Clean Air and Urban Landscapes Hub

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Seasonal prevalence of the chytrid fungus in populations of the motorbike frog *Litoria moorei*

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About the Clean Air and Urban Landscapes Hub

The Clean Air and Urban Landscapes Hub (CAUL) is a consortium of four universities: the University of Melbourne, RMIT University, the University of Western Australia and the University of Wollongong. The CAUL Hub is funded under the National Environmental Science Program of the Australian Government's Department of Agriculture, Water and the Environment. The task of the CAUL Hub is to undertake research to support environmental quality in our urban areas, especially in the areas of air quality, urban greening, liveability and biodiversity, and with a focus on applying research to develop practical solutions.

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Introduction

Amphibian chytrid fungus *Batrachochytrium dendrobatidis (*hereafter *Bd)*, has caused at least 500 amphibian species to decline globally (Scheele *et al.* 2019). While some amphibians are highly susceptible to developing the disease chytridiomycosis, when infected by *Bd*, other species appear to cope despite an association with the pathogen (West 2015). Understanding the environmental factors that influence *Bd* prevalence and disease manifestation is crucial for developing strategies to reduce the impact of the pathogen on natural populations (Blaustein & Kiesecker 2002).

In some cases, environmental conditions may influence the ability of species to cope with *Bd. Bd* infection prevalence – the proportion of individual frogs in a population infected with the pathogen – can be influenced by environmental conditions (Becker *et al.* 2012; Becker & Zamudio 2011; Clemann *et al.* 2013; Heard *et al.* 2014). Temperature, humidity, salinity, and water pH can influence *Bd* prevalence by limiting the pathogen's growth and survival rates (Bramwell 2011; Stockwell *et al.* 2012; Stockwell *et al.* 2015). For example, optimal pathogen growth occurs at 17–25 ^{o}C, growth slows outside of this range, and the pathogen dies at ≥28 ^{o}C (Piotrowski *et al.* 2004). Wetland salinity may also act to protect frogs against chytridiomycosis, by reducing the transmission rates within populations (Clulow *et al.* 2018). The prevalence and pathogenicity of *Bd* increase in mild and humid climates (Becker & Zamudio 2011; Berger *et al.* 2004). Consequently, *Bd* prevalence can be higher during the cool winter months than during the warm-hot summer months (Woodhams & Alford 2005).

Environmental conditions are known or predicted to be highly suitable for *Bd* in multiple regions throughout Australia including the Wet Tropics in Queensland, coastal regions of Queensland and NSW, a vast expanse of Victoria and Tasmania and south-west Western Australia (Murray *et al.* 2010; Murray *et al.* 2011). Amphibians in these areas of Australia are therefore considered at a higher risk of infection than other regions. For example, two related threatened species in south-eastern Australia, the growling grass frog *Litoria raniformis* and green and golden bell frog *Litoria aurea* have both suffered population declines that are associated with *Bd* (Heard *et al.* 2013; Heard *et al.* 2015; Klop-Toker *et al.* 2018a; Stockwell *et al.* 2010). Logically, other related species that occur in highly *Bd* suitable regions in Australia might also be assumed to be susceptible to *Bd*. Another species of related bell frog, the motorbike frog *Litoria moorei*, is known to occur in the highly *Bd* suitable south-west region of Western Australia. Nevertheless, *L. moorei* is not known to have declined despite exposure to this pathogen.

Bd is currently not perceived as a critical management issue (i.e., it is not known to have caused significant population declines) for any amphibians in south-west Western Australia even though this is a highly suitable region, and the pathogen has been present since at least 1985 (Riley et al. 2013). However, few studies have evaluated *Bd* prevalence or impacts on Western Australian amphibians. If *L. moorei* can persist despite the association with *Bd*, then understanding the factors that influence infection prevalence and pathogenicity could help inform management of threatened eastern Australian bell frogs. Conversely, if the *L. moorei* is at risk of *Bd*-mediated declines, then pathogen management in south-western Australia will need to become a higher priority issue. As a first step to address these uncertainties, we conducted a study to determine if the environmental conditions in south-west Western Australia could influence infection risk in *L. moorei* populations. To achieve this, we examined environmental conditions and evaluated the prevalence of *Bd* infection at 45 wetlands throughout the Swan Coastal Plain region of south-western Australia from December 2016 to March 2018.

Materials and Methods

Study sites

The study was conducted at 45 wetlands on the Swan Coastal Plain, Western Australia (Fig. 1). The wetlands surveyed included ponds, swamps and lakes that were influenced by urbanisation to varying degrees.

