

Large-scale seasonal variation in the prevalence and severity of chytridiomycosis

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Keywords

Batrachochytrium dendrobatidis; amphibian declines; chytridiomycosis; seasonality; chytrid; *Litoria wilcoxii*.

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Abstract

The chytrid fungus *Batrachochytrium dendrobatidis* has been implicated as the causative agent of mass mortalities, population declines and the extinctions of amphibian species worldwide. Although several studies have shown that the prevalence of chytridiomycosis (the disease caused by the fungus) increases in cooler months, the magnitude and timing of these seasonal fluctuations have yet to be accurately quantified. We conducted disease sampling in a single population of stony creek frogs *Litoria wilcoxii* on 13 occasions over a 21-month period and used quantitative real-time polymerase chain reaction to detect and quantify the number of *B. dendrobatidis* zoospores present on samples. Disease prevalence varied significantly across sampling sessions, peaking at 58.3% (in early spring) and dropping to as low as 0% on two occasions (late summer and early autumn). There was a significant negative relationship between disease prevalence and mean air temperature in the 30 days prior to sampling. These large-scale seasonal fluctuations in chytridiomycosis levels will strongly influence conservation programs and amphibian disease research.

Introduction

Chytridiomycosis is a potentially lethal skin infection of amphibians caused by the chytrid fungus *Batrachochytrium dendrobatidis*. This emerging infectious disease is known to affect at least 200 amphibian species worldwide (Speare & Berger, 2004; updated with recently published accounts) and is associated with population declines and mass-mortality events on several continents (Berger *et al.*, 1998; Lips, 1999; Bosch, Martinez-Solano & Garcia-Paris, 2001; Weldon & du Preez, 2004; La Marca *et al.*, 2005), making it an important factor in the current global biodiversity crisis (Daszak *et al.*, 1999; Daszak, Cunningham & Hyatt, 2000). The last known individuals of *Taudactylus acutirostris* and *Eleutherodactylus karlshmidti* were found infected with *B. dendrobatidis* (Daszak, Cunningham & Hyatt, 2003; Burrows, Joglar & Green, 2004), highlighting the potentially devastating effects that chytridiomycosis may have on amphibian populations.

Batrachochytrium dendrobatidis, the only member of its phylum (Chytridiomycota) known to parasitize vertebrates, infects the heavily keratinized stratum corneum and stratum granulosum of the amphibian's pelvic patch, digits and ventral body, and the keratinized mouthparts of tadpoles. Associated epidermal changes include irregular cell loss, hyperkeratosis and excessive sloughing of the skin (Berger *et al.*, 1998). Fungal strains collected from infected amphibians in disparate regions of the world show low genetic

variation (Morehouse *et al.*, 2003), and several studies point toward a sudden appearance of *B. dendrobatidis* in Australia (Aplin & Kirkpatrick, 2000), Central America (Lips, Green & Papendick, 2003; Lips *et al.*, 2006) and North America (Ouellet *et al.*, 2005), suggesting the fungus has been introduced to many regions via anthropogenic means. Likely vectors for transcontinental introductions of the disease are infected amphibians involved in the global food trade (Mazzoni *et al.*, 2003), pet trade (Aplin & Kirkpatrick, 1999), zoo trade (Pessier *et al.*, 1999) and laboratory animal trade (Parker *et al.*, 2002).

Numerous laboratory studies have explored the thermal preferences of *B. dendrobatidis*, and there now exists a large body of experimental evidence pointing toward the fungus favoring cooler temperatures. For instance, Longcore, Pessier & Nichols (1999) found that *B. dendrobatidis* could grow *in vitro* at temperatures between 6 and 28 °C. Cultures at 29 °C failed to achieve substantial growth; however, at 23 °C growth was renewed. Although the fungus can survive freezing, it is killed within 96 h at 32 °C and within 4 h at 37 °C (Johnson *et al.*, 2003). In live frogs, experiments have demonstrated that the pathogenicity of *B. dendrobatidis* decreases above 23 °C: 100% ($n = 16$) of experimentally infected great barred frog *Mixophyes fasciolatus* juveniles held at 17 or 23 °C died, whereas only 50% ($n = 8$) of individuals held at 27 °C died (Berger *et al.*, 2004). In a similar experiment, captive-bred blue-and-yellow poison dart frog *Dendrobates tinctorius* larvae were experimentally

infected; tadpoles raised at 25 °C tended to live longer (1–23 days) after metamorphosis than did those raised at 23 °C (0–15 days; Lamirande & Nichols, 2002). At the more extreme end of the spectrum, infected *Litoria chloris* held at 37 °C for 16 h were completely cured of infection (Woodhams, Alford & Marantelli, 2003). As the immune defenses of amphibians decrease with corresponding decreases in temperature (Mattute *et al.*, 2000; Rollins-Smith *et al.*, 2002), frogs are likely more susceptible to chytridiomycosis at the temperatures at which the chytrid fungus reaches its highest pathogenicity.

Temperatures change dramatically across seasons, altitudes and latitudes; therefore, the thermal restrictions on *B. dendrobatidis* and the amphibian immune system have important implications for the host–parasite ecology of the disease and the effect of chytridiomycosis on wild amphibian populations. Herein we examine the potentially seasonal nature of chytridiomycosis in wild frog populations.

