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# Latitudinal Variation in the Prevalence and Intensity of Chytrid (*Batrachochytrium dendrobatidis*) Infection in Eastern Australia

KERRY M. KRIGER,\*‡ FELICIA PEREOGLOU,† AND JEAN-MARC HERO\*

\*Griffith University, Centre for Innovative Conservation Strategies, PMB 50 Gold Coast Mail Centre, QLD 9726, Australia

†Biolink Pty Ltd, P.O. Box 196, Uki, NSW 2484, Australia

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**Abstract:** *Chytridiomycosis is a recently emerged, infectious skin disease of amphibians that has been linked directly to mass mortalities, population declines, and species extinctions worldwide. An understanding of the factors that limit the distribution and abundance of Batrachochytrium dendrobatidis (the etiological agent of chytridiomycosis) is urgently required. We conducted disease surveys at 31 lowland sites distributed north-south along 2315 km of the Australian east coast that encompassed 20.8 degrees of latitude. A total of 863 adult male stony creek frogs (Litoria lesueuri complex) were sampled, and the overall prevalence of B. dendrobatidis infection was 26%. B. dendrobatidis was detected at 77% of the sites, including sites at the northern and southern limits of the latitudinal transect. Frogs from temperate regions, however, had significantly more intense infections than did their tropical counterparts, often carrying an order of magnitude more B. dendrobatidis zoospores, suggesting that at low elevations, temperate frogs are at higher risk of chytridiomycosis-induced mortality than are tropical frogs. The prevalence and intensity of B. dendrobatidis infections were significantly greater at sites with high rainfall (>33 mm in the 30 days prior to sampling) and cool temperatures (stream temperature 1 h after sunset < 23° C). Although climatic variables explained much of the variation in the prevalence and intensity of B. dendrobatidis infections between infected and uninfected sites, frog snout-vent length was consistently the best predictor of infection levels across infected sites. Small frogs were more likely to be infected and carried more intense infections than larger frogs, suggesting either that frogs can outgrow their chytrid infections or that the disease induces developmental stress that limits growth. Our results will directly assist amphibian disease researchers and wildlife managers, whose conservation efforts should focus on those amphibian populations living within the B. dendrobatidis climatic envelope that we have described.*

**Keywords:** amphibian decline, *Batrachochytrium dendrobatidis*, chytridiomycosis, chytrid infection, latitudinal gradient, *Litoria lesueuri*, wildlife diseases

Variación Latitudinal en la Prevalencia e Intensidad de Infección por Quitridios (*Batrachochytrium dendrobatidis*) en Australia Oriental

**Resumen:** *La quitridiomycosis es una enfermedad infecciosa de la piel de anfibios, emergida recientemente, que ha sido relacionada directamente con mortalidades masivas, declinaciones poblacionales y extinciones de especies en todo el mundo. La comprensión de los factores que limitan la distribución y abundancia de Batrachochytrium dendrobatidis (el agente etiológico de la quitridiomycosis) se requiere urgentemente. Realizamos muestreos de la enfermedad en 31 sitios distribuidos, de norte a sur, a lo largo de 2315 km de la costa oriental de Australia que comprende 20.8 grados de latitud. Muestreamos un total de 863 machos adultos de ranas del complejo Litoria lesueuri, y la prevalencia total de infección por B. dendrobatidis fue de 26%. B. dendrobatidis ocurrió en 77% de los sitios, incluyendo sitios en los límites norte y sur del transecto latitudinal. Sin embargo, las ranas de regiones templadas tenían infecciones significativamente más intensas que sus contrapartes tropicales, a menudo con un orden de magnitud más de carga de zoosporas de B. dendrobatidis,*

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‡email [kmk5g@yahoo.com](mailto:kmk5g@yahoo.com)

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lo que sugiere que, en elevaciones bajas, las ranas templadas tienen mayor riesgo de mortalidad inducida por quitridiomycosis que las ranas tropicales. La prevalencia e intensidad de infecciones de *B. dendrobatidis* fueron significativamente mayores en sitios con precipitación alta (>33 mm 30 días antes del muestreo) y temperaturas frescas (temperatura del arroyo 1 hora después del ocaso: < 23° C). Aunque las variables climáticas explicaron mucha de la variación en la prevalencia e intensidad de *B. dendrobatidis* entre sitios infectados y no infectados, la longitud hocico-cloaca consistentemente fue el mejor predictor de los niveles de infección en los sitios muestreados. Las ranas pequeñas tuvieron mayor probabilidad de infección y tenían infecciones más intensas que las ranas más grandes, lo que sugiere que las ranas pueden superar las infecciones con la edad o que la enfermedad induce estrés que limita el crecimiento. Nuestros resultados directamente asistirán a investigadores de enfermedades en anfibios y a manejadores de vida silvestre, cuyos esfuerzos de conservación deberían enfocar a las poblaciones de anfibios que viven dentro del ámbito climático de *B. dendrobatidis* que hemos descrito.

**Palabras Clave:** *Batrachochytrium dendrobatidis*, declinación de anfibios, enfermedades de vida silvestre, gradiente latitudinal, infección por chytridio, *Litoria lesueuri*, quitridiomycosis

## Introduction

Rapid amphibian population declines have occurred worldwide in recent decades (e.g., Kagarise Sherman & Morton 1993; Houlahan et al. 2000; La Marca et al. 2005). At present, nearly one-third of amphibian species are considered threatened (Stuart et al. 2004). Many amphibian declines and disappearances have taken place in protected wilderness areas, where no obvious cause has been identified (e.g., Bradford 1991; Pounds & Crump 1994; Lips 1999; Hero & Morrison 2004), and unidentified processes threaten 48% of rapidly declining species (Stuart et al. 2004). The disappearance of amphibian species from seemingly pristine, protected areas has posed great difficulties for conservation biologists, whose success depends on the mitigation of identifiable causative agents (Caughley 1994).

