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Disentangling causes of seasonal infection prevalence patterns: tropical tadpoles and chytridiomycosis as a model system

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ABSTRACT: Identifying the factors that affect pathogen prevalence is critical to understanding the effects of wildlife diseases. We aimed to examine drivers of seasonal changes in the prevalence of infection by the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in tadpoles. Because tadpoles may be important reservoirs for this disease, examining them will aid in understanding how chytridiomycosis affects entire amphibian populations. We hypothesized that temperature is a strong driver of prevalence of *Bd* in tadpoles, and the accumulation of infection as tadpoles become larger and older also drives prevalence in this system. We studied *Litoria rheocola*, a tropical rainforest stream frog with seasonal recruitment of annual tadpoles, and surveyed 6 streams in northeastern Queensland, Australia. Comparisons among models relating infection status to stream type, season, their interaction, tadpole age, and water temperature showed that age explained a large portion of the variance in infection status. Across sites and seasons, larger, older tadpoles had increased mean probabilities of infection, indicating that a large component of the variation among individuals was related to age, and thus to cumulative infection risk. Our results indicate that in systems with annual tadpoles, seasonal changes in infection prevalence may be strongly affected by seasonal patterns of tadpole growth and development in addition to stream type, season, and water temperature. These effects may then influence prevalence of infection in terrestrial individuals in species that have relatively frequent contact with water. This reinforces the need to integrate studies of the drivers of pathogen prevalence across all host life history stages.

KEY WORDS: *Batrachochytrium dendrobatidis* · Cumulative risk · Disease dynamics · Life stage · Stream ecology · Tropical systems

INTRODUCTION

The prevalence of infection by parasites and pathogens often varies seasonally (Wilson et al. 2002, Altizer et al. 2006). The effects of season may be caused by seasonal changes in transmission rates (Altizer et al. 2006, Dushoff et al. 2004), or by effects of environmental temperatures on the immune systems of hosts or on the growth rates of pathogens and parasites (Schoebel et al. 2011, Zamora-Vilchis et al. 2012). Host age also commonly affects prevalence, and the nature of these effects can differ among systems. Older individuals typically have greater cumulative exposure to the risk of infection (Wilson et al. 2002, Smith et al. 2007), but younger individuals may be more susceptible to infection (Zuk & Stoehr 2002). Thus, the biology of hosts and pathogens can interact in complex, age-specific ways (Johnson et al. 2011).

The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has caused amphibian population declines and extinctions worldwide (e.g. McDonald & Alford 1999, Stuart et al. 2004, Woodhams & Alford 2005,

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Alford 2010). The fungus infects keratin in adult amphibian epidermis and tadpole mouthparts (Berger et al. 1998, Longcore et al. 1999, Simoncelli et al. 2005), killing adults by severely disrupting electrolyte balance across the skin (Voyles et al. 2009). Tadpoles can survive with damaged mouthparts and may even regrow them, so the fungus does not usually kill tadpoles (Blaustein et al. 2005, Cashins 2009). Because infected individuals do not necessarily die, and transmission can occur between tadpoles (i.e. between individuals and among tadpole cohorts) and to adults when adults enter the water (Rachowicz & Vredenburg 2004), larval amphibians can act as reservoir hosts for *Bd* (Brunner et al. 2004, Woodhams & Alford 2005, Rowley & Alford 2007, Sapsford et al. 2013). Reservoir hosts greatly enhance the ability of disease to cause extinctions (de Castro & Bolker 2005), so an understanding of the dynamics of *Bd* prevalence in tadpoles can provide insight into disease-related amphibian declines and extirpations.

The dynamics of *Bd* are often strongly seasonal; this has been attributed to temperature effects on pathogen growth rates (Woodhams et al. 2003, Piotrowski et al. 2004, Stevenson et al. 2013), and to climate-driven changes in the resistance of frogs to infection (Andre et al. 2008, Daskin et al. 2014). In the laboratory, *Bd* grows optimally between 15 and 25°C and dies above 28–30°C (Piotrowski et al. 2004, Stevenson et al. 2013). However, recent evidence has demonstrated that some strains of *Bd* have a wider range of thermal tolerance; some can survive after a freeze (–12°C) or heat shock (28°C) treatment (Voyles et al. 2017). Thus, environmental temperatures may often drive prevalence of infection; in the wild, individual frogs that maintain body temperatures outside the thermal optimum of *Bd* can be less likely to be infected (Rowley & Alford 2013, Greenspan et al. 2017), and prevalence of *Bd* infection in adult frogs is typically higher when environmental temperatures are within the optimal thermal range of the pathogen (Woodhams & Alford 2005, Brem & Lips 2008, Sapsford et al. 2013). Similarly, prevalence of *Bd* infection in adult frogs often differs among elevations; these patterns may also be driven by the availability of temperatures that favour the pathogen's growth (Woodhams & Alford 2005, Brem & Lips 2008, Sapsford et al. 2013). Also, in the tropics, cool water flowing from high elevations may reduce lowland stream temperatures, increasing prevalence of *Bd* even though lowland streams tend to be warmer and less conducive to *Bd* growth (Pilliod et al. 2010, Sapsford et al. 2013). Most studies on the effects of temperature on prevalence have been conducted in adult frogs, but prevalence of *Bd* in tadpoles is also affected by changes in temperature (Cashins 2009, Valencia-Aguilar et al. 2016, Geiger et al. 2017).

