Wetland characteristics influence disease risk for a threatened amphibian

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Abstract. Identifying determinants of the probability and intensity of infections is important for understanding the epidemiology of wildlife diseases, and for managing their impact on threatened species. Chytridiomycosis, caused by the fungal pathogen Batrachochytrium dendrobatidis, has decimated populations of some amphibians. However, recent studies have identified important environmental constraints on the disease, related to the pathogen's physiological tolerances. In this study, we identified several intrinsic and extrinsic determinants of the probability and intensity of chytrid infections for the threatened growling grass frog (Litoria raniformis) in southeastern Australia, and used mark–recapture to estimate the effect of chytrid infections on the probability of survival of these frogs. Water temperature and salinity had negative effects on both the probability and intensity of chytrid infections. We coupled models of the infection process with a model of the effect of chytrid infections on the probability of survival to assess variation in the impact of chytridiomycosis between wetlands with differing temperature and salinity profiles. Our results suggest that warm, saline wetlands may be refuges from chytridiomycosis for L. raniformis, and should be priorities for protection. Our results also suggest that management actions that increase water temperature (e.g., reducing canopy shading) and salinity (e.g., complementing inflows with groundwater) could be trialed to reduce the impacts of chytridiomycosis on this species. This and other recent studies highlight the value of research on environmental risk factors for chytridiomycosis.

Key words: Batrachochytrium dendrobatidis; chytrid fungus; disease; Litoria raniformis; management; mark–recapture; risk factors; survival.

INTRODUCTION

The management of infectious diseases is a major issue for wildlife conservation (Daszak et al. 2000). Although the role that disease plays in the regulation of host density is still the subject of debate (Tompkins et al. 2011), several emerging pathogenic diseases have been shown to be the cause of abrupt declines in wildlife populations. Examples include the Tasmanian devil facial tumor disease (McCallum et al. 2009), chytridiomycosis in amphibians (Skerratt et al. 2007), canine distemper in wolves and ferrets (Laurenson et al. 1998), and white-nose syndrome in bats (Frick et al. 2010). Human-mediated spread and host naïvety have precipitated some epidemics; however, it is also apparent that infectious pathogens are more likely to suppress the abundance of their hosts in situations where population growth rates are being affected by other stressors, suggesting that the interactions between wildlife disease and other threatening processes are important for determining demographic outcomes (Daszak et al. 2001, Tompkins et al. 2011, Brearley et al. 2013). Understanding the epidemiology of infectious diseases and the ecology of their hosts is therefore fundamental to managing the impacts of these diseases on wildlife populations.

From an epidemiological perspective, identifying determinants of the probability and intensity of infections represents an important step. These determinants, or risk factors, may include both intrinsic and extrinsic variables. Properties affecting individual susceptibility, such as sex, age, body condition, and immunocompetence, provide examples of intrinsic risk factors (Nolan et al. 1998, Plowright et al. 2008, Hawley and Altizer 2011). Human-mediated spread and host naïvety have precipitated some epidemics; however, it is also apparent that infectious pathogens are more likely to suppress the abundance of their hosts in situations where population growth rates are being affected by other stressors, suggesting that the interactions between wildlife disease and other threatening processes are important for determining demographic outcomes (Daszak et al. 2001, Tompkins et al. 2011, Brearley et al. 2013). Understanding the epidemiology of infectious diseases and the ecology of their hosts is therefore fundamental to managing the impacts of these diseases on wildlife populations.
disease risks for particular individuals, cohorts, or populations (Ward et al. 2009).

Chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (hereafter chytrid fungus or chytrid), has decimated populations of amphibians in Australia, the Americas, and Europe over recent decades (Skerratt et al. 2007). Chytrid fungus is a member of the phylum Chytridiomycota, a group of heterotrophic fungi that feed on chitin, keratin, or plant detritus (Berger et al. 1998). Chytrid fungus is waterborne, and infects the outer layers of the epidermis of amphibians (Berger et al. 1998, Longcore et al. 1999). Severe infection impairs osmoregulation and electrolyte balance, and can rapidly kill susceptible amphibians (Volos et al. 2009).

Population declines attributed to chytridiomycosis have been recorded across a phylogenetically and ecologically diverse group of amphibians (Skerratt et al. 2007). The pathogen is also known to occur in a wide range of ecosystems, including high-altitude lakes in temperate regions, high- and lowland tropical forests, and coastal plains (Berger et al. 2004, Woodhams and Alford 2005, Rachowicz et al. 2006). However, recent studies have identified important environmental constraints on chytridiomycosis. Murray et al. (2011) modeled the spatial occurrence of chytrid across Australia. They found a strong positive relationship between chytrid occurrence and precipitation, and a strong negative relationship between chytrid occurrence and diurnal temperature range. The first result is consistent with the waterborne nature of the fungus, and its sensitivity to desiccation (Johnson et al. 2003). The second concurs with the sensitivity of chytrid to high environmental temperatures; the growth, survival, and pathogenicity of this fungus is significantly reduced in temperatures above 27°C (Longcore et al. 1999, Johnson et al. 2003, Woodhams et al. 2003, Piotrowski et al. 2004, Andre et al. 2008, Geiger et al. 2011). Other physiological thresholds for chytrid are also emerging as important determinants of its pathogenicity. Piotrowski et al. (2004) report that chytrid is sensitive to pH, displaying markedly higher growth and survival in a narrow band of slightly acidic to neutral conditions (pH of 6–7). Similarly, Stockwell et al. (2012) tested the sensitivity of chytrid to salinity. They observed considerably lower growth and infective capacity of the pathogen in higher salinity treatments (3–4 mg/L NaCl); a result that concurs with the negative relationship between wetland salinity and chytrid prevalence in natterjack toads (*Epidalea calamita*) reported by Bramwell (2011).

