Effects of individual behaviour on host-pathogen interactions: Australian rainforest frogs and the chytrid fungus *Batrachochytrium dendrobatidis*

Thesis submitted by

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Title page photographs

TOP LEFT: Waterfall frog, *Litoria nannotis*

TOP RIGHT: Common mistfrog, *Litoria rheocola*

BOTTOM: Green-eyed treefrog, *Litoria serrata*

Photo credit: Elizabeth A. Roznik

For my parents: Frank and Mary Roznik;

Thanks for inspiring and encouraging my passion for our natural world,

and for always believing in me. Dad, you can look up to me now! ☺

Contributions of others

My PhD thesis would not have been possible without the assistance of my advisors and a number of other people who helped with various aspects of my project. My primary advisor, Ross Alford, provided advice on experimental design and data analysis, editorial assistance on my thesis, and most of the funding for my project. Ross is a co-author on all eight data chapters. My co-advisor, Lin Schwarzkopf, provided advice on my project, some funding for my project, and helpful comments on thesis chapters, especially those in which she is a co-author (Chapters 8-9). David Pike is a co-author on Chapters 8-9, and he collaborated on these projects by assisting with experimental design, fieldwork, data analysis, and editing. David also provided advice and editorial assistance on other thesis chapters. Sarah Sapsford is also a co-author on Chapters 8-9, and she collaborated by contributing data and commenting on drafts of those chapters. Rhondda Jones also improved my thesis by providing statistical advice for several chapters.

The following people assisted me during eight solid months of fieldwork: Sara Bell, Richard Duffy, David Pike, Rob Puschendorf, and Sarah Sapsford. In addition, David Pike, Lisa Stevenson, and Kiyomi Yasumiba assisted with laboratory experiments. Diagnostic quantitative PCR assays were performed by the Amphibian Disease Diagnostic Laboratory at Washington State University (for Chapters 4-6, and 8-9), and by Sara Bell in the School of Public Health, Tropical Medicine and Rehabilitation Sciences at James Cook University (for Chapter 7).

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Ethics approvals and permits

The research presented in this thesis was conducted within the guidelines for research ethics outlined in the NHMRC/AVCC Statement and Guidelines on Research Practice (1997), the James Cook University Policy on Experimentation Ethics: Standard Practices and Guidelines (2001), and the James Cook University Statement and Guidelines on Research Practice (2001). This research was conducted under protocols A1316, A1420, and A1673 approved by the Animal Ethics Committee at James Cook University, and permits WITK03070508 and WITK10651912 issued by the Queensland Department of Environment and Resource Management.

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General abstract

Diseases are strongly influenced by host behaviour, which affects pathogen transmission and the microclimatic conditions that are experienced by both hosts and pathogens. The amphibian disease chytridiomycosis, which is caused by the chytrid fungus *Batrachochytrium dendrobatidis*, has caused severe population declines in many regions of the world. This pathogen is transmitted by contact with water and is highly sensitive to thermal and hydric conditions; it requires relatively cool, moist conditions to survive and reproduce (15-25°C optimal, >28°C lethal). This thesis focuses on the behaviour of stream-breeding frogs that occur in tropical rainforests of northeastern Australia. This thesis demonstrates that the behaviour of individual frogs plays an important role in this host-pathogen system. Patterns of microenvironment use, microhabitat use, and movement by individual hosts can affect their interactions with the pathogen. Frogs that used cooler, moister microenvironments were more likely to be infected than frogs that experienced warmer, drier conditions, likely as a result of differences in rates of pathogen transmission and growth rates associated with these microenvironments. Differences in the microenvironments experienced by infected and uninfected frogs are caused by their patterns of movement and microhabitat use. Laboratory experiments revealed that *B. dendrobatidis* infections can change the behaviour of some, but not all, species. This suggests that in some species, behavioural differences between infected and uninfected frogs reflect effects of innate behaviour on the probability of acquiring or retaining infections, but in other species, such differences can be caused by changes in the behaviour of infected frogs.

This thesis also provides the first demonstration that *B. dendrobatidis* infections can have sublethal effects that interact with male body condition to influence calling probability, a major fitness determinant in frogs. These effects involve complex, potentially adaptive tradeoffs; infected frogs in poor body condition were less likely to

call than uninfected frogs in similar condition, but infected frogs in good condition often had a higher probability of calling than uninfected frogs. These effects should influence fitness and may serve to maximise the lifetime reproductive success of infected frogs. Finally, this thesis demonstrates the important role of habitat heterogeneity in reducing the impact of *B. dendrobatidis*. A severe tropical cyclone dramatically reduced rainforest canopy cover at some study sites, which increased temperatures and decreased moisture levels in frog microhabitats. These microclimatic changes reduced infection risk in frogs, presumably by slowing pathogen growth rates. The effects of this natural experiment suggest that targeted manipulations of canopy cover may reduce the intensity of epidemic outbreaks of chytridiomycosis. Overall, this thesis highlights the importance of individual behaviour in this host-pathogen system, and the complexity of the relationships between *B. dendrobatidis* and different host species. This thesis also demonstrates that *B. dendrobatidis* infection dynamics are strongly driven by environmental conditions, and that habitat characteristics play an important role in influencing the conditions available to individual amphibians.

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Chapter 1: General introduction

Infectious disease ecology

Overview

Infectious diseases exert strong evolutionary forces on wildlife populations by causing host mortality directly, or by having sublethal effects on hosts that affect their fitness (Scott 1988, Hudson et al. 2001, Poulin 2007, Ostfeld et al. 2008). Through these effects, diseases can regulate population size (Tompkins et al. 2001), or can cause population declines (Smith et al. 2009, Daszak et al. 2000) or changes in population structure (e.g., age structure; van Rensburg et al. 1987). The structure and function of ecological communities and ecosystems can also be influenced by diseases (Scott 1988, Ostfeld et al. 2008). The complex effects of infectious diseases on populations, communities, and ecosystems are driven by the interactions between individual hosts, pathogens, and their environments. These interactions determine whether individuals become exposed to pathogens. Once exposed, these interactions determine whether disease develops, and if it does, they also influence its rate of progress and outcome. Most diseases are typically in equilibrium with their hosts as a result of long-term co-evolution, but emerging infectious diseases are not, and can have significant effects on host populations (Daszak et al. 2000). Although disease emergence can result from ecological changes (e.g., climate, translocations, structure of host populations or communities) or from evolutionary changes in pathogens or hosts, ecological factors play an important moderating role in most emerging diseases (Schrag and Wiener 1995).

Effects of environmental conditions

Environmental conditions can strongly influence disease dynamics. The

seasonal cycles of many infectious diseases are caused directly or indirectly by changes in temperature, precipitation, and humidity (Dowell 2001, Woodhams and Alford 2005, Altizer et al. 2006, Murray et al. 2013, Sapsford et al. 2013). Despite environmental conditions affecting the biology of both hosts and pathogens, the mechanisms linking environmental conditions to infection dynamics are poorly understood in many host-pathogen systems. Many pathogens are sensitive to temperature and moisture, and small changes in these conditions can have important implications for their rates of growth and survival (Harvell et al. 2002, Murray et al. 2013, Stevenson et al. 2013). Environmental variation can also influence host susceptibility by affecting the immune responses of hosts (Wright and Cooper 1981, Zapata et al. 1992, Carey et al. 1999, Raffel et al. 2006) or their exposure to pathogens through changes in behaviour (Dowell 2001, Altizer et al. 2006, Rowley and Alford 2007a). Effects of environmental conditions on disease dynamics are especially important for ectothermic hosts because their body temperatures are regulated by ambient temperatures, which can vary daily, seasonally, and geographically (Rowley and Alford 2009).