The results of past disease surveys of wild amphibian populations suggest that the prevalence of chytridiomycosis is higher in cooler months, as would be expected based on the findings of the above-mentioned laboratory studies. For example, surveys of apparently healthy frogs in Western Australia (Aplin & Kirkpatrick, 2000) and North Queensland (Retallick, McCallum & Speare, 2004; McDonald *et al.*, 2005; Woodhams & Alford, 2005) found a significantly higher prevalence of chytridiomycosis in the winter months of June, July and August. Similarly, Berger *et al.* (2004) examined dead and dying frogs from New South Wales and Queensland and determined that 53% ($n = 241$) of the chytridiomycosis-related deaths took place in the winter months of July and August. This trend is not restricted to Australia: in three separate years, Bradley *et al.* (2002) witnessed winter die-offs of *Rana yavapaiensis*, *Rana chiricahuensis* and *Hyla arenicolor* at multiple sites in southern Arizona, and Ouellet *et al.* (2005) found lower chytrid prevalence in Quebec in the summer months.

However, Green, Converse & Schrader (2002) found no seasonality in chytridiomycosis epizootics, with seven North American morbidity and mortality events beginning in 6 different months (January, May, July, August, September and November). This conflicting result highlights our need to clarify the potentially seasonal nature of the disease. To date, the majority of chytridiomycosis field studies have used survey methodologies that lacked rigorous experimental design and were generally restricted to opportunistic sampling across a broad range of species, age classes, altitudes, latitudes, habitat types, seasons and years. Confounding between these various causal factors impedes our ability to disentangle their relative effects (Yoccoz, Nichols & Boulinier, 2001), and thus it has been difficult to quantify the effects of season on the prevalence of chytridiomycosis in wild amphibian populations. Further, all of the above-mentioned studies utilized histological diagnosis of chytridiomycosis, the sensitivity of which has been shown to be low (Kriger *et al.*, 2006). In this study, we utilize highly sensitive molecular diagnostic techniques to examine the seasonal variation in the prevalence and severity of chytri-

diomycosis in a single population of stony creek frogs *Litoria wilcoxii* in the Numinbah Valley in south-east Queensland, Australia.

Methods

Study species

The stony creek frog *L. wilcoxii* (Anura: Hylidae; formerly *Litoria lesueuri*) is one of the most common frogs found along streams in eastern Australia. The species is nocturnal, sexually dichromatic (breeding males have a conspicuous yellow coloration) and sexually dimorphic (females are often four times heavier and 50% longer than males). Breeding males aggregate along rocky sections of the stream at night in the spring and summer months. The species is most common along streams running through open forest or farmland, but is also regularly encountered in closed rainforest. There is little evidence of any population decline in *Litoria wilcoxii* in recent decades, even in locations where sympatric species have disappeared (Gillespie & Hines, 1999). It has a very large geographical distribution, covering much of the eastern seaboard of Australia (Donnellan & Mahony, 2004), and has been implicated as a reservoir host for chytridiomycosis that may serve to maintain and spread the infection throughout the region (Retallick *et al.*, 2004). We thus consider it an ideal focal species for this study, as the epizootiology of the disease in this species may have wide-ranging effects on many species across eastern Australia.

Study site

Frogs were sampled along a 1 km stretch of the Nerang River (28.177°S, 153.228°E, 155 m altitude) in Numinbah Valley, south-east Queensland, Australia. The site is on private property and encompasses both forested areas and cleared areas where cattle graze and utilize the riparian zone. The site supports a diverse amphibian assemblage that includes *Adelotus brevis*, *Limnodynastes peronii*, *Litoria peronii*, *Litoria pearsoniana*, *Litoria latopalmata*, *Litoria gracilentata*, *Mixophyes fasciolatus*, the endangered *Mixophyes iteratus* and the introduced *Bufo marinus*.

Field methods

Sampling took place at *c.* 6-week intervals between 13 April 2004 and 14 January 2006. *Litoria wilcoxii* were captured using clean, unused 20 × 25 cm plastic bags. We sampled each frog for chytridiomycosis by firmly running a cotton swab (Kriger *et al.*, 2006) 10 times over (1) the frog's dorsal surface, (2) each of the frog's sides, from groin to armpit, (3) the ventral surface and (4) the undersides of each thigh. Additionally, five outward strokes of the swab were used on the undersides of each frog's feet, for a total of 70 strokes. Swabs were then replaced in their original container and were frozen at –20 °C upon return from the field (within 8 h of sampling). All frogs were handled with unused non-powdered latex gloves so as to prevent disease transmission

between animals. The snout–vent length of all frogs was measured to the nearest 0.1 mm using vernier calipers.

Sampling was biased toward adult male frogs, as they tend to be most abundant at the stream, although females and juveniles were sampled opportunistically. We attempted to capture 30 frogs at each sampling session, but winter sample sizes were small due to the difficulty in finding animals. The recapture rate of individual frogs between sampling sessions was very low (12%), and thus we treated each sampling session as independent.