Emerging infectious diseases have been implicated in many recent declines (Daszak et al. 2003), and the chytrid fungus *Batrachochytrium dendrobatidis*, causative agent of the potentially lethal skin disease chytridiomycosis (Berger et al. 1998; Longcore et al. 1999), has become a major focus of amphibian conservation efforts. *B. dendrobatidis* has been found on dead and dying amphibians concurrent with population declines in Australia (Berger et al. 1998), South America (Ron et al. 2003), North America (Rachowicz et al. 2006), Central America (Lips et al. 2003, 2006), Europe (Bosch et al. 2001), and Africa (Weldon & du Preez 2004). Thus, it is the pathogen most directly linked to amphibian declines (Green et al. 2002).

Our understanding of chytridiomycosis is limited. Although high levels of chytrid infection are associated with amphibian die-offs in Central America (Lips et al. 2006), few published reports have quantified the prevalence of *B. dendrobatidis* infections in apparently healthy amphibians from wild populations. Furthermore, those studies that do exist (Retallick et al. 2004; McDonald et al. 2005; Ouellet et al. 2005; Woodhams & Alford 2005) likely

underestimate the prevalence of the disease because of their reliance on low-sensitivity histological techniques to diagnose infections (Kriger et al. 2006a). An understanding of how infection levels vary geographically would allow wildlife managers to prioritize disease monitoring in locations where the infection status of amphibian populations is unknown.

*B. dendrobatidis* occurs as far north as British Columbia (54°N; Raverty & Reynolds 2001) and as far south as Tasmania (43°S; Obendorf 2005) and is predicted to occupy large portions of the western and eastern hemispheres (Ron 2005). To date, no one has examined the extent to which the prevalence and intensity of amphibian chytrid infections vary with respect to latitude, and there is no empirical evidence to explain the variation in infection levels between conspecific amphibian populations at disparate sites. Because a large body of evidence points toward *B. dendrobatidis* favoring cooler temperatures (Longcore et al. 1999; Johnson et al. 2003; Piotrowski et al. 2004) in the laboratory, we hypothesized that the prevalence and intensity of chytrid infections in frog populations would increase at cooler latitudes. In addition, *B. dendrobatidis* has waterborne zoospores (Longcore et al. 1999; Piotrowski et al. 2004) and cannot survive desiccation (Johnson et al. 2003). Thus, we also hypothesized that chytrid infections would be less likely to occur in drier areas.

To test these hypotheses, we sampled for *B. dendrobatidis* infections in stony creek frogs (*Litoria lesueuri* complex) at 31 sites along a latitudinal gradient encompassing 2315 km of the Australian east coast. We then examined short- and long-term temperature and rainfall data for all sites to identify factors that might be responsible for the variation in infection levels among sites and to quantify the relative importance of each. Finally, the results of Kriger et al. (2006a) demonstrated a significant negative relationship between the snout-vent length (SVL) of juvenile frogs and their likelihood of being infected with *B. dendrobatidis*. Frogs in our current study

varied considerably in length, so we were able to examine this relationship in adult frogs.

## Methods

### Field Methods

We caught stony creek frogs in clean, unused 20 × 25-cm plastic bags. We sampled each frog for *B. dendrobatidis* infection by firmly running a sterile cotton swab (Kriger et al. 2006a) 10 times over each of the following locations on the frog: (1) dorsal surface; (2) sides, from groin to armpit; (3) ventral surface; and (4) undersides of the thighs. In addition, we made five outward strokes of the swab on the undersides of each foot, for a total of 70 strokes. Swabs were then placed in their original container (a plastic tube), stored on ice in a cooler on return from the field, and later frozen at  $-20^{\circ}\text{C}$ . To ensure consistency in swabbing technique, all frogs were swabbed by K.M.K. Each frog was handled with unused, unpowdered latex gloves to prevent disease transmission among animals. We measured SVL of all frogs to the nearest 0.1 mm with Vernier calipers. Visible signs of disease (e.g., lethargy, lack of righting reflex, reddening or sloughing of skin; Nichols et al. 2001) were noted, and all animals were released immediately after the procedure was finished. Stream water temperature at all sites was measured before sampling began (roughly 1 hour after sunset; hereafter, sunset water temperature) and after sampling was finished (roughly midnight; hereafter, midnight water temperature).

We sampled 31 sites distributed north-south along 2315 km of Australia's east coast that encompassed  $20.8^{\circ}$  of latitude (Fig. 1). We sampled an average of 28 frogs at each site (range: 5–42). Study sites were chosen to maximize our chances of finding frogs, not disease: at only two sites did we have prior knowledge of *B. dendrobatidis* presence or absence. To avoid the potentially confounding effects of elevation, we sampled only lowland sites (25–195 m asl). To avoid the potentially confounding effects of longitude, all sites were on the eastern slope of the Great Dividing Range, and to avoid the potentially confounding effects of age or sex, we restricted our sampling to adult males, the group of frogs most often encountered at sites. Species can vary in both their innate susceptibility and their likelihood of exposure to *B. dendrobatidis* (Bosch et al. 2001; Daszak et al. 2004). By sampling only stony creek frogs, we avoided the problems associated with comparing results obtained from sampling multiple species.

Levels of *B. dendrobatidis* infection in stony creek frogs vary drastically across seasons, with infection prevalence peaking in the spring (Kriger & Hero 2007). To minimize the effect of disease seasonality, all sampling took place within a 42-day period (20 September 2005–1 November 2005). By sampling in the spring, we maxi-

