determine which calls belonged to the focal male.
756 Call recordings were analysed using the sound analysis software Raven Pro v 1.5 (Cornell Lab of
757 Ornithology, Bioacoustics Research Program, 2014). Temporal properties (call duration and pulse
758 repetition rate) were obtained from oscillograms. Frequency information was obtained using Fast
759 Fourier transformation, with a Blackmann window function (window size = 1024 samples, 3 db
760 bandwidth = 70.7 Hz, DFT = 2048 samples, grid spacing = 21.5 Hz, with 50% overlap). For each
761 male, multiple individual calls were analysed (2-6 calls per male), beginning with the first call on
762 the recording and then the next (1-5) successive calls. Individual call measurements were calculated
763 as follows: average call duration (average length from the beginning of the first pulse to the end of
764 the last pulse of the call in seconds), average pulse repetition rate (average number of pulses per
765 second) and average call peak frequency (frequency occurring at the highest amplitude, Hz). Calls
766 were recorded at varying air temperatures (ranging between 6.6-22°C degrees, with 84% of calls
767 recorded between 10°C and 13°C), however temperature did not influence any of the call
768 parameters (see Tables A3-A5).
769
770 *Colour analysis*
771 To determine whether aspects of male coloration influence mating success in *P. pengilleyi*, we
772 obtained average red (R), green (G) and blue (B) values from the yellow stripes on the entire dorsal
773 surface (head, body and legs) of each male using a custom Matlab script (Mathworks Inc., Natick,
774 MA, USA) written by J.A.E. Detailed descriptions for calculating the average R,G,B values using
775 this custom script are described elsewhere (see Smith et al. 2016; Cadena et al. 2017; Cadena et al.

776 2018). In brief, raw RGB values were first calibrated against the known reflectance of 6 gray
777 standards in the X-rite ColourChecker passport, which appeared in every photo, under the same
778 camera settings and lighting conditions; see Appendix Figure A2. This procedure ensured a
779 calibrated relationship between the camera responses (R,G,B) and reflectance (Smith et al. 2016;
780 Cadena et al. 2017; Cadena et al. 2018). Calibrated and standardized RGB values from each digital
781 photo where then used to determine a two-dimensional representation of colour space; by
782 calculating hue, chroma and luminance values using a separate custom MATLAB script written by
783 J.A.E (for detailed descriptions of hue, chroma and luminance calculations please see Smith et al.
784 2016, Cadena et al. 2017 and Cadena et al. 2018). In a two-dimensional representation of colour,
785 physical hue represents the colour, measured as the angle relative to the axis (Endler 1990, Cadena
786 et al. 2018) and chroma is the 'purity' or 'saturation' of the colouration, and is measured as the
787 distance from the origin (Endler 1990; Cadena et al. 2018). Luminance represents the brightness of
788 the colouration and was calculated as the sum of the standardized RGB values (Endler 1990;
789 Cadena et al. 2018). These are first approximations of appearance when we do not have data on the
790 colour vision of the frogs or their (unknown) predators.
791
792 *Age determination*
793 Skeletochronology was used to determine the age of male *P. pengilleyi*. Skeletochronology
794 involved decalcifying whole digits in 10% formic acid for 14 hours, followed by rinsing in running
795 water for 3 hours. Samples were then vertically embedded in paraffin wax and sectioned using a
796 rotary microtome to cut 10µm sections. The entire second phalange was sectioned to ensure that the
797 mid diaphysis region, which contains the best sections for aging, was identified. Sections were
798 mounted on slides and stained for 30 minutes using Harris's haematoxylin and mounted with a 60
799 mm cover-slip using D.P.X. mounting fluid to create a permanent mount. Lines of arrested growth
800 were counted under 400x magnification using a light microscope. To improve age estimation

accuracy, each individual was aged twice without reference to the previous result (with complete congruence). 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 (Smirina, 1994), such as our study region, where *P. pengilleyi* exhibits an extended period of inactivity during winter. Skeletochronology has been successfully applied and validated with repeat sampling and individual identification in the closely related *P. corroboree* (Hunter, 2000).

