Article:

Heard, Geoffrey W., Thomas, Chris D. orcid.org/0000-0003-2822-1334, Hodgson, Jenny A. et al. (3 more authors) (2015) Refugia and connectivity sustain amphibian metapopulations afflicted by disease. Ecology Letters. pp. 853-863. ISSN: 1461-023X

https://doi.org/10.1111/ele.12463

This is the accepted version of the following article: Heard, G.W.**,** Thomas, C.D., Hodgson, J.A., Scroggie, M.P., Ramsey, D.S.L and Clemann, N. (2015). Refugia and connectivity sustain amphibian metapopulations afflicted by disease. *Ecology Letters* **18**, 853-863, which has been published in final form at http://onlinelibrary.wiley.com/doi/10.1111/ele.12463/abstract

Refugia and connectivity sustain amphibian metapopulations afflicted by disease

Geoffrey W. Heard

School of BioSciences, University of Melbourne, Victoria, 3010, Australia. heardg@unimelb.edu.au

Department of Biology, University of York, Wentworth Way, York, YO10 5DD, United Kingdom.

Chris D. Thomas

Department of Biology, University of York, Heslington, York, YO10 5DD, United Kingdom.

chris.thomas@york.ac.uk

Jenny A. Hodgson

Department of Evolution, Ecology and Behaviour, University of Liverpool, Liverpool, L69 7ZB, United Kingdom. jenny.hodgson@liverpool.ac.uk

Michael P. Scroggie

Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, P.O. Box 137, Heidelberg, Victoria, 3084, Australia. Michael.Scroggie@delwp.vic.gov.au

David S. L. Ramsey

Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, P.O. Box 137, Heidelberg, Victoria, 3084, Australia. David.Ramsey@delwp.vic.gov.au

Nick Clemann

Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, P.O. Box 137, Heidelberg, Victoria, 3084, Australia. Nick.Clemann@delwp.vic.gov.au

Corresponding author:

Geoffrey W. Heard

Department of Biology (J2), University of York, Wentworth Way, York, YO10 5DD, United Kingdom.

Email: heardg@unimelb.edu.au

Statement of authorship: GWH, MPS, DSLR, CDT and JAH designed the study, GWH conducted fieldwork and GWH, MPS and DSLR completed the modelling. All authors contributed to interpretation of results. GWH led the writing, with contributions from all authors.

Keywords: amphibian, chytrid, colonisation, conservation, extinction, microclimate, *Litoria raniformis*, pathogen, reservoir host.

Abstract

Metapopulation persistence in fragmented landscapes depends on habitat patches that can support resilient local populations and sufficient connectivity between patches. Yet epidemiological theory for metapopulations has largely overlooked the capacity of particular patches to act as refuges from disease, and has suggested that connectivity can undermine persistence. Here, we show that relatively warm and saline wetlands are environmental refuges from chytridiomycosis for an endangered Australian frog, and act jointly with connectivity to sustain frog metapopulations. We coupled models of microclimate and infection probability to map chytrid prevalence, and demonstrate a strong negative relationship between chytrid prevalence and the persistence of frog populations. Simulations confirm that frog metapopulations are likely to go extinct when they lack environmental refuges from disease and lose connectivity between patches. This study demonstrates that environmental heterogeneity can mediate host-pathogen interactions in fragmented landscapes, and provides evidence that connectivity principally supports host metapopulations afflicted by facultative pathogens.

Introduction

Metapopulation persistence in fragmented landscapes depends on habitat patches that can support resilient local populations, plus sufficient connectivity within the patch network. Metapopulation theory, the source-sink concept and adaptations of island biogeography theory all stress that habitat patches vary in their capacity to maintain populations (due to variation in their size, resources, microclimate, etc.), and that a subset of patches may be pivotal to network viability (Brown & Kodric-Brown 1977; Pulliam 1988; Hanski 1999; Whittaker & Fernández-Palacios 2007). To varying degrees, each paradigm also emphasises the demographic and genetic benefits of connectivity, including the rescue of declining populations by immigrants and recolonisation of patches where local extinction has occurred (Brown & Kodric-Brown 1977; Pulliam 1988; Hanski 1999; Whittaker & Fernández-Palacios 2007). Yet epidemiological theory for metapopulations has largely overlooked variation in disease risk between patches, and has led to uncertainty about the influence of connectivity on persistence.

Since the influential work of Hess (1996), epidemiological models for metapopulations have focussed on dispersal and transmission dynamics (e.g., Keeling & Gilligan 2000; Fulford *et al.* 2002; McCallum & Dobson 2002; Jesse *et al.* 2008; Harding *et al.* 2012; Schmid *et al.* 2012). Most omit relationships between patch characteristics and disease risk, despite pathogen fitness and host susceptibility being often dependent on local environmental conditions (Colhoun 1973; Harvell *et al.* 2002). Examples of environmental drivers of disease risk include effects of forest fragment size on the prevalence of Lyme bacterium among deer ticks (Allan *et al.* 2003), associations between vegetation type and bacterial infections in coyotes (Foley *et al.* 2005), more intense fungal infections of salamander eggs in shadier ponds (Urban *et al.* 2015), and a higher prevalence of malaria infections in birds at warmer locations with nearby waterbodies (Gonzalez-Quevedo *et al.* 2014).

Effects of temperature and moisture availability may be particularly relevant in fragmented systems, because they can vary considerably between habitat patches (Laurance 2004; Bennie *et al.* 2013), and this can produce significant spatial heterogeneity in pathogen fitness (Jousimo *et al.* 2014). Understanding these relationships enables their incorporation into models of spatial disease risk and metapopulation persistence.

Hess (1996) also sparked an enduring debate about the role of connectivity in supporting metapopulations threatened by disease. For species afflicted by an obligate pathogen, Hess showed that the demographic benefits of migration could be over-ridden by increased transmission, leading to an increased risk of extinction for highly connected metapopulations. This result has been challenged for obligate host-pathogen systems (McCallum & Dobson 2002; Jousimo *et al.* 2014), but there remains uncertainty about the role of connectivity for hosts afflicted by facultative pathogens, defined here as pathogens that are maintained and transmitted by reservoir hosts and/or the environment. Gog *et al.* (2002) showed that contagion between susceptible hosts may play a relatively minor role in pathogen distribution and infection rates in these systems, in which case migration should reduce extinction risk through the normal demographic processes. However, subsequent theoretical studies that included more complex transmission and metapopulation dynamics suggest that an important trade-off between migration and transmission could exist for hosts threatened by facultative pathogens (McCallum & Dobson 2002; Harding *et al.* 2012; Park 2012).

Chytridiomycosis in amphibians, caused by the fungus *Batrachochytrium dendrobatidis*, is a useful case study for assessing the role of environmental refugia and connectivity in the persistence of fragmented populations threatened by a facultative pathogen. Chytridiomycosis is capable of decimating amphibian populations (Lips *et al.* 2006; Pounds *et al.* 2006; Vredenburg *et al.* 2010), and has exterminated numerous species in recent decades (Skerratt *et al.* 2007). The efficiency of the pathogen (hereafter 'chytrid') is related to its facultative biology. Chytrid can spread quickly through naive frog communities, but is usually pathogenic in only a subset of species (Woodhams *et al.* 2006), leaving the remainder to act as reservoir hosts that may stifle the recovery of susceptible species (Skerratt *et al.* 2007). Chytrid zoospores are also water-borne and can remain viable in moist substrates for at least three months (Johnson & Speare 2005). Hence, environmental latency and transmission can be an important source of infections (Skerratt *et al.* 2007).

Nevertheless, chytrid has several environmental frailties. It terminates zoospore production at 28^{o}C and zoospores die at temperatures of 30^{o}C or above (Piotrowski *et al.* 2004). Exposure to moderate salinity also reduces the production and survival of zoospores (Stockwell *et al.* 2012), as does exposure to pH < 6 and > 7 (Piotrowski *et al.* 2004). These sensitivities cause spatial variation in the impact of chytrid when there is sufficient variation in the thermal and physicochemical environment (Puschendorf *et al.* 2011; Savage *et al.* 2011; Scheele *et al.* 2015; Stockwell *et al.* 2015a; Stockwell *et al.* 2015b).