Effects of host behaviour

Diseases are strongly influenced by host behaviour, which affects pathogen transmission and the microclimatic conditions that are experienced by both hosts and pathogens (Barber et al. 2000, Wilson et al. 2001, Moore 2002). Host social behaviour has long been recognized as an important factor in the transmission of many infectious diseases; social interactions, especially the formation of groups, promote contact between infected and susceptible individuals (Ezenwa 2004, Rowley and Alford 2007a, Dizney and Dearing 2013). The microhabitats selected by hosts can also influence their exposure to pathogens that persist in the environment by either facilitating or hindering pathogen transmission (Moore 2002). In addition, the thermal and hydric conditions of selected microhabitats can influence host immune responses (Wright and

Cooper 1981, Zapata et al. 1992, Carey et al. 1999, Raffel et al. 2006) and the growth rates of pathogens inhabiting hosts. Host movement patterns can also influence rates of pathogen transmission and the buildup of infections within hosts. Because pathogens often accumulate in an animal's environment over time, more sedentary animals can be more vulnerable to infection (Foldstad et al. 1991, Altizer et al. 2011, Koprivnikar et al. 2012). For example, individuals that change locations infrequently or move short distances between locations may have a higher risk of infection than more active individuals, and once infected, may be more vulnerable to increases in infection loads through re-infection (Briggs et al. 2010).

Chytridiomycosis

Overview

Amphibians have experienced rapid population declines and species extinctions in recent decades, and one-third of extant amphibians are classified as threatened (Stuart et al. 2004). Although there are numerous causes for these losses, including land use change, contaminants, overexploitation, introduced species, and climate change (Collins and Crump 2009, Alford 2010), emerging infectious diseases pose a great threat to global amphibian diversity (Lips et al. 2006, Collins and Crump 2009, Alford 2010). One of the most significant wildlife diseases ever recorded is chytridiomycosis, a recently emerged disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Longcore et al. 1999) that has caused severe amphibian declines and extinctions in many regions of the world (Kilpatrick et al. 2009). This parasitic fungus attacks the skin cells of amphibians and disrupts their osmoregulatory and transport functions, altering electrolyte concentrations in the blood, which can ultimately cause cardiac arrest if the fungal population on the host reaches a high density (Voyles et al. 2009). Aquatic fungal zoospores are transmitted by contact with infected individuals or with contaminated water (Rachowicz and Vredenburg 2004). Infected frogs and tadpoles release aquatic *B. dendrobatidis*

zoospores, which can re-infect the host, thereby maintaining or increasing its fungal load. Zoospores can also be released into the environment, where they can persist outside of hosts and potentially infect other individuals; zoospores can survive in lake water for up to seven weeks (Johnson and Speare 2003), and in sterile moist river sand without nutrients for up to three months (Johnson and Speare 2005).

Environmental conditions and chytridiomycosis

Batrachochytrium dendrobatidis infection dynamics are strongly influenced by environmental conditions. In many regions, amphibians are infected by *B. dendrobatidis* year-round, but the prevalence and intensity of infections, as well as mortality rates due to chytridiomycosis, often vary seasonally. These are typically highest during cooler months and at higher elevations (Woodhams and Alford 2005, Kriger and Hero 2007, Phillott et al. 2013, Sapsford et al. 2013). These patterns have been attributed to the strong thermal and hydric sensitivity of *B. dendrobatidis* growth rates; this fungus requires relatively cool, moist conditions to survive and reproduce (15-25°C optimal, >28°C lethal; Johnson et al. 2003, Piotrowski et al. 2004, Stevenson et al. 2013). In the laboratory, infected frogs maintained in warmer and drier conditions survive longer than those in cool and wet conditions (Bustamante et al. 2010, Murphy et al. 2011). Frogs that are exposed to warm temperatures that are lethal to *B. dendrobatidis* (>28°C) can lose infections entirely (Woodhams et al. 2003, Chatfield and Richards-Zawacki 2011, Geiger et al. 2011). Temperature may also influence infection dynamics directly by affecting host immune responses (Carey et al. 1999, Raffel et al. 2006, Ribas et al. 2009, Rollins-Smith et al. 2011), or indirectly by changing amphibian behaviour, which can alter pathogen transmission rates (Rowley and Alford 2007a).

Habitat characteristics and chytridiomycosis

Habitat characteristics play an important role in influencing *B. dendrobatidis*

infection risk, including salinity of water bodies in coastal areas (Stockwell et al. 2012, Heard et al. 2014), and forest canopy cover, which influences the microclimates experienced by both hosts and pathogens. In forests, large trees block wind and regulate the amount of solar radiation that penetrates through the canopy, causing microclimatic conditions under the canopy to be cooler, more humid, and less variable in these conditions than above the canopy (Whitmore 1998, Madigosky 2004). Therefore, forests with less canopy cover can reduce the risk of *B. dendrobatidis* infection in amphibians by exposing them to warmer, drier conditions that are unfavourable for pathogen growth (Johnson et al. 2003, Piotrowski et al. 2004, Stevenson et al. 2013). Stream-breeding frogs are less susceptible to infection in deforested areas than in natural forest habitats (Van Sluys and Hero 2009, Becker and Zamudio 2011), and some species are able to persist in open-canopy dry forest, but not in nearby closed-canopy rainforest (Daskin et al. 2011, Puschendorf et al. 2011). The risk of infection is also lower for pond-breeding frogs in anthropogenically disturbed areas than in habitats with natural vegetation (Becker et al. 2012). Natural habitat disturbances, such as wildfire, can also reduce vegetation density, thereby causing microclimatic conditions to be warmer and drier (Hossack et al. 2009), which lowers *B. dendrobatidis* infection risk (Hossack et al. 2013).

Amphibian behaviour and chytridiomycosis

Amphibian species vary considerably in their susceptibility to *B. dendrobatidis*. In nearly all communities where amphibian species have disappeared or declined due to chytridiomycosis, other amphibian species have persisted unaffected (McDonald and Alford 1999, Retallick et al. 2004, Lips et al. 2006). Even in areas where *B. dendrobatidis* is endemic, some species consistently have lower prevalence of infection than other species (Rowley and Alford 2009). Species-specific variation in susceptibility can often be explained by differences in behaviour. The most vulnerable species are highly aquatic (Lips et al. 2003, Rowley and Alford 2007a, Bancroft et al.

2011), have a higher frequency of body temperatures in the thermal range optimal for *B. dendrobatidis* growth (<25°C; Rowley and Alford 2013), and/or form aggregations with high levels of physical contact between individuals (Rowley and Alford 2007a). These ecological characteristics can explain differences in rates of pathogen transmission, reproduction, and survival.