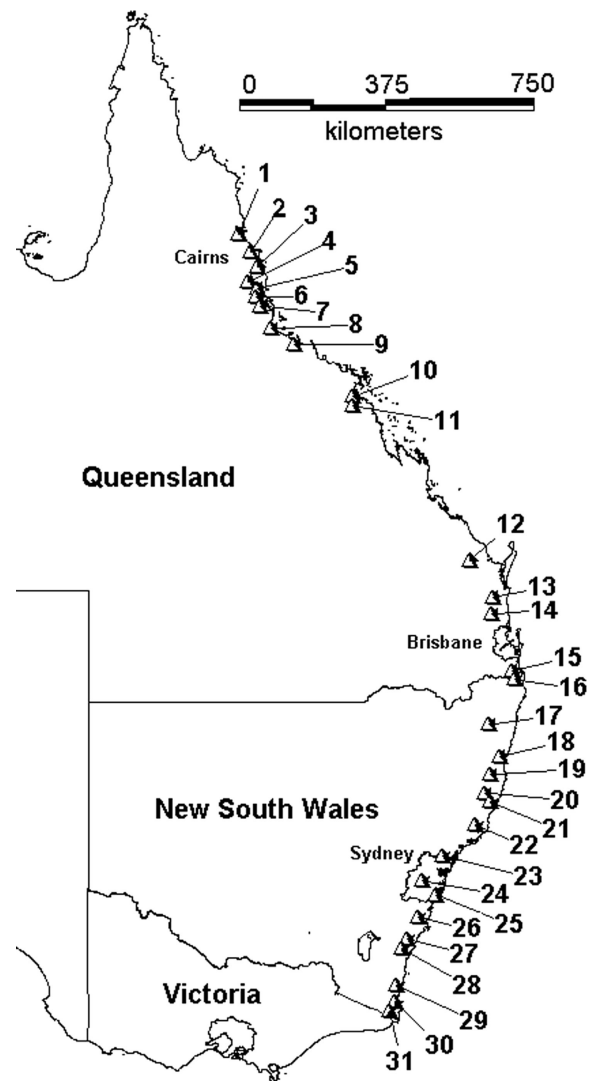


Figure 1. Map of eastern Australia showing locations of sites sampled. Numbers refer to sites as detailed in Table 1.

mized our chance of finding sufficient numbers of frogs and minimized the chance that we would fail to detect *B. dendrobatidis* at infected sites.

Because the study encompassed such a large geographic area, it was not logistically possible to sample the 31 sites in a temporally randomized fashion. We minimized the chance of inadvertently introducing a sampling bias into the study in several ways. Sampling was initiated and completed at the central latitudes of the gradient, which reduced the time difference (and corresponding seasonal variation in disease levels) between sampling sessions at the northern and southern extremes of the gradient (those sites that have results with the most statistical leverage). We sampled the northern half of the transect prior to the southern half. This ensured that the time that had elapsed between the onset of spring at a given location and our sampling that location was similar across

**Table 1.** Location of sampling sites, sample sizes, chytrid infection levels, mean snout-vent length (SVL), and relevant climatic variables in a survey of *Batrachochytrium dendrobatidis* infection in *Litoria lesueuri* in eastern Australia.

Site no.	Location	Latitude (°S) <sup>a</sup>	Longitude (°E) <sup>a</sup>	Elevation (m)	n	Prevalence (%)	Zoospores per frog <sup>b</sup>	SVL (mm)	SWT <sup>c</sup> (°C)	30-day rainfall (mm)	MTWQ <sup>d</sup> (°C)	PDQ <sup>e</sup> (mm)	Date, 2005
1	Mossmann Gorge	16.470	145.331	100	34	8.8	62	41.0	19.0	37.5	26.7	94	25 September
2	Cairns: Crystal Cascades	16.962	145.680	110	39	20.5	29	40.5	20.5	39.1	26.6	94	26 September
3	Babinda Boulders/Double Barrel Ck	17.342	145.869	70	34	44.1	2139	39.3	19.5	111.1	26.4	313	23 September
4	Tully Gorge	17.751	145.611	50	19	15.8	17	38.8	19.0	100.8	26.9	218	22 September
5	Rocky Creek, Tully	17.925	145.878	70	19	31.6	86	39.8	21.5	58.9	26.5	290	28 September
6	Murray Falls	18.153	145.816	95	28	35.7	50	39.7	21.5	30.5	26.7	112	28 September
7	Broadwater Ck, Abergowrie SF	18.417	145.944	70	36	61.1	162	38.1	22.5	9.8	26.9	93	29 September
8	Big Crystal Ck	18.980	146.255	70	5	0	0	33.8	24.0	5.1	27.1	63	30 September
9	Alligator Ck, Mt. Elliot	19.435	146.947	25	35	0	0	32.8	23.5	0.9	27.1	43	21 September
10	O'Connell R.	20.826	148.613	105	38	0	0	31.7	24.0	31.8	26.3	84	1 October
11	Eungella	21.080	148.640	150	35	2.9	24	32.6	18.0	9.0	26.1	105	20 September
12	Burnett R., Booyal Crossing	25.230	152.010	35	42	0	0	31.9	24.2	2.1	25.3	103	5 October
13	Mary R., Gympie	26.207	152.672	40	29	0	0	33.5	25.0	16.0	24.7	133	6 October
14	Little Yabba Ck, Kenilworth SF	26.617	152.653	160	34	29.4	103	35.2	18.5	27.0	23.7	136	6 October
15	Nerang R., Numimbah Valley	28.177	153.228	155	36	58.3	7406	36.3	20.5	23.7	22.8	185	11 October
16	Korumbyn Ck, Mt. Warning	28.388	153.324	25	35	22.9	192	38.3	21.5	72.5	23.3	194	1 November
17	Mann R.	29.579	152.554	105	35	28.6	333	35.0	19.0	11.4	23.2	139	12 October
18	Kalang R., Pearns Bridge	30.467	152.856	30	36	25.0	391	40.2	19.0	71.5	22.9	194	18 October
19	Macleay R.	30.919	152.584	45	32	0	0	37.9	23.0	32.3	22.7	143	13 October
20	Ellenborough R., Tom's Creek Rd.	31.439	152.461	55	12	16.7	235	38.6	21.0	87.9	22.1	166	31 October
21	Camden Haven R.	31.652	152.585	35	7	14.3	14	42.9	16.5	56.0	21.8	208	19 October
22	Myall R.	32.258	152.175	60	29	3.4	507	44.4	15.7	75.5	21.5	196	21 October
23	Basin Camping Area, Olney S.E.	33.100	151.230	190	11	0	0	46.7	13.5	27.4	21.3	175	15 October
24	Glenbrook Gorge	33.780	150.626	70	31	48.4	173	32.4	19.0	58.0	22.1	124	22 October
25	Hacking R., Royal N.P.	34.153	151.029	40	11	63.6	1854	36.1	17.0	82.4	21.2	211	23 October
26	Kangaroo R., Kangaroo Valley	34.727	150.521	95	34	8.8	18	41.7	19.0	23.9	20.8	222	30 October
27	Yadboro R., Budawang N.P.	35.341	150.216	60	10	40.0	840	34.4	16.5	68.8	20.3	181	24 October
28	Currowan Creek	35.575	150.059	80	20	70.0	2207	34.0	16.0	69.9	19.8	177	29 October
29	Mumbulla Falls, Biamanga N.P.	36.575	149.894	195	34	67.6	3160	32.9	15.0	38.8	18.5	177	28 October
30	Yowaka R., Broadwater Picnic Area	36.994	149.859	55	33	45.5	5035	31.4	17.5	82.3	18.8	165	27 October
31	Wallagaraugh R., Newton's Crossing	37.269	149.676	175	30	56.7	10153	31.8	17.5	76.5	17.9	209	26 October