Here, we demonstrate the importance of environmental refugia and connectivity for the persistence of growling grass frog (*Litoria raniformis*) metapopulations in south-eastern Australia that are afflicted by chytridiomycosis. We developed a model of the thermal regime of wetlands across our study area and coupled it with a model of the negative effects of water temperature and salinity on the probability of chytrid infections among *L. raniformis* to predict wetland-level variation in pathogen prevalence. In turn, we used 11 years of monitoring to quantify the effects of chytrid prevalence and wetland connectivity on the probabilities of population persistence and colonisation for *L. raniformis*. Lastly, we brought these analyses together to simulate metapopulation viability, showing that metapopulations have a high risk of extinction when they lack relatively warm and saline wetlands in which chytrid prevalence is low and lose connectivity between neighbouring wetlands.

Materials and methods

Study system

Litoria raniformis is a largely aquatic frog distributed across south-eastern Australia. Formerly abundant, it is now listed as endangered on the IUCN Red List following the disappearance of numerous populations last century (Hero *et al.* 2004). Chytridiomycosis is suspected to have been a key driver of this decline.

In our study area north of Melbourne, in southern Victoria, remnant populations of *L. raniformis* display dynamics consistent with metapopulation theory (Heard *et al.* 2012). Populations are distributed patchily in pools along streams and drainages, as well as lentic wetlands (Fig. 1a). Dispersal is distance-limited, with occupancy, mark-recapture and genetic data suggesting that populations separated by > 1 km share few migrants (Heard *et al.* 2012; Hale *et al.* 2013). Populations are distributed as semi-independent networks, which we refer to as metapopulations (Fig. 1a). Population extinction and (re)colonisation occurs with some frequency (Heard *et al.* 2012; 2013), generating occasional metapopulation collapse and absence from some networks. Extinctions have several proximate causes (Heard *et al.* 2012), but chytridiomycosis is an important underlying stressor.

Chytrid infections are common among *L. raniformis* in the study area and severe infections reduce the monthly probability of survival by 1/3 (Heard *et al.* 2014). Infections have also proven ubiquitous among the sampled wetlands (Heard *et al.* 2014); a fact we attribute to the pathogen's capacity for environmental transmission and the presence of reservoir hosts. Seven sympatric frog species have been recorded in the study area (Appendix S1 of the Supporting Information), at least one of which has been detected at 80% of the wetlands where *L. raniformis* has been observed (Fig. 1b). This rises to 90% when excluding sites surveyed only in summer or autumn, when detection rates of the sympatric species are low. Six of these frogs are known to carry chytrid in the wild and may display a high prevalence of infections; however, they are less susceptible to chytridiomycosis (some are asymptomatic) and can maintain very high population densities (Appendix S1).

With widespread reservoir hosts and environmental transmission also possible, contagion from migrants appears a relatively unimportant source of chytrid infections for *L. raniformis* (see '*Estimating chytrid prevalence*' below). Instead, these frogs appear regularly exposed, but the probability of infection varies through space and time according to local environmental conditions, declining with increasing water temperature and increasing wetland salinity (Heard *et al.* 2014). This provides the opportunity to predict variation in the prevalence of infections among wetlands based on variation in their microclimate and physicochemistry.

Surveys

Two-hundred and fourteen sites across the study area (Fig. 1, Appendix S2) were surveyed a total of 2,076 times between the 2001/2002 and 2011/2012 active seasons of *L. raniformis* (October – April). For simplicity, we refer to these seasons as 'years' hereafter. Sites included pools along streams and drainages, plus farm dams, lakes, water-treatment ponds, swamps and flooded quarries (Appendix S2). As lotic and lentic sites are functionally equivalent for *L. raniformis*, we use 'wetland' or 'site' interchangeably hereafter when referring to either.

Between one and four surveys were undertaken at each site that was present and could be accessed in 2001/2002 (287 surveys), 2006/2007 (542 surveys) and 2011/2012 (338 surveys). Site presence varied due to wetland destruction and creation (Appendix S2). Surveys entailed diurnal or nocturnal searches, relying on visual detection or recognition of advertisement calls (Heard *et al.* 2012). A further 909 surveys were completed in the intervening years, with up to 10 repeat surveys completed at a wetland in one year. Surveys were primarily completed by GWH or colleagues (n = 1,746). The remainder (n = 330) were conducted by other field-workers, data from which were collated by GWH (Appendix S3).

Water temperature model

We used 1,233 measurements of night-time water surface temperature taken during surveys to build a linear model of spatio-temporal variation in water temperature between October and April. Temporal drivers were added first. The seasonal rise and fall of water temperature was modelled using a quadratic effect of days since September 1 (the start of the Austral spring). The effect of ambient temperature was modelled as a linear function of daily maximum temperature recorded at Melbourne Airport (-37.67 S, 144.83 E), ~13 km west of the centre of the study area (Australian Bureau of Meteorology, http://www.bom.gov.au/climate/data/index.shtml). We then added five wetland attributes to explain the remaining variation in water temperature: (1) wetland size, approximated by wetland surface area ($\log_e$ m^2) × maximum depth (cm); (2) site type (lotic or lentic); (3) elevation (m asl); (4) percent cover of emergent vegetation; (5) percent cover of riparian trees and shrubs. We also investigated three interaction terms: (1) wetland size × days since September 1 (hypothesising that larger wetlands display thermal inertia later in the season); (2) maximum daily temperature × days since September 1 (hypothesising that the effect of ambient temperature varies through time); (3) maximum daily temperature × wetland size (hypothesising that the effect of ambient temperature is mediated by wetland size). A site-level random effect was added to account for repeated measures. Model fitting was completed using Markov Chain Monte Carlo (MCMC) sampling in *OpenBUGS* v. 3.2.3 (Thomas *et al.* 2006), called from *R* v. 3.0.3 (R Core Team 2014). Parameter estimates were derived from 40,000 MCMC samples using uninformative priors (burn-in = 5,000 samples).

After excluding effects with credible intervals overlapping zero, the model was used to predict the average night-time water surface temperature at each wetland in each month between October and April in each year. For each of 1,000 estimates of the model coefficients drawn from their joint posterior distribution, we estimated the water surface temperature for each night at each wetland in each year using daily maximum air temperature data from Melbourne Airport. The monthly average was taken from these estimates, giving 1,000 estimates of the average water temperature at each wetland in each month of each year. The mean and standard deviation of these estimates was retained. We validated the model by assessing the correspondence between the predicted average monthly temperature and the actual average monthly temperature at 11 wetlands monitored using data-loggers (Thermocron iButton®, Maxim Integrated, USA) in 2004/2005 and 2005/2006 (n = 78 month-by-site samples; data from Heard *et al.* 2014). *R* code and data are provided in Appendix S4.

Estimating chytrid prevalence

Our previous research, based on swabbing 521 *L. raniformis* across the study area in 2004/2005 and 2005/2006, yielded a hierarchical model in which the probability of chytrid infection is negatively related to water temperature at capture and wetland salinity, and is lower in juveniles than adults (Heard *et al.* 2014). We refitted the model to these data for two reasons. Firstly, to align the spatial pattern of chytrid sampling with our wider water temperature and occupancy dataset. Heard *et al.* (2014) sampled wetlands in three metapopulations (6, 8 and 9 in Fig. 1a), but grouped adjacent pools along streams for analytical purposes (11 pools into 3 transects). We re-partitioned frogs and salinity samples between all 17 sites at which chytrid sampling was undertaken. Salinity (as electrical conductivity, $\log_e[\mu\text{S cm}^{-1}]$) was measured during surveys using an Oakton® CON11 water-quality meter (Oakton Instruments, Vernon Hills, USA).

Secondly, we sought to test whether contagion from migrants was an important source of chytrid infections for *L. raniformis*. We hypothesised that if this was true, the probability of infection would be related to the number and proximity of infected conspecifics in neighbouring wetlands. For frog i in year t, the number and proximity of infected neighbours was defined as $\log_e(\text{sum}_j(infect_{jt} \times w_{ij}) + 1)$, where $infect_{jt}$ is the infection status of frog j also captured in year t at a wetland in the same metapopulation as frog i, and $w_{ij} = (0.10026 \times d_{ij})^{-0.719877}$ (a negative power function giving the probability of dispersal by *L. raniformis* over the distance d_{ij}, derived from mark-recapture data by Heard *et al.* 2012). In this case, d_{ij} was the distance between frog captures. The model was fitted to the data using *OpenBUGS* called from *R*, with parameters estimated from 40,000 MCMC samples using uninformative priors (burn-in = 10,000 samples). Refitting demonstrated that the probability of infection was not influenced by the number and proximity of infected conspecific neighbours (mean effect = 0.02, 95% CI = -0.74, 0.64).