We used a Thermochron iButton DS1921G temperature logger (Dallas Semiconductor, sourced from Alfatek, Bayswater, Victoria, Australia) to record air temperature at the site every 90 min. The temperature logger was placed at ground level in a shaded area ~10 m from the river's edge, so as to best approximate the temperature in a frog's diurnal refugium. The Nerang River regularly experiences large floods, making our attempts to secure a data logger in the river unsuccessful. Thus, water temperature was manually recorded at the start and end of each sampling session (roughly 19:00 and 24:00 h). Rainfall data were obtained from the Bureau of Meteorology weather station at Hinze Dam (28.048°E, 153.288°S, 110 m altitude), 15.5 km north of the study site.

Laboratory methods

Swabs were analyzed for the presence of *B. dendrobatidis* using established quantitative (real-time) polymerase chain reaction (qPCR) techniques (Boyle *et al.*, 2004) and the changes described by Kriger, Hero & Ashton (2006).

Data analysis

Disease prevalence during each sampling period was calculated by dividing the number of positive frogs by the total number of frogs sampled. Quantitation of chytrid zoospores on infected frogs is given as the mean value of *B. dendrobatidis* genome equivalents detected in the three replicates of the triplicate PCR analysis. We use this number as an index of the severity of a frog's infection (parasite load). As the number of zoospores detected varied over five orders of magnitude, data were log transformed before statistical analyses, and geometric means were used as required. We used logistic regression to determine whether snout–vent length was related to an adult male frog's disease status.

As daily temperatures can fluctuate greatly, we used the mean of the 480 temperature recordings in the 30 days before a sampling session to represent temperature for that session (hereafter referred to as 30-day air temperature). Similarly, we used the total accumulated rainfall in the 30 days before a sampling session (30-day rainfall) to represent the precipitation for that session. The life cycle of *B. dendrobatidis* is 4–5 days (Berger *et al.*, 2005), and a single chytrid zoosporangium can produce up to 150 zoospores (Annis *et al.*, 2004). Thus, 30 days should be ample time for chytrid population numbers to track climatic changes and to significantly affect disease prevalence at a site. Furthermore,

it encompasses the timeframe in which experimentally infected laboratory frogs often develop clinical/lethal infections (Nichols *et al.*, 2001; Daszak *et al.*, 2004). We used the mean of the two water temperature measurements taken on the night of sampling to represent water temperature for a given sampling session; this likely underestimates the true mean water temperature, as daytime temperatures are not accounted for. We used logistic regression to determine the relationships between the independent variables air temperature, water temperature and rainfall, and the likelihood of an individual frog being infected with chytridiomycosis. We used linear regression to determine the relationship between climatic variables and the number of zoospores detected on infected frogs.

As the response of juvenile frogs to chytrid infections can differ from that of adults (juveniles are more likely to succumb to infections; Lamirande & Nichols, 2002), and as sample sizes in juveniles were low, we restricted all statistical analyses to adult frogs.

Results

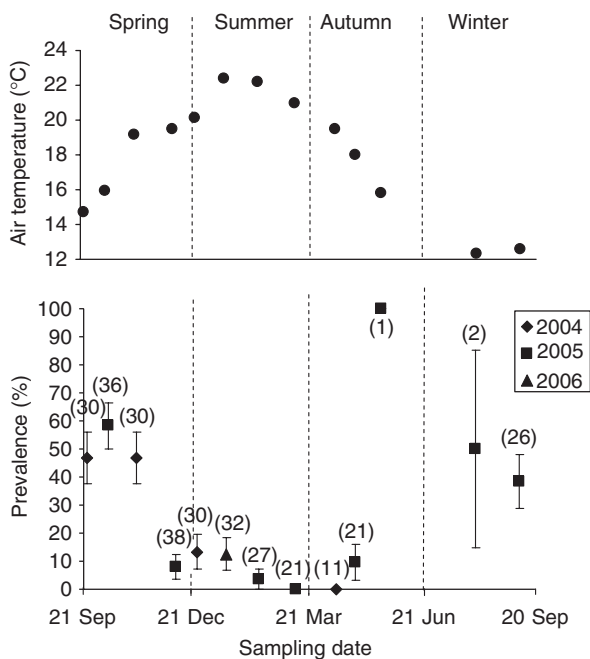
Batrachochytrium dendrobatidis was detected on 24.1% of the 319 *L. wilcoxii* sampled during this study. Disease levels varied significantly across seasons, with the winter and early spring months supporting the highest infection prevalence (Table 1; Fig. 1). This trend was repeated in multiple years. Prevalence during sampling periods ranged from as high as 58.3% (11 October 2005) to as low as 0% on two occasions (13 April 2004 and 12 March 2005). Although it is not possible to conclude that disease was completely absent from the population during these latter two sampling sessions, we can say with 95% confidence that prevalence was as low as 14.3% (DiGiacomo & Koepsell, 1986), and this difference is highly significant ($\chi^2_{1,0.05} = 10.6$; $P = 0.001$). The most striking shift in prevalence occurred in late 2005, when prevalence dropped from 58.3 to 7.9% over a 54-day period (from 11 October to 4 December).