The behaviour of individual amphibians within a species is also related to *B. dendrobatidis* infection status. The thermoregulatory behaviour of individual frogs plays an important role in their interactions with this pathogen. A study on the Panamanian frog *Atelopus zeteki* found that at the population level, the mean body temperature of populations of infected frogs was higher than in populations of uninfected frogs, which suggests that infected frogs behaviourally elevated their body temperatures in response to the pathogen ("behavioural fever"; Richards-Zawacki 2009). However, a study of three species of Australian rainforest frogs found that both within and across species, individuals with a higher percentage of body temperatures above 25°C were more likely to be uninfected (Rowley and Alford 2013). This suggests that individuals that chose warmer body temperatures for reasons other than infection were less prone to acquire or retain infections than those that did not, unless behavioural fever persists long after infections have been lost. These divergent patterns could both be important in the interactions between individual frog behaviour and *B. dendrobatidis*; it is possible that individuals that choose warm, dry microclimates are less likely to acquire and maintain infections, but at some stage of infection buildup, individuals alter their behaviour to seek out warmer or drier conditions.

Research needs

There is a clear need to further explore how individual behaviour affects the interactions of frogs with *B. dendrobatidis*. In particular, very little is known about how body temperatures below the thermal optimum for *B. dendrobatidis* growth (15°C; Stevenson et al. 2013) influence infection risk, or how water use and desiccation rates

of individual frogs are related to infection status and intensity. Contact with water can expose amphibians to aquatic *B. dendrobatidis* zoospores and can also influence their body temperatures and therefore the thermal environment experienced by *B. dendrobatidis* inhabiting their skin. Studies have shown empirically that dry conditions can reduce infection risk. For example, drought can reduce *B. dendrobatidis* infection intensity and mortality in nature (Terrell et al. 2014), and in the laboratory, infected frogs maintained in drier conditions survive longer than those in wetter conditions (Bustamante et al. 2010, Murphy et al. 2011). Understanding how the combined effects of thermal and hydric conditions are related to *B. dendrobatidis* infection probability and intensity will significantly advance our knowledge of this host-pathogen system.

Infections by pathogens can change the behaviour of hosts; however, the behaviour of hosts can also affect the incidence of infections and their course if they are acquired (Barber et al. 2000, Moore 2002, Poulin 2010). The behaviour of frogs that are infected or uninfected by *B. dendrobatidis* can differ (Richards-Zawacki 2009, Rowley and Alford 2013), but it is not known whether these behavioural differences reflect effects of innate behaviour on the probability of acquiring or retaining infections, or if they are a result of changes in the behaviour of infected frogs in response to their infections. Investigating the nature of these relationships is very difficult using field data. Therefore, to disentangle these hypotheses, it will be necessary to conduct laboratory experiments that involve comparisons of frog behaviour before and after frogs are infected. Understanding the causal relationships between amphibian behaviour and *B. dendrobatidis* infection is important for understanding and ultimately managing this important host-pathogen system.

Further understanding how habitat characteristics at different scales influence *B. dendrobatidis* infection risk is also important. Studies have demonstrated relationships between the microclimatic conditions experienced by frogs and their infection risk, but the behavioural mechanisms underlying these differences are

unknown. Because amphibians use their environment to regulate body temperatures and desiccation rates behaviourally, patterns of microhabitat selection and movement by individual amphibians may explain these patterns. Further understanding how large-scale habitat heterogeneity caused by natural or anthropogenic disturbances influences infection risk is also important. Studies have shown that areas with low levels of canopy cover can reduce the risk of *B. dendrobatidis* infection in amphibians by exposing them to warmer, drier conditions that are unfavourable for pathogen growth (Van Sluys and Hero 2009, Becker and Zamudio 2011, Puschendorf et al. 2011, Becker et al. 2012, Hossack et al. 2013). A fuller understanding of these relationships, particularly in natural areas, can be used to identify amphibian populations most at risk to chytridiomycosis, locate potential refuges from the disease, and manage amphibian habitats.

Disease can influence host fitness directly by reducing survival, but it can also have sublethal effects on reproductive success. *Batrachochytrium dendrobatidis* infections cause weight loss (Retallick and Miera 2007, Harris et al. 2009, Murphy et al. 2011) and changes in behaviour (Parris et al. 2006, Venesky et al. 2009, Han et al. 2011), and could potentially affect traits closely tied to fitness, such as calling effort or investment in gamete production. To minimise the negative effects of an infection, hosts may respond adaptively by either increasing or decreasing their reproductive effort (Clutton-Brock 1984, Forbes 1993, Agnew et al. 2000). Very little is known about effects of *B. dendrobatidis* infections on host reproduction and fitness, but one study found that male frogs infected by *B. dendrobatidis* had larger testes that contained more sperm than uninfected males (Chatfield et al. 2013), suggesting that infected frogs can increase their reproductive effort in response to infection. Elucidating the sublethal effects of *B. dendrobatidis* on amphibians is important for understanding how it can alter fitness, which has important evolutionary implications for amphibian populations that co-exist with this pathogen.

Study system

Study species

My research focuses on three species of sympatric treefrogs that occur near tropical rainforest streams in northeastern Queensland, Australia (Figure 1.1). These species are the waterfall frog (*Litoria nannotis*), the common mistfrog (*L. rheocola*), and the green-eyed treefrog (*L. serrata*). Despite often occurring at the same sites, these species differ substantially in behaviour; they have different thermal and hydric preferences, and different patterns of movement and microhabitat use (Rowley and Alford 2007a, b, Rowley and Alford 2013). *Litoria nannotis* typically perch on boulders near waterfalls and fast-flowing sections of stream (Hodgkison and Hero 2001, Rowley and Alford 2007b, Puschendorf et al. 2012), *Litoria rheocola* use rocks and streamside vegetation in faster-flowing sections of stream (Dennis 2012), and *Litoria serrata* are more arboreal than the other species and usually perch on vegetation near slower-flowing sections of stream (Rowley and Alford 2007b).

These three species also differ in conservation status. *Litoria nannotis* and *L. rheocola* are currently classified as Endangered (IUCN 2013) and were extirpated by chytridiomycosis at higher elevations (>400 m ASL) throughout their range by the mid-1990s (Richards et al.1993, McDonald and Alford 1999); however, many populations have subsequently recovered or recolonised areas where they had been extirpated (McDonald et al. 2005) and are currently co-existing with the pathogen (Sapsford 2012). *Litoria serrata* populations occurring >400 m ASL also suffered declines during initial outbreaks of chytridiomycosis, but none were known to be extirpated and all have subsequently recovered to pre-decline abundances (McDonald and Alford 1999), and this species is currently classified as Least Concern (IUCN 2013). *Batrachochytrium dendrobatidis* is still present in all populations of all three species that have been sampled, sometimes reaching high prevalences (Puschendorf et al. 2011; Sapsford et al. 2013).

Study sites

I conducted my study at 11 rainforest streams located in the Wet Tropics World Heritage Area in northeastern Queensland, Australia (Figure 1.2). I selected study sites at different elevations to ensure that my study included frogs that encountered the full range of environmental conditions available throughout their geographic range during the time of sampling. For some analyses, I distinguished between low-elevation (<400 m ASL) and high-elevation (>600 m ASL) sites. Tropical rainforest surrounded all streams; it was characterised by dense vegetation composed of large trees (10 m in height), vines, epiphytes, shrubs, and herbaceous plants. Although most sites were in relatively undisturbed rainforest, several sites were damaged by a tropical cyclone in February 2011 (see Chapter 9). Stream width varied from 5-10 m and streambeds were composed of rocks ranging in size from small pebbles to large boulders (10 m in diameter). All streams contained pools, runs, and riffles, and most had several waterfalls.