In addition to temperature, the prevalence of *Bd* in tadpoles can be affected by age or size (Merilä et al. 2000, Rachowicz & Briggs 2007, Valencia-Aguilar et al. 2016). The body size of tadpoles increases with age, except if they are starving, and although many factors can alter age and size relationships, when members of the same species and cohort share a habitat, older individuals are usually larger than younger ones (reviewed by Alford 1999). Particularly for comparisons within cohorts of tadpoles, body size can therefore serve as a proxy for mean age. The prevalence of *Bd* increases with age in tadpoles of some species (Smith et al. 2007, Cashins 2009, Catenazzi et al. 2013, Vieira et al. 2013). In these species, older, larger tadpoles, with greater cumulative exposure to the risk of infection, have a higher prevalence of infection by *Bd* than do younger, smaller tadpoles. In many tropical stream systems, tadpoles can take a year or more to reach metamorphosis (Alford 1999), so tadpoles are in the water year-round and cohorts can overlap across years. If cumulative exposure is a major driver of infection status (Smith et al. 2007, Cashins 2009, Vieira et al. 2013), an annual cycle of prevalence of *Bd* infection could be driven by tadpole age. Prevalence would be low when there is an abundance of younger recruits, should increase as tadpoles age, grow, and accumulate infections, and then should decrease as overwintering individuals metamorphose and a new cohort of younger, smaller, uninfected individuals enters the system. Because water temperatures are often substantially cooler than air temperatures (Mohseni & Stefan 1999), *Bd* infections may persist in populations of tadpoles through summer, even in locations where air temperatures cause prevalence in terrestrial amphibians to reach zero (Rachowicz & Vredenburg 2004, Sapsford et al. 2013). In systems with annual tadpoles, tadpole cohort dynamics and seasonal patterns of water temperature could interact to drive seasonal patterns of prevalence of *Bd* in tadpoles. As an important reservoir, changes in prevalence of *Bd* in annual tadpoles might directly affect rates of incidence of infection in adult frogs and thus affect the prevalence of infection and ultimately the survival rates of adults.

We examined the hypothesis that annual cycles of tadpole cohort recruitment and ageing may be a driver of seasonal variation in prevalence of *Bd* infection in tadpoles, by studying larvae of the common mistfrog *Litoria rheocola.* This species is endemic to the Wet Tropics Bioregion of Australia, and popula-

tions at high elevations suffered severe declines in the early to mid-1990s due to chytridiomycosis (Berger et al. 1998, McDonald & Alford 1999). Since then, *L. rheocola* have recovered or recolonized at some high-elevation sites (McDonald & Alford 1999, Roznik & Alford 2015). Common mistfrogs breed all year; there is a major peak of breeding in summer, and their tadpoles are present throughout the year and can take up to 1 yr to reach metamorphosis (Richards 1992, Alford 1999, Cashins 2009, Roznik & Alford 2015).

The aim of our study was to examine seasonal patterns of prevalence of *Bd* infection in larval *L. rheocola*. We also aimed to determine how those patterns are affected by tadpole size (as a proxy for age), water temperature, season, and elevation. Although many factors could possibly influence prevalence in these tadpoles, we were particularly interested in the relative role of tadpole size, because although the phenomenon of an increase in infection prevalence with age had been observed in many species, the importance of this factor in long-term patterns of *Bd* prevalence in the field had not been explored.

MATERIALS AND METHODS

Study sites

We sampled tadpoles at 6 sites in the Wet Tropics Bioregion in northeastern Queensland, Australia. We sampled on 5 occasions over 1 full annual cycle: austral winter (June/July 2010), spring (October 2010), summer (January 2011), autumn (March/April 2011), and the following winter (June/July 2011). The sites were of 3 types, and full site descriptions can be found in Sapsford et al. (2013). Briefly, 2 sites were at high (>400 m) elevations, 2 were at contiguous low elevations (i.e. contiguous with, and downstream from, high-elevation sites), and 2 were at non-contiguous low elevations (i.e. not downstream from high-elevation sites) (Table 1). The 2 contiguous low-elevation sites were contiguous with high-elevation sites; however, they were independent of the high-elevation sites we studied.

Field methods

We sampled every 10 m along a 400 m transect at each field site. Sampling at each site in each season took place over 4 d. Tadpoles were sampled during the day using a dip net pressed against and slowly moved along the stream substrate, or held steady while rocks upstream from the net were displaced manually to dislodge tadpoles attached to rock surfaces. Tadpoles of *Litoria rheocola* are part of the 'lotic-suctorial' ecomorphological guild and are adapted to fast-flowing (torrent) sections of streams (Liem & Hosmer 1973, Altig & Johnston 1989, Richards 1992). Their large ventral oral discs, depressed body shape and muscular tails allow them to adhere to rock surfaces (Richards 2002). Tadpoles were captured and held in individual 50 ml vials filled with stream water until they were processed. A pair of new, well-rinsed vinyl gloves was worn while processing each tadpole (Cashins et al. 2008). During processing, tadpoles were identified to species (Anstis 2013). After identification, they were measured and swabbed for PCR assays of *Bd* infection status. Body length was measured to the nearest 0.5 mm from the tip of the snout to the base of the tail where the axis of the tail myotomes contacts the body wall (Altig 2007). Developmental stage (Gosner 1960) was not recorded because the hindlimbs develop in sheaths beneath the epidermis until late in development (Cashins 2009), preventing determination of developmental stage using hindlimb characteristics. After processing, tadpoles were released where they were captured. To reduce the likelihood of capturing tadpoles already processed

Table 1. Sites at which tadpoles were sampled within the Wet Tropics Bioregion in northeastern Queensland, Australia. Temperature data are mean ± SD

Site	National Park	Site type	Location	Elevation (m)	Annual temp. (°C) Air	Water
Windin Creek	Wooroonooran	High	17° 22′ 04″ S, 145° 42′ 52″ E	718	18.90 ± 2.62	18.75 ± 1.42
Bobbin Bobbin Falls	Wooroonooran	High	17° 22′ 44″ S, 145° 46′ 22″ E	700	19.13 ± 2.93	18.05 ± 3.82
Frenchman Creek	Wooroonooran	Contiguous low	17° 18′ 29″ S, 145° 55′ 16″ E	59	22.24 ± 2.94	21.06 ± 2.53
Tully Creek	Tully Gorge	Contiguous low	17° 46′ 30″ S, 145° 38′ 38″ E	114	22.21 ± 3.37	20.36 ± 2.68
Mena Creek	Private land	Non-contiguous low	17° 39′ 00″ S, 145° 59′ 14″ E	59	21.86 ± 3.65	22.40 ± 2.18
Stoney Creek	Hull River	Non-contiguous low	17° 55′ 18″ S, 146° 04′ 07″ E	18	22.79 ± 3.57	21.62 ± 2.84

during the sampling period, sampling always resumed upstream from all locations at which tadpoles were released.

