Austral ECOLOGY A Journal of ecology in the Southern Hemisphere

Austral Ecology (2008) **33**, 1022–1032

Altitudinal distribution of chytrid (*Batrachochytrium dendrobatidis*) infection in subtropical Australian frogs

KERRY M. KRIGER* AND JEAN-MARC HERO

Griffith University, Centre for Innovative Conservation Strategies, PMB 50 Gold Coast Mail Centre, Queensland 9726, Australia (Email: kerry@savethefrogs.com)

Abstract The disappearance of amphibian populations from seemingly pristine upland areas worldwide has become a major focus of conservation efforts in the last two decades, and a parasitic chytrid fungus, *Batrachochytrium dendrobatidis*, is thought to be the causative agent of the population declines. We examined the altitudinal distribution of chytrid infections in three stream-dwelling frog species (*Litoria wilcoxii*, *L. pearsoniana* and *L. chloris*) in southeast Queensland, Australia, and hypothesized that if *B. dendrobatidis* were responsible for the disappearance of high-altitude frog populations, infection prevalence and intensity would be greatest at higher altitudes. Overall, 37.7% of the 798 adult frogs we sampled were infected with *B. dendrobatidis*, and infections were found in all the populations we examined. Contrary to our initial hypothesis, we found no consistent evidence that high-altitude frogs were more likely to be infected than were lowland frogs. Further, the intensity of fungal infections (number of zoospores) on high-altitude frogs did not differ significantly from that of lowland frogs. *Batrachochytrium dendrobatidis* appears to be capable of infecting frogs at all altitudes in the subtropics, suggesting that all populations are at risk of decline when conditions favour disease outbreaks. We did find evidence, however, that chytrid infections persist longer into summer in upland as compared with lowland areas, suggesting that montane amphibian populations remain susceptible to disease outbreaks for longer periods than do lowland populations. Further, we found that at high altitudes, temperatures optimal for chytrid growth and reproduction coincide with frog metamorphosis, the life-stage at which frogs are most susceptible to chytrid infections. While these altitudinal differences may account for the differential population-level responses to the presence of *B. dendrobatidis*, the reason why many of southeast Queensland's montane frog populations declined precipitously while lowland populations remained stable has yet to be resolved.

Key words: altitudinal transect, amphibian decline, *Batrachochytrium dendrobatidis*, chytridiomycosis, disease ecology, Queensland.

INTRODUCTION

The accelerating loss of biodiversity worldwide in recent decades is exemplified by the current status of the world's amphibian species, nearly one-third of which are threatened with extinction (Stuart *et al.* 2004), and many of which have not been seen in decades (La Marca *et al.* 2005). While habitat destruction and over-exploitation are the primary threats to much of the world's fauna (Baillie *et al.* 2004; Li & Wilcove 2005), many amphibian declines and disappearances have taken place in protected wilderness areas where no obvious cause can be identified (Bradford 1991; Kagarise Sherman & Morton 1993; Pounds & Crump 1994; Hero & Morrison 2004; La Marca *et al.* 2005), and unidentified processes threaten 48% of rapidly declining species (Stuart *et al.* 2004).

Amphibian population declines and extinctions worldwide have been concentrated in montane regions (Bradford 1991; Richards *et al.* 1993; Lips 1998; Bosch *et al.* 2001; Muths *et al.* 2003; La Marca *et al.* 2005; Rachowicz *et al.* 2006), and 85% of the world's threatened frog species occur at high altitudes (Hero & Morrison 2004). In Central America, declines have generally occurred above 500 m altitude, and in the Andes above 1000 m (Young *et al.* 2001). Population declines of montane harlequin frogs (Bufonidae: *Atelopus*) have been particularly severe: all 28 upland species with sufficient population trend data have suffered declines, and 21 (75%) of these species are presumed extinct (La Marca *et al.* 2005). In Australia, 41% of upland species (predominantly distributed above 400 m a.s.l.) are threatened, versus only 8% of lowland species (Hero & Morrison 2004), and there are at least four tropical species (*Litoria nannotis*, *L. rheocola*, *Nyctimystes dayi* and *Taudactylus eungellensis*) whose upland populations have declined precipitously, while lowland populations have remained stable (McDonald & Alford 1999). Conservation programmes urgently require the accurate identification of the causal agent(s) responsible for these high-

*Corresponding author.

Accepted for publication December 2007.

doi:10.1111/j.1442-9993.2008.01872.x

altitude amphibian declines and extinctions. Though the cooler temperatures associated with montane areas have been shown to increase the sensitivity of larval and embryonic amphibians to UV-B radiation (van Uitregt *et al.* 2007), it is unlikely that the detrimental effects of UV-B would be a significant factor in the decline of montane rainforest amphibians worldwide (e.g. Heyer *et al.* 1988; Richards *et al.* 1993; Lips *et al.* 2006), as the thick rainforest canopy provides substantial protection from harmful UV-B radiation.

Batrachochytrium dendrobatidis is a pathogenic chytrid fungus that has been recovered from dead and dying amphibians concurrent with mass mortalities, population declines and species extinctions at montane sites worldwide (Berger *et al.* 1998; Bosch *et al.* 2001; Green *et al.* 2002; Burrowes *et al.* 2004; Weldon & du Preez 2004; Lips *et al.* 2006; Schloegel *et al.* 2006), and multiple lines of evidence suggest the fungus has recently been introduced to many regions (including Australia) via anthropogenic means (Mazzoni *et al.* 2003; Morehouse *et al.* 2003; Rachowicz *et al.* 2005; Lips *et al.* 2006). The thermal requirements of *B. dendrobatidis* have been well documented: in the laboratory, the fungus can survive freezing, grows best from 17 to 25°C, fails to make substantial growth above 28°C, and is killed after 96 h at 32°C (Longcore *et al.* 1999; Johnson *et al.* 2003; Piotrowski *et al.* 2004). Results from disease surveys of wild amphibian populations parallel laboratory findings, with infection prevalence and intensity increasing in cooler months (Berger *et al.* 2004; Retallick *et al.* 2004; Ouellet *et al.* 2005; Woodhams & Alford 2005; Kriger & Hero 2007), and at cooler latitudes (Kriger *et al.* 2007a). As *B. dendrobatidis* appears to have a clear preference for the cool temperatures generally associated with montane regions, chytridiomycosis (the cutaneous infection caused by the fungus: Berger *et al.* (1998)) has become the leading explanation put forth for the global decline of montane amphibian populations (Daszak *et al.* 1999; La Marca *et al.* 2005; Pounds *et al.* 2006). However, while *B. dendrobatidis* has been detected in alpine regions as high as 5348 m a.s.l. (Seimon *et al.* 2007), it has also been detected at approximately sea level (Kriger *et al.* 2007a).

