Thermoregulatory behaviour affects prevalence of chytrid fungal infection in a wild population of Panamanian golden frogs

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Predicting how climate change will affect disease dynamics requires an understanding of how the environment affects host–pathogen interactions. For amphibians, global declines and extinctions have been linked to a pathogenic chytrid fungus, Batrachochytrium dendrobatidis. Using a combination of body temperature measurements and disease assays conducted before and after the arrival of B. dendrobatidis, this study tested the hypothesis that body temperature affects the prevalence of infection in a wild population of Panamanian golden frogs (Atelopus zeteki). The timing of first detection of the fungus was consistent with that of a wave of epidemic infections spreading south and eastward through Central America. During the epidemic, many golden frogs modified their thermoregulatory behaviour, raising body temperatures above their normal set point. Odds of infection decreased with increasing body temperature, demonstrating that even slight environmental or behavioural changes have the potential to affect an individual’s vulnerability to infection. The thermal dependency of the relationship between B. dendrobatidis and its amphibian hosts demonstrates how the progression of an epidemic can be influenced by complex interactions between host and pathogen phenotypes and the environments in which they are found.

Keywords: thermoregulation; behavioural fever; Batrachochytrium dendrobatidis; chytridiomycosis; amphibian declines; host–pathogen interactions

1. INTRODUCTION

The idea that environmental variation can affect the outcome of host–pathogen interactions is not new. The seasonal cycles exhibited by many infectious diseases, including important human pathogens, have often been attributed to annual changes in weather (Dowell 2001; Berger et al. 2004; Lowen et al. 2008). However, the physiological mechanism(s) linking ambient conditions to epidemiological patterns remain poorly understood for many host–pathogen systems. An exception is human influenza, where recent work has demonstrated that temperature and relative humidity influence the dynamics of the virus by altering the effectiveness of airborne transmission (Lowen et al. 2008). However, links between environmental conditions and disease dynamics could arise in a number of other ways as well. For example, environmental changes could alter the presence, virulence or latency of a pathogen, the behaviour or resistance of its host, or generate less-predictable outcomes due to complex interactions among factors (Dowell 2001; Jackson & Tinsley 2002; Blanford et al. 2003).

Temperature appears to be a key factor in determining the outcome of host–pathogen interactions. This is especially true for ectotherms where ambient conditions constrain the physiological temperatures of both host and pathogen (Carruthers et al. 1992; Blanford & Thomas 1999, 2000; Jackson & Tinsley 2002; Blanford et al. 2003; Klass et al. 2007; Laine 2008; Lazarro et al. 2008). Several experimental studies have now demonstrated an effect of temperature on the susceptibility and behaviour of ectotherm hosts and/or the virulence of their pathogens (e.g. Carruthers et al. 1992; Blanford & Thomas 1999; Ferguson & Read 2002; Jackson & Tinsley 2002; Wilson et al. 2002; Yourth et al. 2002; Woodhams et al. 2003, 2008; Lazarro et al. 2008). However, the functional mechanisms underlying these effects and their potential to affect the outcomes of infections under natural conditions are not well understood (Carey et al. 1999; Jackson & Tinsley 2002). A clearer understanding of the links between environmental factors and the outcomes of host–pathogen interactions will probably provide valuable insights into host–pathogen coevolution and epidemiology, as well as more fundamental aspects of the ecology and evolution of interspecific interactions (Lambrechts et al. 2006; Klass et al. 2007). Given the potential impact of ongoing climate change on disease risk for humans (McMichael 2006; Estrada-Peña 2009) and other organisms (e.g. Ghini et al. 2008; Gale et al. 2009), such information will undoubtedly be important, especially for threatened or endangered taxa (Smith et al. 2009).