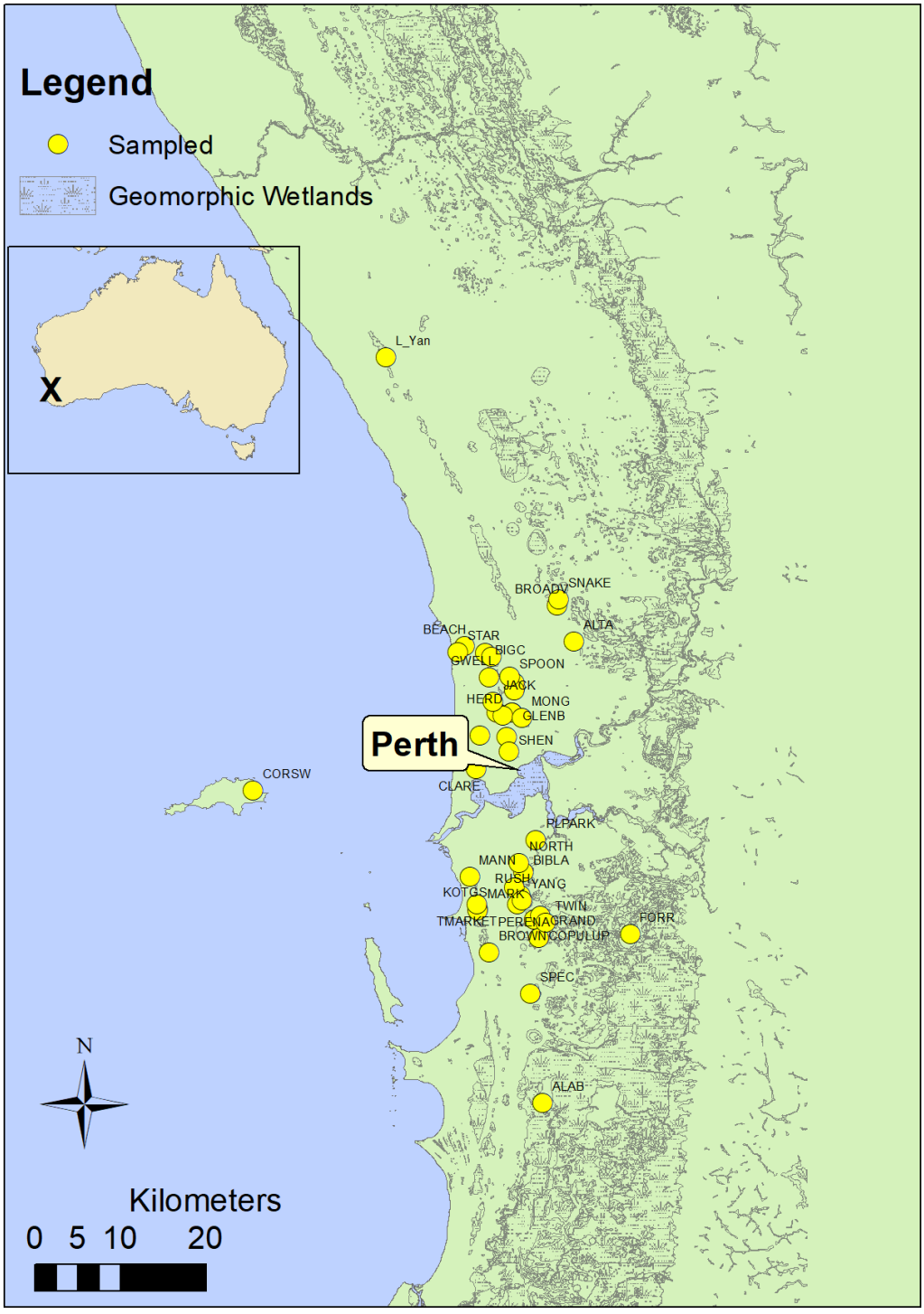

Figure 1. The distribution of the 45 wetland sites (yellow dots) surveyed across the Swan Coastal Plain.

Environmental data recording

We deployed data loggers at 11 wetlands (~24% of the sites surveyed) to monitor water temperature throughout the study period. Data loggers were positioned near the shoreline and floated 5 cm below the water surface in areas where frogs were frequently observed. Data loggers recorded water temperature (C) every 15 minutes from September 2017 to March 2018. Loggers were checked fortnightly to ensure they remained at 5 cm below the water surface level in case of wetland drying.

Frog surveys and chytrid sampling

Each wetland site was surveyed on 1–9 occasions (mean 4 occasions) between December 2016 and March 2018. During each visit to a site, two or three researchers listened for frog calls (call survey) for five minutes and then performed a visual search (visual survey) for 45 minutes (Parris et al., 1999). The calling activity of each frog species was scored on a scale where 0 = no calling, 1 = calling but no overlap, 2 = calling with overlap but breaks and 3 = continuous calling. Visual surveys involved two researchers walking side by side using head torches to search for and record the number of *L. moorei* detected. All surveys were performed at night between 1800 and 0100. The weather conditions were recorded immediately after the call survey and prior to the visual survey, and included wet- and dry-bulb temperature, rain, wind, cloud cover and moonlight. We also collected data on water temperature (C), water depth (cm), salinity (ppm), electrical conductivity (μS/cm), pH and total dissolved solids (ppm).