<sup>a</sup>GPS coordinates based on map datum WGS 84.

<sup>b</sup>Mean numbers of zoospores detected on all frogs sampled at a site.

<sup>c</sup>Sunset water temperature.

<sup>d</sup>Mean temperature of the warmest quarter.

<sup>e</sup>Precipitation of the driest quarter.

sites (spring arrives later at more southerly latitudes in Australia). Finally, only half the sites were sampled while headed north and half were sampled while headed south. Generally every other site was bypassed while traveling in a given direction.

### Laboratory Analysis

We analyzed swabs for the presence of *B. dendrobatidis* with the quantitative (real-time) PCR techniques (qPCR) described by Boyle et al. (2004), except we used the changes to Boyle et al. (2004) described by Kriger et al. (2006b). Although the qPCR assay can detect and quantify the amount of *B. dendrobatidis* present on samples, it cannot be used to determine the degree of sickness in individuals unless appropriate pathological studies on the species in question have been performed.

### Choice of Climatic Variables

Infection levels at sites are likely to be influenced by both short- and long-term climatic conditions. Long-term climatic conditions likely determine the persistence of *B. dendrobatidis* at sites and thus the geographical distribution of the disease, whereas short-term conditions likely influence the prevalence and intensity of the disease at infected sites. Because the life cycle of *B. dendrobatidis* is 4–5 days (Berger et al. 2005) and a single chytrid zoospore can produce up to 150 zoospores (Annis et al. 2004), we believed that 30 days would be ample time for chytrid population numbers to track climatic changes and to significantly affect infection levels at sites. Furthermore, 30 days encompasses the timeframe in which experimentally infected laboratory frogs often develop clinical or lethal infections (Nichols et al. 2001; Daszak et al. 2004).

We examined six variables that reflected short-term climatic conditions at a site: sunset water temperature; mean value of the sunset and midnight water temperatures; mean of the daily maximum temperatures in the 30 days prior to sampling; mean of the daily minimum temperatures in the 30 days prior to sampling; mean of the combined daily maximum and minimum temperatures in the 30 days prior to sampling; and total rainfall in the 30 days prior to sampling (hereafter 30-day rainfall). We obtained all 30-day climatic data from the two Bureau of Meteorology weather stations closest to each site and averaged the data from the two stations (distance to temperature stations: mean [SD] = 34.0 km [16.3], range = 4.1–97.2; distance to rainfall stations: mean = 13.6 km [17.9], range = 0.1–101.9). A comparison of univariate linear regressions of the five short-term temperature variables revealed that sunset water temperature explained the greatest amount of the variation in infection levels at sites; thus, we used it in subsequent analyses.

Long-term climatic data were obtained from BIOCLIM (Nix 1986), a bioclimatic prediction system within the

program ANUCLIM. We restricted our analyses to the two climatic parameters most likely to influence the distribution and abundance of *B. dendrobatidis*: mean temperature of the warmest quarter (hereafter warm-quarter temperature) and precipitation of the driest quarter (hereafter dry-quarter precipitation). Whereas temperatures in the lowlands of eastern Australia in the winter are unlikely to be so low as to kill *B. dendrobatidis*, which can survive freezing (Johnson et al. 2003), high temperatures in many locations often exceed the thermal limits of the fungus. Thus, warm-quarter temperature is likely the most important long-term thermal determinant of fungal persistence at a site. Similarly, *B. dendrobatidis*, being a waterborne pathogen, is unlikely to persist at sites that experience regular drying of streams; thus, dry-quarter precipitation is likely the rainfall variable most responsible for the presence or absence of the fungus at sites.

### Data Analysis

We assigned a positive infection status to any frog on whose swab at least one *B. dendrobatidis* zoospore equivalent was detected (Kriger et al. 2007). Disease prevalence at each site was calculated by dividing the number of positive frogs by the total number of frogs sampled. We used the mean value of *B. dendrobatidis* zoospore equivalents detected in the three replicates of a swab's triplicate PCR analysis as an index of the intensity of an individual frog's infection (the frog's parasite load; see Kriger et al. [2007] for a discussion on quantification with the qPCR assay).

We used the mean number of zoospores detected on all frogs at a given site to represent the intensity of *B. dendrobatidis* infection at that site (hereafter site intensity). This value serves as an index of the *B. dendrobatidis* abundance at a site and is thus more pertinent than the mean load of infected frogs only. The mean number of zoospores detected at sites varied over several orders of magnitude and included zero values. To meet the assumptions of the regression analyses, it was necessary to log+1 transform the data.