(a) Temperature and the amphibian chytrid

Amphibian populations have recently undergone rapid, global declines and extinctions such that over 30 per cent of amphibian species are now threatened and as many as 122 species may be extinct (Stuart et al. 2004). In many
cases this can be attributed to threats amphibians share with other taxa, including land-use change, overexploitation and the introduction of exotic species. However, the declines and extinctions of as many as 200 frog species across the globe have been linked to the fungal pathogen Batrachochytrium dendrobatidis (Skerratt et al. 2007). This chytrid fungus infects the keratinized layers of amphibians’ skins, causing a potentially fatal disease called chytridiomycosis (Longcore et al. 1999). Chytridiomycosis can be transmitted by direct contact with infected frogs or indirectly via contaminated substrates or water (Berger et al. 1998; Parris & Cornelius 2004; Rachowicz & Vredenburg 2004; Rowley et al. 2007). In Central America, an epidemic wave of this disease has been linked to declines and mass die-offs, resulting in dramatic losses of amphibian biodiversity (Lips et al. 2006). A causal link between global climate change and B. dendrobatidis-related amphibian declines has been proposed (Pounds et al. 2006), but supporting evidence is so far weak (Lips et al. 2008; Rohr et al. 2008).

The effect of temperature on the ability of B. dendrobatidis to grow and infect amphibians has been examined in the laboratory, where the fungus grows best from 17 to 25°C and achieves peak growth and pathogenicity at 23°C; at 28°C the fungus stops growing and at 30°C it dies (Johnson et al. 2003; Piotrowski et al. 2004). However, the extent to which these findings hold true under natural conditions remains unknown. Disease surveys of wild amphibians suggest the prevalence and severity of chytridiomycosis infections tend to decrease during warmer months (Berger et al. 2004; Retallick et al. 2004; Woodhams & Alford 2005; Kriger & Hero 2006, 2007; Kriger et al. 2007). However, a direct link between amphibian body temperatures and B. dendrobatidis infection has not been established in the wild.

Because amphibians are ectothermic, their body temperatures are constrained by the temperature of their surroundings. However, by choosing particular microclimates within a spatially and temporally variable environment, they can regulate their body temperature behaviourally and buffer themselves against negative effects of temperature on physiological performance (Bartolomew 1966; Huey 1991). If body temperature affects an amphibian’s vulnerability to B. dendrobatidis, individuals may be able to avoid or reduce the severity of infection by behaviourally manipulating their body temperatures (Woodhams et al. 2003). In laboratory studies, many ectotherms respond to pathogen exposure by inducing a ‘behavioural fever’ (Vaughn et al. 1974; Kluger 1991; Sherman et al. 1998; Gardner & Thomas 2002). By altering thermoregulatory behaviour to sustain a higher-than-normal body temperature, these organisms are better able to fight infection. This is probably due to both direct effects of host temperature on the growth rate and survival of the pathogen as well as effects of temperature on the host’s immune system (Blanford & Thomas 2000). However, behavioural fevers can incur fitness costs (Boorstein & Ewald 1987; Gardner & Thomas 2002; Elliot et al. 2005). In amphibians, behavioural fevers can cause a potentially costly increase in metabolic rate (Sherman & Stephens 1998) or lead to increased predation (Lefcort & Eiger 1993; but see Parris et al. 2004). Nevertheless, the occurrence and effectiveness of behavioural fever in response to natural infections has never been documented in the wild.

Using a combination of mark–recapture population studies and B. dendrobatidis assays, I was able to document the arrival and progression of a B. dendrobatidis epidemic in a Panamanian golden frog (Atelopus zeteki) population in western Panama. The timing of the fungus’s arrival is consistent with a hypothesized wave of epidemic infections in Central America (Lips et al. 2006). In this study, body temperature measurements and B. dendrobatidis assays were used to test the hypotheses that (i) Panamanian golden frogs showed altered thermoregulatory behaviour during an epidemic of B. dendrobatidis and that (ii) this reduced their odds of infection. It also examines (iii) whether the frogs’ body conditions changed during the epidemic and (iv) whether this might indicate a fitness cost associated with an increase in preferred body temperature. This study represents an important step towards understanding whether and how temperature might influence amphibians’ vulnerability to chytridiomycosis in the wild. Such knowledge will probably have applications for many amphibian taxa, and may shed light on the workings of other host–pathogen systems as well.