Litoria moorei observed on transects were captured by hand, and the total number of missed/lost frogs was recorded. A new, clean pair of nitrile gloves was worn to capture and handle each frog to reduce the risk of spreading chytrid between individuals (Phillott *et al.,* 2010). We recorded the following information for each captured frog: body temperature, substrate temperature, reproductive condition, sex, size and age. Adult frogs were sexed using the presence/absence of nuptial pads, although it was also possible to distinguish females if they were gravid. Size was measured as snout-to-vent length (SVL) using DialMax calipers. We cleaned callipers using an ethanol-based disinfectant wipe between each frog. Frogs that measured <53 mm SVL were recorded as juveniles, and frogs still retaining part of their tail were recorded as metamorphs (Tyler & Doughty, 2009).

We collected swab samples from each frog captured to determine its *Bd*-infection status. Following West (2015), a fine-tip sterile cotton swab (Medical Wire & Equipment) was wiped gently over the frog's skin in a standardised protocol that involved four wipes on the palms of the four feet, ventral surface (stomach to chin) and the inner thighs (Figure 2). All swabbing was conducted by the lead investigator to minimise variation in swabbing technique. Following swabbing, the frogs were released at the point of capture. Waypoints for each frog swabbed were recorded at the point of capture using a Garmin GPS unit. Frogs were only captured once during a visit to a site. Some frogs may have been recaptured during subsequent visits to a site, but the frequency of recapture was not a focus of this study.

PCR analysis

We assessed the *Bd*-infection status of each frog using real-time polymerase chain reaction (qPCR). The PCR method has previously been described by Boyle *et al.* (2004) and Hyatt *et al.* (2007), and since modified by West (2015). PCR reactions were run in triplicate and we considered a sample to be positive if at least one of the three wells returned a positive reaction, to maximise sensitivity and maintain specificity (Skerratt et al., 2011).

Prevalence analysis

By repeatedly sampling frogs over an eight-month period (including the breeding season), we aimed to determine: (1) whether *L. moorei* were protected by the high temperatures >28 ^{o}C within the selected wetlands on the Swan Coastal Plain; and (2) whether *Bd* prevalence was further influenced by water chemistry.

We constructed Bayesian logistic regression models to evaluate factors influencing *Bd* prevalence in *L. moorei* populations throughout the Swan Coastal Plain. Fifty-two candidate models were compared to examine combinations of potential explanatory variables that could influence *Bd* prevalence, including age, sex, wetland pH, water temperature, electrical conductivity, daily rainfall, maximum ambient temperature and Julian day of the year. We modelled all explanatory variables as a linear effect except for day of the year, which was modelled as a first order sigmoidal relationship over 366 days.

Figure 2. A motorbike frog being swabbed for the *Bd* fungus © Dom Lim, 2017

Each frog's probability of infection (psi[i]) was modelled as a logistic function, and we included a random effect for site in all models. The candidate models were fitted using JAGS (Plummer, 2013) from R using package jagsUI (Kellner, 2017) with three replicate Markov chains. Each chain was run for a burn in of 20,000 Markov Chain Monte Carlo (MCMC) iterations to ensure convergence. Convergence was verified by checking that the potential scale-reduction factor values (Brooks & Gelman, 1998) were < 1.1. Parameters were estimated from the posterior distribution after running each chain for a further 20,000 iterations, with every 10th iteration retained for inference. The mean, median, and 95% credible interval of each of the monitored parameters were summarised and compared. The derived deviance information criterion (DIC) of each simple model was compared to select a preferred candidate model based on the lowest DIC value.

A final model was then constructed by modifying the preferred candidate model to incorporate uncertainty in each individual frog's infection status, by including an observation model for the three

replicate PCR test results for each swabbed frog. We estimated parameters from the posterior distribution after running each chain for a further 20,000 iterations, with every 10th iteration retained for inferences. We then summarised the mean, median, and 95% credible interval of each of the monitored parameters (probability of detecting infection, beta coefficients and the random effect for site).

Results

Frog surveys

Across the 45 wetland sites, we swabbed 1175 individuals of *L. moorei* to test for *Bd*. PCR analysis indicated 234 frogs (20%) were positive for *Bd*. At each site, 1 to 49 frogs were swabbed during the winter survey period and 1 to 65 frogs were swabbed during the summer period. More *Bd*-infected frogs were detected at sites during the winter period (202 positive individuals representing 57% of frogs sampled over winter) than the summer period (32 positive individuals representing 4% of frogs sampled over summer).