While the epidemiology of chytridiomycosis has been the subject of considerable research over the last 15 years, the dynamics of the disease have received only cursory attention for several amphibians thought to be threatened by it. The growling grass frog (*Litoria raniformis*) is one such species. *Litoria raniformis* (see Plate 1) is a large, semi-aquatic frog that was once widespread across southeastern Australia (Pyke 2002). The species is now listed as endangered, having suffered substantial population declines and extinctions over recent decades (IUCN 2011). Several lines of evidence suggest chytridiomycosis was a driver of these declines. Field and laboratory studies confirm that *L. raniformis* is susceptible to chytridiomycosis (Waldman et al. 2001, Berger et al. 2004). Population losses were also swift in some cases (Hamer et al. 2010), and coincided with chytrid-related declines in other frogs across eastern Australia (Berger et al. 1998). Furthermore, *L. raniformis* appears to have been almost completely extirpated from high-altitude locations (Hamer et al. 2010), where the annual temperature and precipitation regime is particularly suited to chytrid (Murray et al. 2011). The disease has subsequently been listed as a key threatening process for *L. raniformis* (Clemann and Gillespie 2013), but there remains an almost complete dearth of information on the epidemiology of the disease for this species (Vörös et al. 2011).

Our study had two primary aims. First, we sought to identify intrinsic and extrinsic determinants of the probability and intensity of chytrid infections in *L. raniformis*. Second, we sought to quantify the effect of chytrid infections on survival probabilities for this species using mark–recapture data. Our particular interest was the role of chytridiomycosis in the metapopulation dynamics of *L. raniformis*. Heard et al. (2012a) demonstrated a relatively high, but spatially heterogeneous, probability of extinction for populations of *L. raniformis* in the peri-urban landscapes of Melbourne, Victoria, Australia. They concluded that populations of *L. raniformis* are sensitive to a combination of deterministic and stochastic environmental stressors, citing chytridiomycosis as a likely contributor. We coupled statistical models of the infection process with a model of the effect of chytrid infections on the probability of survival to assess variation in the demographic impact of chytridiomycosis between wetlands. We discuss the implications of this work for the management of remnant populations of *L. raniformis*, including identifying and protecting wetlands that represent refuges from chytridiomycosis, and ways of manipulating wetlands to limit the impact of this disease. More generally, this study emphasizes that practical management responses to chytridiomycosis may be available in some cases, and should be a focus of research on the disease (Woodhams et al. 2011).

**Methods**

**Study area**

The study was conducted in the middle reaches of the Merri Creek catchment on the northern outskirts of Melbourne, Victoria (37°37′56″ S, 144°57′24″ E), encompassing the suburbs of Campbellfield, Somerton, and Donnybrook. The landscape is undulating, with a maximum elevation of ~300 m above sea level. The soils are predominantly volcanic basalts. The climate is
temperate, with cool winters (July mean daily temperature range = 6.0°C–13.5°C) and warm summers (January mean daily temperature range = 14.3°C–25.9°C; data available online). Annual rainfall is variable, ranging from 332–967 mm/yr, with a mean of 649 mm/yr (see footnote 4).

Mark–recapture

We undertook a mark–recapture study of 12 populations of L. raniformis during the Austral spring and summer of 2004–2005 and 2005–2006. Populations occupied chains of pools along the Merri Creek and its tributaries (Curly Sedge Creek, Kalkallo Creek), as well as farm dams, quarry pits, and swamps. Site selection proceeded with the aim of monitoring populations subject to varying environmental conditions and levels of urbanization, and the need to detect dispersal within and between clusters of wetlands (Heard et al. 2012a).

Logistical and access constraints did not permit all populations to be surveyed in both seasons. Eight spotlight surveys were conducted at all sites in the 2004–2005 season, and 10 surveys were conducted at five sites in the 2005–2006 season. Surveys were conducted on a two- or three-week rotation, as described by Heard et al. (2012b). Weather variables were recorded during each survey. Air and water temperature at each site was also monitored daily with the aid of a standardized array of temperature data loggers (Thermocron iButton, Maxim Integrated, Sunnyvale, California, USA). Electrical conductivity of water was recorded once per season using an Oakton CON11 water quality meter (Oakton Instruments, Vernon Hills, Illinois, USA).

Capture by hand or net was attempted for all L. raniformis encountered during each survey. Previously unmarked individuals were retained overnight and tagged in the laboratory the following day, using either a Passive Integrated Transponder (PIT) tag (Trovan, Melton, East Yorkshire, UK) or a Visible Implant Alphanumeric (VIA) tag (Northwest Marine Technology, Shaw Island, Washington, USA). Tagging procedures are described in detail by Heard et al. (2008) and Heard et al. (2012b). Frogs retained for tagging were released at their point of capture within 24 h.

Chytrid sampling and analysis

A haphazard sample of 521 L. raniformis from 10 populations was swabbed for chytrid zoospores on first capture. Fine-tip sterile swabs were used throughout. Frogs that were captured but not swabbed (n = 286) were missed for logistical reasons, typically delays in obtaining swabs. Ramifications of the lack of repeat swabbing and missed individuals are covered in Limitations.