Water temperatures were measured at the time and the location where each tadpole was captured, using a glass spirit thermometer with a range of −10 to 50°C.

Assessing infection status

We swabbed each tadpole's mouthparts according to Cashins (2009). Tadpoles were held (mouthparts facing up) between the fingers and thumb. A sterile fine-tip dry rayon swab (no. 113 Dry swabs, Medical Wire and Equipment) was gently passed along the mouthparts in a regular pattern: 8 times horizontally across both the lower and upper tooth rows and jaw sheath, and 8 times vertically across all tooth rows and jaw sheath for a total of 16 strokes.

After sample collection, all swabs were placed in separate vials and refrigerated until the end of sampling. These samples were analysed in triplicate using real-time quantitative PCR assays following the procedures outlined by Boyle et al. (2004). Samples were considered positive for *Bd* if at least 2 of the 3 replicate PCR reactions for that sample had numbers of zoospore equivalents >0 (Hyatt et al. 2007).

We also compared patterns of prevalence of infection in adult *L rheocola* to tadpole prevalence of infection. Data for adult *L. rheocola* were taken from Sapsford et al. (2013) and were used only to compare patterns across the 2 life stages. Data for adults were collected at the same sites at the same time (see Sapsford et al. 2013 for detailed methodology).

Statistical analysis

We performed detailed statistical analysis only on data for *L. rheocola* tadpoles, which were the dominant species in the tadpole assemblage, making up 64% of the individuals in our samples. We initially examined the overall relationship of probability of infection by *Bd* to tadpole size. We built a candidate set of generalized linear mixed models with a binomial link function using the glmer function in R version 3.5.0 (R Core Team 2017) that allowed us to examine influences on the infection status of each tadpole. Individual infection status (infected or not infected) was the response variable. Predictor variables were the continuous standardized covariates individual tadpole size and water temperature at the capture locality of each tadpole, as well as the categorical effects of season, site type (combining elevation and connectivity), and the season by site type interaction. We used the 2 sites of each type (high, contiguous low, non-contiguous low) as replicates, and all models included site as a random effect, to account for site-specific differences in infection status not related to the effects of interest. Collinearity of variables was checked using variance inflation factors, as we were concerned about the relationship between temperature and season; values were on the cusp of the cut-off value of 3, but Pearson correlation coefficients were well within reasonable range (<0.6, Zuur et al. 2010). Taking this into consideration, we decided to keep both temperature and season in the model. To verify that the model complied with underlying model assumptions, residuals were plotted against fitted values (Zuur & Ieno 2016). We evaluated the fit of models using Akaike's information criterion corrected for small sample size (AICc). As well as selecting the best model using AICc, we compared the best model with simpler models, and compared simpler models among themselves to examine the importance of particular variables as influences on tadpole infection status, as suggested by Burnham et al. (2011). We graphically compared the fit of the best-fitting model and the best single fixed-effect model, and compared the predictions of both models to the body size distributions of tadpoles in each season for each site type.

We also used a generalized linear mixed model using the lme function in R to examine infection intensity of each tadpole. Only using individuals positive for *Bd* infection, we used the log-transformed zoospore genomic equivalents as the response variable. Predictor variables and methods used to evaluate the fit of the model were as described above.

RESULTS

We obtained data on 721 *Litoria rheocola* tadpoles. Large numbers of *L. rheocola* were captured in each season: 174 in the first winter, 145 in spring, 122 in summer, 102 in autumn and 178 in the second winter. Preliminary examination of the data (Fig. 1) strongly supported the hypothesis that prevalence of infection increases with size, and thus with tadpole age. Every individual ranging from 15–17 mm was infected. The increasingly wide confidence intervals were caused by increasingly small sample sizes, which, in turn, occurred because most individuals have completed metamorphosis before they reach those sizes (Fig. 1).

Infection status

Of our candidate models (Table 2), the most complex, 18-parameter model was optimal as indicated by AICc, and accounted for 61.2% of the variation in infection status of tadpoles. This model suggested body size was positively correlated with infection status, indicating that larger tadpoles were more likely to be infected. Conversely, water temperature was negatively correlated with infection status (Table 3), indicating that as water temperature increased, infection decreased. As we were interested in the role of body size in *Bd* dynamics, we wanted to know how well variation in body size explained infection status. The model indicating only size as a predictor variable explained 37.5% of the variation, suggesting that body size is an important determinant of infection status in these systems. Furthermore, body size was included in the top 10 models (Table 2).

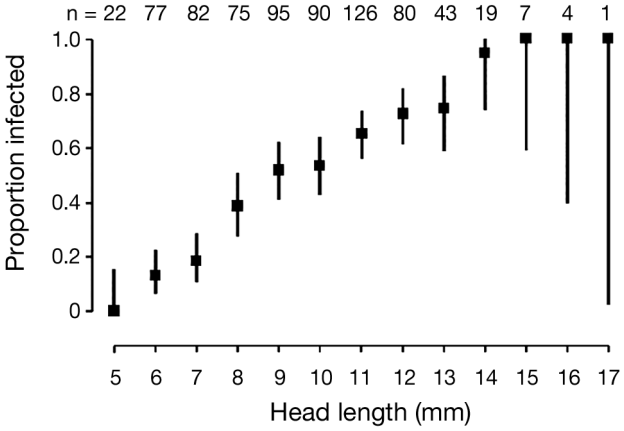

Fig. 1. Proportion of *Litoria rheocola* tadpoles in each 1 mm size interval infected by *Batrachochytrium dendrobatidis*, with 95% Clopper-Pearson confidence limits. Numbers at the top indicate total numbers of tadpoles in each 1 mm interval; small numbers and associated wide confidence intervals in the 3 largest intervals reflect variability in size at metamorphosis