Water temperature

Data from the temperature loggers indicated water temperature varied seasonally and was lowest in October 2017 (mean temperature 22 ^{o}C), peaking in January 2018 (mean temperature 28 ^{o}C). The lowest minimum water temperature registered was 11.9 ^{o}C (October), while the highest recorded maximum water temperature was 42.7 ^{o}C (Perry Lakes, March 2018). There was a slow increase in water temperature from October 2017 to March 2018.

Bd Prevalence

The best-supported model of *Bd* prevalence included a negative effect of electrical conductivity, a negative effect of mean maximum temperature during the 30 days prior to sampling, a sigmoidal relationship for day of year, and a random effect for wetland site (Fig. 3). *Bd* prevalence was lowest when wetlands had a high electrical conductivity (20,000 μS/cm) and a mean maximum ambient temperature of 30–35 C during the 30 days prior to sampling (Figs 4 & 5). In addition, *Bd* prevalence had a clear sigmoidal relationship with Julian day of the year (Figs 6 & 7). *Bd* prevalence was predicted to be highest from mid-September to early November (~day 250–300) and lowest from early April to late May (~day 90–150). The preferred model indicated that the mean probability of detecting *Bd* infection on a swab using PCR analysis was 0.88 (95% CI: 0.85–0.9).

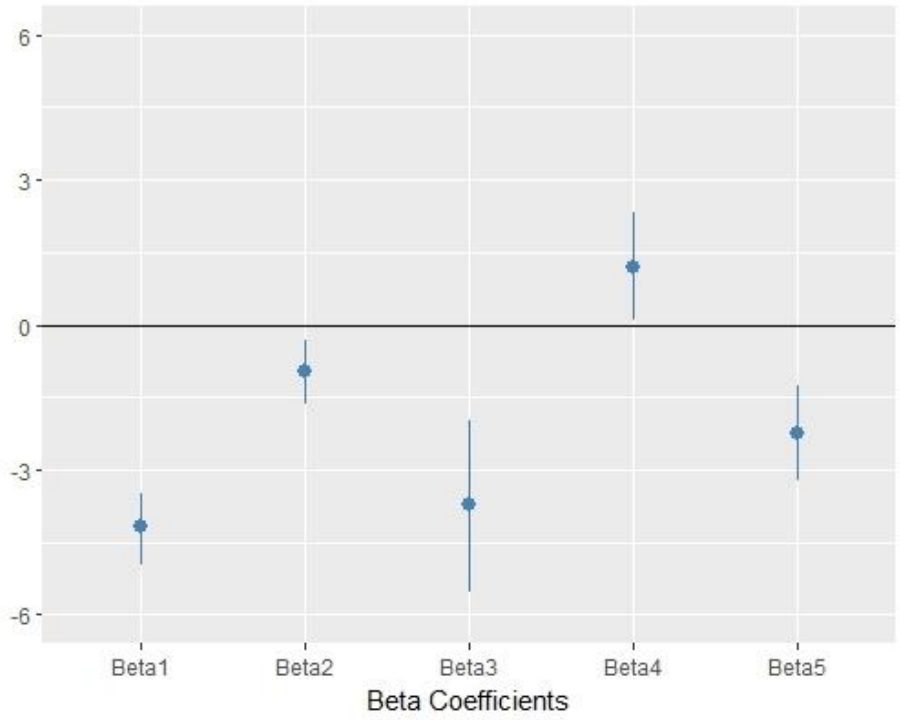

Figure 3. Mean (point) and 95% CI (line) for each of the beta coefficients in the logistic regression model of *Bd* prevalence. Beta1 = intercept, Beta2 = coefficient for EC, Beta3 = coefficient for mean maximum temperature (during the 30 days prior to survey), Beta4 = coefficient for cosine effect on day of survey, Beta5 =coefficient for sine effect on day of survey.