After excluding the spatial infection term, the model was used to predict the seasonal prevalence of infections among adult *L. raniformis* at all 214 wetlands, based on the local water temperature and salinity regime. The occupancy dynamics of *L. raniformis* were ignored for these predictions; instead, we simply sought an estimate of the prevalence of infections among adults if a population was present and sampled randomly across the season. Chytrid was assumed to be ubiquitous among wetlands, in line with our existing swabbing data, the presence of reservoir hosts and the pathogen's capacity for environmental latency and transmission.

For each wetland in each year, we simulated sampling 22 adult *L. raniformis* for chytrid (the average number swabbed per wetland by Heard *et al.* 2014), with capture times allocated randomly in the months October–April. The probability of infection for each frog in each simulation was estimated using the coefficients of the infection model, with water temperature at capture being the predicted average water temperature for the relevant month and wetland, and wetland salinity being the average recorded between 2001 and 2012 (missing measurements were replaced by the mean across sites). We ran 1,000 simulations for each wetland in each year, each time using a random draw of the parameters of the infection model from their joint posterior distribution, and allocating water temperature for each frog as a random draw from the distribution estimated above for the relevant month and wetland. The prevalence of infections in each simulation was simply the sum of the estimated probabilities of infection for each frog divided by the number sampled (Hosmer *et al.* 2013). We retained the mean and standard deviation across prevalence estimates for each wetland (1,000 estimates / year = 11,000 total).

The simulation approach was tested using the relationship between the predicted and observed prevalence of infections at 10 wetlands where at least 15 adults were swabbed by Heard *et al.* (2014). We used the identical procedure as that used in the simulations to estimate the probability of infection for each adult sampled at these 10 wetlands (replaced water temperature at capture with the predicted average monthly temperature), and estimated prevalence among the swabbed frogs as the sum of their probabilities of infection divided by the number sampled. *R* code and data are provided in Appendix S5.

Occupancy modelling

A dynamic occupancy model (Royle & Kéry 2007) was used to estimate the effect of predicted chytrid prevalence on the annual probability of persistence of *L. raniformis* populations, as well as the effect of connectivity on the probabilities of persistence and colonisation for the frog. The occupancy model was fitted to detection data for 190 wetlands that received the majority of surveys (2,011 surveys, 97 % of all those conducted). The probability of occupancy in the first year (ψ) and the annual probability of population persistence (ϕ) were modelled as logistic functions of predicted chytrid prevalence and connectivity, while the probability of colonisation (γ) was modelled as a logistic function of connectivity alone. Connectivity (a measure of immigration rate) was calculated as the distance weighted number of surrounding wetlands with source populations, with the distance weight (w_{ij}) being the probability of dispersal over the intervening distance derived from the negative power function given above. Specifically, connectivity of wetland i for the interval $t-1$ to t was defined as $\log_e(\mathrm{sum}_j(o_{jt-1} \times w_{ij}) + 1)$, where o_{jt-1} is the occupancy status of wetland j in year $t-1$ and w_{ij} is calculated using the edge-to-edge distance between wetlands i and j (Heard *et al.* 2012; 2013). Only wetlands j within a 1 km radius were included (Heard *et al.* 2012; 2013). Rare longer-distance migration can still influence ϕ and γ under this parameterisation, via the intercepts of the logistic models (hence, for example, γ may be > 0 even when connectivity = 0).

Uncertainty in predicted chytrid prevalence at each wetland was propagated by allowing the MCMC algorithm to sample prevalence (on the logit scale) from a normal distribution defined by the site-specific mean and standard deviation estimated above. Connectivity was also estimated stochastically after the first year, to propagate uncertainty in neighbour occupancy due to imperfect detection and missed surveys. The probability of detection (p) was modelled as a function of survey effort, days since September 1 and time of day (Heard *et al.* 2012; 2013). Model fitting was completed using *OpenBUGS* called from *R*, with parameter estimates drawn from 80,000 MCMC samples using uninformative priors (burn-in = 40,000 samples).

The fit and predictive capacity of the occupancy model was assessed using the correlation between the predicted and observed number of years that wetlands were occupied. We first selected a reliable set of presences and absences (n = 525) by identifying, for each wetland, survey years after the first in which detection of *L. raniformis* either occurred, or the cumulative probability of detection was ≥ 0.9. Within this set, we tallied the number of years that *L. raniformis* was present at each wetland and calculated the predicted number as the sum of the mean posterior probabilities of occupancy for each wetland in the corresponding years (Hosmer *et al.* 2013). Model fit was assessed using the correlation between the observed and predicted number of years occupied when the full dataset was used. The predictive capacity of the model was assessed using three-fold cross-validation, where, on each occasion, the model was fitted while withholding detection data from years 2-11 for wetlands in one of three metapopulations (6, 8 and 9 in Fig. 1a; n = 84 wetlands), forcing the model to estimate the annual probabilities of occupancy of these wetlands from the dynamics of the remaining sites. *R* code and data are provided in Appendix S6.

Metapopulation viability

The influence of environmental refugia and connectivity on metapopulation viability was assessed by simulating from the occupancy model (Heard *et al.* 2013). Simulations were run for nine metapopulations with five or more wetlands and at least one extant population in 2011/2012 (Fig. 1a). For each of four scenarios we simulated the extinction and colonisation dynamics in each metapopulation for 30 years, starting from the most recent occupancy pattern. The four scenarios were: (1) metapopulation remained unchanged ('current conditions'); (2) predicted chytrid prevalence at each wetland was set to the metapopulation maximum, eliminating the contribution of relatively warm and saline wetlands with low prevalence (‘no refugia’); (3) connectivity of each wetland was set to zero (‘no connectivity’); (4) both changes were applied (‘no refugia or connectivity’). We ran 5,000 simulations for each metapopulation and scenario; one simulation for each of 5,000 samples from the joint posterior distribution of the occupancy model parameters. The probability of metapopulation extinction was the proportion of simulations in which wetland occupancy declined to zero. *R* code and data are provided in Appendix S7.

Results

Water temperature and salinity

There was considerable environmental variation among sites. Elevation, emergent vegetation cover and riparian tree and shrub cover all had clear negative effects on night-time water surface temperature, after accounting for the temporal effects of time-of-year and daily maximum air temperature (Table 1). Wetland size had both a positive main effect and positive interactive effect with survey date (confirming that larger wetlands stayed warmer late in the season; Table 1). The predicted average monthly temperature at the wetlands monitored using data-loggers was strongly correlated with the actual average temperature (Pearson’s $r = 0.83$, root mean square error [RMSE] = 1.55 ^{o}C, $n = 78$; Appendix S8). Predicted night-time water temperatures displayed considerable heterogeneity across the study area, but displayed a general south to north decline with increasing elevation (Fig. 2a). Wetland salinity ranged from 164 – 13,820 µS cm^{-1} (~0.08 – 8 ppt) and varied considerably in some sections of the study area due to localised ground water inputs, including natural upwellings or, in the case of quarry pits, puncturing of shallow aquifers (Fig. 2b).

Predicted chytrid prevalence

Refitting the infection model for *L. raniformis* confirmed clear negative effects of water temperature and wetland salinity on the probability of chytrid infection (Table 1). Among wetlands with adequate swabbing data, the predicted prevalence of infections (obtained by substituting water temperature at capture with the corresponding predicted water temperature) was strongly correlated with the observed prevalence (Pearson's $r = 0.88$, RMSE = 0.09, $n = 10$; Appendix S8). Resulting predictions of seasonal chytrid prevalence ranged from 0.19 to 0.69 (Fig. 2c), with an increasing trend from south to north across the study area following decreasing water temperatures (cf. Fig. 2a & 2c). However, there was considerable local variation in predicted prevalence, with the extreme being a trebling (0.21 to 0.64) between wetlands separated by only 900 m in the south of the study area (in metapopulation 6; see Fig. 1a & 2c)

Occupancy dynamics

Observed turnover in wetland occupancy by *L. raniformis* included 62 extinctions and 45 colonisations, against 184 persistence events and 264 continued vacancies (mean survey interval = 4 years). The incidence of *L. raniformis* (proportion of years with surveys in which the species was detected at a given wetland) displayed strong spatial clustering, generally around wetlands with low-moderate predicted chytrid prevalence (cf. Fig. 2c & 2d). Incidence declined from 1 for wetlands with the lowest predicted prevalence to 0.13 for wetlands with the highest (Fig. 3a), and rose from 0.12 to 1 over the observed range of connectivity (Fig. 3b).