We used the dependent variables site prevalence and site intensity (analyzed separately) and the independent variables latitude, SVL, sunset water temperature, 30-day rainfall, warm-quarter temperature and dry-quarter precipitation in linear and multiple regressions. Separate multiple regressions were used to examine the effects of short- and long-term climatic data. Because the climatic variables examined may not necessarily be responsible for the absence of *B. dendrobatidis* at uninfected sites (i.e., lack of infection may be due to the pathogen never having been introduced to a site), all regressions were performed both including and excluding sites where no infection was detected (hereafter uninfected sites). Variables were examined for collinearity prior to performing analyses to ensure that highly correlated variables were not included

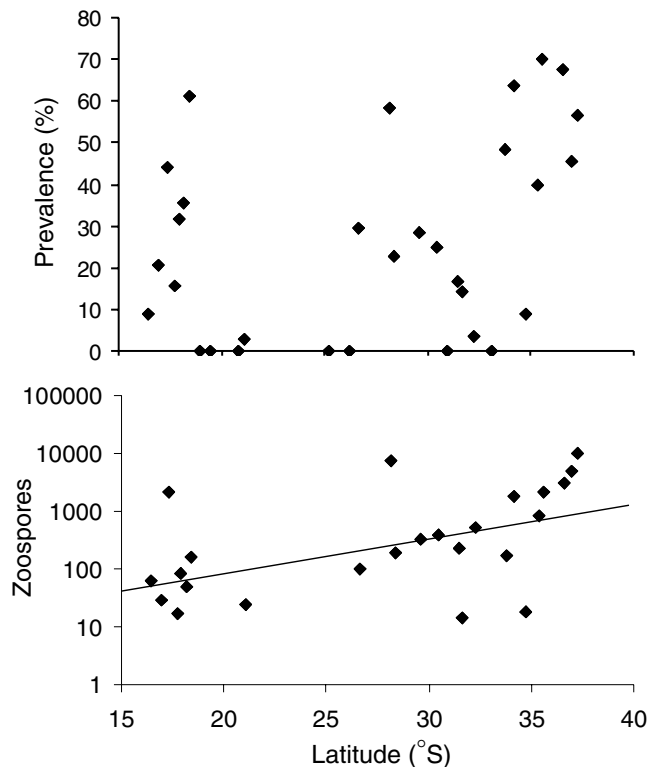
in the final model. Data were examined to ensure homogeneity of variance, and all analyses were performed in STATISTICA 6.0 (StatSoft, Tulsa, Oklahoma).

## Results

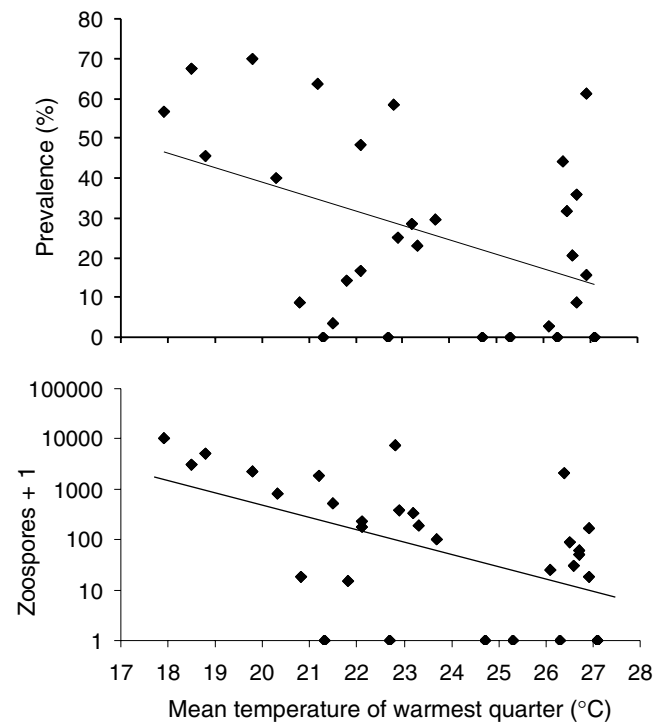
### Prevalence of *B. dendrobatidis* Infections

Our results confirm that *B. dendrobatidis* is widespread in eastern Australia. Infected *L. lesueuri* occurred at 24 of 31 sites, including sites at the northern and southern ends of the transect. Overall, 228 (26.4%) of 863 sampled frogs were infected with *B. dendrobatidis*, and prevalence at sites varied from 0% to 70% (Table 1). Although there was no statistically significant latitudinal variation in infection prevalence, there was a trend toward increasing prevalence in the south ( $p = 0.061$ ; Fig. 2).

There were significant negative relationships between the infection prevalence at a site and both sunset water temperature ( $p = 0.032$ ) and warm-quarter temperature ( $p = 0.012$ ; Fig. 3), although when uninfected sites were excluded from the analyses, these relationships were not significant (sunset water temperature:  $p = 0.54$ ; warm-



**Figure 2.** Relationship between latitude and either the prevalence of *Batrachochytrium dendrobatidis* infections in *Litoria lesueuri* at 31 sites along the east coast of Australia ( $p = 0.061$ ,  $r^2 = 0.116$ ) or the mean number of *B. dendrobatidis* zoospores detected on frogs at 24 infected sites ( $p = 0.0079$ ,  $r^2 = 0.280$ ).