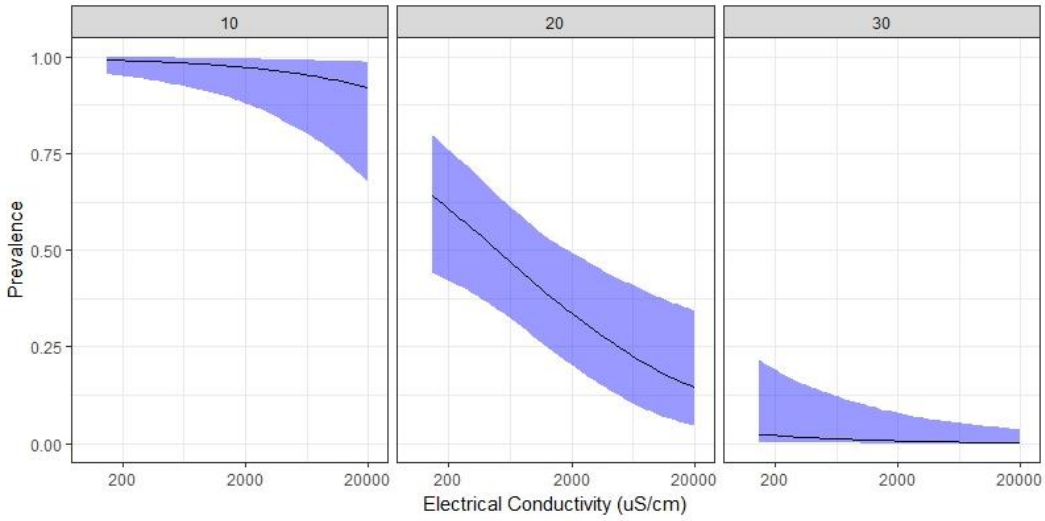

Figure 4. Influence of wetland electrical conductivity on *Bd* prevalence in motorbike frogs on the Swan Coastal Plain, assuming sampling on day 250 and a mean maximum ambient temperature of either 10, 20 or 30 C during the preceding 30 days. Electrical conductivity (µS/cm) is transformed to $\log_e$. Black line indicates the median prevalence and purple shading the 95% credible intervals.

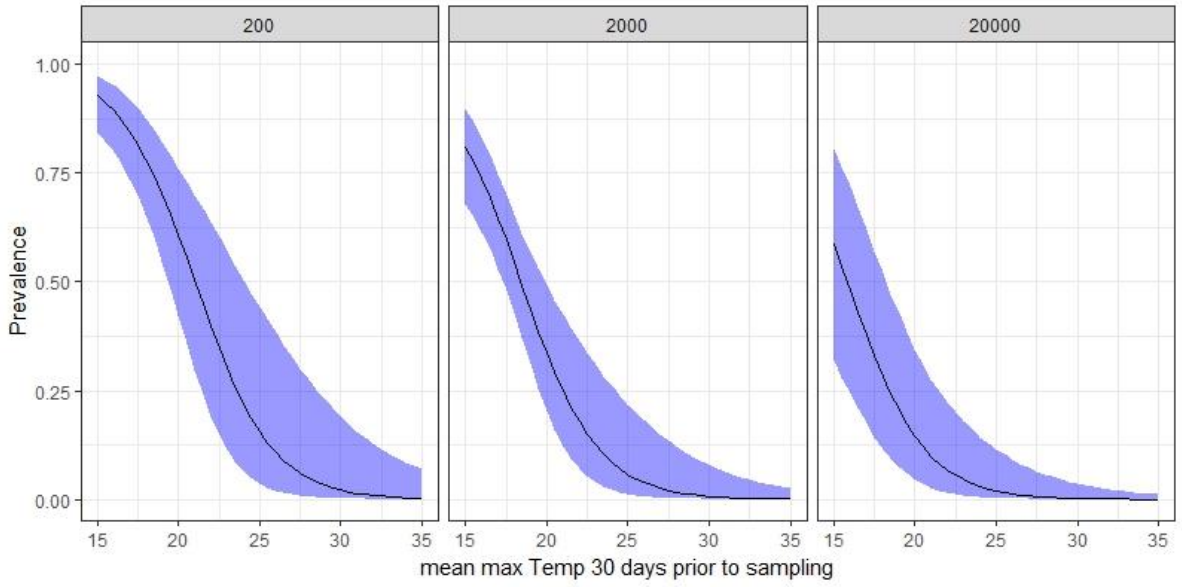

Figure 5. Influence of mean maximum air temperature on *Bd* prevalence in motorbike frogs on the Swan Coastal Plain, assuming sampling on day 250 and a wetland electrical conductivity of 200, 2,000 or 20,000 μS/cm. Black line indicates the median predicted *Bd* prevalence and purple shading the 95% credible intervals.