Statistical Analyses – Model selection

To investigate variables influencing male mating success, we used zero-altered negative binomial (ZANB) generalized linear models, using the function *hurdle* in the R package *pscl* version 1.5.2 (Zeileis et al. 2008). Data were modeled in two parts: 1) a binomial logistic regression with male mating success (yes or no) as the response variable, and 2) a negative binomial GLM of the count data, with the number of eggs as the response variable, excluding zeros (Zuur 2009). The two parts of the model explain; 1) the effect of the predictors on whether or not a male mated at all, and 2) the effect of the predictors on mating success for the males that did mate (the total number of eggs). Because our sample size was smaller than anticipated (n = 25), limiting the number of explanatory variables that could be incorporated in the same model, we first built eight separate models each with one of the following explanatory variables: (1) chroma, (2) luminance, (3) age, (4) average call frequency, (5) average call duration, (6) average pulse repetition rate, (7) soil moisture, and (8) male *Bd* status. All explanatory variables, except male *Bd* status (binary, 0 = not infected, 1 = infected), were fit as continuous variables. We then compared these eight models using the AIC function in R and also examined the model coefficients to determine whether the variable in question was important in the binomial or count part (or both) of the ZANB model. Based on the

lowest AIC values, we retained the following variables for further investigation: luminance, age, average pulse repetition rate, and male *Bd* status (see Appendix Table A6). We then constructed a small set of candidate models informed by previous research to investigate associations with mating success (Appendix Table A7). Based on the initial single variable models, age and *Bd* status were included in both the binomial and count parts of the models. Average pulse repetition rate and luminance were included in the count part only. Because we previously found male *Bd* status was associated with male average pulse repetition rate (see Results section 3.3), we also included an interaction between these two variables. No other interactions were fit given our small sample size. We then compared this subset of candidate ZANB models based on their AIC values using the AIC function in R. The model with the lowest AIC value was considered to provide the best fit. Predictions were calculated using the top ranked model using the function *predict.hurdle* from the package *pscl* version 1.5.2 (Zeileis et al. 2008). We investigated model fit by visually examining diagnostic plots of residual versus fitted values for each predictor variable. All statistical analyses were completed in R version 3.5.1 (The R Foundation for Statistical Computing, 2018).

852 **Table 1.** Coefficient estimates of variables from the best fitting zero-altered negative binomial

853 (ZANB) generalized linear model of male mating success in *P. pengilleyi* (*N* = 25).

Model Terms				
Negative binomial count model	β	SE	z-value	*P*
Intercept	6.060	0.405	14.946	<0.001
Bd	-1.471	0.571	-2.574	**0.010**
Average pulse repetition rate	-0.023	0.004	-5.144	**<0.001**
Bd * Average pulse repetition rate	0.020	0.006	3.526	**<0.001**
Binomial logistic probability model				
Intercept	-3.766	2.173	-1.733	0.080
Age	2.306	1.109	2.078	**0.038**

854 Significant effects are indicated in bold.

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872 **Table A1.** Details of parentage assignment in *Pseudophryne pengilleyi* population (*N* = 36).

Nest	ID of resident male	ID of genetic sire	Number of eggs	Number of contributing females (estimate)
3	3	3	25	1
4	4	4	60	1
5	5	5	164	At least 2
8	8	8	160	At least 2
17	17	17	20	1
18	18	18	84	At least 2
19	19	19	76	At least 2
24	24	24	56	1
25	25	25	18	1
28	28	28	61	1
29	29	29	89	Unknown
33	33	33	20	1
36	36	36	57	1
44	44	44	40	1
46	46	46	83	At least 2
47	47	Unknown male	21	1
49	49	58	32	1
52	52	52	82	1
53	53	53	32	1
55	55	55	96	At least 2
58	58	58	17	1
59	59	59	60	At least 2
60	60	60	108	At least 2
61	61	61	74	At least 2
2	2	N/A	0	N/A
13	13	N/A	0	N/A
14	14	N/A	0	N/A
16	16a	N/A	0	N/A
16	16b	N/A	0	N/A
26	26	N/A	0	N/A

34	34	N/A	0	N/A
45	45	N/A	0	N/A
50	50	N/A	0	N/A
54	54	N/A	0	N/A
56	56	N/A	0	N/A
57	57	N/A	0	N/A

Table A2. Summary of descriptive statistics for call characteristics (call duration, pulse repetition rate and frequency) and colour variables (average red (R), blue (B) green (G) values, hue, chroma and luminance) in male *P. pengilleyi* ($N = 25$).