2. MATERIAL AND METHODS

Mark–recapture studies of A. zeteki were conducted on three 200 m transects along a 3 km stretch of the Rio Mata Ahogado, Panama Province, Panama (elevation 290 m), where mean air temperature is 26.0°C with an annual range of 21.4–31.6°C (Hijmans et al. 2005). Each transect was surveyed between the hours of 10:00 and 18:00 on five days during five time periods: 20 January 2004–2 February 2004, 10–16 December 2004, 20–27 January 2005, 8–15 December 2005 and 22–28 January 2006. Sampling periods were chosen to correspond with this diurnal frog’s breeding season, which occurs from early December to late January. Minimum, maximum and average air temperatures for each survey day were measured using a Kestrel4000 weather meter. Air temperature, relative humidity, atmospheric pressure and wind speed were measured with the Kestrel4000, and stream temperature was measured with a digital thermometer at the start of each transect. Stream temperatures varied from 21.1 to 24.2°C (average 22.7°C).

Body temperature, substrate temperature, weight, body size, sex, location and microhabitat type were recorded for each frog encountered. Body temperature was measured prior to capture using a non-contact infrared thermometer (Rowley & Alford 2007). The temperature of the centre of the dorsum was measured from within 0.5 m of the frog. Substrate temperature (the spot where the frog had been, prior to capture) was also measured with an infrared thermometer from within 0.5 m. Weight was measured using a spring scale and body size (snout–vent length) was measured using dial callipers. The sex of each adult frog was assessed based on the presence (male) or absence (female) of muscular forearms and cornified pads on the first finger (Lötters 1996). Each frog’s position on the transect (to within 5 m) was recorded along with the microhabitat (e.g. exposed rock, leaf litter, streamside gravel, etc.) it was encountered on. Each frog was given an identifying mark (unique toe-clip combination, up to three clips per frog) upon first capture and released at the point of capture. Frogs were also photographed at first capture so that the pattern of dorsal black markings could be used as a secondary method of individual identification.
During January 2005, December 2005 and January 2006, the dorsum, venter and feet of each frog were swabbed with a sterile cotton swab. During January 2006, 86 randomly chosen, moist environmental substrates were also swabbed (see appendix S1 in electronic supplementary material). Swabs were stored in a salt-saturated DMSO solution at room temperature prior to extraction. The 482 A. zeteki samples, 86 environmental samples and 100 negative controls (sterile swabs) were tested in random order for the presence of B. dendrobatidis using Taqman diagnostic quantitative PCR (q-PCR) (Boyle et al. 2004). DNA was extracted from each sample following Hyatt et al. (2007) and q-PCR assays were performed in triplicate following Boyle et al. (2004). Samples containing PCR inhibitors were detected using VIC exogenous internal positive controls (Applied Biosystems) and inhibition was overcome by dilution following Hyatt et al. (2007). Samples were scored as positive if all three replicates indicated the presence of B. dendrobatidis. Samples testing positive in one or two replicates were re-assayed once. If the second assay produced a negative or positive result in all three replicates the sample was scored as negative or positive, respectively. Samples testing positive in only one or two replicates of the second assay were considered ambiguous and not included in subsequent analyses. One of 100 negative controls tested positive for B. dendrobatidis, indicating a false positive rate of 1 per cent for the DNA extraction and q-PCR assay.

Statistical analyses were performed in SPPS 11.0. Because body temperatures and body conditions were not always normally distributed (Shapiro–Wilk, $p < 0.05$), non-parametric tests were used to compare means across sampling periods, infection classes and body conditions.

3. RESULTS

Over the five sampling periods, 1077 individual A. zeteki were captured (227 in January 2004, 299 in December 2004, 123 in January 2005, 167 in December 2005 and 261 in January 2006). Recapture rates within a sampling period (e.g. frogs captured twice during December 2005) varied from 28–41% (average 34%). Recaptures among months within breeding periods (e.g. frogs captured in December 2005 and January 2006) were lower, with 40 frogs (9%) captured in both December 2004 and January 2005 and 19 frogs (4%) captured in both December 2005 and January 2006. This suggests that, soon after breeding, most golden frogs at this site either (i) die or (ii) return to the grassy, upland habitat that they inhabit the remainder of the year. Only 11 frogs (1%) were captured in two breeding periods (e.g. frogs captured in December 2004–January 2005 and in December 2005–January 2006), suggesting they either (i) do not often survive a full year after reproduction, (ii) do not often reproduce in consecutive years or (iii) do not return to the same breeding sites each year.