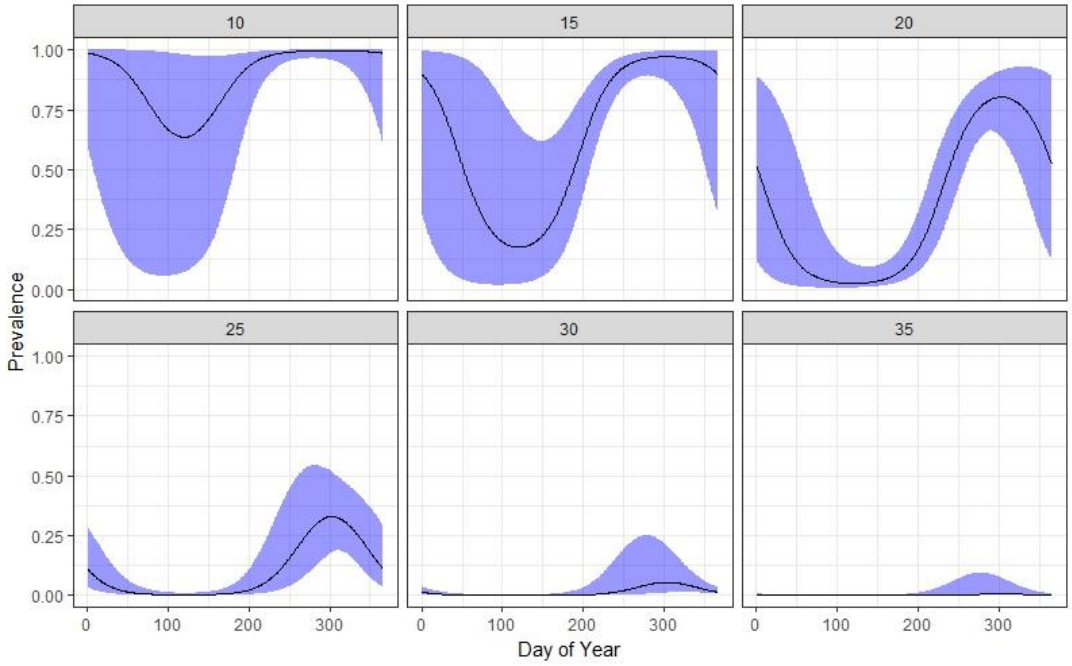

Figure 6. Seasonal *Bd* prevalence in motorbike frogs on the Swan Coastal Plain. Results assume a $\log_e$ electrical conductivity (μS/cm) of 5 and a mean max temperature during the 30 days prior to sampling of 10, 15, 20, 25, 30 or 35 C. The black line indicates the median prevalence and purple shading the 95% credible intervals.

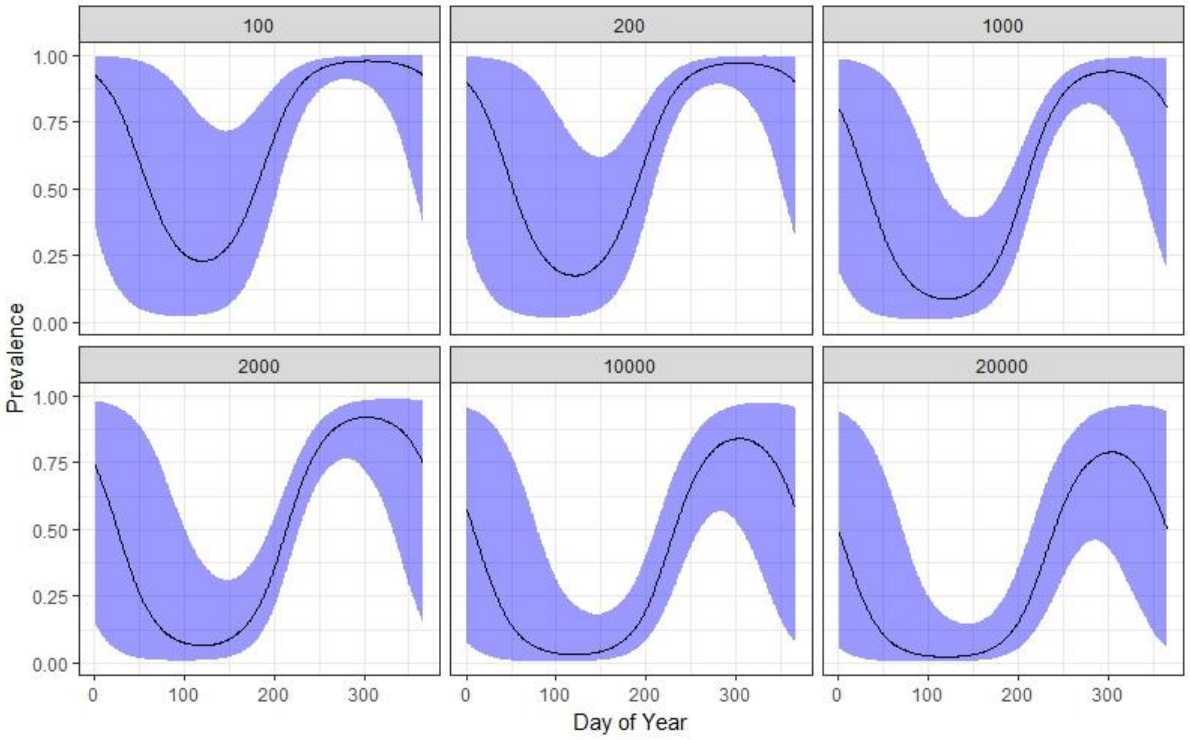

Figure 7. Seasonal *Bd* prevalence in motorbike frogs on the Swan Coastal Plain. Results assume a mean maximum temperature of 15 C and a $\log_e$ electrical conductivity (μS/cm) of 5, 6, 7, 8, 9 or 10. Black line indicates the median prevalence and purple shading the 95% credible intervals.