	Minimum	Mean ± SEM	Maximum
Call duration (sec)	0.3778	0.6119 ± 0.04	1.0685
Pulse repetition rate (pulses/sec)	33.69	95.30 ± 6.70	148.79
Call frequency (Hz)	2334.22	2911.80 ± 44.05	3179.73
R	0.4319	0.4655 ± 0.004	0.5064
G	0.4464	0.4740 ± 0.003	0.4962
B	0.0434	0.0605 ± 0.002	0.0778
Hue	-66.74	-58.90 ± 0.78	-52.10
Chroma	0.4369	0.4102 ± 0.002	0.3833
Luminance	0.4392	0.3934 ± 0.006	0.3118

Values for call characteristics are based on averages per individual (2-6 calls per individual).

924 **Table A3**. Akaike's information criterion model rankings for the different candidate models to
925 investigate the effect of male age, *Bd* status, nest soil moisture and air temperature on average call
926 pulse repetition rate.

Model Terms	**AIC_c**	**ΔAICc**	***W***
Age + Bd + Soil moisture	244.9	0.00	0.383
Bd + Soil moisture	247.4	2.45	0.113
Age + Soil moisture	247.5	2.54	0.108
Soil moisture	248.0	3.07	0.083
Age + Bd + Soil moisture + Temperature	248.2	3.33	0.072
Age + Soil moisture	249.3	4.43	0.042
Bd + Soil moisture + Temperature	249.5	4.57	0.039
Bd	249.9	4.98	0.032
Intercept only	250.1	5.16	0.029
Age + Soil moisture + Temperature	250.5	5.56	0.024
Age	250.6	5.67	0.022
Soil moisture + Temperature	250.8	5.86	0.020
Bd + Temperature	252.1	7.18	0.011
Age + Bd + Temperature	252.4	7.49	0.009
Temperature	252.6	7.73	0.008
Age + Temperature	253.3	8.40	0.006

927 ΔAICc is the difference in AICc compared with the model with the lowest AICc. *W* is the weight
928 the model carries, presented as a proportion.
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940 **Table A4**. Akaike's information criterion model rankings for the different candidate models to
941 investigate the effect of male age, *Bd* status, nest soil moisture and air temperature on average call
942 duration.

Model Terms	**AIC$_c$**	**ΔAICc**	***W***
Bd	6.9	0.00	0.156
Intercept only	7.4	0.45	0.125
Temperature	7.6	0.63	0.114
Age	8.0	1.08	0.091
Age + Bd	8.3	1.38	0.078
Bd + Temperature	8.7	1.73	0.066
Bd + Soil moisture	8.7	1.74	0.065
Soil moisture	9.0	2.03	0.056
Soil moisture + Temperature	9.2	2.22	0.051
Age + Soil moisture	9.4	2.50	0.045
Age + Temperature	9.4	2.51	0.045
Age + Bd + Soil moisture	10.0	3.05	0.034
Bd + Soil moisture + Temperature	10.6	3.61	0.026
Age + Bd + Temperature	10.9	3.97	0.021
Age + Soil moisture + Temperature	11.1	4.12	0.020
Age + Bd + Soil moisture + Temperature	12.9	5.92	0.008

943 ΔAICc is the difference in AICc compared with the model with the lowest AICc. *W* is the weight
944 the model carries, presented as a proportion.
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Table A5. Akaike's information criterion model rankings for the different candidate models to investigate the effect of male age, *Bd* status, nest soil moisture and air temperature on the average call frequency.