Thesis structure and overview

My thesis is presented as a series of eight stand-alone, but interrelated, manuscripts that are either published or will be submitted for publication. This format has resulted in some unavoidable repetition, mainly in the background material and methods. Because all chapters will include multiple co-authors when submitted for publication, the text for these chapters uses personal pronouns that are plural (i.e., “we” and “our”), rather than singular (i.e., “I” and “my”). At the start of each data chapter, I have listed all co-authors in order of their contribution to the work. Chapters 2-3 describe and test techniques used in other chapters, Chapters 4-7 investigate relationships between the behaviour of individual frogs (microenvironment use, microhabtiat use, and movements) and risk of infection by *Batrachochytrium dendrobatidis*, Chapter 8 investigates sublethal effects of *B. dendrobatidis* infections on frog reproductive effort, Chapter 9 investigates population-level effects of a large-

scale habitat disturbance on infection risk, and Chapter 10 provides an overall summary of my thesis, implications, and directions for future research. A brief overview of each chapter is provided below.

Chapter 2 tests a recently published technique for measuring amphibian body temperatures. This technique requires a pair of agar models (each model embedded with a temperature datalogger) that mimic amphibians with 0% and 100% resistance to evaporative water loss; the temperatures of the models together define the upper and lower boundaries of possible amphibian body temperatures for the location in which they are placed. We tested the ability of these models to accurately estimate the body temperatures of frogs under field conditions by comparing two-week thermal profiles of frogs using data collected semi-continuously using (1) agar model pairs and (2) temperature-sensitive radiotransmitters with an automated datalogging receiver, and discrete thermal data using (3) a non-contact infrared thermometer.

Chapter 3 tests an improvement to the physical models described in Chapter 2. One important limitation is that the dataloggers embedded in these moist agar models are not waterproof, which can lead to device failure and loss of data. To increase their water resistance, we waterproofed dataloggers using a plastic coating. This coating could potentially affect the accuracy of dataloggers by biasing temperatures or altering rates of warming and cooling. We tested whether the coating affects the accuracy of recorded temperatures, and whether it prevents failure of dataloggers under field conditions.

Chapter 4 investigates the ecology and behaviour of the common mistfrog *Litoria rheocola*. Little is known about this endangered rainforest stream frog, which has declined due to chytridiomycosis. We tracked *L. rheocola* to examine patterns of movement, microenvironment use, and microhabitat use, and increase our understanding of the behaviour of this species, and how it varies by season and elevation. We use this information to suggest ecological mechanisms for observed patterns of infection dynamics and decline in this endangered species.

Chapter 5 investigates how the thermal and hydric conditions selected by individual frogs influence their susceptibility to *B. dendrobatidis*. We tracked infected and uninfected individuals of three species of rainforest stream frogs (*Litoria nannotis, L. rheocola,* and *L. serrata*), recorded their body temperatures semi-continuously, and used these data to quantify the proportion of body temperatures above, within, and below the optimal temperature range for *B. dendrobatidis* growth (15-25°C) for each individual frog. We also measured the relative desiccation rates experienced by individual frogs at their selected locations. We used these data to model the effects of thermal and hydric conditions experienced by individual frogs on their *B. dendrobatidis* infection probability, and on the infection intensity of infected frogs.

Chapter 6 investigates how the movements and microhabitat use of individual frogs influence their susceptibility to *B. dendrobatidis*. We tracked infected and uninfected individuals of three species of rainforest stream frogs (*Litoria nannotis, L. rheocola,* and *L. serrata*), and examined the types of substrates they used, their positions in relation to the stream, movement distances, and movement probabilities. We used these data to model the effects of patterns of movement and microhabitat use by individual frogs on their *B. dendrobatidis* infection probability, and on the infection intensity of infected frogs. The results here provide the behavioural mechanisms for patterns described in Chapter 5.

Chapter 7 investigates whether the behavioural differences between infected and uninfected frogs that we documented in Chapters 5-6 reflect effects of innate behaviour on the probability of acquiring or retaining infections, or if they are a result of changes in the behaviour of infected frogs in response to their infections. To do this, we performed a laboratory experiment designed to discriminate between these alternatives. We recorded selected body temperatures and water use of naturally infected and uninfected individuals of two frog species (*Litoria nannotis* and *L. serrata*) in thermal gradients, and we re-tested the same individuals after the infected frogs had lost their infections. Understanding the causal relationships between amphibian

behaviour and *B. dendrobatidis* infection is important for understanding and ultimately managing this host-pathogen system.

Chapter 8 investigates whether infection by *B. dendrobatidis* influences the probability of advertisement calling in male *Litoria rheocola*. To minimise the negative effects of a pathogenic infection, hosts may respond adaptively by either increasing or decreasing their reproductive effort. Because calling requires substantial energy, the host's body condition may also mediate calling behaviour. We sampled frog behaviour and infection status both spatially (across six sites varying in elevation) and temporally (seasonally). Our analysis therefore provides a robust test of the hypothesis that infection by *B. dendrobatidis* has sublethal effects that interact with body condition to influence calling probability, and therefore fitness. These effects may have important evolutionary implications for amphibian populations co-existing with this pathogen.

Chapter 9 investigates how a severe tropical cyclone in northeastern Australia influenced *B. dendrobatidis* infection risk in a stream-breeding frog (*Litoria rheocola*). Tropical cyclones are fundamental drivers of rainforest ecosystem dynamics through their impacts on canopy structure, which directly influence microclimates present in the understory and all layers of the canopy. Therefore, cyclones may be an important driver of *B. dendrobatidis* infection dynamics. We investigated how Severe Tropical Cyclone Yasi (2011) affected rainforest canopy cover, and how these changes influenced microclimatic conditions and *B. dendrobatidis* infection risk in frogs. An understanding of these relationships may be useful for identifying amphibian populations most at risk to chytridiomycosis, for locating potential refuges from the disease, and for testing potential habitat manipulation strategies.

Chapter 10 summarises the findings presented in Chapters 2-9, outlines important ecological and conservation implications, and recommends directions for future research.