The mean air temperature during the study was 17.8 °C (range = 2.5–33.5 °C, $SD = 5.0$), a value considered optimal for the growth of *B. dendrobatidis* (Longcore *et al.*, 1999; Piotrowski, Annis & Longcore, 2004). Disease prevalence was inversely related to 30-day air temperature: all six sampling sessions with 30-day air temperatures above 19.4 °C had a prevalence less than 14%, whereas six of seven sampling sessions with 30-day air temperatures less than 19.4 °C had a prevalence greater than 38% (Table 1; Fig. 2). This negative relationship between 30-day air temperature and the likelihood of a frog being infected was highly significant (Wald = 37.2, $P < 0.000001$, $r^2 = 0.126$), as were the negative relationships between 30-day rainfall and the likelihood of a frog being infected (Wald = 15.6, $P = 0.00008$, $r^2 = 0.056$; Table 1), and between water temperature and the likelihood of a frog being infected (Wald = 13.3, $P < 0.0003$, $r^2 = 0.041$; Table 1). The significant correlation between the three independent variables precluded their inclusion in multiple logistic regression, due to the colinearity effect.

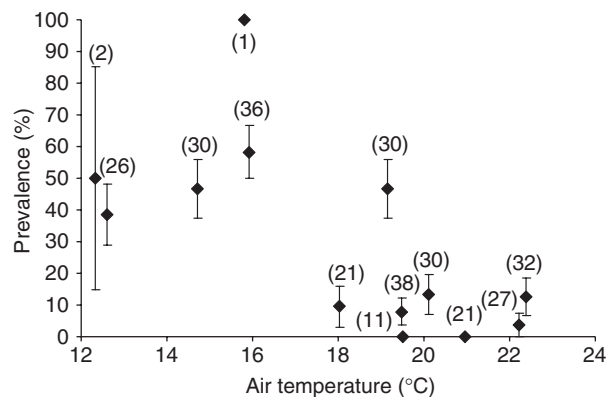
Table 1 Prevalence of chytridiomycosis and number of *Batrachochytrium dendrobatidis* zoospores on adult *Litoria wilcoxii* in Numinbah Valley, and relevant climatic variables

Season	Date	<i>n</i>	Prevalence (%)	Zoospores	30-day air temperature (°C)	Water temperature (°C)	30-day rainfall (mm)
Autumn	13-Apr-04	11	0	0	19.5	19.1	67
Spring	24-Sep-04	30	46.7	96	14.7	17.5	37
Spring	3-Nov-04	30	46.7	74	19.2	22.0	126
Summer	22-Dec-04	30	13.3	170	20.1	22.3	185
Summer	10-Feb-05	27	3.7	100	22.2	23.0	108
Summer	12-Mar-05	21	0	0	21.0	21.0	23
Autumn	29-Apr-05	21	9.5	794	18.0	17.5	52
Autumn	20-May-05	1	100	12 462	15.8	14.0	50
Winter	4-Aug-05	2	50.0	1995	12.3	14.3	9
Winter	8-Sep-05	26	38.5	372	12.6	15.8	18
Spring	11-Oct-05	36	58.3	955	15.9	20.0	17
Spring	4-Dec-05	38	7.9	11	19.5	21.2	160
Summer	14-Jan-06	32	12.5	65	22.4	23.5	129

Zoospores expressed as the geometric mean of all infected frogs at a sampling session.

**Figure 1** Seasonal variation in the prevalence of chytridiomycosis in adult *Litoria wilcoxii* in Numinbah Valley, and mean air temperature for the 30 days before sampling. Bars represent standard error based on a binomial distribution. Sample size shown in parentheses.

We found *B. dendrobatidis* on 28.0% of males sampled ($n = 268$) and on 14.3% of juveniles ($n = 14$). We did not find the fungus on any of the 37 female frogs we sampled. The difference in infection levels between males and females is significant ($\chi^2_{1,0.05} = 13.9$, $P = 0.002$) though only six of the 37 females were sampled during the cooler sampling sessions (when 30-day air temperatures were below 19.4 °C). There was no relationship between an adult male frog's snout-vent length and its disease status (Wald = 0.02, $P = 0.89$).

**Figure 2** Prevalence of chytridiomycosis in adult *Litoria wilcoxii* in Numinbah Valley, and mean air temperature for the 30 days before sampling. Bars represent standard error based on a binomial distribution. Sample size shown in parentheses.