Model Terms	**AIC$_c$**	**ΔAICc**	***W***
Intercept only	344.2	0.00	0.205
Temperature	344.5	0.25	0.181
Soil moisture	345.6	1.43	0.100
Bd + Temperature	345.8	1.64	0.090
Soil moisture + Temperature	345.9	1.66	0.089
Bd	346.5	2.30	0.065
Age	346.7	2.45	0.060
Age + Temperature	347.3	3.09	0.044
Bd + Soil moisture + Temperature	347.4	3.19	0.042
Bd + Soil moisture	348.2	3.96	0.028
Age + Soil moisture	348.2	4.04	0.027
Age + Bd + Temperature	349.0	4.79	0.019
Age + Soil moisture + Temperature	349.0	4.82	0.018
Age + Bd	349.1	4.93	0.017
Age + Bd + Soil moisture + Temperature	350.9	6.69	0.007
Age + Bd + Soil moisture	351.0	6.76	0.007

ΔAICc is the difference in AICc compared with the model with the lowest AICc. *W* is the weight the model carries, presented as a proportion.

Table A6. Akaike's information criterion model rankings for the different candidate models to investigate the effect of male age, *Bd* status, chroma, luminance, average call duration, average call frequency, average pulse repetition rate and nest soil moisture separately on male mating success in *P. pengilleyi*.

Model terms	**AIC**	**ΔAIC**
Age	200.5195	0.00
Luminance	203.2219	2.7024
Average pulse repetition rate	204.6031	4.0836
Bd	204.8449	4.3254
Soil moisture	206.5194	5.9999
Average call duration	207.9469	7.4274
Average call frequency	207.9564	7.4369
Chroma	208.5164	7.9969

ΔAIC is the difference in AIC compared with the model with the lowest AIC.

Table A7. Akaike's information criterion model rankings for the different candidate models to investigate the effect of male age, *Bd* status, average pulse repetition rate and luminance on male mating success in *P. pengilleyi*. Variables to the left of | are included in the binomial portion of the model only. ΔAIC is the difference in AIC compared with the model with the lowest AIC.

Model terms	**AIC**	**ΔAIC**
Age | Bd * Average pulse repetition rate	188.9951	0.00
Age | Luminance	196.2672	7.2721
Age | Bd + Average pulse repetition rate	196.3170	7.3219
Age | Bd + Average pulse repetition rate + Luminance	197.1575	8.1624
Age | Average pulse repetition rate	197.9692	8.9741
Age | Bd + Luminance	198.0519	9.0568
Bd | Luminance	199.2315	10.2364
Bd | Average pulse repetition rate + Luminance	200.1881	11.193
Bd | Age + Average pulse repetition rate + Luminance	200.7628	11.7677
Bd | Average pulse repetition rate	200.9334	11.9383
Age | Bd	201.8807	12.8856
Bd | Age + Average pulse repetition rate	202.5291	13.534
Bd | Age	203.4838	14.4887

Variables to the left of | are included in the binomial portion of the model only. ΔAIC is the difference in AIC compared with the model with the lowest AIC.

Figure 1. Male adult northern corroboree frog on a nest of eggs. Photo credit – Rohan Bilney