(a) Batrachochytrium dendrobatidis infections in space and time

Batrachochytrium dendrobatidis was not detected on any of the 123 frogs sampled in January 2005. However, in December 2005, 19/141 (14%) frogs were infected and by late January 2006, infection prevalence had risen to 47 per cent (94/200). No dead A. zeteki were found in December 2005, but eight were found in January 2006, all of which tested positive for B. dendrobatidis.

Golden frogs at this study site were encountered in two microhabitat types: (i) exposed on rocks or gravel along the stream or (ii) hidden in leaf litter further (less than 5 m) from the stream. During each breeding season, the majority of frogs (92% in 2004 and 85% in 2005) were encountered on rocks or gravel in December whereas by January, after most breeding had occurred, more frogs (55% in 2004, 52% in 2005 and 65% in 2006) were encountered hidden in leaf litter. Infection rates of frogs found on rocks or gravel were not different from those found in leaf litter (December 2005: 18/78 infected on rocks or gravel, 4/39 infected in leaf litter, $n = 117$, $\chi^2 = 0.20$, $p = 0.15$; January 2006: 24/43 infected on rocks or gravel, 62/97 infected in leaf litter, $n = 140$, $\chi^2 = 0.52$, $p = 0.47$). The spatial pattern of infection was random with respect to the frog’s position along each of the three transects ($n = 20$/transect, $-0.38 < $Moran’s $I < 1.6$, $p > 0.05$). Five (6%) of 86 environmental samples tested positive for B. dendrobatidis in January 2006 (see appendix S1 in electronic supplementary material) suggesting that during the height of the epidemic, the fungus was common enough in the ecosystem that chytridiomycosis could potentially have been transmitted to frogs directly from contaminated substrates. A total of 11 frogs were captured and swabbed during both December 2005 and January 2006. None of these were infected in December, but by January, six (55%) had developed B. dendrobatidis infections.

(b) Batrachochytrium dendrobatidis infection and body temperature

Mean frog body temperatures were higher during the epidemic than during three previous sampling periods (Kruskal–Wallis: $n = 1225$, $\chi^2 = 537$, $p < 0.001$; Dunnett’s C: $p < 0.05$; see also figure 1). Infected frogs had lower body temperatures than uninfected frogs during both December 2005 (infected: $n = 19$, avg. $= 24.30^\circ$C, s.d. = 0.64°C; uninfected: $n = 100$, avg. $= 24.70^\circ$C, s.d. = 0.82°C; Mann–Whitney: $U = 1241$, $p = 0.036$) and January 2006 (infected: $n = 83$, avg. $= 25.67^\circ$C, s.d. = 1.39°C; uninfected: $n = 45$, avg. $= 26.39^\circ$C, s.d. = 1.86°C; Mann–Whitney: $U = 2352$, $p = 0.016$). Furthermore, the odds being infected decreased by 61 per cent with each 1°C increase in body temperature in December 2005 (logistic regression: $n = 119$, $\chi^2 = 4.10$, $p = 0.025$) and by 28 per cent with each 1°C increase in body temperature in January 2006 (logistic regression: $n = 128$, $\chi^2 = 5.89$, $p = 0.01$). Frogs encountered in leaf litter had higher body temperatures than those encountered exposed on rocks or gravel (ANOVA: $n = 301$, $F_{1,297} = 13.94$, $p < 0.001$). However, a 2-way ANOVA found no interaction between infection status and microhabitat, ($n = 247$, $F_{1,243} < 0.001$, $p = 1$) indicating that the magnitude of the difference in body temperatures between infected and uninfected frogs did not differ between the two most common microhabitats.