A standardized swabbing protocol was implemented, in which the swab was rubbed gently over the frog’s skin on the dorsal, lateral, and ventral surfaces, as well as the inner thighs and the palms of the front feet. Swabs were stored at −18°C. Protocols employed to minimize the chance of contaminating samples or cross-infecting individual frogs are described in Appendix A.

Swabs were analyzed using quantitative polymerase chain reaction (qPCR) assays by Ecogene (Auckland, New Zealand), following Hyatt et al. (2007). These assays not only provide a sensitive test for detection of the pathogen, but also estimate the number of zoospores present on the swab (zoospore equivalents). Ecogene applied a repeated sampling scheme to each swab to account for per-test probabilities of detection that were less than one. However, only a single estimate of zoospore equivalents was provided. We refer to zoospore equivalents as zoospore load, making the assumption that the estimated number of zoospores per swab was proportional to infection intensity.

Statistical modeling

Analyses were undertaken within a Bayesian framework using OpenBUGS v. 3.1.2 (Thomas et al. 2006). Parameter estimates and their 95% credible intervals (95% CI) were estimated using Markov Chain Monte Carlo (MCMC) sampling. Convergence was assessed by analysis of two replicate Markov chains with over-dispersed starting values, and by visual inspection of chain histories. To aid convergence, all continuous covariates were centered by subtracting the mean and dividing by two standard deviations. Convergence was achieved within 20 000 MCMC samples in all cases, and parameter estimates derived from a subsequent 40 000 samples from each chain. OpenBUGS code and data for each analysis are provided in the supplement.

Following McClintock et al. (2010), we used a Bayesian formulation of a single-season occupancy model (MacKenzie et al. 2002) to model relationships between the probability of infection at first capture and covariates thought to influence that probability. These models were developed to allow estimation of site occupancy rates when the probability of detection (p) is <1. Given a survey design that entails multiple visits to each site, in which detection (coded as 1 in the analysis) or nondetection (coded as 0 in the analysis) is recorded, the approach of MacKenzie et al. (2002) allows the probability of occupancy (ψ) to be estimated while jointly estimating (and therefore accounting for) p. Our sampling design was equivalent to a multi-visit occupancy survey, because the per-test probability of detecting chytrid for each swab was <1, and three replicate tests were conducted. Applying the approach of MacKenzie et al. (2002) to these data simply requires re-interpretation of ψ and p: ψ becomes the probability of infection at first capture and p becomes the probability of detecting the pathogen during each replicate test of the swab taken at first capture.

The approach of MacKenzie et al. (2002) provides considerable flexibility to model the effects of covariates
on $\psi$. We used a linear equation and logistic link function to constrain the probability of infection of each frog $i$ ($\psi_i$) to be a function of age at first capture (age [0 = juvenile, 1 = sub-adult/adult]), water temperature at first capture (wttemp [in °C]), and electrical conductivity (ec) of the wetland of capture (ec [log$_e$ transformed, originally measured in $\mu$S/cm]). We hypothesized that juvenile *L. raniformis* (those $\leq$40 mm snout–vent length; Heard et al. 2008) would have a lower probability of infection, as the keratinized tissue that chytrid fungus requires is only found in the mouthparts of tadpoles (Knapp and Morgan 2006), which should limit the chance of infection of recently metamorphosed individuals. On the basis of past studies on the physiological tolerances of chytrid fungus, we hypothesized that increasing water temperatures would reduce the probability of infection, as increasing water temperatures would reduce the probability defined as $\psi_i$ to be the power of the number of days in the interval $s - 1$ to $s$ (see Heard et al. 2012b for further details).

A linear equation and logistic link function were used to model the effect of zoospore load on the probability of survival after first capture (Lebreton et al. 1992). Uncertainty in the infection status of each individual was propagated by embedding the model for infection status within the CJS model. As several populations were affected by a severe flood in the first year of the study, we also included a binary flood effect for the appropriate interval. Thus, $\phi_{is}$ was modeled as

$$\logit(\psi_i) = \alpha_{ij} + \beta_{age} \times \text{age}_i + \beta_{wttemp} \times \text{wttemp}_i$$

$$\alpha_{ij} = \text{Normal}(a + b \times \text{ec}_j, \sigma)$$

If infected, $= \text{Bernoulli}(\psi_i)$

(1)

where $\alpha_{ij}$ (the intercept term for frog $i$ from site $j$) is a normally distributed random effect defined by the hyper-parameters $a$ and $b$, and standard deviation ($\sigma$) to be estimated from the data, $\beta_{age}$ and $\beta_{wttemp}$ are regression coefficients, and infected $i$ is the infection status (a Bernoulli random variable with probability $\psi_i$). Uninformative priors were used for all parameters: $a$, $b$, $\beta_{age}$, $\beta_{wttemp}$ = Normal(0, 100); $\sigma$ = Uniform(0, 100); $p$ = Uniform(0, 1).