Infection intensity

Of our candidate models (Table 2), the most complex, 19-parameter model was optimal and accounted for 30% of the variation in infection intensity of tadpoles. Infection intensity was positively correlated with body size and negatively correlated with water temperature. This corresponds with the results from

Table 2. Results of modelling. The intercept-only model included an overall intercept and separate intercepts for the random effect of each of our 6 sites. More complex models included all possible combinations of the effects of season (Season), site type (Type: high elevation, contiguous low elevation, non-contiguous low elevation), and their interaction, with body size (Size) of *Litoria rheocola* and temperature (Temp) at the site where each individual was captured as possible covariates. Models of particular interest appear in **bold**. The top 10 models are included for 2 types of models: (1) infection status (infected or not infected) as the response and (2) intensity of infection (number of zoospore equivalents for infected individuals only) as the response. AICc: Akaike's information criterion corrected for small sample size

Response	Model	df	Log likelihood	AICc	ΔAICc	Akaike weight	Nagelkerke R^2
Infection status	**Type + Size + Season + Temp + Type×Season**	**18**	**−278.17**	**593.30**		**0.998**	**0.612**
	Type + Size + Season + Type×Season	17	−285.42	605.70	12.41	0.002	0.597
	Type + Size + Season + Temp	10	−313.12	646.60	53.25	<0.001	0.539
	Type + Size + Season	9	−321.76	661.80	68.47	<0.001	0.519
	Size + Season + Temp	8	−323.21	662.60	69.32	<0.001	0.516
	Type + Size + Temp	6	−331.76	675.60	82.33	<0.001	0.496
	Size + Season	7	−331.03	676.20	82.91	<0.001	0.498
	Size + Temp	4	−340.85	689.70	96.44	<0.001	0.475
	Type + Size	5	−370.91	751.90	158.59	<0.001	0.400
	Size	**3**	**−380.66**	**767.30**	**174.04**	<0.001	**0.375**
Infection intensity	**Type + Size + Season + Temp + Type×Season**	**19**	**−468.72**	**977.70**		**0.943**	**0.300**
	Type + Size + Season + Temp	11	−480.28	983.30	5.62	0.057	0.249
	Size + Season + Temp	9	−490.75	1000.00	22.31	<0.001	0.200
	Type + Season + Temp + Type×Season	18	−482.05	1002.20	24.43	<0.001	0.241
	Type + Season + Temp	10	−491.95	1004.50	26.82	<0.001	0.194
	Season + Temp	8	−500.32	1017.10	39.34	<0.001	0.152
	Type + Size + Season + Type×Season	18	−492.44	1022.90	45.21	<0.001	0.192
	Size + Season	8	−505.97	1028.40	50.63	<0.001	0.123
	Type + Size + Season	10	−505.93	1032.50	54.79	<0.001	0.123
	Size	**4**	**−512.32**	**1032.80**	**55.03**	<0.001	**0.089**

Table 3. Estimated regression parameters, standard errors, *z*-values (or *t*-values), and p-values for (1) binomial generalized linear mixed model where infection status was the response (*z*-values) and body size of *Litoria rheocola*, season, and site type were the predictors, and (2) generalized linear mixed model where intensity of infection was the response (*t*-values) and body size, season, and site type were the predictors. **Bold** values indicate predictor variables that were significant ($p < 0.05$)

Response	Predictor	Estimate	SE	*z* (or *t*)	p
Disease status	Intercept	0.271	0.692	0.391	0.696
	Size	1.714	0.156	11.020	**<0.001**
	Water temperature	−1.660	0.470	−3.536	**<0.001**
	Autumn	−2.418	0.765	−3.162	**0.002**
	Spring	−0.869	0.854	−1.018	0.308
	Summer	−2.185	0.940	−2.325	**0.020**
	Winter	−1.579	0.807	−1.958	0.050
	Contiguous	0.898	0.759	1.184	0.237
	Non-contiguous	−3.269	0.935	−3.496	**<0.001**
	Autumn × Contiguous	1.698	0.962	1.765	0.078
	Spring × Contiguous	1.710	0.963	1.777	0.076
	Summer × Contiguous	3.476	1.009	3.447	**<0.001**
	Winter × Contiguous	0.373	0.922	0.405	0.686
	Autumn × Non-contiguous	4.820	1.095	4.400	**<0.001**
	Spring × Non-contiguous	4.808	0.970	4.960	**<0.001**
	Summer × Non-contiguous	6.142	1.065	5.770	**<0.001**
	Winter × Non-contiguous	4.259	0.970	4.390	**<0.001**
Infection intensity	Intercept	0.070	0.179	0.390	0.697
	Size	0.287	0.056	5.134	**<0.001**
	Water temperature	−1.220	0.172	−7.093	**<0.001**
	Autumn	0.329	0.325	1.013	0.312
	Spring	0.138	0.297	0.463	0.644
	Summer	1.311	0.470	2.792	**0.006**
	Winter	−1.828	0.276	−6.620	**<0.001**
	Contiguous	0.474	0.259	1.830	0.165
	Non-contiguous	0.626	0.493	1.270	0.294
	Autumn × Contiguous	0.690	0.461	1.496	0.136
	Spring × Contiguous	1.199	0.371	3.228	**0.001**
	Summer × Contiguous	0.604	0.525	1.150	0.251
	Winter × Contiguous	0.158	0.316	0.500	0.617
	Autumn × Non-contiguous	−0.453	0.653	−0.693	0.489
	Spring × Non-contiguous	1.101	0.520	2.117	**0.035**
	Summer × Non-contiguous	0.714	0.689	1.037	0.301
	Winter × Non-contiguous	0.681	0.495	1.376	0.170

the infection status model; however, the estimated regression parameter of body size was lower than in the infection status model. To determine if body size played a large role in the variation of infection intensity, we also examined the model with size as the only predictor variable; this model only explained 8.9% of the variation, and only 7 out of the top 10 models included size as an explanatory variable. Our modelling of infection intensity suggests that it is highly variable among individuals and is less subject to both seasonal and body size effects than is infection status.