(c) Environment and body temperature

Amphibian body temperatures are influenced by a host of environmental factors, including air temperature, relative
humidity, wind speed, absorbed solar and thermal radiation and substrate temperature (Tracy 1976). However, over the course of this study, golden frog body temperatures (daily mean, min. and max.) were not significantly correlated with atmospheric pressure, relative humidity or wind speed (Spearman’s rank: $-0.350 < R < 0.046$, Bonferroni-corrected $p > 0.05$) (figure 2). The lowest body temperature (min.) and mean body temperature (mean) for each sampling day were not significantly correlated with any air temperature measurements (Spearman’s rank: $-0.05 < R < 0.40$, Bonferroni-corrected $P > 0.05$). In contrast, the highest body temperature (max.) for each sampling day was significantly correlated with both daily mean air temperature ($R = 0.45$, $p = 0.003$) and air temperatures measured at the start of each transect ($R = 0.49$, $p = 0.001$). However, neither the daily mean air temperature (Mann–Whitney: $n = 36$, $U = 140$, $p = 0.33$) nor the temperature at the start of each transect (Mann–Whitney: $n = 72$, $U = 161$, $p = 0.44$) differed significantly between the pre-epidemic and epidemic sampling periods. Moreover, the frogs’ maximum body temperatures were still warmer during the epidemic than prior to the epidemic after removing the variance due to changes in air temperature (measured at start of transect: ANCOVA: $F_{1,72} = 6.22$, $p = 0.015$; mean daily air temperature: ANCOVA: $F_{1,33} = 10.07$, $p = 0.003$). This suggests the increase in body temperatures during the epidemic was not caused by differences in air temperature.

Before the epidemic, body temperatures were, on average, 4.0°C below the mean daily air temperature and only 1 per cent of frogs (7/649) had body temperatures above the mean daily air temperature. However, during the epidemic, body temperatures were closer to the mean air temperature (average 2.1°C below) and 9 per cent of frogs (39/428) had body temperatures above the mean air temperature. The difference between daily mean air temperature and body temperatures (body–air temperature) was smaller during the epidemic than before it (Kruskal–Wallis: $n = 1077$, $\chi^2 = 562$, $p < 0.001$; Dunnett’s C: $p < 0.01$), further indicating that the increase in body temperatures was not due to environmental differences during the epidemic. Instead, the relationship between air temperatures and frog body temperatures appears to have changed after the fungus arrived.

The five frogs that were uninfected in December 2005 and still uninfected when recaptured in January 2006 had increased their body temperatures with respect to air temperature (paired-difference $t$-test: $t_4 = 3.33$, $p = 0.01$; figure 3a). This difference was significant despite the small sample size ($n = 5$) and thus, low power to detect a difference in means. Of the six frogs that were uninfected in December 2005 but infected when recaptured in January 2006, two showed similar increases in body temperature to the five uninfected frogs. However, the other four showed slight decreases in body temperature. Taken together, the body temperatures of these six frogs did not differ significantly between sampling periods (paired-difference $t$-test: $t_5 = 0.002$, $p = 0.499$; figure 3b). However, because of the small sample size ($n = 6$) the power to detect such a difference was small. Compared with the five uninfected frogs (figure 3a), the six infected frogs (figure 3b) had a much larger variance in body temperature.

(d) Body temperature / time of day relationships
Frog body temperatures tended to be cooler in the morning (between the hours of 10.00 and 12.00) than later in the day. Body temperatures were positively correlated with time of day, regardless of whether the entire day was considered ($R = 0.115$, $T_{1489} = 4.51$, $p < 0.001$) or just frogs captured before 12.00 ($R = 0.266$, $T_{641} = 6.98$, $p < 0.001$). The time of day at which frogs were sampled differed between pre-epidemic and epidemic sampling periods ($F$-test: $T_{1489} = 5.41$, $p < 0.001$). The average sampling time was 13.12 hours prior to the epidemic and 13.41 hours during the epidemic.
(a difference of 29 min). However, body temperatures still differed before and during the epidemic after the variance due to time of day was removed (all day ANCOVA: $F_{905.618} = 569, p < 0.001$; before noon ANCOVA: $F_{552.199} = 129, p < 0.001$). This indicates that the difference in body temperatures before and during the epidemic was not due to differences in the time of day that they were measured.