If chytridiomycosis were the primary cause of amphibian population declines in montane regions, we would expect that the prevalence and/or intensity of chytrid infections would increase with corresponding increases in altitude. McDonald *et al.* (2005) examined chytrid infections in four north Queensland frog species and determined a significant overall increase in infection prevalence above 300 m. However, their statistical analysis was based on data pooled from multiple species that were sampled in different seasons and from a wide geographic range (37 sites from an undisclosed number of catchments). Thus it was not possible to determine the magnitude of the altitudinal effect in frog populations in the individual catchments, or whether the effect existed in all four frog species or in all catchments. To date, there have been only two published studies that have examined the altitudinal variation in the prevalence of amphibian chytrid infections within individual catchments, and those studies yielded conflicting results. Woodhams & Alford (2005) determined that upland green-eyed treefrogs (*Litoria genimaculata*) were 2.5 times more likely to carry chytrid infections than were their lowland counterparts. In contrast, Retallick *et al.* (2004) found no evidence of increased infection prevalence in upland Eungella torrent frogs (*T. eungellensis*). Furthermore, owing to their having used histology to diagnose chytrid infections, neither of the above studies was able to quantify the number of *B. dendrobatidis* zoospores present on infected samples, and thus there exists no published information regarding the altitudinal variation in the intensity of chytrid infections. In this study, we use molecular diagnostic techniques to examine the altitudinal variation in both the prevalence and intensity of chytrid infections in three stream-dwelling frog species of subtropical southeast Queensland, Australia.

METHODS

Frog surveys

Sampling was conducted in the Nerang River, Canungra Creek and Mary River catchments of southeast Queensland, and most sites were within protected areas of Springbrook, Lamington and Conondale National Parks (Table 1). Declines in Australia and abroad have been concentrated in stream-dwelling species (Williams & Hero 1998; Stuart *et al.* 2004; Hero *et al.* 2005), so we restricted our sampling to the southern orange-eyed treefrog (*Litoria chloris*), cascade treefrog (*L. pearsoniana*) and stony creek frog (*L. wilcoxii*, formerly *L. lesueuri*), three species that have aquatic larvae and are generally encountered within 5 m of streams. Furthermore, these species have large altitudinal ranges (Morrison *et al.* 2004), and chytrid infections are known to occur in frog populations from all three catchments (Kriger *et al.* 2006a).

Sampling took place over the course of two field seasons (Year 1: 22 October 2004 to 26 January 2005; Year 2: 3 November 2005 to 25 January 2006). We sampled frogs along altitudinal transects within each catchment, and attempted to capture at least 100 adults of each species per transect. Rather than restricting our sampling to solely an upland and lowland site in each catchment, we sampled frogs from as many altitudes within a catchment as was logistically possible. *Litoria pearsoniana* was sufficiently

doi:10.1111/j.1442-9993.2008.01872.x

Table 1. Location of sampling sites

Location	Latitude (S) deg min sec	Longitude (E) deg min sec	Altitude (m)	Species sampled
Nerang River Catchment				
Mundora Creek	28°13′26″	153°17′06″	790	LC, LP
Rush Creek – Tallanbana	28°13′30″	153°16′19″	785	LC
Boy-Ull Creek – above Twin Falls	28°13′34″	153°16′34″	760	LC, LP
Boy-Ull Creek – below Twin Falls	28°13′26″	153°16′30″	720	LP
Purling Brook – road crossing	28°11′42″	153°16′19″	590	LC, LP
Purling Brook – below falls	28°11′20″	153°16′16″	460	LP
Warringa Pools	28°10′59″	153°16′05″	360	LC, LP
Natural Bridge	28°13′59″	153°14′31″	270	LC, LP
Waterfall Creek	28°09′47″	153°14′53″	250	LC
Dave's Creek	28°13′30″	153°13′23″	235	LC, LP
Austinville	28°10′48″	153°18′25″	110	LC, LP
Austinville – Moffat Crossing	28°10′16″	153°18′25″	90	LC, LP
Canungra Creek Catchment				
Toolona Creek – Gwongurai Falls	28°14′42″	153°09′36″	885	LP
Canungra Creek – Picnic Rock	28°13′26″	153°09′11″	810	LP
Canungra Creek – Darragumai Falls	28°14′28″	153°09′07″	730	LP
Canungra Creek – Yanbacoochie Falls	28°14′13″	153°08′53″	680	LP
Canungra Creek – Blue Pools	28°13′01″	153°08′24″	475	LP
Canungra Creek – Yandooya	28°08′13″	153°08′02″	240	LP
Mary River Catchment				
Six Mile Creek headwaters	26°43′12″	152°33′22″	680	LP
Summer Creek	26°39′47″	152°34′44″	600	LP
Six Mile Creek – Middle Road	26°44′13″	152°30′47″	545	LW
Bundaroo Creek	26°41′35″	152°36′40″	495	LP
Peter's Creek	26°40′52″	152°36′22″	475	LP
Booloumba Falls	26°41′10″	152°37′12″	430	LP, LW
Booloumba Creek – Campground #3	26°39′04″	152°38′17″	130	LP, LW
East Cedar Creek	26°33′40″	152°50′53″	120	LP

Sampling took place within 40 vertical meters of given altitude. LC = *Litoria chloris*; LP = *L. pearsoniana*; LW = *L. wilcoxii*.

abundant across the altitudinal gradients of all three catchments to allow transects to be completed, but *L. chloris* only in the Nerang River catchment, and *L. wilcoxii* only in the Mary River catchment. To determine whether the qualitative results of individual transects would remain consistent through time, *L. pearsoniana* in the Canungra Creek catchment and *L. chloris* in the Nerang River catchment were sampled in two consecutive years, and transects for *L. pearsoniana* in the Nerang River catchment were conducted twice within Year 2, once in spring and once in summer. The main breeding seasons for our three study species end by late January (K.M.K. pers. obs. 2004, 2005, 2006), and attempts to conduct transects after this point resulted in insufficient numbers of frogs. Thus, eight complete altitudinal transects were conducted.

Chytrid infection levels in the region can change drastically over short time periods (Kriger & Hero 2007). To avoid introducing a confounding effect of season, we attempted to complete sampling within each transect in as short a timeframe as possible, and every effort was made to ensure that sampling at different altitudes was undertaken in a randomized fashion with respect to time.