Among the frogs that were known to be infected (those with at least one positive test), we assessed the influence of frog age, conductivity, and water temperature on zoospore load (zoospores, log$_e$ transformed, originally measured in zoospores per swab) using a hierarchical linear model. We hypothesized that these variables would also influence the intensity of infection via the mechanisms cited above. Simple additive effects of frog age and water temperature at the time of capture were included. As above, the intercept was allowed to differ between sites according to their conductivity. Thus, zoospore load for each frog $i$ was modeled as

$$\log_{10}(\text{zoospores}_i) = \theta_{ij} + \beta_{zoage} \times \text{age}_i$$

$$+ \beta_{zo wttemp} \times \text{wttemp}_i + \epsilon_i$$

$$\theta_{ij} = \text{Normal}(c + d \times \text{ec}_j, \sigma_{site})$$

$\epsilon_i = \text{Normal}(0, \sigma_{resid})$ (2)

where $\theta_{ij}$ (the intercept term for frog $i$ from site $j$) is a normally distributed random effect with a mean defined by the hyper-parameters $c$ and $d$, and standard deviation ($\sigma_{site}$) to be estimated from the data. $\beta_{zoage}$ and $\beta_{zo wttemp}$ are regression coefficients, and $\epsilon_i$ is a normally distributed error term with mean of zero and standard deviation ($\sigma_{resid}$) to be estimated from the data. Parameters $c$, $d$, $\beta_{zo age}$, and $\beta_{wttemp}$ were given uninformative normal priors (Normal[0, 100]); $\sigma_{site}$ and $\sigma_{resid}$ were given uninformative uniform priors (Uniform[0, 100]).

The effect of zoospore load on the probability of survival of *L. raniformis* was assessed using a Bayesian Cormack-Jolly-Seber (CJS) model. Similar to the occupancy approach described above, CJS models are built on the assumption that the detection (1) or non-detection (0) of each individual $i$ on each survey $s$ during a mark–recapture study results from two processes: (1) the probability of survival of individual $i$ between surveys $s - 1$ and $s$ ($\phi_{is}$), and (2) the probability of recapture of individual $i$ on survey $s$ ($r_{is}$, Lebreton et al. 1992). Given variation in the length of the interval between capture occasions, we rescaled $\phi_{is}$ to be the daily probability of survival by raising it to the power of the number of days in the interval $s - 1$ to $s$ (see Heard et al. 2012b for further details).

A linear equation and logistic link function were used to model the effect of zoospore load on the probability of survival after first capture (Lebreton et al. 1992). Uncertainty in the infection status of each individual was propagated by embedding the model for infection status within the CJS model. As several populations were affected by a severe flood in the first year of the study, we also included a binary flood effect for the appropriate interval. Thus, $\phi_{is}$ was modeled as

$$\logit(\phi_{is}) = \gamma + \beta_{ch surv} \times (\text{infected}_i \times \text{zoospores}_i)$$

$$+ \beta_{flood} \times \text{flood}_{is}$$

$$\text{alive}_{is} = \text{Bernoulli}(\phi_{is}^{days \in [s-1, s]})$$

(3)

where $\gamma$ is the intercept term, $\beta_{ch surv}$ and $\beta_{flood}$ are regression coefficients, and $\phi_{is}$ is the survival status at each time step (a Bernoulli random variable with a probability defined as $\phi_{is}$ raised to the power of the number of days in the interval $s - 1$ to $s$). Uninformative normal priors (Normal[0, 100]) were used for $\gamma$, $\beta_{ch surv}$, and $\beta_{flood}$.

As well as an effect of zoospore load on the probability of survival, we hypothesized that infection intensity would influence the probability of recapture of infected individuals, due to changes in the alertness, agility, or behavior of heavily infected frogs. The probability of recapture ($r_{is}$) was modeled as
\[ \text{logit}(r_{is}) = \tau + \beta_{\text{ch.recap}} \times (\text{infected}, \times \text{zoospores}) \]

\[ \text{recapture}_{is} = \text{Bernoulli}(r_{is} \times \text{alive}_{is}) \]  

(4)

where \( \tau \) is the intercept term, \( \beta_{\text{ch.recap}} \) is a regression coefficient, and \( \text{recapture}_{is} \) is the recapture status (a Bernoulli random variable with probability \( r_{is} \), contingent on individual \( i \) remaining alive at survey \( s \)). Uninformative normal priors (Normal\([0, 100]\)) were again used for \( \tau \) and \( \beta_{\text{ch.recap}} \).

Simulations

We used the preceding statistical models to simulate variation between wetlands in the probability and intensity of chytrid infections for \( L. \ raniformis \), dependent on variation in wetland salinity and water temperature regime. Resulting impacts on survival were also simulated, enabling estimation of wetland-level variation in the effect of chytridiomycosis on the probability of survival. In each of three wetlands from our initial 10 sampling sites, and for each month of the active season (October–March), we ran 1000 simulations of infection status and intensity for an adult \( L. \ raniformis \), and recorded the survival rate (dependent on infection status and intensity) across these 1000 simulations. Matching simulations were run assuming no exposure to chytrid, enabling the change in the monthly probability of survival of an adult \( L. \ raniformis \) due to chytrid to be estimated at each wetland. Survival rate in the absence of chytrid was estimated by simply fixing the probability of infection to zero in all simulations.

For simulations assuming exposure to chytrid, frogs were randomly infected with the pathogen at the start of the relevant month by setting infection status as a Bernoulli variable with a probability defined by the fitted parameters of Eq. 1. For each wetland in each month, water temperature was set as the mean midnight water temperature recorded using the data logger array described in Mark–recapture. For infected individuals, the logarithm of zoospore load was set as a random normal variate, defined by the fitted parameters of Eq. 2. Infection status and zoospore load remained constant across the month.

Survival or death over the course of the relevant month was set as a Bernoulli variable with a probability calculated as the daily probability of survival (as per the fitted parameters of Eq. 3), raised to the power of the number of days over which the month extends. For each month in each wetland, the probability of survival (surv) was estimated as the proportion of simulations in which survival was predicted. The change in the probability of survival due to exposure to chytrid (Δsurv) for each month in each wetland was then

\[ \Delta \text{surv} = \text{surv}_c - \text{surv}_{cf} \]  

where \( \text{surv}_c \) is the estimated probability of survival in the presence of chytrid, and \( \text{surv}_{cf} \) is the estimated probability of survival for the chytrid-free scenario.