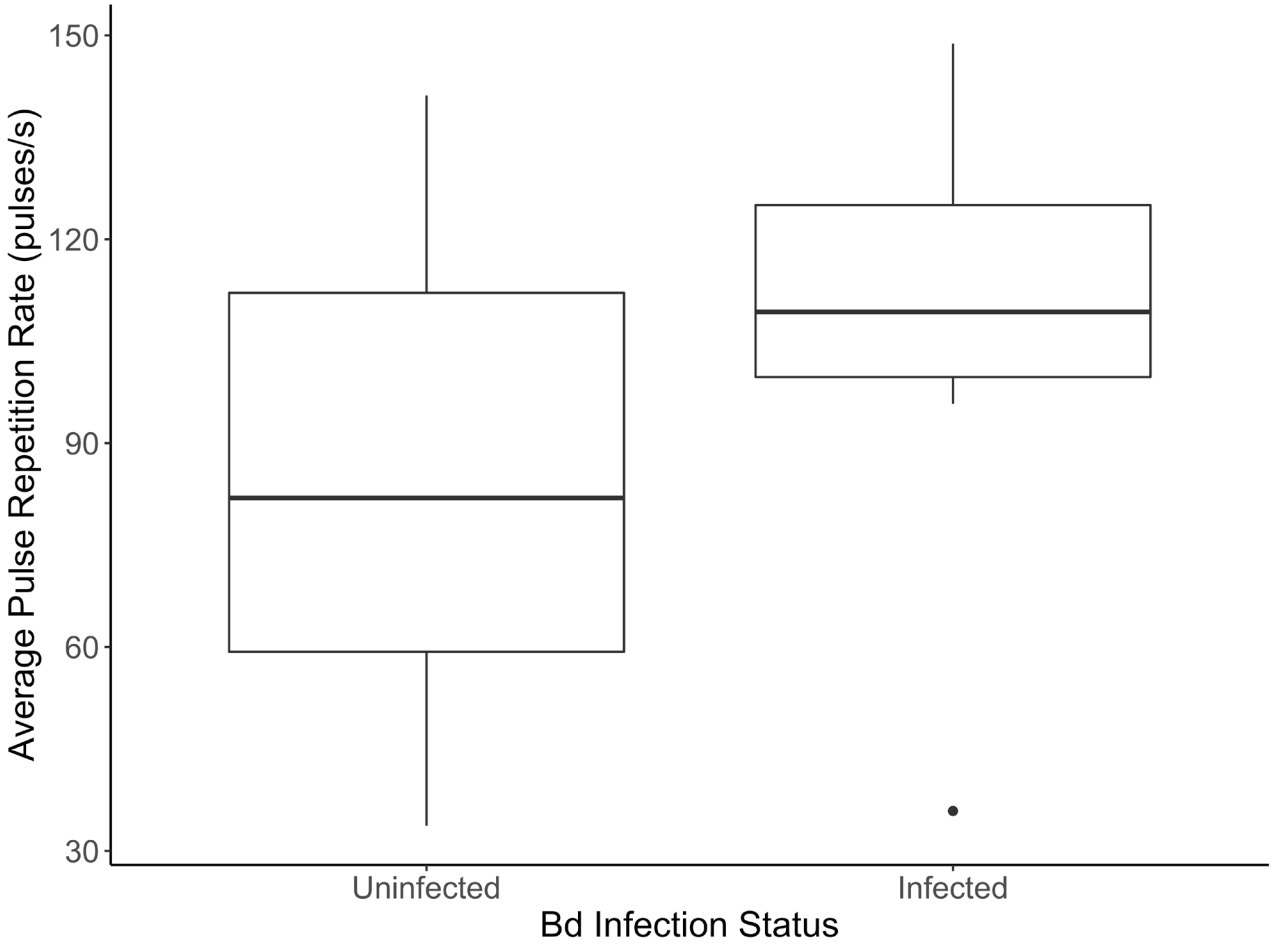

Figure 2. The effect of *Bd* infection status (uninfected, $N = 15$ and infected, $N = 10$) on average call pulse repetition rate (pulses/second) in male *P. pengilleyi*. Bars in box and whisker plots indicate sample medians, boxes indicate upper and lower quartiles, whiskers show sample minimum and maximum, and closed circles indicate outliers.