Because the best-fitting models for both infection status and infection intensity incorporated the interaction of site type and season, we did separate graphical comparisons for each site type of selected model fits with each other and with the body size distributions of tadpoles (Fig. 2). We calculated prevalence of infection using the predicted values of infection status from the top model and the tadpole body size only model and compared these to the actual prevalence of infection recorded at each season at each site. At sites of each type, the best-fitting model performed very well at predicting seasonal mean prevalence of infection by *Bd* (Fig. 2). At high-elevation sites (Fig. 2a), although it was not as good a fit, the predictions of the model incorporating tadpole body size as the only fixed factor followed a very similar pattern to those of the data and the best-fitting model; however, the model for tadpole size (as the only fixed factor) overpredicted prevalence in spring and summer and underpredicted it in winter. At

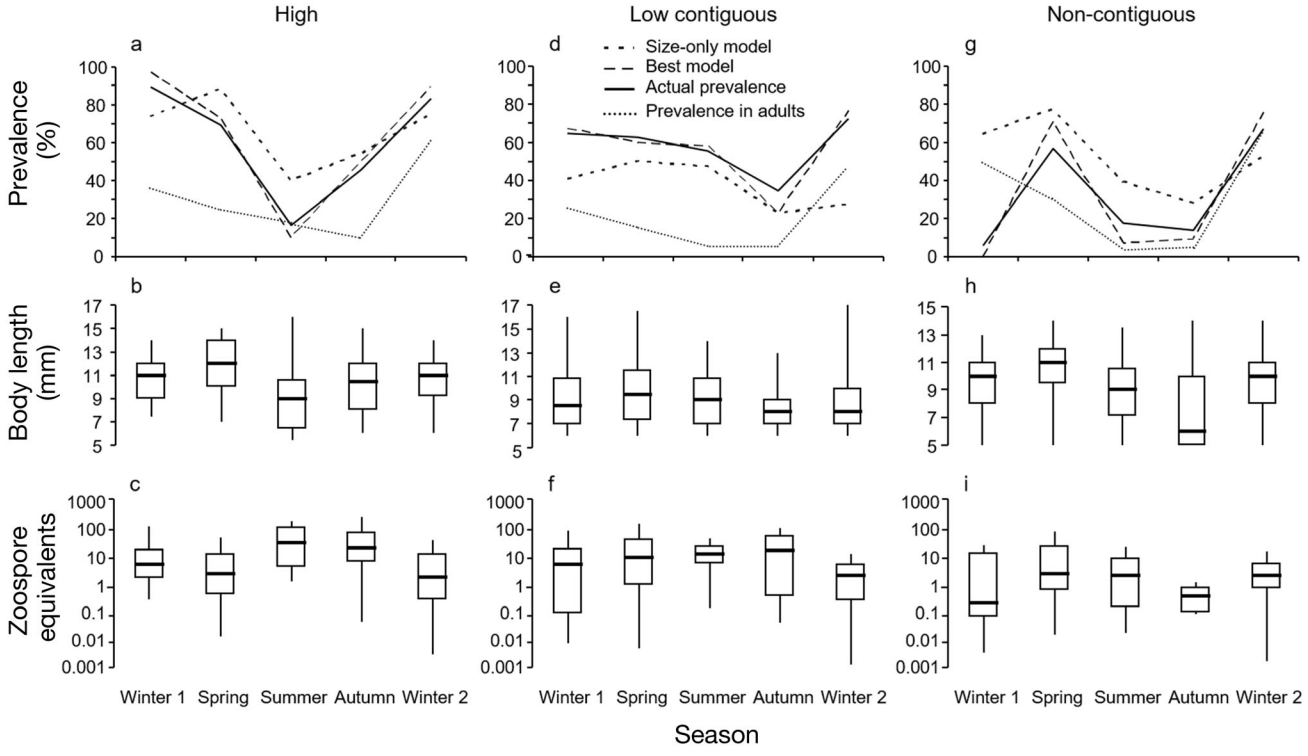

Fig. 2. (a,d,g) Prevalence of infection by *Batrachochytrium dendrobatidis* (*Bd*) (solid line) in *Litoria rheocola* tadpoles and adults across seasons at high-elevation sites, low-elevation sites contiguous with high-elevation streams, and non-contiguous sampling sites, and prevalence of infection in tadpoles predicted at those sites by a model incorporating only size (body size) and random site effects only (dotted line) and by the best fitting 18-parameter model (dashed line, see Table 2). Note that the prediction using only size and random site effects is quite similar to the empirical observations, suggesting that size contributes strongly to the observed patterns. (b,e,h) Body size distributions across seasons at high-elevation, low-elevation, and non-contiguous sites (heavy lines: medians; boxes: 25 and 75% quartiles; whiskers: 95% confidence intervals). (c,f,i) Infection intensity distributions across seasons at high-elevation, low-elevation, and non-contiguous sites

these sites, the seasonal pattern of prevalence in adults was very similar to that in tadpoles, although prevalence in adults was always lower. At least in direction, changes between seasons in the body size distribution of tadpoles (Fig. 2b) matched changes in prevalence; however, the median body size did not appear to change proportionally as much as did prevalence. Median infection intensity was weakly associated with body size and season at high elevation, and did not follow a similar pattern to that shown by prevalence (Fig. 2c). Results at contiguous low (Fig. 2d,e) and non-contiguous low sites (Fig. 2g,h) were similar, with somewhat different seasonal patterns of prevalence, and median infection intensity seemed to follow similar patterns to prevalence (Fig. 2f,i). As at high-elevation sites, patterns of prevalence in adults were similar to those in tadpoles, although adult prevalence was lower. The sole exception to this pattern was in the first winter at non-contiguous sites, where adult prevalence was many times higher than prevalence in tadpoles. The values predicted by the size-only model consistently underpredicted prevalence in the contiguous low sites and overpredicted it at the non-contiguous low sites in all seasons except the final winter. Patterns of body size distributions with season (Fig. 2b,e,h) show that, in general, body size behaved as predicted, with an influx of small recruits in summer (or late summer, leading to a minimum in autumn) and progression through to the largest individuals in spring, before many have metamorphosed.