Frogs were captured using clean, unused 20 × 25 cm plastic bags. We sampled each frog for *B. dendrobatidis* by firmly running a cotton swab (Medical Wire & Equipment, MW 100-100; Kriger *et al.* (2006b)) 10 times over each of the following locations: (i) the frog's dorsal surface; (ii) the frog's sides, from groin to armpit; (iii) the ventral surface; and (iv) the undersides of the thighs. Additionally, five outward strokes of the swab were employed on the undersides of each frog's feet, for a total of 70 strokes. Swabs were then replaced in their original container (a plastic tube), stored on ice in a cooler upon return from the field, and frozen at –20°C. All frogs were handled with unused non-powdered latex gloves to prevent disease transmission between animals, and were released immediately after sampling. To ensure that no frogs were inadvertently sampled twice, sampling of frogs did not commence until all frogs at a given section of stream were caught, and no further sampling took place at that section of stream after frogs were released.

doi:10.1111/j.1442-9993.2008.01872.x

Stream water temperature was recorded at the start and end of each sampling session (roughly 1900 and 2400 h), and an average of the two readings was used to represent water temperature for the sampling session. In the Nerang River catchment, we used Thermochron iButton DS1921G temperature loggers (Dallas Semiconductor, sourced from Alfatek, Bayswater, Victoria, Australia) to record air temperature at five altitudes (90 m, 155 m, 400 m, 600 m, 770 m) every 90 min. We placed the temperature loggers at ground level in shaded areas, so as to best approximate the thermal range experienced by the nocturnal frogs we were studying.

Laboratory analysis

Swabs were analysed for the presence of *B. dendrobatidis* using quantitative (real-time) polymerase chain-reaction techniques (qPCR) described by Boyle *et al.* (2004), and employing the changes described by Kriger *et al.* (2006a). Thus, all samples that tested positive in the initial singlicate qPCR assay were re-analysed using a triplicate assay and a full set of *B. dendrobatidis* standards, in order to confirm the initial result and accurately quantify the number of *B. dendrobatidis* zoospores present.

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.* 2007b). Disease prevalence for each transect was calculated by dividing the number of frogs positive for *B. dendrobatidis* 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. For simplicity, zoospore equivalents are hereafter referred to as zoospores. We used logistic regression to determine if the likelihood of a frog being infected with *B. dendrobatidis* varied significantly with altitude. Each of the eight transects was analysed individually. For each transect, we used linear regression to examine the relationship between altitude and the number of *B. dendrobatidis* zoospores (log-transformed) detected on infected frogs. To determine the relationship between water temperature and infection levels, we performed the above-mentioned analyses with water temperature replacing altitude as the predictor variable. We also combined all frogs from Year 1, all frogs from Year 2, and all frogs from both years, and performed the logistic and linear regressions mentioned above. We used an ANCOVA (with transect as the independent variable, altitude as the covariate and the number of zoospores on infected frogs as the dependent variable) to test for an interaction between transect and altitude with respect to the number of zoospores detected on infected frogs.

To test for a potential interaction between season and altitude with respect to the prevalence and intensity of *B. dendrobatidis* infections, we categorized all Year 2 frogs into either upland (above 400 m) or lowland (below 400 m) frogs. We then used the number of days elapsed since the date of the season's first sampling session as an independent variable and either a frog's PCR result (positive/negative) or the number of zoospores on infected frogs as the dependent variable, and compared results of logistic and linear regressions for each group of frogs (upland or lowland).

The failure of a researcher to detect a statistically significant effect can indicate either that no significant effect existed, or that the researcher had insufficient power to detect the effect. For all transects that yielded non-significant results, and for the analyses of all frogs from either Year 1, Year 2 or Years 1 & 2 combined, we used PASS 2008 (Hintze 2008) to calculate the power of our logistic and linear regressions to detect significant effects of altitude on *B. dendrobatidis* levels. Few data exist regarding the variation in *B. dendrobatidis* infection levels across environmental gradients. Kriger *et al.* (2007a) demonstrated that the prevalence of chytrid infection in *L. wilcoxii* could increase by as much as 24% along a latitudinal gradient and Kriger and Hero (2007) demonstrated that infection prevalence in a single *L. wilcoxii* population could vary by as much as 58.3% depending on recent ambient temperatures. We took a conservative approach and chose a 15% increase in prevalence as the effect size we wished to detect across the altitudinal transects examined. Voyles *et al.* (2007) found that clinically infected *Litoria caerulea* carried approximately 100 times as many *B. dendrobatidis* zoospores as did aclinically infected conspecifics, and Kriger *et al.* (2007a) detected a 20-fold increase in the number of *B. dendrobatidis* zoospores at sites along a latitudinal gradient. For our power calculations, we conservatively chose the latter of these two values to represent the increase in *B. dendrobatidis* zoospores we wished to detect across our altitudinal transects.

RESULTS

Batrachochytrium dendrobatidis was detected on 37.7% of the 798 frogs we sampled, and in all the populations and species we examined. The chytrid fungus is widely distributed across the altitudinal gradient in southeast Queensland, and we found no consistent evidence that either the prevalence or intensity of chytrid infections increases with altitude. Chytrid infection prevalence was significantly higher in upland frogs on two

doi:10.1111/j.1442-9993.2008.01872.x

transects, but significantly higher in lowland frogs on two other transects (Table 2). There were non-significant trends towards increasing prevalence in upland frogs for two transects, and two transects showed no relationship between chytrid prevalence and altitude. When analyses were repeated on all Year 1 frogs, all Year 2 frogs, or all frogs from the entire study, we found no relationships between altitude and prevalence (Table 3). Power analyses demonstrated that all of the above-mentioned analyses had over 80% power to detect a 15% increase in infection prevalence across the altitudinal gradient.

The number of *B. dendrobatidis* zoospores detected on infected frogs ranged from 1 to 51 082 (arithmetic mean = 1689; geometric mean = 136; median = 124; $n = 301$). There was a significant interaction between altitude and transect with respect to the number of zoospores detected on infected frogs (ANCOVA: $F = 2.58$, d.f. = 6, $P = 0.019$). On two transects the number of *B. dendrobatidis* zoospores detected on infected frogs increased significantly with altitude, but on five transects it did not (Table 2, Fig. 1). One altitudinal transect (Nerang River *L. chloris*, Year 1) did not have sufficient numbers of infected frogs to allow an analysis to be performed. Contrasting results were also obtained from linear regressions using all frogs from Year 1, Year 2, or both years combined (Table 3). In Year 1 there were significantly more zoospores infecting frogs at higher altitudes ($P = 0.034$), but for Year 2 and both years combined there was no such relationship ($P = 0.48$, $P = 0.060$, respectively; Fig. 1h). The independent variable altitude was unable to explain more than 5% of the variation in zoospore levels in any of the three analyses. While the relationship between altitude and zoospores for both years combined approached significance ($P = 0.060$), the corresponding r^2 value was so low (0.012) that we do not consider the relationship to be biologically significant. Power analyses demonstrated that all but two of the above-mentioned analyses would have had over 87% power to detect a 20-fold increase in the number of *B. dendrobatidis* zoospores along the altitudinal transect. The Canungra Creek *L. pearsoniana* (Year 1) and the Mary River *L. wilcoxii* transects had 79.3% and 43.7% power, respectively. The low power on these transects was likely due to the extreme variation in infection levels: the number of zoospores detected on infected frogs varied by over four orders of magnitude (Fig. 1a and b).