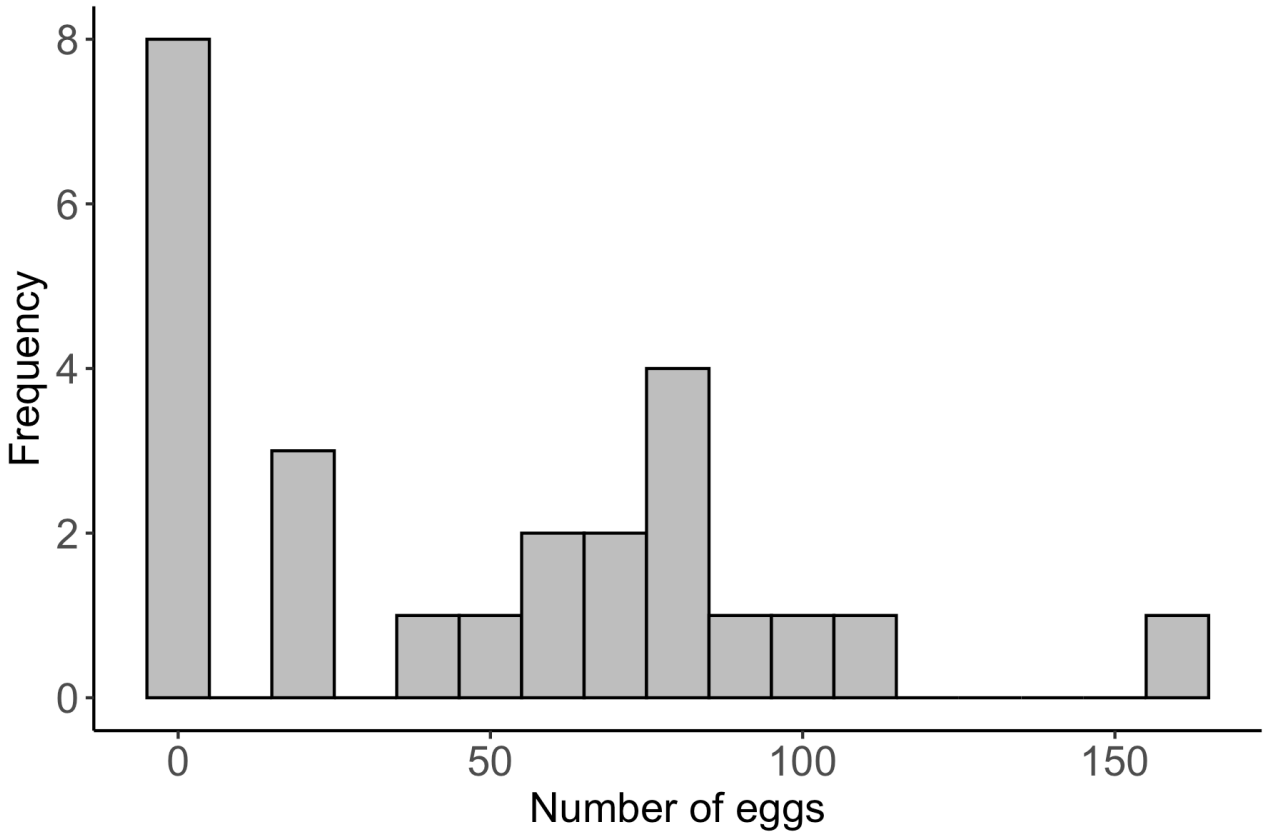

Figure 3. Frequency histogram of the distribution of eggs in individual male *P. pengilleyi* nests ($N = 25$).