The number of chytrid zoospores detected on infected frogs ranged from 2 to 235 287 (arithmetic mean = 9504; median = 272; geometric mean = 295). The negative relationship between 30-day air temperature and the number of zoospores found on infected frogs was significant ($P = 0.011$, $r^2 = 0.085$, $n = 75$; Fig. 3), as were the negative relationships between water temperature and the number of zoospores on infected frogs ($P = 0.034$, $r^2 = 0.060$, $n = 75$) and between 30-day rainfall and the number of zoospores on infected frogs ($P = 0.002$, $r^2 = 0.126$, $n = 75$). Although there was a great deal of variation within sampling sessions, maximum infection levels during summer were orders of magnitude lower than those from other seasons. The 17 most infected frogs were caught outside the summer months, and the only three frogs yielding over 100 000 zoospores were sampled in September, October and early November. One of these frogs (100 489 zoospores, sampled 3 November 2004) was discolored, anorexic and had lost its

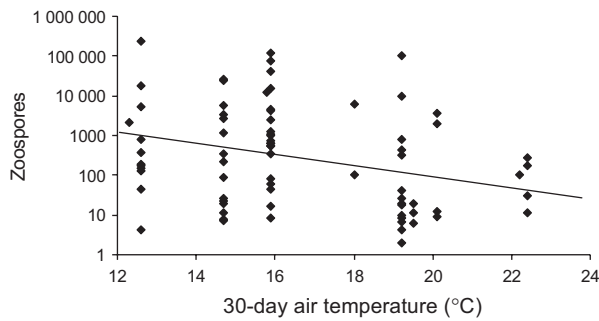


Figure 3 Number of *Batrachochytrium dendrobatidis* zoospores detected on infected male *Litoria wilcoxii*, and mean air temperature 30 days before sampling ($r^2 = 0.085$, $P = 0.011$, $n = 75$).

righting reflex, representing the sole dead or dying frog sighted during our study. All other frogs sighted during the study lacked clinical signs of infection.

Discussion

Seasonal variation in disease prevalence is common in both humans and wildlife (Hosseini, Dhondt & Dobson, 2004; Comrie, 2005; Pascual & Dobson, 2005), and may be due to a variety of factors, including thermal requirements of the parasite, changes in host immunity over time, or variation in the density and abundance of intermediate hosts (Van Riper *et al.*, 1986; Schaubert & Ostfeld, 2002; Hosseini *et al.*, 2004). We have demonstrated that the prevalence of chytridiomycosis in a single frog population can vary dramatically throughout the year, with disease levels closely tracking climatic changes. Peak disease levels at our site were recorded when mean air temperatures fell below 19.4 °C, and levels were still high at 12.3 °C, the coolest mean temperature experienced during our sampling. These results closely parallel laboratory studies, in which *B. dendrobatidis* grows best and is most pathogenic to frogs in cool conditions (Longcore *et al.*, 1999; Lamirande & Nichols, 2002; Berger *et al.*, 2004; Piotrowski *et al.*, 2004). It also concurs with the results of several field studies (Aplin & Kirkpatrick, 2000; Bradley *et al.*, 2002; Woodhams & Alford, 2005).

Disease levels in adults of our study population varied from as high as 58.3% (one of the highest chytrid prevalences ever reported for a wild amphibian population) in springtime to as low as 0% at two sampling sessions, one in late summer and one in early autumn. This was unexpected, and has important implications for amphibian disease researchers. First, many surveys are currently being planned to determine the presence or absence of *B. dendrobatidis* across large geographical areas. Those designing protocols for these surveys can reduce the number of samples required (and therefore costs) by ensuring that sampling takes place at the time of year when disease prevalence is at its maximum (DiGiacomo & Koepsell, 1986). Furthermore, sampling at a time of year when disease levels are at their lowest could result in a researcher falsely concluding that a location is free of disease. We therefore recommend that

these chytrid surveys take place when mean air temperatures are between 12 and 19 °C, and chytrid infections are most likely to be detected. It is currently unknown how disease prevalence is affected by temperatures less than 12 °C.

The findings of our study also imply that the results of past chytrid surveys that have taken place in the warmer months may not be comparable with those that have taken place in cooler months, as surveys undertaken at times when temperatures did not coincide with growth optima of *B. dendrobatidis* may have severely underestimated the potential prevalence of disease in the sampled populations or failed to detect the disease altogether, even though it was present.

Captive-breeding programs rely on accurate knowledge regarding the disease status of potential release sites for endangered captive-bred animals (Viggers, Lindenmayer & Spratt, 1993). If captive-breeding programs are unable to find a chytrid-free location for the capture or release of amphibians, we recommend that efforts to capture disease-free individuals take place when average temperatures exceed 20 °C, in order to minimize the chance of bringing infected individuals into captivity. The release of healthy captive-bred individuals into the wild should also focus on the warmest months, although the precise date of release needs to be weighed against the potentially decreased probabilities that an individual released late in the season will reproduce or will have had adequate time to acclimatize to the wild in order to survive a winter.

It is unclear how general the seasonal disease pattern we have documented is across species, altitudes and latitudes. Pounds *et al.* (2006) found a significant relationship between warm years and the extinctions of high-altitude harlequin frogs (Bufonidae: *Atelopus*) in Latin America, and hypothesized that contemporary climate warming had created (rather than diminished) thermally optimal conditions for *B. dendrobatidis*. While our finding that the prevalence and severity of chytridiomycosis increase at cooler temperatures may initially appear to contradict the findings of Pounds *et al.* (2006), the two studies can be reconciled by viewing them in an altitude-dependent context. At low altitudes, *B. dendrobatidis* growth is probably limited by high summer temperatures, which in many subtropical and tropical regions should reach levels lethal to the chytrid fungus (hence the lack of extinctions in low-altitude harlequin frogs). We can expect that global warming will further reduce the impacts of chytridiomycosis on low-altitude frog populations in subtropical and tropical regions. However, in montane regions and temperate lowlands, maximum temperatures may never attain levels lethal to *B. dendrobatidis*. Instead, fungal growth may be limited primarily by low temperatures (10 °C or less) which, while they may not kill the fungus, hinder its growth (Piotrowski *et al.*, 2004). Warming in these regions would thus be expected to favor the chytrid fungus and exacerbate amphibian population declines.