The three wetlands selected for the simulations represented the spectrum of water temperature regimes and electrical conductivity observed across our sampling sites. Pools on the Kalkallo Creek (Donnybrook) represented a cool and fresh environment, with a mean midnight water temperature across the active season of 16.1°C and conductivity of 396 \( \mu \)S/cm. Pools on the neighboring Merri Creek represented an intermediate scenario, with a mean midnight water temperature of 17.6°C and conductivity of 1145 \( \mu \)S/cm. A spring-fed quarry in Campbellfield represented warm and saline conditions, with a mean midnight water temperature of 20.8°C and conductivity of 4922 \( \mu \)S/cm. Each site is depicted in Appendix B.

Simulations were run in R version 2.15.1 (R Development Core Team 2012). Uncertainty in the fitted parameters of Eqs. 1–3 was propagated by repeating the simulations for 10000 samples of the parameters drawn from their joint posterior distribution. Code used for running the simulations is provided in the Supplement.

Limitations

Our methodology had three limitations that require further discussion. The first was the haphazard nature of swabbing, with some 286 frogs that were captured during this study going unswabbed. This occurred throughout the study, without any particular spatial or temporal pattern, and was mostly due to delays in obtaining swabs. While the reduced sample size no doubt inflated the uncertainty around our parameter estimates, we are confident that the estimates themselves are unbiased, because the individuals that were swabbed were representative of the overall sample of individuals captured during this study (Appendix C). The second limitation was the lack of repeat swabbing, necessitating the assumption of no change in disease status after first capture. In line with the results of Murray et al. (2009), we suspect that this would have inflated uncertainty around the effect of chytrid on survival and recapture probabilities, and possibly weakened the effect size. While we could not address the latter explicitly, our simulations represent the resulting uncertainty faithfully, because we propagated the error in the relevant parameter estimates through to our estimates of Δsurv.

Lastly, our use of a Cormack-Jolly-Seber model to assess the effect of chytrid infections on the probability of survival did not account for the possibility that infections could influence the emigration rate rather than the survival rate (Schmidt 2010). We acknowledge this possibility, but consider it unlikely to have biased our results, because we intensively sampled clusters of wetlands to observe dispersal events (Heard et al. 2012a), and there was no relationship between zoospore...
load and dispersal distance among the recaptured individuals ($r_S = 0.098$).

**RESULTS**

Electrical conductivity of the wetland of capture had a negative effect on the probability of infection ($\psi$) as hypothesized, although the 95% CI for this effect overlapped zero (Table 1). For a sub-adult or adult frog experiencing the mean water temperature, $\psi$ was estimated at 0.408 (95% CI = 0.283–0.538) at the wetland with the lowest conductivity among our set of sites, compared with 0.262 (95% CI = 0.155–0.396) at the wetland with the highest conductivity (Fig. 1). Juvenile frogs were also less likely to be infected than sub-adults or adults; however, water temperature at the time of capture clearly had the strongest effect on $\psi$ (Table 1). For the site with the lowest conductivity, $\psi$ for a sub-adult or adult frog captured at 13°C was estimated to be almost four times that at 27°C (13°C mean = 0.782, 95% CI = 0.627–0.895; 27°C mean = 0.201, 95% CI = 0.104–0.328; Fig. 1).

Water temperature at the time of capture also displayed the strongest effect on the zoospore load of infected individuals (Table 2). Electrical conductivity of the wetland of origin was negatively related to zoospore load (mean coefficient estimate $= -0.983$), but the 95% CI for this effect again encompassed zero (Table 2), and there was only a modest difference in the estimated zoospore load of infected frogs at the least and most saline wetlands (Fig. 2). The regression coefficient for the effect of frog age on zoospore load was also small (Table 2), and the estimated zoospore load among infected individuals was almost identical for the two age classes (Fig. 2). In contrast, at the wetland with the

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Mean</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>$a$</td>
<td>-2.039</td>
<td>-2.626, -1.419</td>
</tr>
<tr>
<td>$b$ (conductivity effect)</td>
<td>-0.611</td>
<td>-1.431, 0.156</td>
</tr>
<tr>
<td>$\beta_{\text{age}}$ (age effect)</td>
<td>1.364</td>
<td>0.801, 1.933</td>
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<tr>
<td>$\beta_{\text{temp}}$ (temperature effect)</td>
<td>-1.082</td>
<td>-1.512, -0.659</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>0.395</td>
<td>0.030, 0.986</td>
</tr>
</tbody>
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**Table 1.** Coefficients for the effect of log(electrical conductivity), frog age (juvenile [0] vs. sub-adult or adult[1]), and water temperature (°C) on the probability of infection ($\psi$).

Notes: Electrical conductivity (ec) was originally measured in $\mu$S/cm. The posterior means of all model parameters are shown, along with their 95% credible intervals (95% CI). Hyper-parameters $a$ and $b$ define the intercept for the probability of infection given wetland salinity, $\beta_{\text{age}}$ and $\beta_{\text{temp}}$ are regression coefficients, and $\sigma$ is the standard deviation of the intercept.