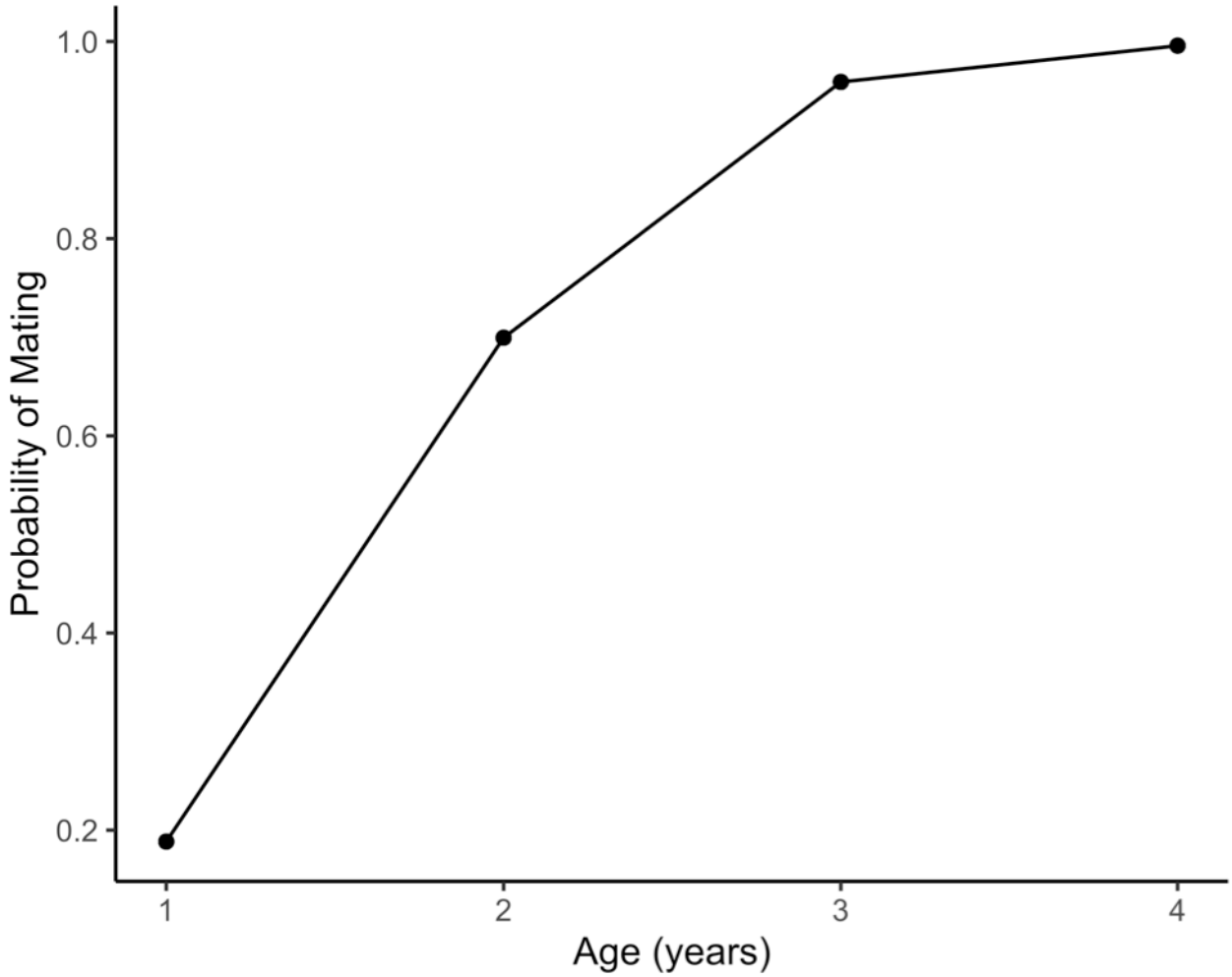

Figure 4. Model predictions showing the effect of male age on the probability of a male *P. pengilleyi* mating (number of eggs > 0) or not mating (no eggs).