DISCUSSION

We found that, while many factors influence infection status in tadpoles, tadpole age was a very important factor, indicating that cumulative infection risk may be as important as temperature in driving prevalence of *Bd*. This result is notable because most studies have assumed that temperature is the most important factor driving prevalence (e.g. Hamilton et al.

2012, Fernández-Beaskoetxea et al. 2015). In our study, infection probability increased strongly with body size, indicating that the probability of becoming infected by *Bd* was cumulative, so that larger, older individuals were more likely to be infected (Smith et al. 2007, Cashins 2009). The full model incorporating the effects of tadpole size, individual temperature, site type, season, and the site type by season interaction, was the best predictor of infection status in tadpoles. However, our planned comparisons of simpler models indicated that tadpole body size alone accounted for a substantial proportion (37.5%) of the variation in probability of infection. These comparisons strongly suggest that temperature effects, at least those separable from general seasonal changes in temperature, were not the only drivers of probability of infection, but that probability of infection is also affected by age, and thus cumulative exposure to the risk of transmission.

We suggest that the annual cycle in tadpole body size worked together with changes in season and temperature, and differences among site types, to produce the patterns of prevalence of *Bd* that we observed. *Litoria rheocola* were numerically dominant in our streams, and the other species present have similar annual life cycles (Woodhams & Alford 2005, Cashins 2009), so it is unlikely that the patterns we observed were strongly influenced by different dynamics in tadpoles of other species. *L. rheocola* breed mostly in summer, so in summer there was an influx of newly hatched tadpoles that were initially uninfected. It is likely there were effects of seasonal mean temperature; in summer, mean water temperatures were warm, which would have reduced the prevalence of *Bd* because the growth of *Bd* slows at temperatures above 25°C (Piotrowski et al. 2004, Stevenson et al. 2013). As temperatures decreased in autumn and winter, the tadpole cohorts aged and their cumulative risk of transmission increased; however, we cannot separate these effects entirely. Our graphical comparisons showed that considered alone, body size tended to slightly overpredict prevalence at high-elevation sites, to more strongly overpredict it at the non-contiguous low sites, and to underpredict prevalence at the contiguous low sites, suggesting that environmental factors influence the predictions, with different influences in different environments.

The effects of elevation were similar to those of season, in that prevalence was higher at the cooler, higher elevations and lower at the warmer, lower elevations. In addition to the elevational effect, prevalence of infection tended to be lowest at non-contiguous low-elevation sites. Mean water temperatures at these sites were warmer than water temperatures at contiguous low-elevation sites, probably because water flows from higher, cooler elevation sites into contiguous low-elevation sites. In summer, water temperatures at non-contiguous low-elevation sites were between 25 and 28°C, whereas water temperatures were always under 25°C at contiguous low-elevation sites. The higher prevalence of *Bd* infections in tadpoles at contiguous low-elevation sites may be accounted for by this thermal effect, or by transport in stream flow of *Bd* zoospores from high-elevation sites, or both, as suggested for differences in *Bd* prevalence in terrestrial frogs by Sapsford et al. (2013). The fact that infection intensity was relatively weakly affected by season and body size indicates that transmission of infections from tadpoles to other tadpoles and to adults, which should be related to the density of infective zoospores in the environment, is likely to be more strongly affected by prevalence in tadpoles than by changes in intensity. Furthermore, the fact that prevalence in adults was substantially higher than that in tadpoles at non-contiguous low-elevation sites in the first winter of the study suggests that in more isolated systems with greater seasonal temperature fluctuations, adults may serve as a reservoir for *Bd* infections in tadpoles, as well as tadpoles serving as a reservoir for adults.

Elevation and season affect the prevalence of *Bd* infections (Woodhams & Alford 2005, Phillott et al. 2013, Sapsford et al. 2013). The mechanism driving these effects is usually thought to be temperature. However, in the present study, we have provided evidence to support the hypothesis that the probability of infection as tadpoles age may also have a major role in driving prevalence in tadpole assemblages. In systems in which tadpoles contribute to infection by *Bd* in terrestrial frogs, the dynamics of tadpole age and density could, therefore, have strong effects on temporal patterns of *Bd* prevalence in amphibian populations more generally (Valencia-Aguilar et al. 2016). In our Australian Wet Tropics streams, and in similar streams in Central and South America, many species have annual tadpoles that are present throughout the year (Woodhams & Alford 2005, Whiles et al. 2006). In any of these systems, effects of tadpole age dynamics on seasonal cycles of *Bd* prevalence could affect seasonal patterns of prevalence in terrestrial amphibians. For example, populations of *Telmatobius jelskii* in the Peruvian Andes may have multiple breeding events throughout the year, and thus infected tadpoles are present all year in streams; older tadpoles in this South American system also had a

higher prevalence of infection by *Bd* than younger tadpoles (Catenazzi et al. 2013). The patterns of prevalence that have been detected in tadpoles at Australian sites are a close match to patterns of prevalence in adult frogs (Woodhams & Alford 2005, Sapsford et al. 2013). In our case, prevalence of *Bd* in tadpoles was almost always higher than prevalence of *Bd* in adults. Some adult populations can lose their infections in warm summer months (Sapsford et al. 2013); however, high prevalence among tadpoles enables reinfection of adult populations. *L. rheocola* spend the majority of their time in and around streams (Hoskin & Hero 2008, Dennis 2012), and thus have frequent contact with water where infected tadpoles reside. This pattern provides evidence that tadpoles act as effective reservoirs for *Bd*, especially in systems with annual tadpoles, and is becoming evident in other systems (e.g. Catenazzi et al. 2013). Interestingly, this trend is being detected in more temperate regions; for example, perennially overwintering larvae of the midwife toad *Alytes obstetricans* in a lake in France may act as reservoirs of infection depending on the time of spring thaws (Clare et al. 2016). In addition, larval populations of *A. obstetricans* in ponds in Switzerland also maintained their infections while overwintering (Geiger et al. 2017).