**Fig. 1.** Effect of electrical conductivity (ec) of the wetland of capture, frog age, and water temperature at capture on the probability of infection ($\psi$). Electrical conductivity was originally measured in $\mu$S/cm. The upper plot shows the probability of infection for a sub-adult or adult frog captured at the mean water temperature for each of the 10 sampling sites, plotted against the conductivity of those sites. The middle plot compares $\psi$ for a juvenile frog against that for a sub-adult or adult frog at the site with the lowest ec, assuming the mean water temperature. The lower plot depicts the relationship between $\psi$ and water temperature for a sub-adult or adult frog at the site with the lowest ec. Mean estimates are shown in black (points, bars, and lines) and the 95% credible intervals are shown in gray.
lowest conductivity, zoospore load for a sub-adult or adult frog was estimated to fall by a factor of almost 30 across the range of temperatures sampled, from a mean of 1005 zoospore equivalents at 13°C to 36 zoospore equivalents at 27°C (Fig. 2).

Zoospore load had a negative effect on the daily probability of survival (φ) of L. raniformis during the mark–recapture study. The mean estimate of the regression coefficient for this effect was −0.478, with a 95% CI that did not overlap zero (Table 3). Mean estimates of φ across the observed range of zoospore loads (Fig. 3) show a decrease from >0.99 given a light infection (20 zoospore equivalents or less), to 0.978 with a heavy infection (~22 000 zoospore equivalents).

Zoospore load also had a strong positive effect on the probability of recapture (r; Table 3). Using the above definitions of infection intensity, the probability of recapture for a heavily infected frog was estimated to be more than double that of a lightly infected individual (Fig. 3).

Fig. 4 depicts the estimated change in the monthly probability of survival due to chytridiomycosis (Δsurv) for an adult L. raniformis at each of the three wetlands considered. There were clear differences in Δsurv between the wetlands classed as cool and fresh, and warm and saline. For the former, the mean estimate of Δsurv was −0.119 (95% CI = −0.268 to −0.066) in the coolest month of the active season (October), and averaged −0.095 across the season. Estimates of Δsurv for the warm and saline wetland were substantially lower, with an October mean of −0.037 (95% CI = −0.110−0.017) and average of −0.028 across the season (Fig. 4). Importantly, the upper 95% CI for Δsurv was lower than zero for the three coldest months of the season in the cool and fresh site, providing clear evidence of an effect of chytridiomycosis on survival rates in those months at that location, whereas all 95% CIs of Δsurv overlapped zero for the warm and saline wetland. In the wetland with an intermediate salinity and water temperature profile, an unequivocal decrease

Table 2. Coefficients for the effect of log$_e$(electrical conductivity), frog age (juvenile [0] vs. sub-adult or adult[1]), and water temperature (°C) on the zoospore load of infected individuals.

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<tr>
<th>Coefficient</th>
<th>Mean</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c$ (conductivity effect)</td>
<td>4.702</td>
<td>3.35, 6.187</td>
</tr>
<tr>
<td>$d$ (age effect)</td>
<td>−0.978</td>
<td>−2.462, 0.516</td>
</tr>
<tr>
<td>$\beta_{age}$ (age effect)</td>
<td>0.716</td>
<td>0.038, 1.881</td>
</tr>
<tr>
<td>$\beta_{wtemp}$ (temperature effect)</td>
<td>0.387</td>
<td>−0.943, 1.717</td>
</tr>
<tr>
<td>$\beta_{wtemp}$ (temperature effect)</td>
<td>−1.486</td>
<td>−2.355, −0.618</td>
</tr>
<tr>
<td>$\sigma_{resid}$</td>
<td>2.445</td>
<td>2.173, 2.759</td>
</tr>
</tbody>
</table>

Notes: Electrical conductivity was originally measured in µS/cm. The posterior means of all model parameters are shown, along with their 95% credible intervals (95% CI). Hyperparameters $c$ and $d$ define the intercept for zoospore load given wetland salinity, $\beta_{zoo,age}$ and $\beta_{zoo,wtemp}$ are regression coefficients, $\sigma_{age}$ is the standard deviation of the intercept term, and $\sigma_{resid}$ is the standard deviation of the residual error term.
in the monthly probability of survival was only recorded in October (95% CI of Δsurv = −0.219 to −0.004), but the estimates of Δsurv were overwhelmingly negative across the season (Fig. 4).

DISCUSSION

Our results suggest that wetland salinity and water temperature regimes jointly influence the impact of chytridiomycosis on populations of *Litoria raniformis*. There were clear negative effects of water temperature on both the probability and intensity of chytrid infections for this species. Although a weaker effect, wetland salinity (as measured by electrical conductivity) was also negatively related to the probability and intensity of chytrid infections. Given these relationships, the estimated reduction in the monthly probability of survival for an adult *L. raniformis* due to chytridiomycosis was considerably lower under warm and saline conditions than either cool and fresh, or intermediate conditions.

Mitigation of the impacts of chytridiomycosis by high environmental temperatures and salinity concurs with past laboratory studies on the physiological tolerances of chytrid fungus (see Introduction), and with studies on the infection dynamics of this pathogen in the field (Retallick et al. 2004, Woodhams and Alford 2005, Kriger et al. 2007, Bramwell 2011, Forrest and Schlaepfer 2011, Kinney et al. 2011, Savage et al. 2011, Becker et al. 2012, Doddington et al. 2013, Rowley and Alford 2013). Our study, however, is one of only a few to assess the demographic consequences of these relationships for amphibian populations.