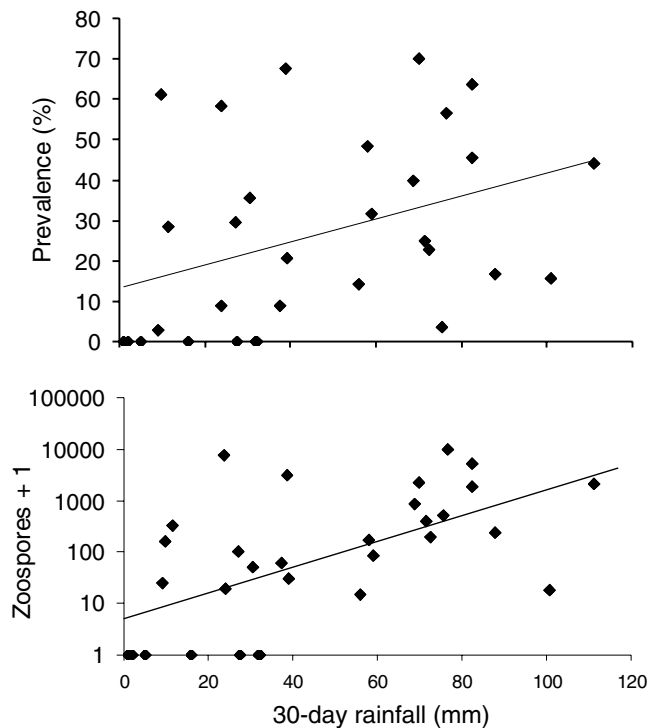


**Figure 3.** Relationship between the mean temperature of the warmest quarter at 31 sites along the east coast of Australia and either the prevalence of *Batrachochytrium dendrobatidis* infections in *Litoria lesueuri* ( $p = 0.012$ ,  $r^2 = 0.197$ ) or the mean number of *B. dendrobatidis* zoospores detected on *L. lesueuri* ( $p = 0.0014$ ,  $r^2 = 0.301$ ).

quarter temperature:  $p = 0.061$ ). Although no infected frogs were found at any of the six sites with sunset water temperature  $23^{\circ}\text{C}$  or higher, all sites with sunset water temperature between  $14^{\circ}\text{C}$  and  $23^{\circ}\text{C}$  ( $n = 24$ ) had infected frogs (Table 1). We did not detect *B. dendrobatidis* at the site with the coolest sunset water temperature ( $13.5^{\circ}\text{C}$ ).

There was a positive relationship between the infection prevalence at a site and the amount of rainfall in the previous 30 days ( $p = 0.038$ ; Fig. 4), but not between prevalence and dry-quarter precipitation (although this relationship approached significance:  $p = 0.055$ ). Excluding uninfected sites from these analyses made both of these relationships nonsignificant (30-day rainfall:  $p = 0.72$ ; dry-quarter precipitation:  $p = 0.69$ ). Whereas only half of the 14 sites with 30-day rainfall  $<33$  mm had infected frogs, all 17 sites with 30-day rainfall  $>33$  mm had infected frogs (Table 1), and this difference was highly significant ( $\chi^2 = 11.0$ ;  $df = 1$ ;  $p = 0.0009$ ).

When all sites were included in the analysis, there was no relationship between the infection prevalence and mean SVL ( $p = 0.18$ ). There was, however, a highly significant negative relationship when only infected sites were analyzed ( $p = 0.0025$ ; Fig. 5). This highly significant



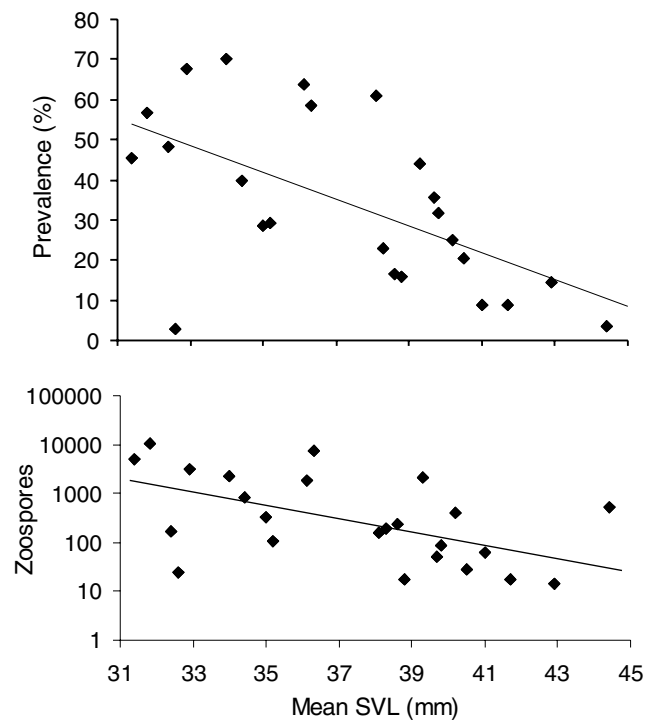
**Figure 4.** Relationship between the 30-day rainfall at 31 sites along the east coast of Australia and either the prevalence of *Batrachochytrium dendrobatidis* infections in *Litoria lesueuri* ( $p = 0.038$ ,  $r^2 = 0.141$ ) or the mean number of *B. dendrobatidis* zoospores detected on *L. lesueuri* ( $p = 0.0006$ ,  $r^2 = 0.339$ ).

negative relationship remained ( $p < 0.000001$ , Wald = 30.4) when the relationship between SVL and PCR result (positive/negative) was analyzed with frogs (as opposed to sites) as independent data points.

Multiple regressions of site prevalence versus mean SVL and either the short- or long-term climatic variables all produced negative relationships between SVL and site prevalence, whether uninfected sites were included or excluded from the analyses (Table 2). Of the climatic variables, however, only the negative relationship between site prevalence and sunset water temperature remained significant in the multiple regressions ( $p = 0.034$ ), and this negative relationship was significant only when uninfected sites were included in the analyses (Table 2). There was a positive relationship approaching significance ( $p = 0.051$ ) between site prevalence and dry-quarter precipitation when uninfected sites were included in the analysis.

#### Intensity of *B. dendrobatidis* Infections

The number of *B. dendrobatidis* zoospores detected on infected frogs ranged 5 orders of magnitude, from 2 to 217,433 (geometric mean = 190, median = 149,  $n = 228$ ). The most intensely infected frog was found at the most southerly site. There was a positive relationship be-



**Figure 5.** Relationship between mean snout-vent length (SVL) of *Litoria lesueuri* at infected sites and either the prevalence of *Batrachochytrium dendrobatidis* infection ( $p = 0.0025$ ,  $r^2 = 0.345$ ) or the mean number of *B. dendrobatidis* zoospores detected on frogs ( $p = 0.0077$ ,  $r^2 = 0.281$ ).

tween site intensity and latitude. The most intensely infected sites were farther from the equator (Fig. 2) irrespective of whether uninfected sites were included ( $p = 0.017$ ) or excluded ( $p = 0.0079$ ) from the analyses.