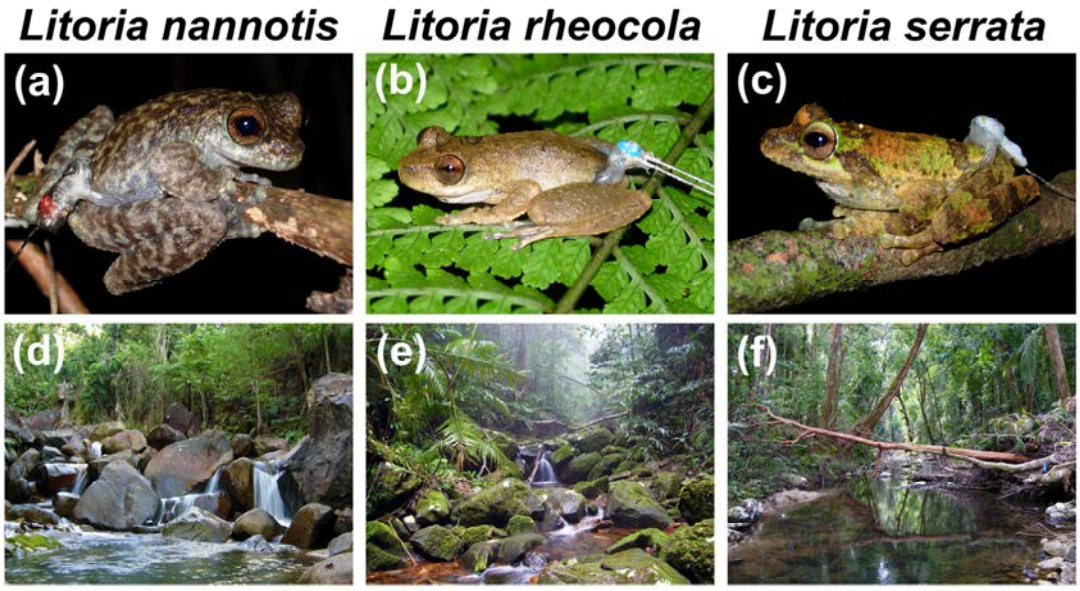

Figure 1.1. Study species and study sites. This thesis focuses on three species of treefrogs that occur near rainforest streams: (a) waterfall frog, *Litoria nannotis*, (b) common mistfrog, *L. rheocola*, and (c) green-eyed treefrog, *L. serrata*. A representative study site for each species is shown below the species image: (d) Tully Creek, (e) Windin Creek, and (f) Birthday Creek.

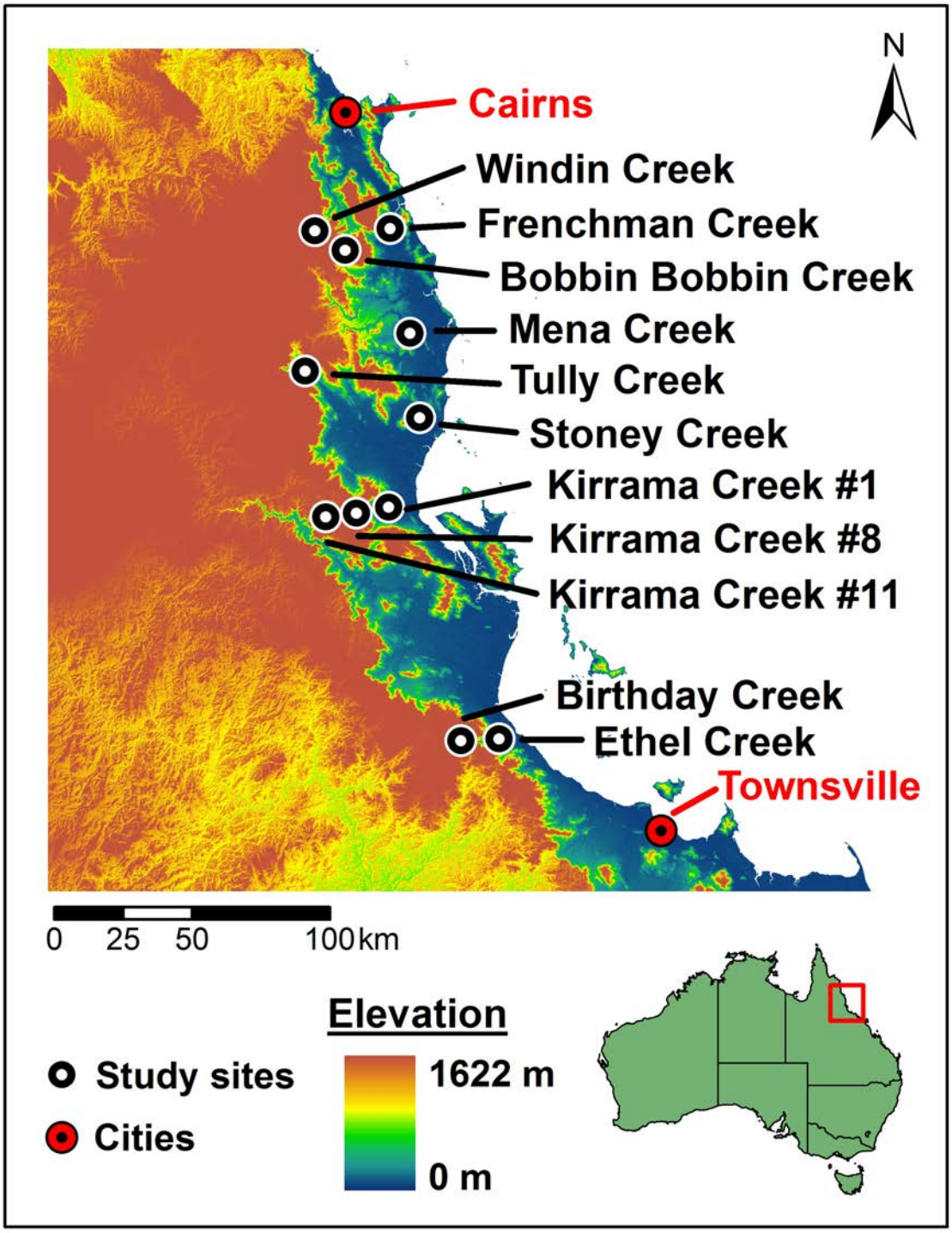

Figure 1.2. Locations of the 11 study sites used for the research presented in this thesis. All sites were streams surrounded by tropical rainforests located within the Wet Tropics World Heritage Area in northeastern Queensland, Australia.

Chapter 2: Using pairs of physiological models to estimate temporal variation in amphibian body temperature

Elizabeth A. Roznik and Ross A. Alford

Abstract

Temperature strongly influences the physiology and behaviour of amphibians, and understanding thermal biology is becoming increasingly important for amphibian conservation. Physical models are often used to estimate ectotherm body temperatures, but designing accurate models for amphibians is difficult because they can vary in cutaneous resistance to evaporative water loss. To account for this variability, a recently published technique requires a pair of agar models that mimic amphibians with 0% and 100% resistance to evaporative water loss; the temperatures of these models define the lower and upper boundaries of possible amphibian body temperatures for the location in which they are placed. The goal of our study was to develop a method for using these pairs of models to estimate parameters describing the distributions of body temperatures of frogs under field conditions, and to gain an overall estimate of the degree of resistance to evaporative water loss. We radiotracked green-eyed treefrogs (*Litoria serrata*) and collected semi-continuous thermal data using agar model pairs, and temperature-sensitive radiotransmitters with an automated datalogging receiver, and discrete thermal data using a non-contact infrared thermometer. We first verified the accuracy of temperature-sensitive transmitters in measuring frog body temperatures by comparing transmitter data with direct temperature measurements taken simultaneously for the same individuals. We then compared thermal profiles (mean, minimum, maximum, standard deviation) of individual frogs using data collected using each of the three methods. We found strong

relationships between thermal data collected using automated radiotelemetry and both types of thermal models. However, thermal parameters estimated for individuals using temperatures measured by transmitters were more highly correlated with those estimated using models that did not lose water than with those that lost water freely, suggesting that *Litoria serrata* has a high resistance to evaporative water loss. We demonstrate how these pairs of models can be used to approximate the level of skin resistance of a species by comparing direct body temperature measurements to model temperatures, and how researchers can use these relationships to selectively use only one type of model or combine information from both model types to obtain the most appropriate temperature estimate. Obtaining accurate thermal data from amphibians using this method can be used to advance many aspects of amphibian biology and address many urgent ecological and conservation questions.