Heightened chytrid-related mortality during relatively cool periods or at relatively cool locations has been reported for amphibians in regions of Australia (Berger et al. 2004) and the Americas (Kinney et al. 2011, Savage et al. 2011) where environmental temperatures commonly exceed the upper temperature tolerances of chytrid. However, a negative relationship between environmental temperature and chytrid prevalence has not translated into a corresponding relationship with mortality in at least two cases (Retallick et al. 2004, Voordouw et al. 2010). Furthermore, Pilliod et al. (2010) and Muths et al. (2011) reported only a weak interaction between annual temperature variation and chytrid-related mortality for populations of the boreal toad (*Anaxyrus boreas*) in North America, while Bosch et al. (2007) reported chytrid outbreaks in central Spain during unusually warm years. These inconsistencies reflect interspecific differences in the susceptibility of amphibians to the pathogen (Woodhams et al. 2007), as well as variation in the temperature gradient assessed. As the required studies accrue, meta-analyses may identify important generalities in the relationship between environmental temperature regimes and chytrid-related mortality among susceptible amphibians.

Generalities in the effect of salinity on the outcomes of chytridiomycosis are also plausible. In addition to the physiological sensitivity of the pathogen to this variable (Piotrowski et al. 2004, Stockwell et al. 2012), it is noteworthy that many of the historical declines of amphibians that have been attributed to chytridiomycosis occurred in upland or high-rainfall regions, particularly upland streams (Berger et al. 1998, Skerratt et al. 2007). Water salinity can be expected to be consistently low through both time and space in such

### Table 3. Coefficients for the effect of zoospore load on the daily probability of survival (φ) and the probability of recapture (r) for infected individuals.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Mean</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ</td>
<td>4.629</td>
<td>4.332, 4.949</td>
</tr>
<tr>
<td>βch.surv (survival effect)</td>
<td>−0.479</td>
<td>−0.873, −0.082</td>
</tr>
<tr>
<td>βflood</td>
<td>−0.618</td>
<td>−1.364, 0.446</td>
</tr>
<tr>
<td>τ</td>
<td>−1.964</td>
<td>−2.238, −1.691</td>
</tr>
<tr>
<td>βch.recap (recapture effect)</td>
<td>0.795</td>
<td>0.338, 1.259</td>
</tr>
</tbody>
</table>

Note: The posterior means of all model parameters are shown, along with their 95% credible intervals (95% CI). The parameter γ defines the intercept for the effect of zoospore load on the daily probability of survival, and βch.surv and βflood are regression coefficients. The parameter τ is the intercept term for the effect of zoospore load on the probability of recapture, and βch.recap is the regression coefficient.

**Fig. 3.** Effect of zoospore load on the daily probability of survival (φ) and the probability of recapture (r) of infected individuals. Mean estimates are shown in black and the 95% credible intervals are shown in gray.
environments. While the thermal biology of chytrid and its sensitivity to desiccation are almost certainly important mechanisms underlying the pathogenicity of the fungus in upland environments (see Introduction), we speculate that these environments may also be particularly suitable with regard to the physiochemical niche of chytrid. Again, meta-analyses of the demographic effects of chytridiomycosis across salinity gradients are ultimately required to test this proposition for susceptible amphibians.

The relationship identified in this study between the probability of capture and infection status is also one that requires further study across taxa. For *L. raniformis*, we observed a doubling in the probability of recapture for individuals with relatively heavy infections (~22,000 zoospore equivalents) to those with light infections (~20 zoospore equivalents). We are aware of only four other studies that have sought to assess the effect of chytrid infections on the probability of capture for amphibians (Retallick et al. 2004, Murray et al. 2009, Pilliod et al. 2010, Phillott et al. 2013). The effect was small in each case, and considered inconsequential. Our finding of differing rates of capture between heavily and lightly infected *L. raniformis* is important, because relationships of this nature can undermine programs that seek to measure or monitor disease dynamics (Jennelle et al. 2007, Cooch et al. 2012). When not accounted for, a higher likelihood of capture among infected individuals will lead to overestimates of chytrid prevalence or infection intensity, while underestimates of these parameters will ensue when infected individuals are less likely to be captured. In addition to further study of the relationship between chytrid infection status and capture probabilities for amphibians, we encourage research on the ramifications of these relationships for programs that seek to measure and monitor chytrid dynamics.

Returning to the demographic effects of chytridiomycosis on populations of *L. raniformis*, further insights could be obtained by assessing the relationship between the probability of population extinction and the risk factors identified here for the probability and intensity of chytrid infections. Heard et al. (2013) modeled the probability of extinction of populations of *L. raniformis* across northern Melbourne. At the wetland level, they demonstrated strong negative relationships between the probability of extinction and wetland area, hydroperiod (the flux in water level through time) and aquatic vegetation cover. In light of our results, it would be useful to test the interactive effect of water temperature regime and electrical conductivity on extinction risk. Confirmation of such an effect would provide additional evidence of a functional role for chytridiomycosis in the metapopulation dynamics of *L. raniformis* in this region.

Assessing the interactive effect of water temperature regime and conductivity on the probability of extinction may also clarify the effects of the variables identified previously as drivers of extinction risk for *L. raniformis*, because these variables could (at least partly) represent proxies for chytrid risk. For example, the negative effect of wetland area and hydroperiod on the probability of extinction could partly reflect correlations between these variables and water temperature, as the thermal inertia of large, deep wetlands should ensure that they also

![Fig. 4](image-url)
display relatively stable water temperatures. Aquatic vegetation cover could also represent a proxy for higher water temperature regimes, because solar insolation is a key determinant of both (Carr et al. 1997, Becker et al. 2012). Likewise, the weak correlation between wetland hydroperiod in this system and conductivity ($r_S = 0.28$) could also contribute to the negative relationship between hydroperiod and extinction risk. While wetland area, hydroperiod, and aquatic vegetation cover each have clear mechanisms linking them to population size and extinction risk for L. raniformis (Heard et al. 2013), any weakening of their effect when accounting for the interactive effect of water temperature regime and conductivity would suggest that they do partially represent proxies for chytrid risk.