Occupancy modelling revealed a strong negative relationship between the annual probability of population persistence for *L. raniformis* and predicted chytrid prevalence (Table 1, Fig. 4a). The posterior mean probability of persistence was 0.98 at wetlands with the lowest predicted chytrid prevalence, dropping to 0.29 for wetlands with the highest (assuming average connectivity; Fig. 4a). The annual probabilities of persistence and colonisation were both influenced by connectivity (Table 1, Fig. 4b & 4c); however, the relationship with colonisation was considerably stronger, with the posterior mean probability of colonisation rising from 0.03 to 0.97 across the observed range of connectivity. The model fitted the data extremely well and displayed high predictive performance (Appendix S8). The Pearson's r correlation between the predicted and observed number of years a wetland was occupied was 0.98 for the full dataset (RMSE = 0.40 years, $n = 190$) and 0.84 for the cross-validation (RMSE = 1.26 years, $n = 84$).

Metapopulation viability

The predicted probability of metapopulation extinction over 30 years was low for all but three metapopulations under current conditions (≤ 0.2; Fig. 5a). When deprived of wetlands with low chytrid prevalence (all wetlands set to have the local maximum prevalence), extinction risk was highest for the smallest metapopulations (Fig. 5b). However, the probability of extinction increased by a factor of 5.9 under this scenario for one moderately-sized metapopulation (no. 6; Fig 5b) that is currently supported by two wetlands with low predicted chytrid prevalence (0.2 & 0.29; see Fig. 1a, 2c & 2d). The effect of removing connectivity between neighbouring wetlands declined with increasing metapopulation size, although again, one large metapopulation proved proportionally sensitive (no. 8, with 23 wetlands; Fig. 1a & 5c). All metapopulations displayed a high risk of extinction when they were deprived of both wetlands with low chytrid prevalence and connectivity (mean = 0.89; Fig. 5d).

Discussion

The primary theoretical frameworks applied to species persistence in fragmented landscapes stress that metapopulation viability depends on the environmental characteristics of the constituent habitat patches (through their effects on local population size and resilience), and that connectivity enhances viability through rescue effects and colonisation of vacant patches (Brown & Kodric-Brown 1977; Pulliam 1988; Hanski 1999; Whittaker & Fernández-Palacios 2007). However, epidemiological theory for metapopulations often ignores the role of environmental heterogeneity in host-pathogen interactions, and has led to some uncertainty about the influence of connectivity on persistence for species afflicted by facultative pathogens (Gog *et al.* 2002; McCallum & Dobson 2002; Harding *et al.* 2012; Park 2012). Our long-term study shows that patches with particular environments can act as refugia from infectious diseases, and sustain threatened host metapopulations. It also represents an important empirical contribution to debate about the role of connectivity in supporting metapopulations threatened by facultative pathogens, providing evidence that connectivity principally aids their viability.

Chytridiomycosis is a leading cause of recent amphibian extinctions (Skerratt *et al.* 2007), but the demographic impact of the disease varies through space and time. Our study is the first to demonstrate conclusively that small-scale heterogeneity in microclimate and physicochemical conditions facilitates the persistence of susceptible amphibians. In warm and relatively saline wetlands in which the prevalence of chytrid infections was predicted to be < 1/3, the annual probability of persistence of *L. raniformis* populations was high (> 0.93). However, the probability of persistence decreased sharply to 0.29 in cool wetlands with low salinity, where chytrid prevalence was at its predicted maximum (0.69). This confirms that the demographic impact of chytrid on *L. raniformis* is considerably lower in warmer and more saline wetlands, as predicted by Heard *et al.*

(2014). Here, we have demonstrated the wider significance of these refugia, showing that four of the nine focal metapopulations of *L. raniformis* have a high probability of extinction (>0.8) when deprived of relatively warm, saline wetlands, and that no metapopulations are likely to persist (11% chance over 30 years on average) if deprived of these refugia and connectivity between wetlands.

In our study system, contagion from migrants evidently has little influence on chytrid infection dynamics among *L. raniformis*, with the probability of infection being unrelated to the abundance and proximity of infected conspecific neighbours. This is mirrored in at least one other Australian frog-chytrid system (Stockwell *et al.* 2015a), and one from the United States (Beyer *et al.* 2015). Connectivity among host populations should facilitate metapopulation persistence in this circumstance (Gog *et al.* 2002). Our occupancy modelling and simulations confirm this for *L. raniformis*. The probability of population persistence increased by a factor of 1.3 across the observed range of connectivity, while the probability of colonisation rose by a factor of 32 (based on the posterior mean estimates). This does not in itself demonstrate the importance of connectivity to metapopulation persistence, because these relationships could simply produce short-lived subsidiary populations that make little contribution to long-term persistence. However, our simulations show that the risk of metapopulation extinction can increase significantly when connectivity is lost. Hence, rather than purely source-sink (Pulliam 1988) or mainland-island dynamics (Whittaker & Fernández-Palacios 2007), the metapopulations of *L. raniformis* studied here evidently exist in a balance between population extinction and colonisation over decadal time-scales (Hanski 1999), in which occasional extinction at refugia must be offset by recolonisation from neighbouring (daughter) populations, from neighbouring refugia, or through rare long-distance migration events (see also Heard *et al.* 2012).

Our study challenges the realism of theoretical models that suggest connectivity may undermine the viability of metapopulations afflicted by facultative pathogens (McCallum & Dobson 2002; Harding *et al.* 2012; Park 2012). Instead, our study provides an empirical example of host metapopulations that benefit substantially from connectivity, because migration-based contagion plays a minor role (if any) in the infection dynamics of the susceptible host, and there appears no significant trade-off between the demographic benefits of migration and the risk of disease transmission (Gog *et al.* 2002). We suspect that the infection dynamics for most amphibians threatened by chytrid will be similarly decoupled from migration once the pathogen is endemic. As in our study system, the distribution of the pathogen is likely to be quite stable given reservoir hosts and environmental transmission, while the prevalence of infections within susceptible species will be determined by environmental variables bearing on pathogenicity (see also Woodhams & Alford 2005; Murray *et al.* 2009; Savage *et al.* 2011; Beyer *et al.* 2015; Scheele *et al.* 2015; Stockwell *et al.* 2015a). Thus, we suspect that maintaining connectivity will only prove beneficial for amphibians threatened by chytrid once the pathogen is endemic.

To close, we return to environmental mediation of disease risk in this and other systems of fragmented host populations. Environmental heterogeneity frequently underpins the co-existence of species that display antagonistic interactions (Chesson 2000). In the case of host-pathogen interactions, understanding the environmental drivers of pathogenicity provides opportunities to manipulate the environment in ways that facilitates host persistence. This is a burgeoning field with regard to chytridiomycosis, and is already providing practical means of environmental manipulation to support threatened amphibians (Woodhams *et al.* 2011; Scheele *et al.* 2014). We foresee similar opportunities in other wildlife disease systems. Deciphering relationships between environmental heterogeneity, pathogenicity and host persistence deserves greater attention.

Acknowledgements

Funding was provided by the Australian Research Council (ARC Centre of Excellence for Environmental Decisions and LP0990161), Australian National Environmental Research Program, Australian Research Centre for Urban Ecology, Victorian Department of Environment, Land, Water and Planning, Growling Grass Frog Trust Fund, Museums Victoria, Melbourne Water and Parks Victoria. GWH was supported by a Victorian Postdoctoral Research Fellowship from the Victorian Department of State Development, Business and Innovation. D. DeAngelis, A. Hamer, C. Keely, P. Robertson and D. Stokeld are thanked for their contribution to surveys, along with numerous other colleagues and volunteers. P. Byrne, S. Canessa, A. Canzano, B. Casey, L. Conole, C. Crane, D. DeAngelis, K. Ficken, D. Gilmore, P. Grenfell, C. Griffith, A. Hamer, P. Homan, S. Koehler, B. Lane, A. Organ, E. Poole, M. Perry, D. Quinn, C. Renowden, P. Robertson, J. Urlus, M. Venosta and L. Williams provided access to additional survey data. M. McCarthy and K. Parris provided statistical advice and guidance. C. Todd and three anonymous reviewers provided valuable comments on an earlier draft.