Introduction

Studying the thermal biology of amphibians is fundamental to understanding their physiology, ecology, behaviour, distribution, and evolution (Feder and Burggren 1992; Wells 2007), and it is becoming increasingly important for understanding, predicting, and managing the effects of diseases and climate change on amphibians (Deutsch et al. 2008, Rohr and Raffel 2010, Rowley and Alford 2013, Chapters 4-6). Several methods have been developed to measure amphibian body temperatures in the field directly, but most are used for point sampling and do not record temperature semi-continuously (i.e., at regular intervals through time). The most commonly used method is direct measurement with a fluid-filled thermometer or a thermocouple probe; these can be used to measure skin, oral, or cloacal temperatures (Brattstrom 1963, Lillywhite 1970, Wygoda 1984, Navas 1996). These methods require handling individuals, which can elevate their body temperature through heat transfer from the researcher's hands (Navas and Araujo 2000, Lillywhite 2010). In addition, the stress associated with handling may alter individuals' behaviour, which could bias data during

longer-term studies that require repeated sampling. Non-invasive methods of measuring amphibian body temperatures that do not require handling include using non-contact infrared thermometers (Rowley and Alford 2007c) or temperature-sensitive radiotransmitters (Lillywhite 2010). In such transmitters, a change in temperature results in a corresponding increase or decrease in transmitter pulse rate; this rate can be recorded and later converted to temperature using calibration curves.

The goal of many studies on the thermal biology of amphibians is to understand how body temperatures are distributed in relation to environmental temperatures and how they change through time (Wells 2007, Lillywhite 2010). This aim is best achieved by measuring body temperatures semi-continuously over time, rather than by sampling individual temperatures at discrete points in time (Taylor et al. 2004). Temperature-sensitive radiotransmitters can be implanted or attached externally to amphibians and used with an automated datalogging receiver to record body temperatures at regular intervals (Lillywhite 1970, Lillywhite 2010, Chapters 5-6). However, such automated telemetry systems are expensive, complex, and typically can only record data from animals within a relatively small area. In addition, because transmitters are too heavy and bulky to be carried by very small amphibians, this method is not feasible for many individuals and species. Another approach to semi-continuous monitoring of body temperatures is the use of physical models. These can be placed in locations used by amphibians and used to estimate body temperatures that would be experienced in those locations by the modelled species over time. Various objects have been used to mimic the thermal properties of amphibians, including dead amphibians (Seebacher and Alford 2002), casts made of agar (Navas and Araujo 2000, Rowley and Alford 2010) and plaster (Tracy et al. 2007), sponges (Hasegawa et al. 2005), and copper casts or tubes covered with wet cotton or cloth (Bradford 1984, Bartelt and Petersen 2005).

Designing accurate thermal models for amphibians is difficult because they vary in cutaneous resistance to evaporative water loss. Although many amphibians

have little resistance to evaporative water loss, some species have much higher levels of resistance, especially arboreal frogs (Wygoda 1984, Young et al. 2005). In some species, resistance to evaporative water loss can vary substantially among individuals and across time within individuals because they can adjust their skin resistance to water loss, depending on their physiological state and behaviour (Wygoda 1989, Tracy et al. 2008). For these species, no models with fixed rates of evaporation can fully reflect the range of body temperatures available. To account for this variability, Rowley and Alford (2010) designed a system in which pairs of agar models are used together; one model mimics an amphibian with 0% resistance to evaporative water loss, and the other model has 100% resistance. Together, the temperatures of the models define the lower and upper boundaries of possible amphibian body temperatures for the location in which they are placed. Rowley and Alford (2010) tested these models in the field with frogs of one species and found that actual body temperatures fell within the thermal ranges defined by model pairs.

The goal of our study was to develop a method for using the pairs of models designed by Rowley and Alford (2010) to accurately estimate the body temperatures of frogs under field conditions. We focused on measuring the distribution of body temperatures among ecologically relevant categories, which is necessary for many ecological studies (Feder and Burggren 1992, Wells 2007). We radiotracked green-eyed treefrogs (*Litoria serrata*) and collected thermal data using several approaches. Semi-continuous data were recorded using both types of models and temperature-sensitive radiotransmitters with an automated datalogging receiver, and discrete measurements were made using a non-contact infrared thermometer. We first determined the accuracy of transmitter temperatures by comparing them to body temperatures measured simultaneously using a non-contact infrared thermometer. We then compared two-week thermal profiles (mean, minimum, maximum, standard deviation) of frogs created using data from each of these three methods. We also examined how data from models pairs can be used to approximate the average

resistance to evaporative water loss of a species under field conditions by comparing body temperature measurements to model temperatures, and how this information can inform the selection of model type to best estimate temperature distributions. To determine the utility of data collected using pairs of thermal models in a broader range of species, we examined data on two additional frog species (*Litoria nannotis* and *Litoria rheocola*) collected using model pairs and a non-contact infrared thermometer.

Methods

Radiotracking

We radiotracked a total of 61 male green-eyed treefrogs (*Litoria serrata*) at two low-elevation (<400 m ASL) and two high-elevation (>600 m ASL) rainforest streams in northeastern Queensland, Australia. We chose this combination of sites to provide the widest possible range of environmental conditions. Tracking took place over a two-week period at each site during the winter (cool/dry season) in 2011. Our sites were at Kirrama Creek #1 in Girramay National Park (18.203°S, 145.886°E; 100 m ASL; 4-18 July), Stoney Creek in Djiru National Park (17.920°S, 146.069°E; 20 m ASL; 12-25 August), Birthday Creek in Paluma Range National Park (18.980°S, 146.168°E; 800 m ASL; 19 July-1 August), and Windin Creek in Wooroonooran National Park (17.365°S, 145.717°E; 750 m ASL; 26 August-8 September).

Frogs were fitted with temperature-sensitive radiotransmitters (model A2414, 0.30 g, Advanced Telemetry Systems, Isanti, Minnesota, USA; factory-calibrated for 15-30°C). Each transmitter was attached to a frog externally by a belt made of silicone tubing (Gourret et al. 2011); a length of cotton thread was passed through the tubing and tied to secure the tubing around the frog's inguinal region (waist). The combined mass of the transmitter and belt never exceeded 8% of the frog's body mass, which is below the recommended maximum 10% transmitter-to-body-mass ratio for amphibians (Richards et al. 1994). We tracked all frogs using a Sika receiver (Biotrack Ltd, Wareham, Dorset, UK) with a handheld three-element Yagi antenna. Frogs were

located once during each day (10:00-17:00) and once each night (20:00-03:00) throughout the tracking period. At the end of the tracking period, we removed the tracking devices from all recaptured frogs. We excluded all data collected during the 24-hr period following attachment of tracking devices due to potential short-term behavioural effects of handling, which are unlikely to persist after the first night of transmitter attachment (Langkilde and Alford 2002, Rowley and Alford 2007d).