**Body condition**

The average adult male body condition—measured as (weight$^{-1/3}$/snout–vent length)—did not differ among pre-epidemic sampling periods (January 2004: $n = 149$, avg. = 0.042, s.d. = 0.002; December 2004: $n = 164$, avg. = 0.040, s.d. = 0.001; January 2005: $n = 85$, avg. = 0.040, s.d. = 0.002) or among the two sampling periods during the epidemic (December 2005: $n = 122$, avg. = 0.039, s.d. = 0.001; January 2006: $n = 130$, avg. = 0.038, s.d. = 0.002) (Kruskal–Wallis: $n = 867$, $x^2 = 60.88$, $p < 0.001$; Dunnett’s $C$: $p > 0.05$).

However, the body condition of male frogs was lower during the epidemic than before it (during: $n = 252$, avg. = 0.039, s.d. = 0.002; before: $n = 398$, avg. = 0.041, s.d. = 0.002; Dunnett’s $C$: $p < 0.05$) and the log of male body condition was inversely related to body temperature (Spearman’s rank: $n = 867$, $R = 0.2809$, $t = 8.92$, $p < 0.001$). Body conditions of infected and uninfected males did not differ in December 2005 (infected: $n = 14$, avg. = 0.039, s.d. = 0.002; uninfected: $n = 62$, avg. = 0.039, s.d. = 0.001; Mann–Whitney: $n = 76$, $U = 486$, $P_{1\text{ sided}} = 0.248$) or January 2006 (infected: $n = 52$, avg. = 0.038, s.d. = 0.002; uninfected: $n = 17$, avg. = 0.039, s.d. = 0.002; Mann–Whitney: $n = 100$, $U = 1419$, $P_{1\text{ sided}} = 0.119$).

### 4. DISCUSSION

The arrival and progression of *B. dendrobatidis* infections at the study site—which is located 60 km east of the epidemic that occurred at El Cope, Panama in late 2004 (Lips *et al*. 2006)—is consistent with the hypothesized wave of epidemic infections in Central America. In the population studied here, golden frogs only spend time along the river during their breeding season, which occurs from early-December until late-January. The

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*Figure 2. Distribution of frog body temperatures with respect to air temperatures. Body temperatures (circles) were almost exclusively below the mean daily air temperature (black line) and often below the daily low temperature prior to the epidemic. However, during the epidemic, a number of frogs’ body temperatures exceeded the mean air temperature, putting them within the range where *B. dendrobatidis* stops growing and dies (grey shaded area). Grey lines show daily air temperature fluctuations.*

*Figure 3. Changes in (air–body temperature) relationships for frogs captured during both months of the epidemic. Each line represents an individual frog. (a) Frogs uninfected during both December 2005 and January 2006 ($n = 5$). (b) Frogs uninfected in December 2005 but infected in January 2006 ($n = 6$). The shaded area indicates ‘normal’ thermoregulatory behaviour as defined by the 95% CI on the mean of (body–air temperature) for the three sampling periods prior to the epidemic.*
remainder of the year, they inhabit dry, grassy uplands on either side of the river. Since B. dendrobatidis was not detected towards the end of the 2004–2005 breeding season, and was detected in only 14 per cent of frogs at the beginning of the 2005–2006 breeding season, it is probable that the golden frogs encountered the pathogen for the first time as they arrived at the river to breed in December 2005. One month later, the infection rate had risen to 47 per cent, which is consistent with the rapid onset of infection observed during other B. dendrobatidis epidemics (Lips et al. 2006). In January 2006, B. dendrobatidis was detected on 6 per cent of randomly selected, moist environmental substrates (e.g., rocks, leaves, sticks) encompassing all three transects. These findings suggest that abiotic reservoirs may play a role in transmission of the fungus during an epidemic (Lips et al. 2006). Exposure to infection did not appear to be strongly location or microhabitat dependent as infection rates did not differ significantly among frogs encountered in different microhabitat types and infected frogs were distributed randomly along the three transects.