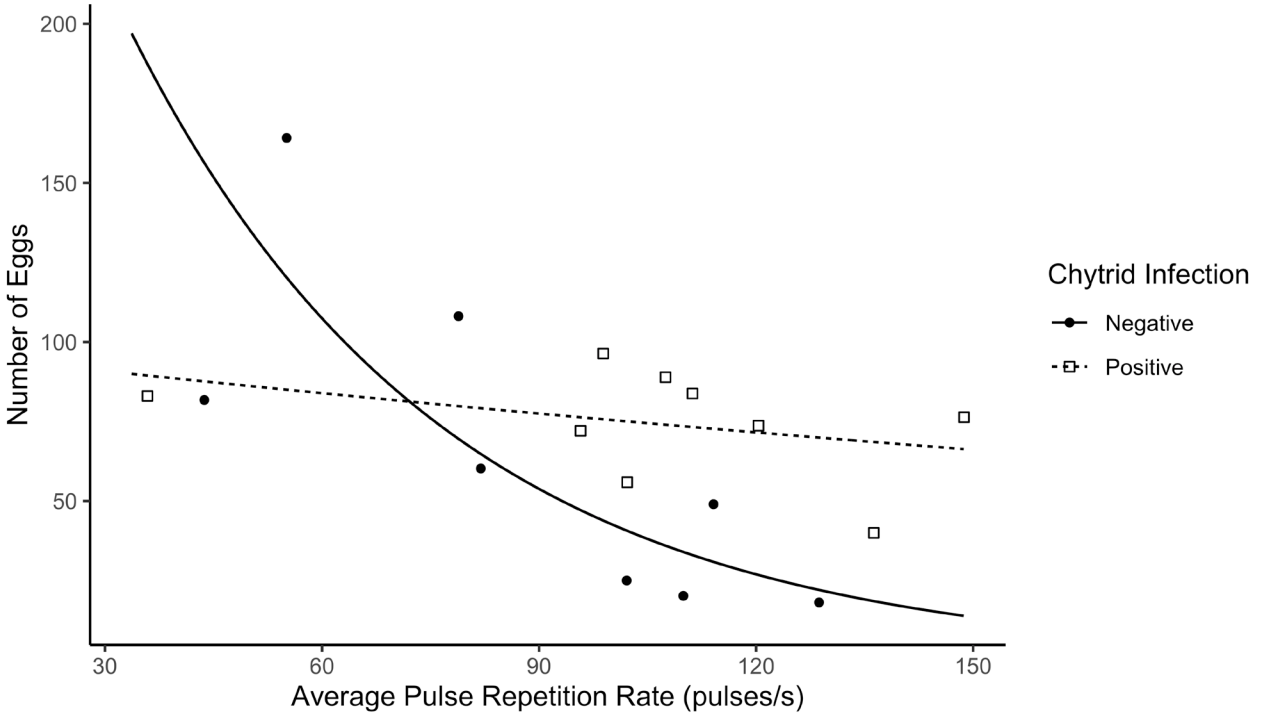

Figure 5. Model predictions showing the effect of average call pulse repetition rate (pulses/second) and *Bd* infection (positive or negative) on male *P. pengilleyi* mating success (number of eggs > 0). Lines are predictions using the top ranked model. The solid line represents predictions for uninfected males and the dashed line represents infected males. Dots are raw data points, solid circles are uninfected males ($N = 8$), open squares are infected males ($N = 9$).

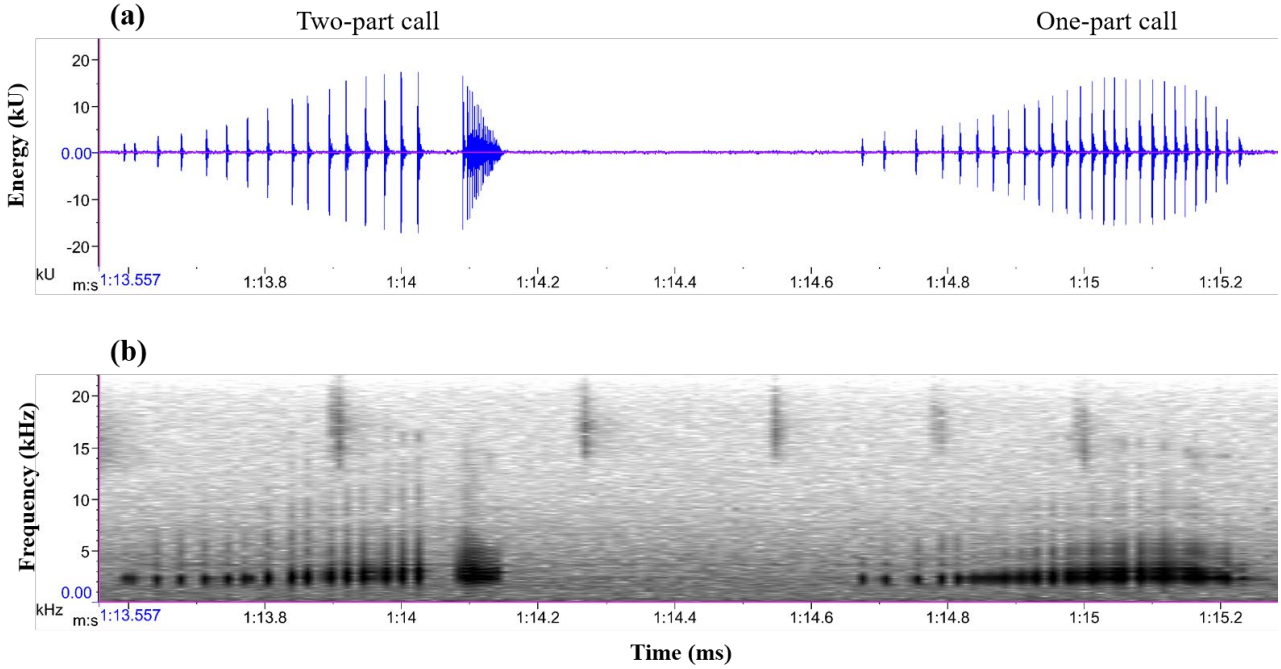

Figure A1. (a) Oscillogram and (b) spectrogram of two *P. pengilleyi* call types; a two-part call and one-part call.

Figure A2. Example of a standardized colour photo of male *P. pengilleyi* used to measure the luminance, hue and chroma of a male's yellow colouration.