Temperature measurements from *Litoria serrata*

We used three different methods to collect data on *Litoria serrata* body temperatures: a non-contact infrared thermometer (Rowley and Alford 2007c), temperature-sensitive radiotransmitters with an automated datalogging receiver, and thermal models (Rowley and Alford 2010). We measured the body temperature of each frog whenever possible during tracking using a non-contact infrared thermometer (OS425-LS, Omega Engineering Ltd, Irlam, Manchester, UK; factory-calibrated and accurate to ±1.0°C). This device had a distance to spot ratio of 50:1, and the area measured was delineated by a circle of laser points. We set the emissivity to 0.95 (Rowley and Alford 2007c). To take a temperature reading, we held the device approximately 5 cm away from the frog and aimed it at the lower dorsal region, sufficiently above the transmitter.

The pulse rate of each transmitter was recorded at 30-min intervals during the study period by an automated datalogging receiver (model SRX400A, Lotek Wireless, Newmarket, Ontario, Canada). During tracking at each field site, two four-element Yagi antennas were mounted in trees at each field site and connected to the receiver to maximize detection of transmitter signals. Recorded pulse rates were downloaded and converted to temperatures using calibration curves provided for each transmitter by the manufacturer. Transmitters were factory-calibrated for 15-30°C, but visual inspection of the data revealed that they were accurate for 10-35°C (Figure 2.1), so only *Litoria serrata* temperatures within this temperature range (from any method) were included in

any analysis.

We also collected thermal data using physical models (Rowley and Alford 2010) that were placed in every day and night location in which each frog was found. The models consisted of paired frog-shaped models made of 3% agar, each with an embedded DS1921G Thermochron iButton temperature datalogger (Maxim Integrated Products, Sunnyvale, California, USA; factory-calibrated and accurate to ±0.5°C) that was waterproofed using a plastic coating (Chapter 3, Roznik and Alford 2012) and programmed to record temperatures at 30-min intervals. Model pairs comprised one model that was permeable to water loss, and one that was impermeable (i.e., coated with plastic); together the temperatures of each pair of models define the lower and upper boundaries of possible frog body temperatures at their location each time temperatures are recorded (Rowley and Alford 2010). All models were placed in frog locations the day after frogs were found in them; we used temperatures measured between 07:00 and 18:30 from models placed in day locations, and we used temperatures measured between 19:00 and 06:30 from models placed in night locations.

Temperature measurements from additional species

We used thermal data from two additional species to further assess the utility of thermal models. Body temperatures of waterfall frogs (*Litoria nannotis*) and common mistfrogs (*Litoria rheocola*) were recorded during tracking studies in northeastern Queensland, Australia. We radiotracked a total of 80 male *Litoria nannotis* (using transmitter model BD-2NT, 0.44 g, Holohil Systems Ltd., Carp, Ontario, Canada) at two low-elevation (<400 m ASL) and two high-elevation (>600 m ASL) rainforest streams. Transmitter attachment and tracking methods were the same as for *Litoria serrata*. Tracking took place over 10-14 days at each site during the winter (cool/dry season) in 2010. The sites used were Kirrama Creek #8 in Girramay National Park (18.196°S, 145.868°E; 170 m ASL; 5-19 June), Kirrama Creek #11 in Girramay

National Park (18.214°S, 145.798°E; 750 m ASL; 18-29 July), Tully Creek in Tully Gorge National Park (17.773°S, 145.645°E; 150 m ASL; 7-17 July), and Windin Creek in Wooroonooran National Park (17.365°S, 145.717°E; 750 m ASL; 20 June – July 4).

We tracked a total of 120 *Litoria rheocola* using harmonic direction finding (Langkilde and Alford 2002, Rowley and Alford 2007d) with self-built tracking devices (Gourret et al. 2011), attached to frogs externally using the same methods as for radiotransmitters (described above) and located using RECCO detectors (models R4 and R8, RECCO Avalanche Rescue System, Lidingö, Sweden). We tracked *Litoria rheocola* for three weeks at each of two rainforest streams in Wooroonooran National Park (Frenchman Creek: 17.307°S, 145.922°E; 40 m ASL; Windin Creek: 17.365°S, 145.717°E; 750 m ASL) during the winter (cool/dry season) and summer (warm/wet season). Tracking took place during the winter in 2009 (Frenchman Creek: 13 July – 6 August; Windin Creek: 18 August – 9 September), and the summer in 2010 (Frenchman Creek: 20 January – 9 February; Windin Creek: 11 February – 3 March). Individuals of each species were located twice daily (each day and night) and their body temperatures were recorded then, when they were in accessible locations, using a non-contact infrared thermometer. We also placed paired thermal models (Rowley and Alford 2010) at each unique location used by each frog using the approach described above.

Data analysis

The main goal of our study was to develop a method for using the pairs of models designed by Rowley and Alford (2010) to estimate the distributions of body temperatures of frogs under field conditions. We first verified the correspondence between *Litoria serrata* body temperatures measured using radiotransmitters and a non-contact infrared thermometer. We used a reduced major axis regression to examine the relationship between simultaneous temperature measurements of the same frog taken using a non-contact infrared thermometer and a temperature-

sensitive transmitter. We considered transmitters to provide good estimates of the infrared thermometer temperature if the values derived by the two devices were highly correlated. If the slope of the relationship differed significantly from one, or the Y-intercept differed significantly from zero, we adjusted transmitter temperatures for all subsequent analyses for *Litoria serrata* using the regression equation.

We compared summaries of the thermal regime experienced by each frog over the entire study period by comparing parameters derived from its transmitter temperatures, point measurements of its temperature using the non-contact thermometer, and temperatures recorded by the permeable and impermeable models placed in its diurnal and nocturnal locations. For each method of measurement, we calculated the mean, minimum, maximum, and standard deviation of temperatures for each frog over the study period. We used a series of reduced major axis regressions to examine the relationships between temperature parameters estimated by (1) transmitters and the non-contact infrared thermometer, (2) transmitters and impermeable models, and (3) transmitters and permeable models. We also examined the slopes and Y-intercepts of the regression lines, as described above.

To determine the utility of data collected using pairs of thermal models in a broader range of species, we examined data on two additional frog species (*Litoria nannotis* and *Litoria rheocola*). We measured the body temperatures of individual frogs using a non-contact infrared thermometer when they were located during tracking. Frogs of these two species frequently sheltered in rock crevices where we were unable to directly measure their body temperatures. Therefore, we obtained relatively few body temperatures for each frog using this method and could not compare temperature parameters between the infrared thermometer and models, as we did for *L. serrata*. Instead, we used reduced major axis regressions to examine the relationship between each temperature measured by the infrared thermometer and the temperature recorded at the same time of day by each type of model placed in that frog's location. Separate analyses were performed for each species, and we did not

estimate P-values for those correlations because individuals were represented more than once in the dataset.