Changes in the vulnerability of individuals to infection with age can cause seasonal changes of prevalence that are driven by the timing of recruitment of juveniles (Altizer et al. 2006), and the risk of infection to juveniles can also differ among seasonal cohorts (Cornell et al. 2008). We suggest that understanding drivers of *Bd* infection in tadpoles is critical to fully understanding what drives *Bd* infection in adult amphibians. Our results reinforce the need to examine the effects of all potential drivers of observed patterns of change of infection prevalence. In particular, it is important to integrate studies of host–pathogen dynamics across all stages of host life histories in conjunction with temperature and elevation as possible drivers of seasonal infection prevalence patterns.

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LITERATURE CITED

Alford RA (1999) Ecology: resource use, competition, and predation. In: McDiarmid RW, Altig R (eds) Tadpoles: the biology of anuran larvae. The University of Chicago Press, Chicago, IL, p 240–278

Alford RA (2010) Declines and the global status of amphibians. In: Sparling DW, Linder G, Bishop CA, Krest SK (eds) Ecotoxicology of amphibians and reptiles, Vol 2. Society of Environmental Toxicology and Chemistry, Pensacola, FL, p 13–46

Altig R (2007) A primer for the morphology of anuran tadpoles. Herpetol Conserv Biol 2:71–74

Altig R, Johnston GF (1989) Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. Herpetol Monogr 3:81–109

Altizer S, Dobson A, Hosseini P, Hudson P, Pascual M, Rohani P (2006) Seasonality and the dynamics of infectious diseases. Ecol Lett 9:467–484

Andre SE, Parker J, Briggs CJ (2008) Effect of temperature on host response to *Batrachochytrium dendrobatidis* infection in the mountain yellow-legged frog (*Rana muscosa*). J Wildl Dis 44:716–720

Anstis M (2013) Tadpoles and frogs of Australia. New Holland Publishers, Chatswood

Berger L, Speare R, Daszak P, Green DE and others (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc Natl Acad Sci USA 95:9031–9036

Blaustein AR, Romansic JM, Scheessele EA, Han BA, Pessier AP, Longcore JE (2005) Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis.* Conserv Biol 19:1460–1468

Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD (2004) Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis Aquat Org 60:141–148

Briggs CJ, Knapp RA, Vredenburg VT (2010) Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. Proc Natl Acad Sci USA 107:9695–9700

Brunner JL, Schock DM, Davidson EW, Collins JP (2004) Intraspecific reservoirs: complex life history and the persistence of a lethal ranavirus. Ecology 85:560–566

Burnham KP, Anderson DR, Huyvaert KP (2011) AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behav Ecol Sociobiol 65:23–35

Cashins SD (2009) Epidemiology of chytridiomycosis in rainforest stream tadpoles. PhD thesis, James Cook University, Townsville

Cashins SD, Alford RA, Skerratt LF (2008) Lethal effect of latex, nitrile, and vinyl gloves on tadpoles. Herpetol Rev 39:298–301

Catenazzi A, von May R, Vredenburg VT (2013) High prevalence of infection in tadpoles increases vulnerability to fungal pathogen in high-Andean amphibians. Biol Conserv 159:413–421

Clare FC, Halder JB, Daniel O, Bielby J and others (2016) Climate forcing of an emerging pathogenic fungus across a montane multi-host community. Philos Trans R Soc B 371:20150454

Cornell SJ, Bjornstad ON, Cattadori IM, Boag B, Hudson PJ (2008) Seasonality, cohort-dependence and the development of immunity in a natural host–nematode system. Proc R Soc B 275:511–518

Daskin JH, Bell SC, Schwarzkopf L, Alford RA (2014) Cool temperatures reduce antifungal activity of symbiotic bacteria of threatened amphibians–implications for disease management and patterns of decline. PLOS ONE 9: e100378

de Castro F, Bolker B (2005) Mechanisms of disease-induced extinction. Ecol Lett 8:117–126

Dennis A (2012) Common mistfrog, *Litoria rheocola*. In: Curtis LK, Dennis AJ, McDonald KR, Kyne PM, Debus SJS (eds) Queensland's threatened animals. CSIRO Publishing, Collingwood, p 166–167

Dushoff J, Plotkin JB, Levin SA, Earn DJ (2004) Dynamical resonance can account for seasonality of influenza epidemics. Proc Natl Acad Sci USA 101:16915–16916

Fernández-Beaskoetxea S, Carrascal LM, Fernández-Loras A, Fisher MC, Bosch J (2015) Short term minimum water temperatures determine levels of infection by the amphibian chytrid fungus in *Alytes obstetricans* tadpoles. PLOS ONE 10:e0120237

Geiger CC, Bregnard C, Maluenda E, Voordouw MJ, Schmidt BR (2017) Antifungal treatment of wild amphibian populations caused a transient reduction in the prevalence of the fungal pathogen, *Batrachochytrium dendrobatidis*. Sci Rep 7:5956

Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190

Greenspan SE, Bower DS, Webb RJ, Roznik EA and others (2017) Realistic heat pulses protect frogs from disease under simulated rainforest frog thermal regimes. Funct Ecol 31:2274–2286

Hamilton PT, Richardson JML, Govindarajulu P, Anholt BR (2012) Higher temperature variability increases the impact of *Batrachochytrium dendrobatidis* and shifts interspecific interactions in tadpole mesocosms. Ecol Evol 2:2450–2459

Hoskin C, Hero JM (2008) Rainforest frogs of the wet tropics, north-east Australia. Griffith University, Southport