Three transects were replicated in two different time periods. For all three pairs of transects, the results obtained differed between the first and second replicates (Table 2). In Year 1, there was no relationship between altitude and either the prevalence ($P = 0.74$) or intensity ($P = 0.21$) of chytrid infections in Canungra Creek *L. pearsoniana*, but in Year 2 significant positive relationships existed for both analyses ($P =$

Table 2. Summary information for altitudinal transects: number of frogs sampled for *Batrachochytrium dendrobatidis*, altitudinal range, time period of sampling, overall infection prevalence along transect (%), and results for tests of significant variation with altitude

Catchment	Species	n	Altitude (m)	Sampling Dates		Prevalence			Log Zoospores		
				Mean	SD	(%)	P	Wald	r^2	P	b
Year 1											
Canungra Creek	*Litoria pearsoniana*	106	240–885	31-Oct-04	12.8	48.1	0.735	0.11	0.033	0.205	0.0009
Nerang River	*L. chloris*	111	90–790	17-Dec-04	35.2	6.3	**0.033**	(+) 4.54	n/a	n/a	n/a
Mary River	*L. wilcoxii*	73	110–545	21-Nov-04	8.3	53.4	0.156	2.01	0.065	0.119	0.002
Year 2											
Canungra Creek	*L. pearsoniana*	90	245–885	8-Dec-05	2.2	40.0	**0.00005**	(+) 12.1	0.266	**0.001**	0.0038
Nerang River	*L. chloris*	120	90–790	27-Nov-05	6.3	40.0	0.096	(+) 2.76	0.002	0.768	–0.0002
Mary River	*L. pearsoniana*	105	120–680	19-Dec-05	0.9	37.1	**0.003**	(–) 8.88	0.031	0.287	0.0007
Nerang River	*L. pearsoniana*	96	95–760	18-Nov-05	13.1	56.3	**0.011**	(–) 6.51	0.021	0.295	–0.0005
Nerang River	*L. pearsoniana*	97	90–790	23-Jan-06	1.3	35.1	0.078	(+) 3.11	0.117	**0.047**	0.0009

(+) = positive relationship. (–) = negative relationship. SD is measured in days. Significant effects ($P < 0.05$) are shown in bold.

doi:10.1111/j.1442-9993.2008.01872.x

Table 3. Results for tests of significant variation with altitude in either the prevalence of *Batrachochytrium dendrobatidis* infection or the number of *B. dendrobatidis* zoospores detected on infected frogs, combining all frogs sampled in a given time period, irrespective of catchment or species

		Prevalence		Log Zoospores		
Time period	*n*	*P*	Wald	r^2	*P*	B
Year 1	290	0.400	0.71	0.047	**0.034**	0.001
Year 2	508	0.713	0.14	0.002	0.478	0.0002
Years 1 & 2	798	0.478	0.50	0.012	0.060	0.0004

Significant effects ($P < 0.05$) are shown in bold.

0.00005, $P = 0.001$, respectively). In Year 1, there was a significant positive relationship between altitude and the prevalence of chytrid infection in Nerang River *L. chloris* ($P = 0.033$), but in Year 2 no such relationship existed ($P = 0.096$). There appeared to be a seasonal shift in the altitudinal distribution of *B. dendrobatidis* in *L. pearsoniana* on the Nerang River: in the springtime, infection prevalence was significantly greater in the lowlands ($P = 0.011$), but by summer there was a non-significant trend towards increased prevalence at high-altitudes ($P = 0.078$). And while there was no relationship between altitude and the intensity of chytrid infections in the spring ($P = 0.30$, Fig. 1c), by summer high-altitude Nerang River *L. pearsoniana* carried significantly more intense infections than did their lowland counterparts ($P = 0.047$, Fig. 1g).

The prevalence of chytrid infection decreased significantly in lowland frogs (under 400 m) in Year 2 as the summer progressed ($P = 0.0028$, Wald = 8.91), but no such relationship existed in upland frog populations ($P = 0.983$, Wald = 0.0005), where infection prevalence remained high throughout the sampling season. While there was no relationship between the date of sampling and the number of *B. dendrobatidis* zoospores detected on infected lowland frogs ($P = 0.30$, $r^2 = 0.011$), the number of zoospores detected on infected upland frogs increased significantly as the summer progressed ($P = 0.0041$, $r^2 = 0.076$).

Air temperatures in the Nerang River catchment seldom rose higher than 28°C (the temperature above which *B. dendrobatidis* fails to make substantial growth *in vitro*: Longcore *et al.* (1999); Piotrowski *et al.* (2004)), regardless of altitude, and we did not record any temperatures below 1°C (Fig. 2). Even at the lowest altitude for which we have temperature logger data (90 m a.s.l.), only 0.6% of the readings were above 28°C. 30-day mean air temperatures never exceeded 23°C at any of the five sites where we had temperature loggers, and never fell below 9°C (Fig. 3). Stream water temperature on the night of sampling never exceeded 23°C or fell below 13°C at any site in southeast Queensland during our study (Fig. 4). We found no consistent evidence that either the prevalence or intensity of chytrid infections increased with cooler water temperatures. Chytrid infection prevalence on three transects (Nerang River *L. chloris*, Years 1 and 2; Canungra Creek *L. pearsoniana*, Year 2) was significantly higher in frogs associated with cooler water, but significantly higher in frogs associated with warmer water on two other transects (Mary River and Nerang River *L. pearsoniana*, Year 2). The remaining three transects showed no relationship between chytrid prevalence and water temperature. While on one transect (Canungra Creek *L. pearsoniana*, Year 2) the number of *B. dendrobatidis* zoospores detected on infected frogs increased significantly at cooler water temperatures, no relationship existed between zoospores and water temperature on the remaining six transects. One transect (Nerang River *L. chloris*, Year 1) did not have sufficient numbers of infected frogs to allow an analysis to be performed.

Two dead frogs were encountered during this study. An adult male *L. chloris* was found dead at 760 m a.s.l. in the Nerang River catchment on 24 November 2005. We detected 669 *B. dendrobatidis* zoospores on this frog. An adult male great barred frog (*Mixophyes fasciolatus*) was found dead at 720 m a.s.l. in the Conondale Range on 14 November 2004. No *B. dendrobatidis* zoospores were detected on this frog. In both instances, frogs of these and multiple other species were calling in the immediate vicinity.