References

1.

Allan B.F., Keesing F. & Ostfeld R.S. (2003). Effect of forest fragmentation on Lyme disease risk. *Conservation Biology*, 17, 267-272.

2.

Bennie J., Hodgson J.A., Lawson C.R., Holloway C.T.R., Roy D.B., Brereton T., Thomas C.D. & Wilson R.J. (2013). Range expansion through fragmented landscapes under a variable climate. *Ecology Letters*, 16, 921-929.

3.

Beyer S.E., Phillips C.A. & Schooley R.L. (2015). Canopy cover and drought influence the landscape epidemiology of an amphibian chytrid fungus. *Ecosphere*, 6, art78.

4.

Brown J.H. & Kodric-Brown A. (1977). Turnover rates in insular biogeography: effect of immigration on extinction. *Ecology*, 58, 445-449.

5.

Chesson P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, 31, 343-366.

6.

Colhoun J. (1973). Effects of environmental factors on plant disease. *Annual Review of Phytopathology*, 11, 343-364.

7.

Foley J.E., Queen E.V., Sacks B. & Foley P. (2005). GIS-facilitated spatial epidemiology of tick-borne diseases in coyotes (*Canis latrans*) in northern and coastal California. *Comparative Immunology, Microbiology and Infectious Diseases*, 28, 197-212.

8.

Fulford G.R., Roberts M.G. & Heesterbeek J.A.P. (2002). The metapopulation dynamics of an infectious disease: Tuberculosis in possums. *Theoretical Population Biology*, 61, 15-29.

9.

Gog J., Woodroffe R. & Swinton J. (2002). Disease in endangered metapopulations: the importance of alternative hosts. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269, 671-676.

10.

Gonzalez-Quevedo C., Davies R.G. & Richardson D.S. (2014). Predictors of malaria infection in a wild bird population: landscape-level analyses reveal climatic and anthropogenic factors. *Journal of Animal Ecology*, 83, 1091-1102.

11.

Hale J.M., Heard G.W., Smith K.L., Parris K.M., Austin J.J., Kearney M. & Melville J. (2013). Structure and fragmentation of growling grass frog metapopulations. *Conservation Genetics*, 14, 313-322.

12.

Hanski I. (1999). *Metapopulation Ecology*. Oxford University Press, Oxford.

13.

Harding K.C., Begon M., Eriksson A. & Wennberg B. (2012). Increased migration in host–pathogen metapopulations can cause host extinction. *Journal of Theoretical Biology*, 298, 1-7.

14.

Harvell C.D., Mitchell C.E., Ward J.R., Altizer S., Dobson A.P., Ostfeld R.S. & Samuel M.D. (2002). Climate warming and disease risks for terrestrial and marine biota. *Science*, 296, 2158-2162.

15.

Heard G.W., McCarthy M.A., Scroggie M.P., Baumgartner J.B. & Parris K.M. (2013). A Bayesian model of metapopulation viability, with application to an endangered amphibian. *Diversity and Distributions*, 19, 555-566.

16.

Heard G.W., Scroggie M.P. & Malone B.S. (2012). Classical metapopulation theory as a useful paradigm for the conservation of an endangered amphibian. *Biological Conservation*, 148, 156-166.

17.

Heard G.W., Scroggie M.P., Ramsey D.S.L. & Clemann N. (2014). Wetland characteristics influence disease risk for a threatened amphibian. *Ecological Applications*, 24, 650–662.

18.

Hero J.-M., Gillespie G., Lemckert F.L., Littlejohn M.J., Robertson P., Brereton R. et al. (2004). *Litoria raniformis*. The IUCN Red List of Threatened Species. Version 2014.2. Available at: www.iucnredlist.org. Last accessed 30 September 2014.

19.

Hess G. (1996). Disease in metapopulation models: implications for conservation. *Ecology*, 77, 1617-1632.

20.

Hosmer D.W., Lemeshow S. & Sturdivant R.X. (2013). *Applied Logistic Regression*. Third edn. John Wiley and Sons, New Jersey.

21.

Jesse M., Ezanno P., Davis S. & Heesterbeek J.A.P. (2008). A fully coupled, mechanistic model for infectious disease dynamics in a metapopulation: movement and epidemic duration. *Journal of Theoretical Biology*, 254, 331-338.

22.

Johnson M.L. & Speare R. (2005). Possible modes of dissemination of the amphibian chytrid *Batrachochytrium dendrobatidis* in the environment. *Diseases of Aquatic Organisms*, 65, 181-186.

23.

Jousimo J., Tack A.J.M., Ovaskainen O., Mononen T., Susi H., Tollenaere C. & Laine A.-L. (2014). Ecological and evolutionary effects of fragmentation on infectious disease dynamics. *Science*, 344, 1289-1293.

24.

Keeling M.J. & Gilligan C.A. (2000). Metapopulation dynamics of bubonic plague. *Nature*, 407, 903-906.

25.

Laurance W.F. (2004). Forest-climate interactions in fragmented tropical landscapes. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 359, 345-352.

26.

Lips K.R., Brem F., Brenes R., Reeve J.D., Alford R.A., Voyles J., Carey C., Livo L., Pessier A.P. & Collins J.P. (2006). Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 3165-3170.

27.

McCallum H. & Dobson A. (2002). Disease, habitat fragmentation and conservation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269, 2041-2049.

28.

Murray K.A., Skerratt L.F., Speare R. & McCallum H. (2009). Impact and dynamics of disease in a species threatened by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*. *Conservation Biology*, 23, 1242-1252.

29.

Park A.W. (2012). Infectious disease in animal metapopulations: the importance of environmental transmission. *Ecology and Evolution*, 2, 1398-1407.

30.

Piotrowski J.S., Annis S.L. & Longcore J.E. (2004). Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia*, 96, 9-15.

31.

Pounds A.J., Bustamante M.R., Coloma L.A., Consuegra J.A., Fogden M.P.L., Foster P.N., La Marca E., Masters K.L., Merino-Viteri A., Puschendorf R., Ron S.R., Sanchez-Azofeifa G.A., Still C.J. & Young B.E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, 439, 161-167.

32.

Pulliam H.R. (1988). Sources, sinks and population regulation. *The American Naturalist*, 132, 652-661.

33.

Puschendorf R., Hoskin C.J., Cashins S.D., McDonald K., Skerratt L.F., Vanderwal J. & Alford R.A. (2011). Environmental refuge from disease-driven amphibian extinction. *Conservation Biology*, 25, 956-964.

34.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria. http://www.r-project.org/.

35.

Royle J.A. & Kéry M. (2007). A Bayesian state-space formulation of dynamic occupancy models. *Ecology*, 88, 1813-1823.

36.

Savage A.E., Sredl M.J. & Zamudio K.R. (2011). Disease dynamics vary spatially and temporally in a North American amphibian. *Biological Conservation*, 144, 1910-1915.

37.

Scheele B.C., Hunter D.A., Grogan L.F., Berger L.E.E., Kolby J.E., McFadden M.S., Marantelli G., Skerratt L.F. & Driscoll D.A. (2014). Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians. *Conservation Biology*, 28, 1195-1205.

38.

Scheele B.C., Hunter D.A., Skerratt L.F., Brannelly L.A. & Driscoll D.A. (2015). Low impact of chytridiomycosis on frog recruitment enables persistence in refuges despite high adult mortality. *Biological Conservation*, 182, 36-43.

39.

Schmid B.V., Jesse M., Wilschut L.I., Viljugrein H. & Heesterbeek J.A.P. (2012). Local persistence and extinction of plague in a metapopulation of great gerbil burrows, Kazakhstan. *Epidemics*, 4, 211-218.

40.

Skerratt L.F., Berger L., Speare R., Cashins S., McDonald K.R., Phillott A.D., Hines H.B. & Kenyon N. (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *Ecohealth*, 4, 125-134.

41.

Stockwell M.P., Clulow J. & Mahony M.J. (2012). Sodium chloride inhibits the growth and infective capacity of the amphibian chytrid fungus and increases host survival rates. *PLoS ONE*, 7, e36942.

42.