Our preliminary data support the existence of a season–altitude interaction. While seasonal shifts in disease prevalence in *L. pearsoniana* and *L. chloris* at other lowland sites

in the Nerang River catchment closely mirror the results of the current study, we have found high disease levels in these two species at upland sites in the summer months (K. M. Kriger, unpubl. data). A similar interaction may occur between latitude and season, with *B. dendrobatidis* capable of maintaining high population numbers at temperate latitudes even in the warmest months. Future research should examine the seasonality of chytrid infections at high altitudes and temperate latitudes, where the chytrid growth optima may actually occur in summer and be limited by extreme cool temperatures in winter.

The difficulty in finding *L. wilcoxii* during winter (on multiple sampling occasions, we were unable to locate any frogs) precluded our determining May–August disease levels with a high degree of resolution. However, we did find infected individuals during all winter sampling sessions. Combining this fact with our knowledge of *B. dendrobatidis* biology and the fact that 30-day mean temperatures never fell below 12 °C, we feel confident that disease levels at our study site remain high even in the months (June–July) when no sampling could take place.

We found a significant negative relationship between rainfall and disease prevalence, a result that contradicts what would be expected based on our knowledge of *B. dendrobatidis* as a waterborne pathogen (Longcore *et al.*, 1999; Johnson *et al.*, 2003; Ron, 2005). However, as rainfall and temperature were highly correlated (south-east Queensland experiences warm wet summers and cool dry winters), we hesitate to conclude that high chytridiomycosis levels will coincide with drier months in other regions. On the contrary, we expect that long-term rainfall patterns would explain much of the variation in disease levels between sites, with *B. dendrobatidis* favoring wetter regions (Ron, 2005).

Our results show that both 30-day air temperature and water temperature are significant predictors of disease levels, although the former variable yielded a much higher test statistic in the logistic regression than did the latter and explained a greater proportion of variance in the model. This could be attributed to *B. dendrobatidis* being regulated more by terrestrial processes (e.g. fungal growth and reproduction on post-metamorphic frogs or in soil; seasonal variation in the immune response of terrestrial, post-metamorphic frogs) than by aquatic processes (growth of the fungus on tadpoles or stream substrate; waterborne zoospore dispersal ability). Further research is needed to clarify whether chytrid levels are controlled more by aquatic or terrestrial processes.

Finally, there are evolutionary implications with regard to a host–parasite system that favors the parasite during cooler months. Semi-aquatic amphibians are most likely to be exposed to the waterborne zoospores of *B. dendrobatidis* during times of breeding (Muths *et al.*, 2003) and as tadpoles (Rachowicz & Vredenburg, 2004). In many species, breeding occurs in the early spring, the time when chytrid levels are at their peak. We could expect that after the introduction of chytridiomycosis to a naïve stream-breeding amphibian population, evolutionary pressures could force a shift in breeding until later in the year, when temperatures have

risen and are unsuitable for *B. dendrobatidis*. We can also hypothesize that, in its native range, the thermal biology of *B. dendrobatidis* evolved specifically to coincide with the time when amphibians were most likely to make contact with the water.

In conclusion, we have shown drastic seasonal variation in the prevalence of chytridiomycosis within a single amphibian population, and have identified mean air temperature in the 30 days before sampling as a powerful predictor of disease levels, with most chytrid infections occurring in cooler months (<19.4 °C). The results of our study will aid wildlife managers charged with designing disease survey protocols and will also assist conservation biologists involved in captive-breeding programs and research on disease dynamics in natural systems.

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References

- Annis, S.L., Dastoor, F.P., Ziel, H., Daszak, P. & Longcore, J.E. (2004). A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. *J. Wildl. Dis.* **40**, 420–428.
- Aplin, K. & Kirkpatrick, P. (1999). *Progress report on investigations into chytrid fungal outbreak in Western Australia*. Perth: Western Australia Museum.
- Aplin, K. & Kirkpatrick, P. (2000). Chytridiomycosis in southwest Australia: historical sampling documents the date of introduction, rates of spread and seasonal epidemiology, and sheds new light on chytrid ecology. In *Getting the jump! on amphibian disease: conference and workshop compendium*: 24. Williams, K. & Speare, R. (Eds). Cairns, Australia: Rainforest CRC.
- Berger, L., Hyatt, A.D., Speare, R. & Longcore, J.E. (2005). Life cycle stages of the amphibian chytrid *Batrachochytrium dendrobatidis*. *Dis. Aquat. Org.* **68**, 51–63.
- Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G. & Parkes, H. (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.