DISCUSSION

A large body of evidence points towards *Batrachochytrium dendrobatidis* preferring cooler temperatures. In the laboratory the fungus grows best and is most pathogenic to frogs below 25°C (Longcore *et al.* 1999; Lamirande & Nichols 2002; Berger *et al.* 2004; Piotrowski *et al.* 2004), and in wild frog populations the prevalence and intensity of chytrid infections have been shown to increase in cooler months (Ouellet *et al.* 2005; Woodhams & Alford 2005; Kriger & Hero 2007) and at cooler latitudes (Kriger *et al.* 2007a). The thermal requirements of *B. dendrobatidis*, along with the fact that the fungus has been consistently recovered from dead and dying frogs concurrent with

doi:10.1111/j.1442-9993.2008.01872.x

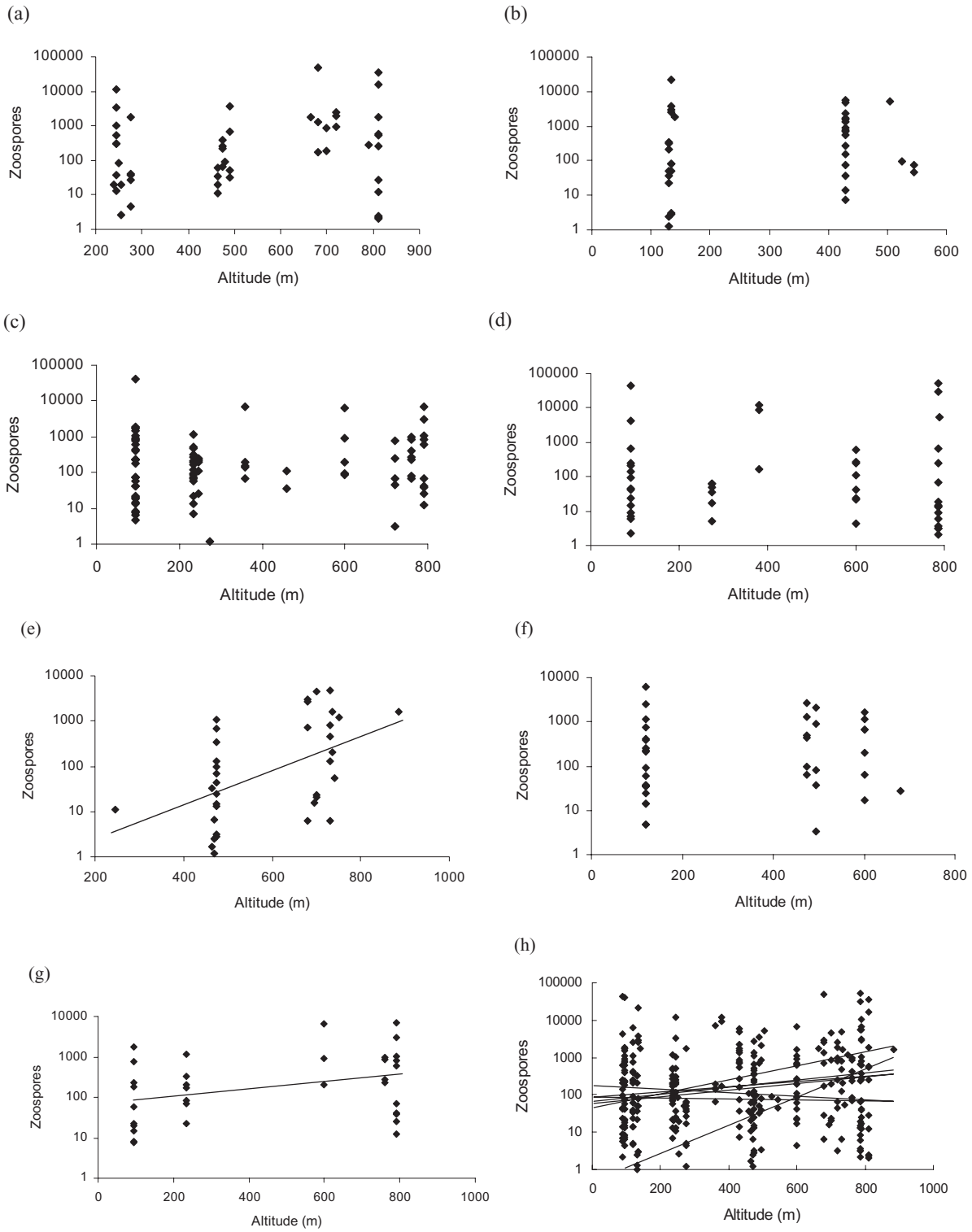

Fig. 1. Relationship between altitude and the number of *Batrachochytrium dendrobatidis* zoospores detected on infected frogs in Year 1: (a) Canungra Creek *Litoria pearsoniana* ($P = 0.205$, $r^2 = 0.033$, $n = 51$), (b) Mary River *L. wilcoxii* ($P = 0.119$, $r^2 = 0.065$, $n = 39$); and Year 2: (c) Nerang River springtime *L. pearsoniana* ($P = 0.295$, $r^2 = 0.021$, $n = 54$), (d) Nerang River *L. chloris* ($P = 0.768$, $r^2 = 0.002$, $n = 48$), (e) Canungra Creek *L. pearsoniana* ($P = 0.001$, $r^2 = 0.266$, $n = 36$), (f) Mary River *L. pearsoniana* ($P = 0.287$, $r^2 = 0.031$, $n = 39$), (g) Nerang River summertime *L. pearsoniana* ($P = 0.047$, $r^2 = 0.117$, $n = 34$); and (h) on all infected frogs in the study ($P = 0.060$, $r^2 = 0.012$, $n = 301$). Trend lines clearly indicate heterogeneity of slopes among transects.

doi:10.1111/j.1442-9993.2008.01872.x

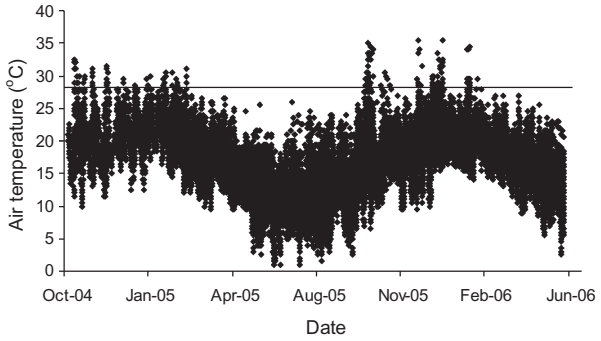

Fig. 2. Range of air temperatures experienced in the Nerang River catchment over a 20-month period (mean = 16.4°C; SD = 4.6°C). Data based on 31 815 temperature readings taken from five data loggers placed in shaded areas between 90 m and 770 m a.s.l. Line represents the temperature (28°C) above which *Batrachochytrium dendrobatidis* fails to make substantial growth *in vitro* (Longcore *et al.* 1999; Piotrowski *et al.* 2004).