Stockwell M.P., Clulow J. & Mahony M.J. (2015a). Evidence of a salt refuge: chytrid infection loads are suppressed in hosts exposed to salt. *Oecologia*, 177, 901-910.

43.

Stockwell M.P., Storrie L.J., Pollard C.J., Clulow J. & Mahony M.J. (2015b). Effects of pond salinization on survival rate of amphibian hosts infected with the chytrid fungus. *Conservation Biology*, 29, 391-399.

44.

Thomas A., O'Hara B., Ligges U. & Sturtz S. (2006). Making BUGS Open. *R News*, 6, 12-17.

45.

Urban M.C., Lewis L.A., Fučíková K. & Cordone A. (2015). Population of origin and environment interact to determine oomycete infections in spotted salamander populations. *Oikos*, 124, 274-284.

46.

Vredenburg V.T., Knapp R.A., Tunstall T.S. & Briggs C.J. (2010). Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proceedings of the National Academy of Sciences*, 107, 9689-9694.

47.

Whittaker R.J. & Fernández-Palacios J.M. (2007). *Island Biogeography: Ecology, Evolution and Conservation*. Oxford University Press, Oxford.

48.

Woodhams D.C. & Alford R.A. (2005). Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. *Conservation Biology*, 19, 1449-1459.

49.

Woodhams D.C., Bosch J., Briggs C.J., Cashins S., Davis L.R., Lauer A., Muths E., Puschendorf R., Schmidt B.R., Sheafor B. & Voyles J. (2011). Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. *Frontiers in Zoology*, 8, 8.

50.

Woodhams D.C., Rollins-Smith L.A., Carey C., Reinert L., Tyler M.J. & Alford R.A. (2006). Population trends associated with skin peptide defenses against chytridiomycosis in Australian frogs. *Oecologia*, 146, 531-540.

Captions to Figures

Figure 1. Map of the study area, showing (a) the distribution of wetlands surveyed for *Litoria raniformis* and all detections of the species between 2001/2002 and 2011/2012, and; (b) the number of sympatric frogs species (reservoir hosts of chytrid) recorded at each wetland. Records of sympatric frogs were opportunistic. Pink shading in (a) shows the nine metapopulations of *L. raniformis* persisting as of 2011/2012 which were used in the simulations (Fig. 5), where metapopulations were defined as networks of wetlands connected by distances of $\leq$ 1 km. Blue lines are major streams and grey lines 10 m contours. For clarity, the extreme north and south of the study area are not shown.

Figure 2. Variation in water surface temperature, salinity, chytrid prevalence and the incidence of *Litoria raniformis* across the study area. Dots depict pools along streams, farm dams, quarries, swamps and water-treatment wetlands, with dot size relative to their surface area (not to scale). (a) Predicted average night-time water surface temperature (^{o}C) across the 2006/2007 active season of *L. raniformis* (October – April). (b) Observed salinity (µS cm^{-1}). (c) Predicted prevalence of chytrid infections across the active season (assuming random sampling), dependent on water temperature and salinity. (d) Observed incidence of *L. raniformis* between 2001/2002 and 2011/2012, where incidence at each wetland is the proportion of years with surveys in which the frog was detected. Blue lines are major streams and grey lines 10 m contours. For clarity, the extreme north and south of the study area are not shown.

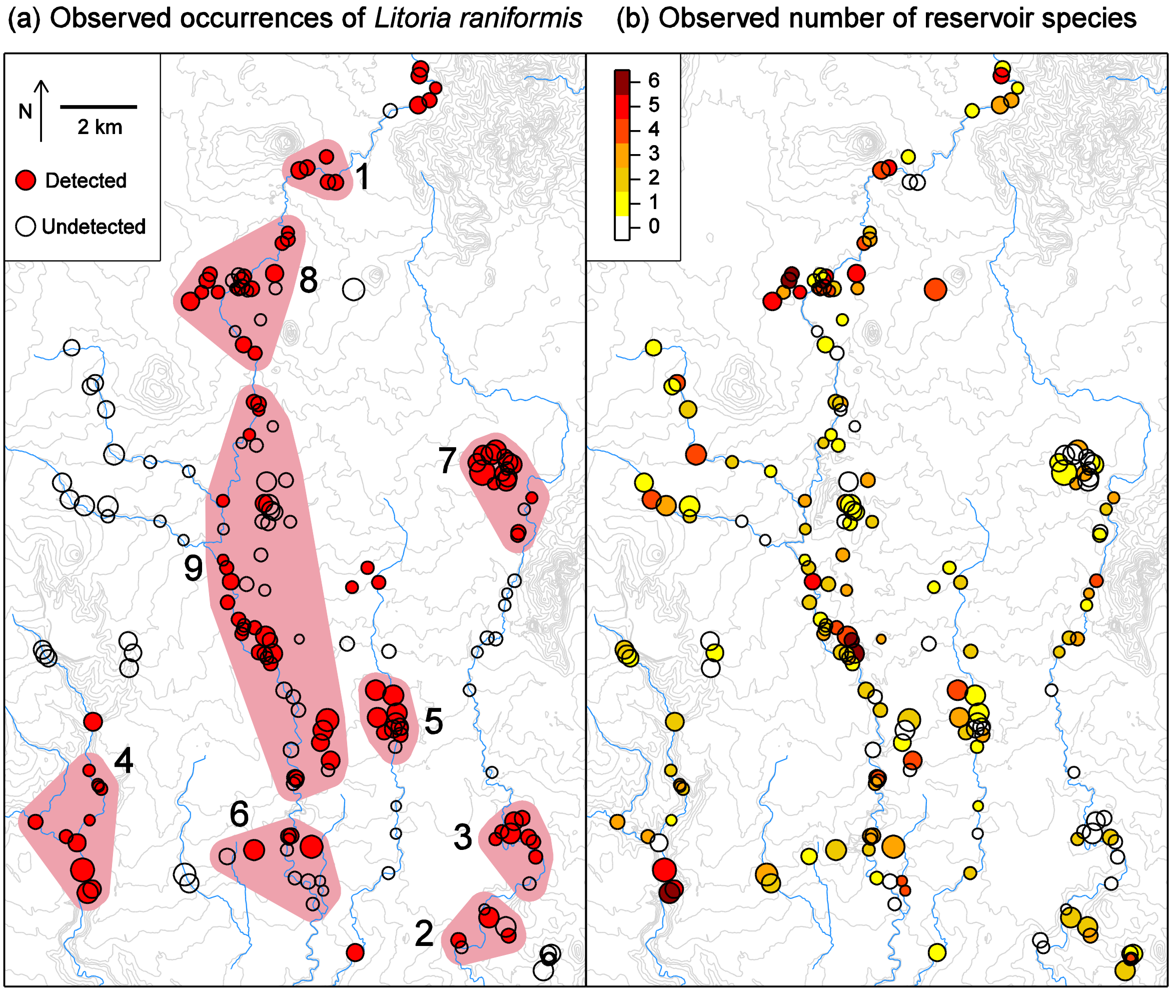
Figure 3. The observed incidence of *Litoria raniformis* at wetlands across the range of predicted chytrid prevalence (a) and connectivity (b). For a given wetland, incidence is the proportion of years with surveys in which the frog was detected. Means for wetlands in each bin are shown, with error bars showing one standard error among wetlands. Connectivity for each wetland is the mean across years as estimated from the occupancy model.

Figure 4. The relationship between the annual probability of persistence of *Litoria raniformis* populations and predicted chytrid prevalence (a) and connectivity (b), and between the annual probability of colonisation of vacant wetlands and connectivity (c). The thick black lines show the posterior mean predictions and dashed lines the 95% credible intervals. The relationships depicted for the probability of persistence assume the other covariate is at its mean value.

Figure 5. The probability of extinction for nine metapopulations of *Litoria raniformis* over 30 years, when: (a) predicted chytrid prevalence and connectivity remained unchanged ('current conditions'); (b) chytrid prevalence at all wetlands was set to the local maximum ('no refugia'); (c) connectivity of each wetland was set to zero ('no connectivity'), or; (d) both changes were applied ('no refugia or connectivity'). The numbering of metapopulations matches that in Fig. 1a. The numbers above the bars in the top plot are the number of wetlands in each metapopulation as of 2011/2012.