Should chytridiomycosis prove an important driver of extinction risk for L. raniformis, further research on the temporal dynamics of this disease would be valuable. We were unable to include the winter inactive season in our simulations, as we did not have the requisite water temperature data, nor did we undertake chytrid sampling and mark–recapture over the winter months. Nevertheless, if the relationships identified here between water temperature and infection rate and intensity hold over the winter months, populations of L. raniformis should be most susceptible to chytridiomycosis during winter. This pattern fits with winter observations of dead and dying L. raniformis in this region (G. Heard, personal observations), and with the seasonal pattern of chytrid-related mortality for some anurans in Australia and elsewhere.

More generally, the temperature dependence of chytrid infection rate and intensity in L. raniformis could drive significant between-year variability in the effects of chytridiomycosis on this species. In concert with the sensitivity of chytrid zoospores to desiccation (Johnson et al. 2003), it is likely that the pathogenicity of chytrid is notably higher in cool, wet years. Annual variation in the pathogenicity and virulence of chytrid fungus has so far received limited attention in the international literature (see Pilliod et al. 2010 and Muths et al. 2011 for examples). It remains a key gap in our understanding of the impacts of chytridiomycosis on amphibian populations.

Our study corroborates recent research on the conservation requirements of L. raniformis (Heard et al. 2012a, Heard et al. 2013), and provides additional direction to managers seeking to prioritize wetlands for protection for this species and to bolster local population sizes. Populations of L. raniformis occupying large wetlands with dense aquatic vegetation and long hydroperiods are considerably less likely to be extirpated.
through time; a fact previously attributed to the direct influence of these factors on carrying capacity (Heard et al. 2013). However, as above, the relatively low rate of extinction of *L. raniformis* in such wetlands could partly reflect their unsuitability for chytrid. Whichever the case, large wetlands with dense aquatic vegetation and long hydroperiods are priorities for protection for this species. To this list, we would add wetlands that are moderately saline (up to 5000 μS/cm), given the lower impact of chytridiomycosis on populations of *L. raniformis* in such wetlands, and the possibility that these wetlands represent refuges from the disease. In our study area, relatively saline wetlands are usually quarry pits, and are often spring fed. These sites represent attractive landfill sites for private industry, and are frequently destroyed for this purpose (Heard and Scroggie 2009). We urge much greater emphasis on the protection of quarry wetlands for *L. raniformis* across southeast Australia.

In line with Geiger et al. (2011), Woodhams et al. (2011), and Becker et al. (2012), our study suggests that reducing canopy cover to increase solar insolation and water temperature could be a practical technique for minimizing the impact of chytridiomycosis on populations of *L. raniformis*. Throughout the range of this species, exotic trees such as willows (*Salix* spp.), poplars (*Populus* spp.), and elms (*Ulmus* spp.) are major riparian weeds that may significantly reduce water temperatures through shading (Zukowski and Gawne 2006, Ryan et al. 2013). Targeted removal or thinning of canopy trees, particularly exotic species, could be trialed at wetlands where cold water temperature regimes heighten the impacts of chytridiomycosis on *L. raniformis*. Likewise, we caution that riparian re-vegetation schemes may ultimately prove detrimental to populations of *L. raniformis*, if those schemes lead to wetland shading and reductions in water temperature.

Manipulation of wetland salinity is more problematic for this species than water temperature, given logistical difficulties and possible effects on other biota. For this reason, and particularly because of its stronger effect on chytrid dynamics, we recommend managers concentrate on manipulating water temperature rather than salinity for *L. raniformis*. Nevertheless, in cases where ground-water of the appropriate conductivity is accessible, and where impacts on other biodiversity will be minimal (see Smith et al. [2007, 2009a, b] for guidance in this regard), trialing the application of bore water to reduce chytrid impacts would be an informative management experiment.

It is becoming increasingly clear that the impact of chytridiomycosis on amphibian populations is mediated by particular environmental variables. Our study is one of several to highlight that this has important implications for management, including the identification of refuges from the disease and the development of manipulative habitat management regimes (Bramwell 2011, Forrest and Schl ae pfer 2011, Puschendorf et al. 2011, Becker et al. 2012, Stockwell et al. 2012, Doddington et al. 2013, Rowley and Alford 2013). Given the absence of any direct means of controlling chytrid for most amphibians, we encourage further research on the environmental drivers of chytridiomycosis, particularly field experiments that assess the efficacy of manipulating habitat variables to mitigate the impacts of this disease.

**Acknowledgments**

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**Literature Cited**


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**SUPPLEMENTAL MATERIAL**

**Appendix A**

Hygiene protocols (*Ecological Archives A024-038-A1*).

**Appendix B**

Photographs of the study sites used for the simulations (*Ecological Archives A024-038-A2*).

**Appendix C**

Representativeness of the swabbed sample of frogs (*Ecological Archives A024-038-A3*).

**Supplement**

R code for simulating the effect of chytridiomycosis on survival, and OpenBUGS code for fitting the hierarchical model of the probability of infection, the hierarchical linear model of zoospore load, and the Cormack-Jolly-Seber model incorporating the hierarchical model of the probability of infection (*Ecological Archives A024-038-S1*).