When all sites were included in the analyses, there were highly significant negative relationships between the number of *B. dendrobatidis* zoospores infecting frogs at sites and both sunset water temperature ( $p = 0.0017$ ) and warm-quarter temperature ( $p = 0.0014$ ; Fig. 3), although after exclusion of the uninfected sites only warm-quarter temperature remained significant (sunset water temperature:  $p = 0.12$ ; warm-quarter temperature:  $p = 0.0015$ ).

There were positive relationships between site intensity and both 30-day rainfall ( $p = 0.0006$ ; Fig. 4) and dry-quarter precipitation ( $p = 0.0034$ ) when all sites were included in the analyses, although after exclusion of the uninfected sites neither variable remained significant (30-day rainfall:  $p = 0.13$ ; dry-quarter precipitation:  $p = 0.21$ ).

There was no relationship between site intensity and SVL ( $p = 0.61$ ) when all sites were included in the analysis, but there was a highly significant negative relationship when only infected sites were analyzed ( $p = 0.0077$ ; Fig. 5). This highly significant negative relationship remained ( $p < 0.032$ ,  $r^2 = 0.02$ ) when the relationship

**Table 2.** Summary of multiple regression analyses for overall effects of snout-vent length (SVL) and either the short- or long-term climatic variables on the prevalence and intensity of *Batrachochytrium dendrobatidis* infection in stony creek frogs, both including and excluding uninfected sites.

Source of variation	Prevalence			Intensity		
	r <sup>2</sup>	p	β (SE)	r <sup>2</sup>	p	β (SE)
All sites included (n = 31)						
SVL		0.014*	-2.4 (0.92)		0.028*	-0.1 (0.04)
30-day rainfall		0.097	0.22 (0.13)		0.004*	0.019 (0.01)
sunset water temperature		0.034*	-3.08 (1.4)		0.006*	-0.19 (0.07)
whole model	0.366	0.0058*		0.536	0.0001*	
SVL		0.045*	-2.09 (0.99)		0.149	-0.074 (0.05)
precipitation driest quarter		0.051	0.15 (0.07)		0.011*	0.0098 (0.00)
mean temperature warmest quarter		0.14	-2.19 (1.4)		0.035*	-0.16 (0.07)
whole model	0.343	0.0092*		0.453	0.00086*	
Infected sites only (n = 24)						
SVL		0.0038*	-3.48 (1.1)		0.0085*	-0.12 (0.04)
30-day rainfall		0.49	0.093 (0.13)		0.074	0.01 (0.005)
sunset water temperature		0.76	0.62 (2.0)		0.52	-0.052 (0.08)
whole model	0.361	0.027*		0.421	0.011*	
SVL		0.010*	-3.31 (1.2)		0.031*	-0.10 (0.04)
precipitation driest quarter		0.33	0.069 (0.07)		0.103	0.004 (0.003)
mean temperature warmest quarter		0.64	-0.69 (1.5)		0.051	-0.11 (0.05)
whole model	0.396	0.016*		0.527	0.0016*	

\*Significant effects.

between SVL and the number of zoospores detected on infected frogs ( $n = 228$ ) was analyzed with frogs (as opposed to sites) as independent data points. Because there was no relationship between SVL and latitude ( $p = 0.55$ ), it is unlikely that the chytrid-SVL relationships we found were simply by-products of latitude-induced changes in morphology.

Multiple regressions of site intensity versus SVL and either the short- or long-term climatic variables both produced negative relationships between SVL and site intensity when uninfected sites were excluded from the analyses, but the relationship was not significant in the multiple regression of long-term climatic variables when uninfected sites were included (Table 2). Although all of the climatic variables were significantly related to site intensity (rainfall positively and temperature negatively) in the multiple regressions that included uninfected sites, none of these relationships were significant when uninfected sites were excluded from the analyses (Table 2). There remained, however, a nearly significant negative relationship between site intensity and warm-quarter temperature ( $p = 0.051$ ).

### Visible Signs of Disease

No dead or dying frogs of any species were found during this study. Sixty *L. lesueuri* exhibited noticeable reddening of either their ventral surface or toe pads, and *B. dendrobatidis* was detected on 25 (41.7%) of these frogs, significantly more than would be expected by chance in the study population ( $\chi^2 = 7.71$ ;  $df = 1$ ;  $p = 0.006$ ). Nevertheless, because *B. dendrobatidis* was not detected on

58.3% of frogs with marked reddening and was detected on 25.2% of frogs that exhibited no reddening ( $n = 803$ ), we do not consider reddening of the skin a reliable indicator of chytrid infection.

### Discussion

This study is the first to describe the latitudinal variation in chytrid infection levels in wild amphibian populations. Our results demonstrate that although there may be a slight increase in the prevalence of *B. dendrobatidis* infections in *L. lesueuri* populations farther from the equator, there is a highly significant increase in the number of *B. dendrobatidis* zoospores infecting frogs farther from the equator, with frogs from temperate populations often carrying an order of magnitude more zoospores than their more equatorial counterparts. Because amphibian populations at cooler latitudes have life-history characteristics that may decrease their resilience to environmental disturbances such as disease outbreaks (i.e., longer age to maturity, fewer clutches per year; Morrison & Hero 2003), the increased pathogen load experienced by these populations may present a serious threat to their long-term persistence.

Our results show that variation in the prevalence and intensity of chytrid infections between infected and uninfected sites is largely due to differing rainfall and thermal regimes, thus providing important empirical evidence that climatic factors function to limit the extent and impact of *B. dendrobatidis* infections in natural amphibian populations. Sites in warmer, drier regions were unlikely to support *B. dendrobatidis* populations, and, when

present, infections on frogs from these regions were less intense than those on frogs from cool, wet climates. This result supports both bioclimatic predictive models (Ron 2005) and laboratory studies (Longcore et al. 1999; Johnson et al. 2003; Piotrowski et al. 2004) that point to *B. dendrobatidis* as being unable to survive high temperatures (above 29° C) or desiccation. Indeed, we did not detect the fungus at any site where the sunset water temperature exceeded 22.5° C. Although this temperature is well within the thermal range of the fungus (Longcore et al. 1999; Johnson et al. 2003), sunset water temperatures taken during our springtime sampling are likely to severely underestimate the maximum temperatures experienced by both tadpoles and chytrid fungi on hot summer days. Infected frogs were found at all sites where the 30-day rainfall exceeded 33 mm, as opposed to only half the sites where 30-day rainfall was below 33 mm. These are the first empirical data to corroborate predictions (Ron 2005) that *B. dendrobatidis* should be more abundant in wetter areas.