- Berger, L., Speare, R., Hines, H.B., Marantelli, G., Hyatt, A.D., McDonald, K.R., Skeratt, L.F., Olsen, V., Clarke, J.M., Gillespie, G.R., Mahony, M.J., Sheppard, N., Williams, C. & Tyler, M.J. (2004). Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Aust. Vet. J.* **82**, 31–36.
- Bosch, J., Martínez-Solano, I. & García-París, M. (2001). Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biol. Conserv.* **97**, 331–337.
- Boyle, D.G., Boyle, D.B., Olsen, V., Morgan, J.A.T. & Hyatt, A.D. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Dis. Aquat. Org.* **60**, 141–148.
- Bradley, G.A., Rosen, P.C., Sredl, M.J., Jones, T.R. & Longcore, J.E. (2002). Chytridiomycosis in native Arizona frogs. *J. Wildl. Dis.* **38**, 206–212.
- Burrowes, P.A., Joglar, R.L. & Green, D.E. (2004). Potential causes for amphibian declines in Puerto Rico. *Herpetologica* **60**, 141–154.
- Comrie, A.C. (2005). Climate factors influencing coccidiodomycosis seasonality and outbreaks. *Environ. Health Perspect.* **113**, 688–692.
- Daszak, P., Berger, L., Cunningham, A.A., Hyatt, A.D., Green, D.E. & Speare, R. (1999). Emerging infectious diseases and amphibian population declines. *Emerg. Infect. Dis.* **5**, 735–748.
- Daszak, P., Cunningham, A.A. & Hyatt, A.D. (2000). Emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science* **287**, 443–449.
- Daszak, P., Cunningham, A.A. & Hyatt, A.D. (2003). Infectious disease and amphibian population declines. *Divers. Distrib.* **9**, 141–150.
- Daszak, P., Striemy, A., Cunningham, A.A., Longcore, J.E., Brown, C.C. & Porter, D. (2004). Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. *Herpetol. J.* **14**, 201–207.
- DiGiacomo, R.F. & Koepsell, T.D. (1986). Sampling for detection of infection or disease in animal populations. *J. Am. Vet. Med. Assoc.* **189**, 22–23.
- Donnellan, S.C. & Mahony, M.J. (2004). Allozyme, chromosomal and morphological variability in the *Litoria lesueuri* species group (Anura: Hylidae), including description of a new species. *Aust. J. Zool.* **52**, 1–28.
- Gillespie, G. & Hines, H. (1999). Status of temperate riverine frogs in south-eastern Australia. In *Declines and disappearances of Australian frogs*: 109–130. Campbell, A. (Ed.). Canberra: Environment Australia.
- Green, D.E., Converse, K.A. & Schrader, A.K. (2002). Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996–2001. *Ann. NY Acad. Sci.* **969**, 323–339.
- Hosseini, P.R., Dhondt, A.A. & Dobson, A. (2004). Seasonality and wildlife disease: how seasonal birth, aggregation and variation in immunity affect the dynamics of *Mycoplasma gallisepticum* in house finches. *Proc. Roy. Soc. Lond. Ser. B* **271**, 2569–2577.
- Johnson, M., Berger, L., Phillips, L. & Speare, R. (2003). Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid, *Batrachochytrium dendrobatidis*. *Dis. Aquat. Org.* **57**, 255–260.
- Kriger, K.M., Hero, J.-M. & Ashton, K.J. (2006). Cost efficiency in the detection of chytridiomycosis using PCR assay. *Dis. Aquat. Org.* **71**, 149–154.
- Kriger, K.M., Hines, H., Hyatt, A.D., Boyle, D.G. & Hero, J.-M. (2006). Techniques for detecting chytridiomycosis in wild frogs: comparing histology with real-time Taqman PCR. *Dis. Aquat. Org.* **71**, 141–148.
- La Marca, E., Lips, K.R., Lotters, S., Puschendorf, R., Ibanez, R., Rueda-Almonacid, J.V., Schulte, R., Marty, C., Castro, F., Manzanilla-Puppo, J., Garcia-Perez, J.E., Bolanos, F., Chaves, G., Pounds, J.A., Toral, E. & Young, B.E. (2005). Catastrophic population declines and extinctions in neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica* **37**, 190–201.
- Lamirande, E.W. & Nichols, D.K. (2002). Effects of host age on susceptibility to cutaneous chytridiomycosis in blue-and-yellow poison dart frogs (*Dendrobates tinctorius*). In *Proceedings of the sixth international symposium on the pathology of reptiles and amphibians*: 3–13. McKinnell, R.G. & Carlson, D.L. (Eds). Minnesota: Saint Paul.
- Lips, K.R. (1999). Mass mortality and population declines of anurans at an upland site in western Panama. *Conserv. Biol.* **13**, 117–125.
- Lips, K.R., Brem, F., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J., Carey, C., Livo, L., Pessier, A.P. & Collins, J.P. (2006). Emerging infectious disease and the loss of biodiversity in a neotropical amphibian community. *Proc. Natl. Acad. Sci. USA* **103**, 3165–3170.
- Lips, K.R., Green, D.E. & Papendick, R. (2003). Chytridiomycosis in wild frogs from southern Costa Rica. *J. Herpetol.* **37**, 215–218.
- Longcore, J.E., Pessier, A.P. & Nichols, D.K. (1999). *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* **91**, 219–227.
- Mattute, B., Storey, K.B., Knoop, F.C. & Conlon, J.M. (2000). Induction of synthesis of an antimicrobial peptide in the skin of the freeze-tolerant frog, *Rana sylvatica*, in response to environmental stimuli. *FEBS Lett.* **483**, 135–138.
- Mazzoni, R., Cunningham, A.C., Daszak, P., Apolo, A., Perdomo, E. & Speranza, G. (2003). Emerging pathogen of wild amphibians in frogs (*Rana catesbeiana*) farmed for international trade. *Emerg. Infect. Dis.* **9**, 995–998.
- McDonald, K.R., Mendez, D., Muller, R., Freeman, A.B. & Speare, R. (2005). Decline in the prevalence of chytridiomycosis in frog populations in North Queensland, Australia. *Pac. Conserv. Biol.* **11**, 114–120.