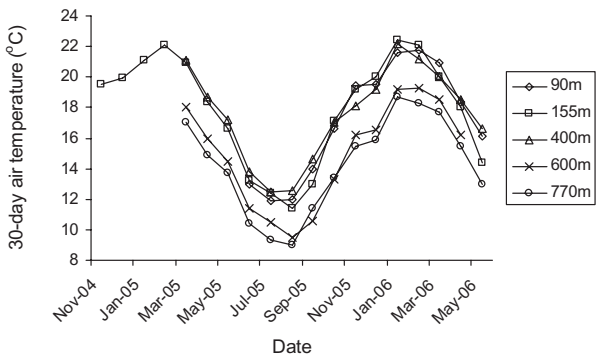

Fig. 3. Air temperatures in the Nerang River catchment at five altitudes. Data points represent the mean air temperature in the 30 days prior.

population declines at montane sites worldwide (Berger *et al.* 1998; Bosch *et al.* 2001; Weldon & du Preez 2004; Lips *et al.* 2006; Rachowicz *et al.* 2006), suggest that infection prevalence and intensity likely increase with altitude. However, we found no consistent evidence in the present study to support this hypothesis. Contrary to our initial expectations, lowland frogs were as likely to be infected with *B. dendrobatidis* as were high-altitude frogs, and carried fungal infections as intense as those of their high-altitude counterparts.

While we found no consistent relationship between altitude and chytrid levels in subtropical southeast Queensland, the effect of altitude on the prevalence and intensity of chytridiomycosis at tropical and temperate latitudes remains unclear. Kriger *et al.* (2007a) found a significant decrease in the intensity of lowland frogs' *B. dendrobatidis* infections in the warmer regions close to the equator, suggesting that tropical lowlands

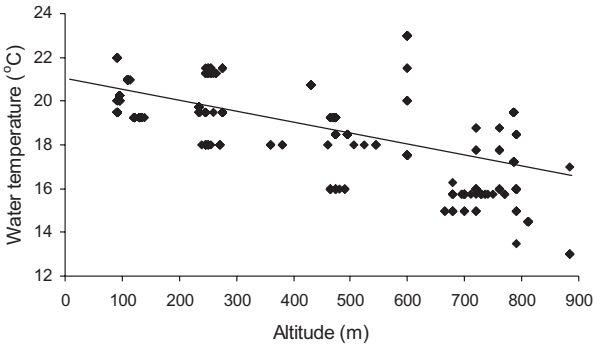

Fig. 4. Relationship between altitude and stream water temperature on night of sampling, at sites in three catchments in southeast Queensland ($P < 0.0001$, $r^2 = 0.384$). All temperatures fall within the thermal growth range of *Batrachochytrium dendrobatidis*.

may be too hot to sustain high *B. dendrobatidis* infection levels. In these regions, there may indeed be a significant increase in chytrid levels at the higher altitudes where it is cool enough for chytrid fungi to thrive.

Whereas certain populations of tropical frog species with large altitudinal ranges may find sanctuary from chytridiomycosis in the hot lowlands (Kriger *et al.* 2007a), our study suggests subtropical frog species have no such safe havens from the disease. Thermal regimes at all of our sites were suitable for chytrid growth and reproduction, regardless of altitude. Air temperatures seldom deviated from the range of temperatures (between 4°C and 28°C: Longcore *et al.* 1999; Piotrowski *et al.* 2004) at which *B. dendrobatidis* is known to grow (Figs 2,3), and stream water temperatures consistently remained within the range of temperatures at which the chytrid fungus can thrive. Kriger and Hero (2007) found that infection prevalence in lowland *L. wilcoxii* in the Nerang River catchment could reach 46% when water temperatures were as low as 14.3°C or as high as 22°C. Only on three occasions during this study did water temperatures depart from this range, regardless of the altitude at which sampling occurred (Fig. 4). As subtropical regions may provide no thermal refuge from *B. dendrobatidis*, frogs at all altitudes are likely to be threatened when conditions favour disease outbreaks. We therefore consider subtropical amphibian populations to be especially at risk of disease-related population decline.

It is possible that the altitudinal range over which our sampling took place was not large enough to allow for an effect of altitude to be detected. We consider this to be unlikely for two reasons. First, our sampling encompassed the altitudinal range over which most frog declines in Australia have occurred. Whereas frog declines in the Americas have often taken place thousands of meters above sea level (Bosch *et al.* 2001;

doi:10.1111/j.1442-9993.2008.01872.x

Muths *et al.* 2003; La Marca *et al.* 2005; Rachowicz *et al.* 2006), declines in Australia (and Queensland in particular) have generally affected frog populations living over 300–400 m a.s.l. (Richards *et al.* 1993; Hero & Morrison 2004). *Batrachochytrium dendrobatidis* has been present in southeast Queensland since at least 1978 (Speare & Berger 2005), and two local frog species (*Taudactylus diurnus* and *Rheobatrachus silus*) went extinct shortly thereafter. Both these species inhabited sites in the Conondale Range at which our sampling took place, and were restricted to altitudes from roughly 400–800 m a.s.l. (Czechura & Ingram 1990; Hines *et al.* 1999). We therefore feel that the altitudinal range over which our sampling took place would be sufficient to detect the differential effects of an unidentified causative agent of high-altitude amphibian population declines in the region. Second, the lack of a consistent altitudinal effect did not appear to be due to our not having sampled at high enough altitudes. Rather, the lack of an effect was due to lowland frog populations (~100 m a.s.l) often having infection prevalence over 50%, and lowland frogs often carrying infection loads of well over 1000 chytrid zoospores. We feel confident that *B. dendrobatidis* is well established at all altitudes in the region, even where amphibian population declines and extinctions have not occurred.

Do our results imply that chytridiomycosis was not the primary cause of amphibian population declines in montane regions of southeast Queensland? Not necessarily. We found a significant interaction between altitude and season, with infection levels remaining high in the upland frog populations well into summer, but dropping off in the lowlands as temperatures warmed. McDonald *et al.* (2005) found a similar interaction in north Queensland. The longer chytrid seasons experienced by high-altitude frog populations: (i) increase the likelihood that conditions suitable for lethal disease outbreaks will occur (i.e. abnormally cool temperatures; arrival of, or increased levels of a co-factor); (ii) allow more time for uninfected frogs to contract infections; and (iii) may exacerbate any sub-lethal effects of chytridiomycosis (Parris & Cornelius 2004), as infected frogs likely sustain their infection for longer periods of time than do lowland frogs (lowland frogs can clear their infections in warm summer months: Kriger and Hero (2006)).