The average body temperature of the golden frog population increased with infection prevalence; it was 1.1°C higher in December 2005 and 2.4°C higher in January 2006 than the average of the pre-infection sampling periods (figure 1). This change in body temperature was not related to differences in air temperature among sampling periods. Instead, the relationship between air temperature and body temperatures appears to have changed with the arrival of the fungus. During the three pre-epidemic sampling periods, very few frogs (less than 1% or 3/649) had body temperatures above the mean air temperature, only 12 per cent of frogs (74/649) had body temperatures above of the range where B. dendrobatidis achieves peak growth and infectivity (17–25°C), and fewer than 1 per cent (5/649) had body temperatures high enough to stop the fungus from growing (28°C) (figure 2). However, by January 2006, many more frogs (17% or 44/261) had body temperatures above the mean air temperature, 73 per cent of frogs (191/261) had body temperatures above 25°C and 11 per cent (29/261) had body temperatures above 28°C. This apparent change in thermoregulatory behaviour is consistent with the idea that the frogs exhibited a population-wide ‘behavioural fever’ response during the epidemic. If so, the pathogen must have become so prevalent in the ecosystem that most, if not all frogs had been exposed by late-January 2006.

Given the relationship between temperature and the standard metabolic rate of amphibians (average Q10 = 2.21, White et al. 2006) the increase in body temperature during the B. dendrobatidis epidemic would have resulted in an 8.7 per cent (December 2005) and a 20.5 per cent (January 2006) increase in average metabolic energy expenditure over pre-infection rates. This might account for the lower body condition of males during the epidemic if they were not able to replace lost energy from available environmental resources (McEwen & Wingfield 2003; Wikelski & Cooke 2006). If so, and if elevated body temperatures decrease the frogs’ vulnerability to infection, this would result in a fitness trade-off between fighting B. dendrobatidis infection and overall physiological performance. Although the infection itself might have led to the decrease in body condition, a previous study failed to find a link between body condition and infection prevalence (Woodhams & Alford 2005) and no difference in body condition between infected and uninfected frogs was seen in this study. A third possibility is that the decrease in body condition preceded the epidemic and was related to other environmental stressors. This would be consistent with data suggesting that prior environmental stress predisposed several Australian frog populations to B. dendrobatidis infection (Alford et al. 2007). However, if body condition did decline prior to the epidemic described here, its onset must have come fewer than 11 months prior to the arrival of B. dendrobatidis, as body condition did not differ among sampling periods prior to the end of January 2005.

While both uninfected and infected frogs had higher average body temperatures during the epidemic than before it, uninfected frogs had higher body temperatures than infected frogs during both December 2005 and January 2006. This, coupled with the decrease in odds-of-infection with increasing body temperature, suggests a strong link between body temperature and vulnerability to B. dendrobatidis. However, this situation (uninfected frogs exhibiting, on average, higher temperatures than infected frogs), appears to run counter to the expectations for a behavioural fever response. This is not necessarily the case, as I outline below.

If golden frogs can clear an infection by raising body temperatures (thereby reducing the growth rate and viability of B. dendrobatidis and/or boosting their immune response), we would expect to find frogs at many different stages of this process in our sample of the population (see figure 4). The first stage (I) would consist of uninfected individuals with normal body temperatures that either have not become infected or have recovered from a previous infection. If these frogs become infected, they would probably continue to exhibit normal thermoregulatory behaviour (II) until they reach a certain level of infection, at which point they would begin to exhibit a behavioural fever (III). Such latency in the behavioural fever response is commonly seen in laboratory studies (e.g. Kluger 1977; Louis et al. 1986; Blanford 2004). If the behavioural fever is effective, and as long as stage III frogs maintain high enough temperatures, we can expect their pathogen load to decrease. Infections can be induced in the laboratory by exposure to even a single zoospore of B. dendrobatidis (Carey et al. 2006), suggesting that frogs that return to normal body temperatures prior to completely eliminating the fungus may experience a resurgence of infection. However, if stage III frogs are able to maintain an elevated body temperature long enough, they may be able to completely clear the infection. At this point, there may be some latency to return to a normal body temperature, and thus, we may find frogs that test negative for the fungus, but still have elevated body temperatures (IV). Previous, laboratory-based, behavioural fever studies (e.g. Kluger 1977; Louis et al. 1986; Blanford et al. 1998; Cabanac & Cabanac 2004) have not monitored the infection status of individuals along with their body temperatures, and therefore, were unable to investigate whether or for how long body temperatures remained elevated once the infection was gone.