- Morehouse, E.A., James, T.Y., Ganley, A.R.D., Vilgaly, R., Berger, L., Murphy, P.J. & Longcore, J.E. (2003). Multi-locus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. *Mol. Ecol.* **12**, 395–403.
- Muths, E., Corn, P.S., Pessier, A.P. & Green, D.E. (2003). Evidence for disease related amphibian decline in Colorado. *Biol. Conserv.* **110**, 357–365.
- Nichols, D.K., Lamirande, E.W., Pessier, A.P. & Longcore, J.E. (2001). Experimental transmission of cutaneous chytridiomycosis in dendrobatid frogs. *J. Wildl. Dis.* **37**, 1–11.
- Ouellet, M., Mikaelian, I., Pauli, B.D., Rodrigue, J. & Green, D.M. (2005). Historical evidence for widespread chytrid infection in North American amphibian populations. *Conserv. Biol.* **19**, 1431–1440.
- Parker, J.M., Mikaelian, I., Hahn, N. & Diggs, H.E. (2002). Clinical diagnosis and treatment of epidermal chytridiomycosis in African clawed frogs (*Xenopus tropicalis*). *Comp. Med.* **52**, 265–268.
- Pascual, M. & Dobson, A. (2005). Seasonal patterns of infectious diseases. *PLOS Med.* **2**, 18–20.
- Pessier, A.P., Nichols, D.K., Longcore, J.E. & Fuller, M.S. (1999). Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frog (*Litoria caerulea*). *J. Vet. Diag. Invest.* **11**, 194–199.
- Piotrowski, J.S., Annis, S.L. & Longcore, J.E. (2004). Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* **96**, 9–15.
- Pounds, J.A., Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.N., La Marca, E., Masters, K.L., Merino-Viteri, A., Puschendorf, R., Ron, S.R., Sanchez-Azofeifa, G.A., Still, C.J. & Young, B.E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* **439**, 161–167.
- Rachowicz, L.J. & Vredenburg, V.T. (2004). Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. *Dis. Aquat. Org.* **61**, 75–83.
- Retallick, R., McCallum, H. & Speare, R. (2004). Endemic infection of the amphibian chytrid fungus in a frog community post-decline. *PLOS Biol.* **2**, 1–7.
- Rollins-Smith, L.A., Reinert, L.K., Miera, V. & Conlon, J.M. (2002). Antimicrobial peptide defenses of the Tarahumara frog, *Rana tarahumarae*. *Biochem. Biophys. Res. Comm.* **297**, 361–367.
- Ron, S.R. (2005). Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the new world. *Biotropica* **37**, 209–221.
- Schauber, E.M. & Ostfeld, R.S. (2002). Modeling the effects of reservoir competence decay and demographic turnover in Lyme disease ecology. *Ecol. Appl.* **12**, 1142–1162.
- Speare, R. & Berger, L. (2004). *Global distribution of chytridiomycosis in amphibians*. Townsville, Australia: Amphibian Diseases Research Group. Available from www.jcu.edu.au/school/phtm/PHTM/frogs/chyglob.htm (accessed 10 February 2006).
- Van Riper, C., Van Riper, S.G., Goff, L.M. & Laird, M. (1986). The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecol. Monogr.* **56**, 327–344.
- Viggers, K.L., Lindenmayer, D.B. & Spratt, D.M. (1993). The importance of disease in reintroduction programmes. *Wildl. Res.* **20**, 687–698.
- Weldon, C. & du Preez, L.H. (2004). Decline of the Kihansi spray toad, *Nectophrynoides asperginis*, from the Udzungwa mountains, Tanzania. *Froglog* **62**, 2–3.
- Woodhams, D.C. & Alford, R.A. (2005). Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. *Conserv. Biol.* **19**, 1449–1459.
- Woodhams, D.C., Alford, R.A. & Marantelli, G. (2003). Emerging disease of amphibians cured by elevated body temperature. *Dis. Aquat. Org.* **55**, 65–67.
- Yoccoz, N.G., Nichols, J.D. & Boulinier, T. (2001). Monitoring of biological diversity in space and time. *Trends Ecol. Evol.* **16**, 446–453.