Furthermore, complex relationships between the timing of metamorphosis and chytrid thermal optima may render montane amphibian populations more susceptible to chytrid-induced mortality than their lowland counterparts. Lamirande and Nichols (2002) found that recently metamorphosed dendrobatid frogs were more susceptible to chytridiomycosis than were conspecifics of other life-stages, suggesting that the population-level effects of chytridiomycosis may be primarily determined by disease levels in the weeks immediately following metamorphosis. Kriger and Hero (2007) found that the prevalence and intensity of chytrid infections in the Nerang catchment increase dramatically when 30-day mean air temperatures fall below 19.4°C. *Litoria chloris*, *L. pearsoniana* and *L. wilcoxii* tend to metamorphose from December to March (a pattern common to many of southeast Queensland's amphibian species: Anstis (2002)). During much of this period, 30-day mean air temperatures in the lowlands of the Nerang catchment exceed 19.4°C (Kriger & Hero 2007), whereas mean temperatures in the uplands never exceed this value (Fig. 3). Thus, while tadpoles of many lowland amphibian populations are likely to emerge when temperatures are too high for lethal infections, metamorphs at high altitudes will inevitably be exposed to temperatures optimal for chytrid growth and development, potentially resulting in differential mortality and recruitment between high and low altitude populations. Further research is needed to test this hypothesis.

If *B. dendrobatidis* was the primary agent responsible for the declines in southeast Queensland's montane frog populations, it is possible that the high-altitude frog populations that survived the initial epidemic experienced a rapid evolutionary response to the fungus. Two possible mechanisms by which this could be accomplished are the increased production or potency of either antimicrobial peptides (many of which have been shown to inhibit *B. dendrobatidis*: Rollins-Smith *et al.* (2002)) or antibodies (Berger *et al.* 2002). The evolutionary response would have been selected for until the montane frogs were at low-risk of lethal chytridiomycosis. In this scenario, no significant altitudinal variation in chytrid levels would be expected in extant frog populations, a result observed in this study. Unfortunately, no empirical data exist regarding the altitudinal variation in frogs' antimicrobial peptides or antibodies, or the evolutionary response of frog populations to chytridiomycosis-induced declines.

Kriger and Hero (2007) and Kriger *et al.* (2007a) demonstrated a significant negative relationship between water temperature at a sampling site and the prevalence and intensity of *B. dendrobatidis* infections on *L. wilcoxii*. In the current study, we found no consistent evidence of such a relationship in the three frog species examined. It is possible that we did not detect such a relationship in the current study because the range of stream water temperatures over which sampling took place was narrow (min: 13°C, max: 23°C, SD: 2.1°C) and all temperatures fell within the thermal growth range of *B. dendrobatidis* (Fig. 4). Alternatively, the abundance of *B. dendrobatidis* (or frogs' resistance to infection) in southeast Queensland may be significantly influenced by factors other than temperature, thus obscuring any effect of temperature in the catchments we examined.

doi:10.1111/j.1442-9993.2008.01872.x

In this study, we found frogs with chytrid infections at virtually every site we sampled, and there was no consistent relationship between altitude and chytrid levels. More research is required to determine the magnitude of the interaction between season, altitude and chytrid levels, the degree to which susceptibility to chytridiomycosis varies across life stages in wild amphibian populations, and the relative influence of life-history traits on the population-level effects of chytridiomycosis. Future research should also focus on accurately identifying potential co-factors that favour the growth and reproduction of the chytrid fungus, or decrease the amphibian immune response, as the presence of *B. dendrobatidis* in and of itself does not necessarily result in amphibian population declines (Daszak *et al.* 2003; 2005; Kriger & Hero 2006; Puschendorf *et al.* 2006).

ACKNOWLEDGEMENTS

Funding for this study was provided by the Eppley Foundation for Research and the National Geographic Society's Committee for Research and Excellence. The Consortium for Conservation Medicine also provided financial assistance. K.M.K was partially supported by the Griffith University School of Environmental and Applied Sciences. We thank the Griffith University Heart Foundation Research Centre for allowing use of their PCR equipment, K. Ashton for providing many helpful insights regarding PCR analysis, R. Campbell for providing positive controls, and D. Boyle and A. Hyatt for providing chytrid standards. The Centre for Aquatic Processes and Pollution generously allowed access to laboratory space and equipment, J. Webley gave helpful comments on an earlier draft of this manuscript, and M. Arthur and J. Vandemerwe provided statistical advice. This study would not have been possible without the excellent field assistance provided by D. Hall, F. Pereoglou, U. Kawai and many other volunteers.

REFERENCES

Anstis M. (2002) *Tadpoles of South-eastern*. New Holland Publishers, Sydney.

Baillie J. E. M., Hilton-Taylor C. & Stuart S. N. (2004) *IUCN Red List of Threatened Species*. A Global Species Assessment. IUCN, Gland, Switzerland and Cambridge.

Berger L., Speare R., Daszak P. *et al.* (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc. Natl. Acad. Sci. USA* **95,** 9031–6.

Berger L., Hyatt A. D., Olsen V *et al.* (2002) Production of polyclonal antibodies to *Batrachochytrium dendrobatidis* and their use in an immunoperoxidase test for chytridiomycosis in amphibians. *Dis. Aquat. Org.* **48,** 213–20.

Berger L., Speare R., Hines H. B. *et al.* (2004) Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Aust. Vet. J.* **82,** 31–6.

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,** 331–7.

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,** 141–8.

Bradford D. F. (1991) Mass mortality and extinction in a high-elevation population of *Rana muscosa*. *J. Herpetol.* **25,** 174–7.

Burrowes P. A., Joglar R. L. & Green D. E. (2004) Potential causes for amphibian declines in Puerto Rico. *Herpetologica*. **60,** 141–54.

Czechura G. V. & Ingram G. J. (1990) *Taudactylus diurnus* and the case of the disappearing frogs. *Memoirs of the Queensland Museum* **29,** 361–5.

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,** 735–48.

Daszak P., Cunningham A. A. & Hyatt A. D. (2003) Infectious disease and amphibian population declines. *Divers. Distrib.* **9,** 141–50.

Daszak P., Scott D. E., Faggioni C., Kilpatrick A. M., Gibbons J. W. & Porter D. (2005) Amphibian population declines at Savannah River site are linked to climate, not chytridiomycosis. *Ecology*. **86,** 3532–7.

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. N. Y. Acad. Sci.* **969,** 323–39.

Hero J.-M. & Morrison C. (2004) Frog declines in Australia: global implications. *Herpetol. J.* **14,** 175–86.

Hero J.-M., Williams S. E. & Magnusson W. E. (2005) Ecological traits of declining amphibians in upland areas of eastern Australia. *J. Zool. (Lond)*. **267,** 221–32.

Heyer W. R., Rand A. S., Goncalvez da Cruz C. A. & Peixoto O. L. (1988) Decimations, extinctions, and colonizations of frog populations in southeast Brazil and their evolutionary implications. *Biotropica* **20,** 230–5.

Hines H., Mahony M. J. & McDonald K. R. (1999) An assessment of frog declines in wet subtropical Australia. In *Declines and Disappearances of Australian Frogs* (ed. A. Campbell) pp. 44–63. Environment Australia: Canberra.

Hintze J. (2008) *PASS 2008*. NCSS, LLC, Kaysville, UT.

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,** 255–60.