Hyatt AD, Boyle DG, Olsen V, Boyle DB and others (2007) Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Dis Aquat Org 73:175–192

Johnson PT, Kellermanns E, Bowerman J (2011) Critical windows of disease risk: amphibian pathology driven by developmental changes in host resistance and tolerance. Funct Ecol 25:726–734

Liem DS, Hosmer W (1973) Frogs of the genus *Taudactylus* (Myobatrachidae) from mid-eastern Queensland with notes on the other species of the genus. Mem Queensl Mus 16:435–457

Longcore JE, Pessier AP, Nichols DK (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219–227

McDonald K, Alford R (1999) A review of declining frogs in northern Queensland. In: Campbell A (ed) Declines and disappearances of Australian frogs. Environment Australia, Canberra, p 14–22

Merilä J, Laurila A, Laugen AT, Räsänen K, Pahkala M (2000) Plasticity in age and size at metamorphosis in *Rana temporaria* comparison of high and low latitude populations. Ecography 23:457–465

Mohseni O, Stefan HG (1999) Stream temperature/air temperature relationship: a physical interpretation. J Hydrol (Amst) 218:128–141

Phillott AD, Grogan LF, Cashins SD, McDonald KR, Berger L, Skerratt LF (2013) Chytridiomycosis and seasonal mortality of tropical stream associated frogs 15 years after introduction of *Batrachochytrium dendrobatidis*. Conserv Biol 27:1058–1068

Pilliod DS, Muths E, Scherer RD, Bartelt PE and others (2010) Effects of amphibian chytrid fungus on individual survival probability in wild boreal toads. Conserv Biol 24: 1259–1267

Piotrowski JS, Annis SL, Longcore JE (2004) Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96:9–15

R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rachowicz LJ, Briggs CJ (2007) Quantifying the disease transmission function: effects of density on *Batrachochytrium dendrobatidis* transmission in the mountain yellow-legged frog *Rana muscosa*. J Anim Ecol 76:711–721

Rachowicz LJ, Vredenburg VT (2004) Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. Dis Aquat Org 61:75–83

Richards S (1992) The tadpole of the Australian frog *Litoria nyakalensis* (Anura, Hylidae), and a key to the torrent tadpoles of northern Queensland. Alytes 10:99–103

Richards SJ (2002) Influence of flow regime on habitat selection by tadpoles in an Australian rainforest stream. J Zool (Lond) 257:273–279

Rowley JJL, Alford RA (2007) Movement patterns and habitat use of rainforest stream frogs in northern Queensland, Australia: implications for extinction vulnerability. Wildl Res 34:371–378

Rowley JJ, Alford RA (2013) Hot bodies protect amphibians against chytrid infection in nature. Sci Rep 3:1515

Roznik EA, Alford RA (2015) Seasonal ecology and behavior of an endangered rainforest frog (*Litoria rheocola*) threatened by disease. PLOS ONE 10:e0127851

Sapsford SJ, Alford RA, Schwarzkopf L (2013) Elevation, temperature, and aquatic connectivity all influence the infection dynamics of the amphibian chytrid fungus in adult frogs. PLOS ONE 8:e82425

Schoebel CN, Tellenbach C, Spaak P, Wolinska J (2011) Temperature effects on parasite prevalence in a natural hybrid complex. Biol Lett 7:108–111

Simoncelli F, Fagotti A, Dall'Olio R, Vagnetti D, Pascolini R, Di Rosa I (2005) Evidence of *Batrachochytrium dendrobatidis* infection in water frogs of the *Rana esculenta* complex in central Italy. EcoHealth 2:307–312

Smith KG, Weldon C, Conradie W, du Preez LH (2007) Relationships among size, development, and *Batrachochytrium dendrobatidis* infection in African tadpoles. Dis Aquat Org 74:159–164

Stevenson LA, Alford RA, Bell SC, Roznik EA, Berger L, Pike DA (2013) Variation in thermal performance of a widespread pathogen, the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. PLOS ONE 8:e73830

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW (2004) Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786

Valencia-Aguilar A, Toledo LF, Vital MV, Mott T (2016) Seasonality, environmental factors, and host behavior linked to disease risk in stream-dwelling tadpoles. Herpetologica 72:98–106

Vieira CA, Toledo LF, Longcore JE, Longcore JR (2013) Body length of *Hylodes* cf. *ornatus* and *Lithobates catesbeianus* tadpoles, depigmentation of mouthparts, and pres-

ence of *Batrachochytrium dendrobatidis* are related. Braz J Biol 73:195–199

Voyles J, Young S, Berger L, Campbell C and others (2009) Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. Science 326:582–585

Voyles J, Johnson LR, Rohr J, Kelly R and others (2017) Diversity in growth patterns among strains of the lethal fungal pathogen *Batrachochytrium dendrobatidis* across extended thermal optima. Oecologia 184:363–373

Whiles MR, Lips KR, Pringle CM, Kilham SS and others (2006) The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. Front Ecol Environ 4:27–34

Wilson K, Bjørnstad O, Dobson A, Merler S and others (2002) Heterogeneities in macroparasite infections: patterns and processes. In: Hudson PJ, Rizzoli A, Grenfell BT, Heesterbeek H, Dobson AP (eds) The ecology of wildlife diseases. Oxford University Press, New York, NY, p 45–62

Woodhams DC, Alford RA (2005) Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. Conserv Biol 19:1449–1459

Woodhams DC, Alford RA, Marantelli G (2003) Emerging disease of amphibians cured by elevated body temperature. Dis Aquat Org 55:65–67

Zamora-Vilchis I, Williams SE, Johnson CN (2012) Environmental temperature affects prevalence of blood parasites of birds on an elevation gradient: implications for disease in a warming climate. PLOS ONE 7:e39208

Zuk M, Stoehr AM (2002) Immune defense and host life history. Am Nat 160:S9–S22

Zuur AF, Ieno EN (2016) A protocol for conducting and presenting results of regression-type analyses. Methods Ecol Evol 7:636–645

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14

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