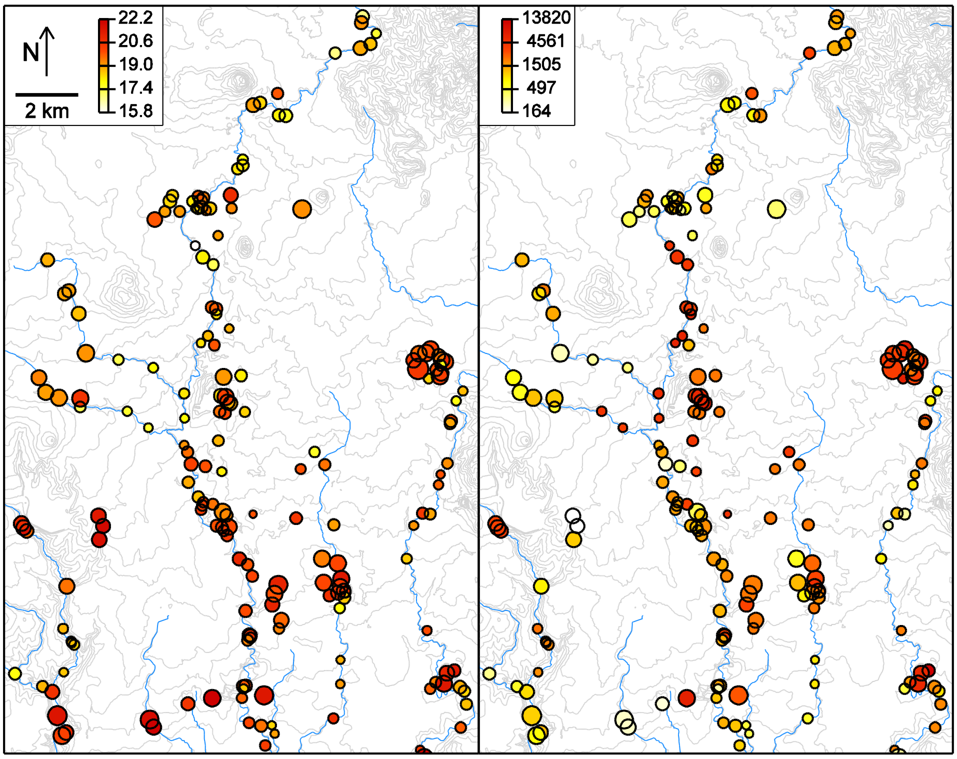
Table 1. Parameter estimates for each statistical model. Only parameters for the final water temperature and infection models are shown. The posterior mean estimate is presented for each parameter, with the 95% credible interval in parentheses. See text for covariate definitions.

Model	**Parameter**	**Estimate**
Water temperature	Intercept	20.24 (20.07, 20.42)
	Date	9.02 (7.73, 10.23)
	Date^2	-8.19 (-9.37, -6.94)
	Daily maximum air temperature	2.62 (2.36, 2.88)
	Wetland size	0.81 (0.42, 1.21)
	Elevation	-1.07 (-1.45, -0.69)
	Emergent vegetation cover	-0.90 (-1.31, -0.49)
	Riparian tree and shrub cover	-0.47 (-0.85, -0.08)
	Date × wetland size	0.45 (0.21, 0.69)
	Site-level standard deviation	0.85 (0.66, 1.04)
Probability of infection	Grand intercept	-3.14 (-3.99, -2.43)
	Salinity	-0.53 (-1.05, -0.03)
	Frog age (adult vs juvenile)	2.54 (1.82, 3.41)
	Water temperature	-1.14 (-1.58, -0.71)
	Site-level standard deviation	0.28 (0.01, 0.72)
Probability of persistence	Intercept	5.86 (3.91, 8.40)
	Predicted chytrid prevalence	-10.05 (-16.31, -5.41)
	Connectivity	1.03 (-0.13, 2.49)
Probability of colonisation	Intercept	-1.86 (-2.37, -1.39)
	Connectivity	4.00 (2.60, 5.70)


(a) Observed occurrences of *Litoria raniformis*

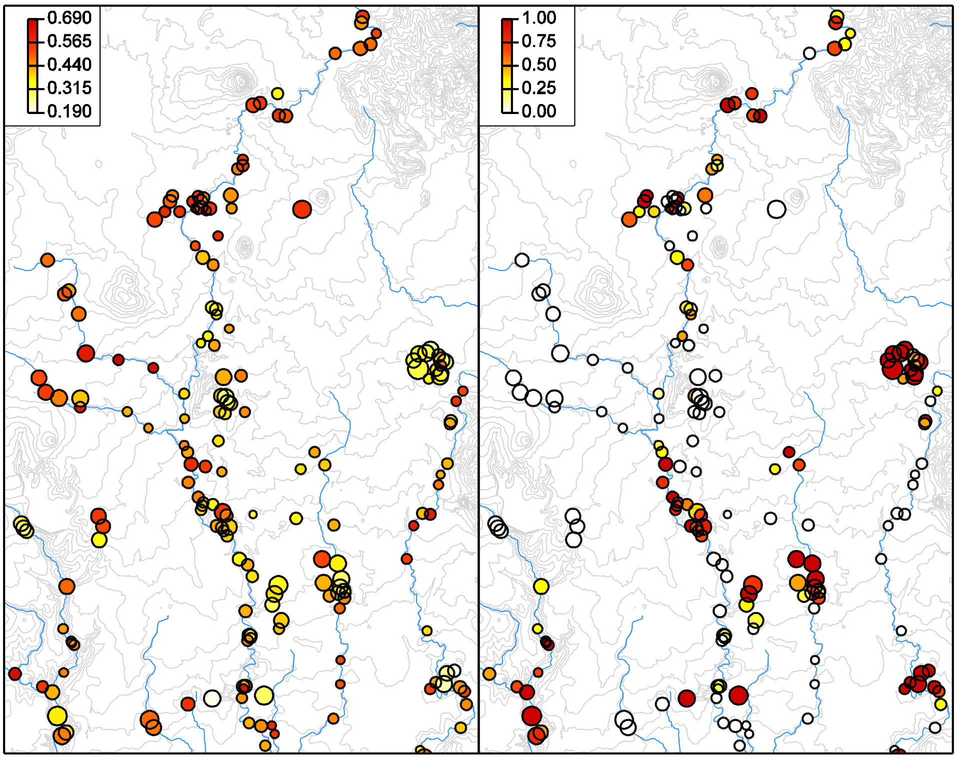
(b) Observed number of reservoir species