Individuals undergoing a successful behavioural fever response in the wild might show up to four different
combinations of infection status and body temperature, depending on where they are in the process of becoming infected and/or mounting a behavioural defence (figure 4). This contrasts with laboratory studies where individuals are exposed at time zero and their body temperatures monitored for some length of time afterward. In these studies, individuals progress through the stages of infection and behavioural defence synchronously such that a clear correlation between infection and body temperature can be seen. In the current study, I encountered frogs with body temperature/infection status combinations consistent with each of the four stages proposed in figure 4. If the 95 per cent CI of the mean of (body–air temperature) for the three pre-epidemic sampling periods (−6.4°C < body–air temperature < −1.5°C) are used to define ‘normal’ thermoregulatory behaviour, then 89 frogs (36%) in stage I, 82 frogs (33%) in stage II, 22 frogs (9%) in stage III and 57 frogs (23%) in stage IV were encountered during the epidemic (figure 5). Four of the five frogs that were uninfected in December 2005 were also uninfected when recaptured in January 2006, suggesting a transition from stage I to stage IV between sampling periods, and one frog appears to have remained in stage IV during both sampling periods (figure 3a). Of the six frogs that were uninfected in December 2005 and infected upon recapture in January 2006, four appear to have transitioned from stage I to stage II, and two appear to have gone from stage I to stage III (figure 3b). The larger variance in January 2006 body temperatures among the six infected frogs, when compared with the five uninfected ones, is consistent with the model described above and depicted in figure 4.

The number of frogs in stage IV (uninfected, high body temperature) was surprising, and seems to have contributed strongly to the counterintuitive relationship between body temperature and infection status seen in this study (i.e. infected frogs with lower, rather than higher average body temperatures). If exposure to B. dendrobatidis was necessary to trigger this change in thermoregulatory behaviour, this suggests that frogs labelled as ‘stage IV’ must have recovered from an infection, but retained their elevated body temperatures for some, unknown length of time prior to capture. However, without knowing their individual infection histories, it is also conceivable that these frogs changed their thermoregulatory behaviour in response to some external, environmental cue or stressor that signals a disease outbreak rather than becoming infected themselves. While certain types of stress have been shown to cause behavioural fevers in lizards (Cabanac & Gosselin 1993) and turtles (Cabanac & Bernieri 2000), the two amphibians tested (Bufo marinus and Bombina bombina) did not develop behavioural fevers in response to stress (Cabanac & Cabanac 2004). Whether these results hold true for all amphibians remains to be determined.

Given its potential to cause drastic amphibian declines, an epidemic of B. dendrobatidis could lead to strong selection on thermoregulatory behaviour and a potential thermal arms race between the fungus and its amphibian hosts. However, the outcome of this interaction is likely to differ among host species due to interspecific variation in thermoregulatory behaviour and other aspects of natural history (e.g. microhabitat associations, climate, breeding phenology). A clearer understanding of the expected relationship between pathogen load and body temperature during a chytridiomycosis infection, and how this might vary among host taxa and B. dendrobatidis strains, could be gained in a laboratory setting by allowing infected frogs to thermoregulate in a temperature gradient while monitoring their infection status. These types of studies have the potential to clarify to what extent and
for how long amphibians must raise their body temperatures in order to combat B. dendrobatidis infection, and as such, will aid predicting where and when outbreaks of chytridiomycosis are likely to impact the viability of amphibian populations and species. In addition, understanding how temperature changes affect the interaction between this fungus and its amphibian hosts will be essential in forecasting how the distribution and severity of the pathogen’s effects might change with the changing climate.

5. CONCLUSIONS

The results of this study demonstrate how even small changes in body temperature can impact an individual’s vulnerability to natural, pathogenic infections in wild populations. Furthermore, this work suggests that wild ectotherms are capable of altering their thermoregulatory behaviour in response to an epizootic, and that this response can be effective in reducing the odds of infection. The thermal dependency of the relationship between B. dendrobatidis and its amphibian hosts demonstrates how the progression of an epidemic can be influenced by complex interactions between host and pathogen phenotypes and the environments in which they occur.

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