Kagarise Sherman C. & Morton M. L. (1993) Population declines of Yosemite toads in the eastern Sierra Nevada of California. *J. Herpetol.* **27,** 186–98.

Kriger K. M. & Hero J.-M. (2006) Survivorship in wild frogs infected with chytridiomycosis. *EcoHealth*. **3,** 171–7.

Kriger K. M. & Hero J.-M. (2007) Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *J. Zool.* **271,** 352–9.

Kriger K. M., Hero J.-M. & Ashton K. J. (2006a) Cost efficiency in the detection of chytridiomycosis using PCR assay. *Dis. Aquat. Org.* **71,** 149–54.

Kriger K. M., Hines H., Hyatt A. D., Boyle D. G. & Hero J.-M. (2006b) Techniques for detecting chytridiomycosis in wild

doi:10.1111/j.1442-9993.2008.01872.x

frogs: comparing histology with real-time Taqman PCR. *Dis. Aquat. Org*. **71,** 141–8.

Kriger K. M., Pereoglou F. & Hero J.-M. (2007a) Latitudinal variation in the prevalence and intensity of chytrid (*Batrachochytrium dendrobatidis*) infection in eastern Australia. *Conserv. Biol*. **21,** 1280–90.

Kriger K. M., Ashton K. J., Hines H. B. & Hero J.-M. (2007b) On the biological relevance of a single *Batrachochytrium dendrobatidis* zoospore: a reply to Smith (2007). *Dis. Aquat. Org*. **73,** 257–60.

La Marca E., Lips K. R., Lotters S *et al*. (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* (eds R.G. McKinnell, D.L. Carlson) pp. 3–13. Saint Paul, Minnesota.

Li Y. M. & Wilcove D. S. (2005) Threats to vertebrate species in China and the United States. *BioScience*. **55,** 147–53.

Lips K. R. (1998) Decline of a tropical montane amphibian fauna. *Conserv. Biol*. **12,** 106–17.

Lips K. R., Brem F., Brenes R. *et al*. (2006) Emerging infectious disease and the loss of biodiversity in a neotropical amphibian community. *Proc. Natl. Acad. Sci. USA* **103,** 3165–70.

Longcore J. E., Pessier A. P. & Nichols D. K. (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia*. **91,** 219–27.

McDonald K. & Alford R. A. (1999) A review of declining frogs in northern Queensland. In *Declines and Disappearances of Australian Frogs* (ed. A. Campbell) pp. 14–22. Environment Australia, Canberra.

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,** 114–20.

Mazzoni R., Cunningham A. C., Daszak P., Apolo A., Perdomo E. & Speranza G. (2003) Emerging pathogen of wild amphibians in frogs (*Rana catesbiana*) farmed for international trade. *Emerg. Infect. Dis*. **9,** 995–8.

Morehouse E. A., James T. Y., Ganley A. R. D. *et al*. (2003) Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. *Mol. Ecol*. **12,** 395–403.

Morrison C., Hero J.-M. & Browning J. (2004) Altitudinal variation in the age at maturity, longevity, and reproductive lifespan of anurans in subtropical Queensland. *Herpetologica* **60,** 34–44.

Muths E., Corn P. S., Pessier A. P. & Green D. E. (2003) Evidence for disease related amphibian decline in Colorado. *Biol. Conserv*. **110,** 357–65.

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,** 1431–40.

Parris M. J. & Cornelius T. O. (2004) Fungal pathogen causes competitive and developmental stress in larval amphibian communities. *Ecology* **85,** 3385–95.

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. & Crump M. L. (1994) Amphibian declines and climate disturbance: the case of the Golden Toad and the Harlequin Frog. *Conserv. Biol*. **8,** 72–85.

Pounds J. A., Bustamante M. R., Coloma L. A. *et al*. (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*. **439,** 161–7.

Puschendorf R., Bolanos F. & Chaves G. (2006) The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica. *Biol. Conserv*. **132,** 136–42.

Rachowicz L. J., Hero J.-M., Alford R. A. *et al*. (2005) The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife. *Conserv. Biol*. **19,** 1441–8.

Rachowicz L. J., Knapp R. A., Morgan J. A. T. *et al*. (2006) Emerging infectious disease as a proximate cause of amphibian mass mortality. *Ecology* **87,** 1671–83.

Retallick R., McCallum H. & Speare R. (2004) Endemic infection of the amphibian chytrid fungus in a frog community post-decline. *Public Lib Sci*. **2,** 1–7.

Richards S. J., McDonald K. R. & Alford R. A. (1993) Declines in populations of Australia's endemic tropical rainforest frogs. *Pac. Conserv. Biol*. **1,** 66–77.

Rollins-Smith L. A., Doersam J. K., Longcore J. E. *et al*. (2002) Antimicrobial peptide defenses against pathogens associated with global amphibian declines. *Dev. Comp. Immunol*. **26,** 63–72.

Schloegel L. M., Hero J.-M., Berger L., Speare R., McDonald K. R. & Daszak P. (2006) The decline of the sharp-snouted day frog (*Taudactylus acutirostris*): the first documented case of extinction by infection in a free-ranging wildlife species? *EcoHealth* **3,** 35–40.

Seimon T. A., Seimon A., Daszak P. *et al*. (2007) Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to tropical deglaciation. *Global Change Biol*. **13,** 288–99.

Speare R. & Berger L. (2005) *Chytridiomycosis in amphibians in Australia*. [Cited 28 March 2006.] Available from URL: http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyspec.htm

Stuart S. N., Chanson J. S., Cox N. A. *et al*. (2004) Status and trends of amphibian declines and extinctions worldwide. *Science* **306,** 1783–6.

van Uitregt V. O., Wilson R. S. & Franklin C. E. (2007) Cooler temperatures increase sensitivity to ultraviolet B radiation in embryos and larvae of the frog Limnodynastes peronii. *Global Change Biol*. **13,** 1114–21.

Voyles J., Berger L., Young S. *et al*. (2007) Electrolyte depletion and osmotic imbalance in amphibians with chytridiomycosis. *Dis. Aquat. Organ*. **77,** 113–8.

Weldon C. & du Preez L. H. (2004) Decline of the Kihansi spray toad, *Nectophrynoides asperginis*, from the Udzungwa mountains, Tanzania. *Froglog* **62,** 2–3.

Williams S. E. & Hero J.-M. (1998) Rainforest frogs of the Australian Wet Tropics: guild classification and the ecological similarity of declining species. *Proc. R. Soc. London* **265,** 597–602.

Woodhams D. C. & Alford R. A. (2005) Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. *Conserv. Biol*. **19,** 1449–59.

Young B. E., Lips K. R., Reaser J. K. *et al*. (2001) Population declines and priorities for amphibian conservation in Latin America. *Conserv. Biol*. **15,** 1213–23.

doi:10.1111/j.1442-9993.2008.01872.x