Figure 1



(a) Predicted mean water temperature, °C

(b) Salinity, $\mu S\ cm^{-1}$



(c) Predicted chytrid prevalence

(d) Observed incidence of *Litoria raniformis*

Figure 2

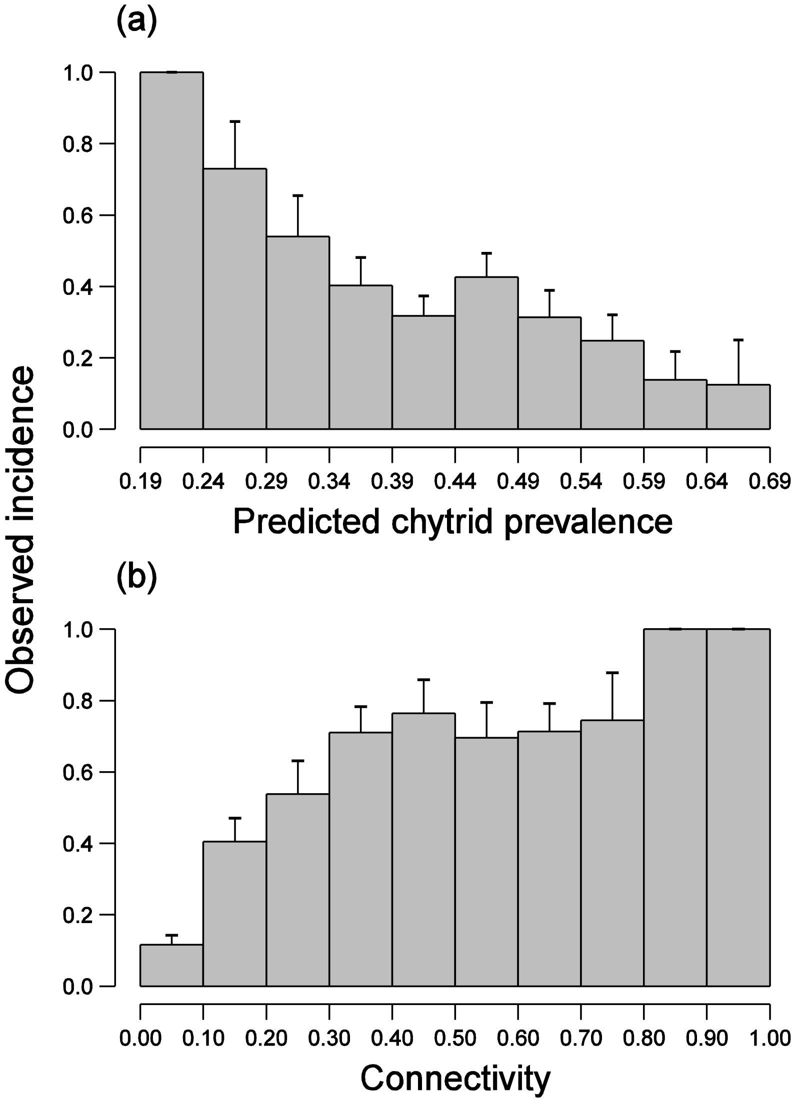

(a)
1.0
0.8
0.6
0.4
0.2
0.0
0.19
0.24
0.29
0.34
0.39
0.44
0.49
0.54
0.59
0.64
0.69
Predicted chytrid prevalence
Observed incidence
(b)
1.0
0.8
0.6
0.4
0.2
0.0
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
Connectivity

Figure 3

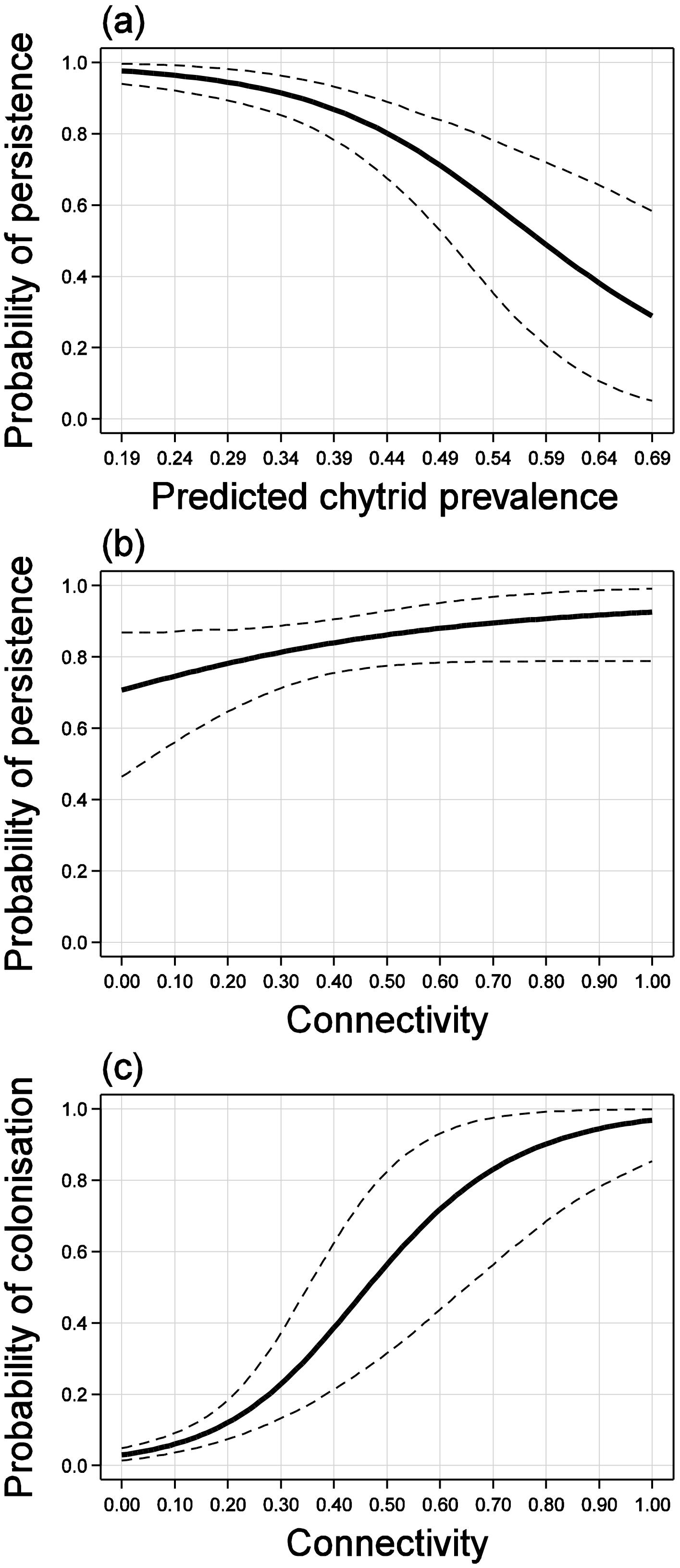

(a)
Probability of persistence
1.0
0.8
0.6
0.4
0.2
0.0
0.19
0.24
0.29
0.34
0.39
0.44
0.49
0.54
0.59
0.64
0.69
Predicted chytrid prevalence
(b)
Probability of persistence
1.0
0.8
0.6
0.4
0.2
0.0
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
Connectivity
(c)
Probability of colonisation
1.0
0.8
0.6
0.4
0.2
0.0
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
Connectivity

Figure 4

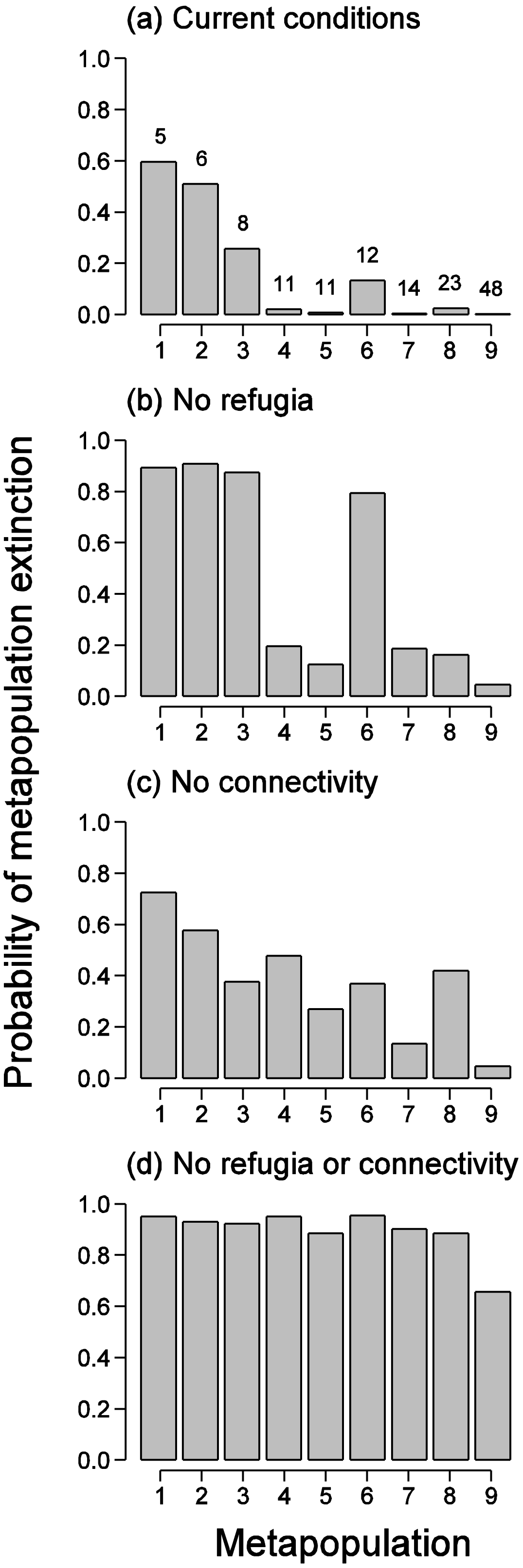

(a) Current conditions
1.0
0.8
0.6
0.4
0.2
0.0
5
6
8
11
11
12
14
23
48
1 2 3 4 5 6 7 8 9
(b) No refugia
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9
(c) No connectivity
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9
(d) No refugia or connectivity
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9
Probability of metapopulation extinction
Metapopulation

Figure 5