Discussion

Our study found that *Bd* is present, widespread, and infects *L. moorei* throughout the assessed wetlands on the Swan Coastal Plain of Western Australia. This finding confirms that the environmental conditions in this region are suitable for *Bd* survival and transmission. However, *Bd* prevalence in *L. moorei* populations at the assessed wetlands varied spatially and temporally with variation in environmental conditions at the site and across seasons.

High ambient temperatures were strongly associated with a low *Bd* prevalence in the sampled *L. moorei* populations. *Bd* prevalence was lowest when ambient temperatures were frequently above 28 °C in the 30-day period prior to sampling. This is consistent with laboratory studies that identified 28 °C as the upper threshold for *Bd* viability and that the rate of *Bd* mortality increases with time exposed to temperatures above 28 °C (Piotrowski et al., 2004). These results are also consistent with findings for the related, threatened bell frog *L. raniformis* in south-eastern Australia (Heard *et al.* 2014). Water temperature can also be an important factor restricting *Bd* prevalence in bell frogs, and water temperatures are influenced by ambient temperature and the vegetation within and surrounding wetlands (Heard *et al.* 2014). We have not yet found a clear relationship between *Bd* prevalence and water temperature for *L. moorei,* and additional research would be useful to verify habitat variables that influence *Bd* prevalence in populations of *L. moorei*. For example, cooler, highly shaded areas may be more conducive to *Bd* survival and growth, hence increasing the transmission rate among individual frogs (Johnson & Speare, 2003; Berger *et al.,* 2004).

Seasonal changes in temperatures can be a critical driver of *Bd* prevalence, and a pattern of low *Bd* prevalence during hot weather and high Bd prevalence during mild temperatures is broadly reported for other amphibian species (Kriger & Hero 2006; Petersen *et al.* 2016; Phillott *et al.* 2013). Water availability, rainfall and humidity can also influence seasonal *Bd* infection rates (e.g., Ruggeri *et al.*

2018). In our study, rainfall was not clearly associated with *Bd* prevalence when the variable was included in statistical models. However, rainfall, humidity, and other factors that vary seasonally could all play a role. Seasonal changes may even be driven by frog behaviour in response to the changing seasons, or the presence/absence of reservoir host species (Rowley & Alford 2007). Our results clearly show that *Bd* prevalence in *L. moorei* populations was low (mean 4%) during our summer survey periods when average ambient temperature was high (mean 32 ^{o}C). During winter, *Bd* prevalence was higher (mean 54%) when ambient temperatures were lower (mean 23 ^{o}C). These results suggest that high summer temperatures in the south-west of Western Australia can help to moderate *Bd* infections in *L. moorei* populations, however winter ambient temperatures are conducive to pathogen growth, survival and transmission.

Electrical conductivity of wetlands was negatively associated with *Bd* prevalence in *L. moorei* populations in the Swan Coastal Plain wetlands of Western Australia. This finding is consistent with studies in related bell frog species in eastern Australia. These other studies have also shown that the electrical conductivity or more specifically, the salinity of wetlands, can inhibit the growth of *Bd* in *L. aurea* (e.g., Stockwell et al., 2012) and *L. raniformis (Heard et al. 2014)*. The properties of salt (NaCl) in inhibiting growth, survival and transmission of *Bd* have been discovered both in the field and under laboratory conditions (Clulow *et al.* 2018; Klop-Toker *et al.* 2018b; Stockwell *et al.* 2012). As results across the three bell frog species have all found that high electro-conductive or saline wetlands can help to reduced *Bd* prevalence, increasing wetland salinity may be a viable management solution (Clulow *et al.* 2018; Heard *et al.* 2014). Saline wetlands in a landscape can be important for ensuring the persistence of species like these bell fogs that exhibit metapopulation dynamics (Heard *et al.* 2015). However, in our study, high ambient temperatures had a stronger influence on *Bd* prevalence than electrical conductivity (see Figs 4 & 5) and clearly, there is an interaction between both factors.

Our results demonstrate that under some circumstances *Bd* prevalence can be high in some populations. This mainly occurs at wetlands with a low conductivity during periods of mild temperature conditions. Anecdotally, some large die-offs of *L. moorei* have been reported during periods of mild temperature conditions and some of the dead frogs were positive for *Bd* (Glen Gaikhorst pers. com.). This suggests that *Bd* may influence *L. moorei* survival. The most important next step for *L. moorei* is to determine if *Bd* is influencing population persistence. Our results also suggest that further research into *Bd* prevalence and impacts in other south-west Western Australian frogs is warranted to clarify if *Bd is* a threat and an important management issue for any other amphibian species in the region.