Our data on the rainfall and thermal requirements of the fungus will prove useful to amphibian disease researchers and wildlife managers, whose conservation efforts should focus on those amphibian populations living within the *B. dendrobatidis* climatic envelope that we have described (sunset water temperature in spring < 23° C; 30-day rainfall > 33 mm). Because the prevalence of chytrid infection in *L. lesueuri* is generally as high as that of many sympatric species (Retallick et al. 2004; Woodhams & Alford 2005; Kriger et al. 2006b), our findings are likely to be applicable to many amphibian species throughout eastern Australia. Long-term data sets do not exist in many regions of the world. For these regions our recommendations (which are based on short-term climatic data easily obtained in the field) may prove especially useful, although further work will be needed to determine the generality of our results on other continents and for other species.

Although climatic variables explained a great deal of the variation in the prevalence and intensity of *B. dendrobatidis* infections between infected and uninfected sites, no climatic variable remained significant in any multiple regression analysis when examining infected sites only (Table 2). Rather, SVL was consistently the best predictor of both infection prevalence and intensity across infected sites, with small frogs more likely to be infected and carrying more intense infections than larger frogs (Fig. 5). A similar negative relationship between chytrid infection levels and SVL was found within a single population of juvenile *Mixophyes iteratus* in southeast Queensland (Kriger et al. 2006a), suggesting this pattern may be common in amphibian populations affected by *B. dendrobatidis*. It is unclear whether size (or age) influenced frogs' abilities to combat infection or whether it was the infection that influenced the size (or age) the frogs could attain. Lamirande and Nichols (2002) demonstrated increased resistance to chytridiomycosis in older (and

thus larger) *Dendrobates tinctorius*, lending support to the first hypothesis, whereas Parris and Cornelius (2004) demonstrated that chytridiomycosis can induce developmental stress in both *Bufo fowleri* and *Hyla chrysoscelis*, a finding that supports the second hypothesis. The two hypotheses have very different implications. The former suggests that frogs can outgrow their chytrid infections, whereas the latter suggests that chytridiomycosis causes sublethal effects (Semlitsch et al. 1988) even in amphibian species that are not declining, such as *L. lesueuri*.

Over one-fourth of the frogs we sampled were infected with *B. dendrobatidis*, and parasite loads on infected individuals were consistently high enough to kill experimentally infected captive frogs (inoculations of only 100 zoospores killed *M. fasciolatus*; Berger et al. 1999). It is surprising then that we did not encounter morbid or moribund individuals of any species at any of the sites, even though search effort was high (~250 person hours). Although *B. dendrobatidis* is highly capable of causing mass mortalities and population declines in naïve amphibian populations (Lips et al. 2006), the extent to which it affects the survivorship of individual frogs in locations in which it has become established is unclear (Retallick et al. 2004; Daszak et al. 2005; Kriger & Hero 2006). *L. lesueuri* remains one of the most abundant frogs in streams of eastern Australia, so it is unlikely that chytridiomycosis is significantly reducing the size of infected populations. It is possible that *L. lesueuri* populations have evolved a high degree of resistance to *B. dendrobatidis* in the over 25 years the fungus has been present in eastern Australia (data on time since introduction: Speare & Berger 2005). Alternatively, the intrinsic reproductive rate of *L. lesueuri* populations may be such that any loss due to disease-related mortality is easily overcome by a high degree of recruitment. In contrast to many Australian species that have experienced population declines, *L. lesueuri* is highly fecund (Hero et al. 2005).

In temperate southeastern Australia substantial population declines have occurred in recent decades in *Litoria spenceri*, *L. castanea*, *L. aurea*, *L. raniformis*, *L. booroolongensis*, *L. verreauxi alpina*, *Adelotus brevis*, *Pseudophryne corroborae*, *P. pengilleyi*, *Philoria frosti*, and *M. balbus* (Hero et al. 2006) and *B. dendrobatidis* has been detected in eight of these species (Speare & Berger 2005). Although our results suggest that at low elevations, temperate amphibians are more at risk of chytridiomycosis-related decline than tropical amphibian populations, the relationship between latitude and chytrid infection levels in montane amphibian populations has yet to be resolved. The majority of the rapidly declining and "enigmatic-decline" species, for whom chytridiomycosis is often implicated as a primary causative agent, occur in tropical upland areas (Stuart et al. 2004). It is possible that at high elevations, the optimal climatic conditions for *B. dendrobatidis* may actually occur in the tropics, with temperate upland areas being

too cold to support significant *B. dendrobatidis* populations. Alternatively, the greater number of amphibian declines in tropical areas may simply be an artifact of high species richness in those areas. As the tropics have higher amphibian biodiversity than temperate regions (Duellman 1999), so do they have the potential for more species to disappear or decline (Morrison & Hero 2003). An analysis of the global distribution of chytridiomycosis in relation to patterns of biodiversity and amphibian declines may shed light on the relative effects of chytridiomycosis on populations living at different elevations and latitudes.

Our results show that the intensity of chytrid infections in lowland *L. lesueuri* populations increases with distance from the equator and that thermal and precipitation regimes influence the distribution and abundance of *B. dendrobatidis*, limiting the fungus' ability to persist in hot, dry regions. We also identified an important relationship between *B. dendrobatidis* infection and SVL, with small frogs more likely to be infected and to have more intense infections than larger frogs. This information is a major addition to the understanding of chytridiomycosis host-pathogen ecology and will prove useful to amphibian conservation programs.

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