Environmental conditions (particularly temperature and wetland electrical conductivity) can moderate *Bd* prevalence in all three bell frog species. We have not yet resolved the mystery of why *L. moorei* can thrive in south-west Western Australia while the two eastern Australian bell frog species are declining, despite all species being exposed to *Bd* and habitat loss, degradation and pollution associated with urbanisation and industrial activities. In future, the differential response of bell frog species to threats will be resolved by examining factors that that can influence the survival of individual frogs and the persistence of populations when exposed to *Bd*. Differences in recruitment rates (West *et al.* 2020), immune responses (Rollins-Smith *et al.* 2009), microbial defences (Harris *et al.* 2009) and behaviour (Karavlan & Venesky 2016) could all be important factors, as could the availability of environmental refuges and species metapopulations dynamics (Heard *et al.* 2015). This could help inform management of threatened bell frogs in eastern Australian and other threatened frogs throughout the country.

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Appendix A. Percentage of *Bd*-infected frogs detected during the winter and summer season at each survey site, across the whole sampling period (2017–2018).

Wetland site	Winter			Summer			TOTAL		
	Count	No. of Frogs infected	%	Count	No. of Frogs infected	%	Count	No. of Frogs infected	%
Alabaster Pond	13	2	15.38	39	1	2.56	52	3	5.77
Alta Laguna Lake	20	15	75.00	32	4	12.50	52	19	36.54
Beach Marmion	0	0	0.00	5	0	0.00	5	0	0.00
Bibra Lake	11	6	54.55	39	0	0.00	50	6	12.00
Big Carine Swamp	5	4	80.00	10	1	10.00	15	5	33.33
Branch Circus	1	1	100.00	5	0	0.00	6	1	16.67
Broadview Park	11	0	0.00	3	0	0.00	14	0	0.00
Bullsbrook Reserve	0	0	0.00	0	0	0.00	0	0	0.00
Candella Square	0	0	0.00	3	0	0.00	3	0	0.00
Copulup Lake	0	0	0.00	6	0	0.00	6	0	0.00
Corio Swamp	0	0	0.00	0	0	0.00	0	0	0.00
Foreshore Herdsman	0	0	0.00	7	0	0.00	7	0	0.00
Forrestdale Lake	5	3	60.00	10	0	0.00	15	3	20.00
Glendalough (A)	12	5	41.67	24	0	0.00	36	5	13.89
Glendalough (B)	5	4	80.00	7	1	14.29	12	5	41.67
Grande Cr	22	12	54.55	33	3	9.09	55	15	27.27
Harrisdale Swamp	1	0	0.00	0	0	0.00	1	0	0.00
Herdsman Lake	26	14	53.85	39	4	10.26	65	18	27.69
Jackadder Lake	17	12	70.59	36	0	0.00	53	12	22.64
Kotisina Gardens	8	5	62.50	1	0	0.00	9	5	55.56
Lake Claremont	4	2	50.00	34	2	5.88	38	4	10.53
Lake Gwelup	12	5	41.67	41	2	4.88	53	7	13.21
Lake Monger	4	3	75.00	8	2	25.00	12	5	41.67
Little Rush Lake	23	16	69.57	29	0	0.00	52	16	30.77
Loch McNess	26	15	57.69	37	2	5.41	63	17	26.98
Mabel Talbot	20	12	60.00	31	2	6.45	51	14	27.45
Manning Lake	19	3	15.79	49	0	0.00	68	3	4.41
Market Garden	6	3	50.00	0	0	0.00	6	3	50.00

Mt Brown Lake	9	9	100.00	46	1	2.17	55	10	18.18
North Lake	1	0	0.00	4	0	0.00	5	0	0.00
Perena Rocchi	0	0	0.00	6	0	0.00	6	0	0.00
Perry Lakes	19	10	52.63	39	1	2.56	58	11	18.97
Piney Lakes	6	1	16.67	7	0	0.00	13	1	7.69
Roselea Lake	0	0	0.00	9	0	0.00	9	0	0.00
Salerno Square	0	0	0.00	7	0	0.00	7	0	0.00
Secret Garden	6	1	16.67	14	0	0.00	20	1	5.00
Shearwater Spoonbill	13	7	53.85	30	0	0.00	43	7	16.28
Shenton Park	11	9	81.82	10	0	0.00	21	9	42.86
Snake Swamp	21	14	66.67	44	4	9.09	65	18	27.69
South Lake	3	0	0.00	13	1	7.69	16	1	6.25
Spectacles North	1	0	0.00	6	0	0.00	7	0	0.00
Star Swamp	5	3	60.00	0	0	0.00	5	3	60.00
Twin Bartram	8	5	62.50	26	1	3.85	34	6	17.65
Twin Market	0	0	0.00	3	0	0.00	3	0	0.00
Yangebup Lake	2	1	50.00	7	0	0.00	9	1	11.11
TOTAL	**376**	**202**	**53.72**	**799**	**32**	**4.01**	**1175**	**234**	**19.91**