Results

There was a strong, significant linear relationship between individual *Litoria serrata* body temperatures measured directly using a non-contact infrared thermometer and temperatures measured simultaneously using temperature-sensitive transmitters carried by the frogs ($R^2 = 0.843$, $P < 0.001$; Figure 2.1). Although temperatures measured using each method were similar, the slope of the regression line (1.214) differed significantly from one, and the Y-intercept (-5.152) was significantly different from zero. Transmitters slightly underestimated body temperatures in the lower portion of the range experienced by frogs as measured by the infrared thermometer (Figure 2.1). For this reason, we used the regression equation to adjust transmitter temperatures to body temperatures before estimating the mean, minimum, maximum, and standard deviation of transmitter-derived body temperatures for each *Litoria serrata*.

After adjustment for the difference between transmitter temperatures and actual body temperatures measured using an infrared thermometer, we found that parameters estimated using transmitter data were more highly correlated with model parameters than were those estimated using temperatures taken twice daily using the infrared thermometer when individuals were located during radiotracking (Table 2.1). Parameters estimated from temperatures measured using the infrared thermometer were relatively poorly correlated with those estimated from transmitter data (Table 2.1). Reduced major axis regressions indicated that, when compared to adjusted transmitter data, thermometer data tended to overestimate minimum temperatures and underestimate maximum temperatures. Estimates of mean temperatures were relatively close to those estimated from transmitter data, but estimates of the standard

deviation of measurements were relatively poorly correlated with estimates from transmitter data.

We found strong, significant relationships between parameters for individual *Litoria serrata* estimated using adjusted transmitter temperatures and parameters estimated using models placed in their diurnal and nocturnal locations (Table 2.1, Figures 2.2-2.3). The mean, minimum, maximum, and standard deviation of adjusted transmitter temperatures were most highly correlated with, and thus better predicted by, parameters estimated from data on impermeable models than those estimated from permeable models (Table 2.1, Figures 2.2-2.3). Two of the four slopes of regressions for impermeable models were significantly different from one, as opposed to four of four for permeable models (Table 2.1 For both types of models, estimates of minimum temperatures tended to overestimate the lowest temperatures derived from transmitters, and to underestimate the highest minimum transmitter temperatures. For permeable models, the estimated mean, maximum, and standard deviation of temperatures were always lower than estimates from transmitter temperatures. Using either type of model produced estimates of thermal parameters that were closer to those obtained from transmitters than were estimates produced using only point temperatures measured with a non-contact infrared thermometer (Table 2.1 Overall, the parameters estimated using data from impermeable models were the most accurate approximations of those obtained from transmitter data (Table 2.1, Figure 2.2).

For *Litoria nannotis*, temperatures measured using both impermeable and permeable models were strongly and significantly correlated with temperatures measured using the non-contact infrared thermometer (impermeable: $R^2 = 0.528$, $P < 0.001$; permeable: $R^2 = 0.517$, $P < 0.001$; Figure 2.4). The slopes for both model types were very close to and did not differ significantly from one, and the Y-intercepts, although slightly higher than zero, were not significantly different from zero. This suggests that both types of models produced reasonably accurate estimates of mean

body temperature in this species. For *Litoria rheocola*, both types of models were also significantly correlated with infrared thermometer temperatures (impermeable: R^2 = 0.812, P < 0.001; permeable: R^2 = 0.828, P < 0.001; Figure 2.4). However, the slopes and Y-intercepts of the regressions for impermeable models were significantly different from one and zero, respectively, while the slopes and Y-intercepts of regressions for permeable models were not significantly different from one and zero, respectively, and were closer to these values. This suggests that for *Litoria rheocola,* permeable models provided better estimates of body temperature than did impermeable models.

Discussion

Studies on the thermal biology of amphibians in the field require sampling methods that provide accurate profiles of the temperatures experienced by animals, in terms of the mean and variation of individuals' body temperatures. We found that both automated telemetry using temperature-sensitive transmitters, and data collected semi-continuously using the pairs of physiological models developed by Rowley and Alford (2010) accurately characterised the thermal regimes experienced by individual *Litoria serrata* (Figures 2.1-2.2). Both methods provided better estimates than did twice-daily point measurements of frog body temperatures. Thermal data from models that were impermeable to water loss provided the best approximation to parameters characterising the temperatures measured using temperature-sensitive transmitters (Table 2.1, Figures 2.2-2.3). Body temperature parameters derived from transmitters were more highly correlated with impermeable models than with permeable models, and impermeable models also provided more accurate measures of transmitter temperatures (Table 2.1, Figures 2.2-2.3). Permeable models tended to underestimate temperature parameters of *Litoria serrata*, whereas impermeable models accurately characterised the mean, maximum, and standard deviation of temperatures. Impermeable model estimates of the minimum temperatures experienced by individuals were the most poorly correlated with transmitter temperatures.

There is considerable variability in cutaneous resistance to evaporative water loss among amphibian species, and individuals of some species are able to adjust their rates of water loss over time, depending on their physiological state and behaviour (Wygoda 1989, Tracy et al. 2008). This greatly increases the range of body temperatures individuals might experience in a given location (Wygoda 1984, Young et al. 2005), which is why model pairs are used to define the lower and upper boundaries of possible body temperatures in a given location by mimicking frogs with 0% or 100% resistance to water loss. Because impermeable models provided the most accurate approximations of transmitter data for *Litoria serrata*, it is likely that this species typically has a high cutaneous resistance to evaporative water loss in the field. High cutaneous resistance to water loss has been found in laboratory studies of arboreal frogs (Wygoda 1984, Young et al. 2005), but this is the first demonstration that it is pervasive in a species in nature.

Models also accurately estimated body temperatures of two additional frog species (*Litoria nannotis* and *Litoria rheocola*; Figure 2.4), and should be useful for a wide range of other frog species. Permeable models were the most accurate for *Litoria rheocola*, and both model types provided accurate estimates for *Litoria nannotis*. Differences in these patterns among the three species we examined probably relate to the behaviour of these species; *Litoria serrata* is the most arboreal species and often uses sunny and dry microhabitats, which change temperature rapidly, whereas *Litoria rheocola* typically uses moist and shady microhabitats with low thermal variation, and *Litoria nannotis* uses very wet, sheltered microhabitats with very little thermal variation (Rowley and Alford 2007b, Chapters 4-6). Researchers can use pairs of models to approximate the level of skin resistance of a study species by comparing direct temperature measurements to models, and then selectively use only one type of model or combine information from both model types to determine the most appropriate temperature estimate.

Temperature parameters obtained semi-continuously from impermeable

models were more highly correlated with those derived from transmitters than readings taken twice daily using a non-contact infrared thermometer (Table 2.1). Although point measurements and semi-continuous measurements both provided relatively accurate measures of the overall mean body temperature experienced by frogs, the variation in body temperatures (minimum, maximum, standard deviation) was more accurately represented by semi-continuous data. Taylor et al. (2004) also found that semi-continuous measures of ectotherm body temperature generated more accurate thermal profiles than body temperatures sampled at random or non-random points in time. The variation in body temperature is considered more important than the mean body temperature in studies of thermal biology because of the relationship between temperature and performance (Angiletta et al. 2002), and because of the importance of temperature variability and extreme temperatures in many ecological studies, including those that examine the effects of climate change and disease on animals (Rohr and Raffel 2010, Paaijmans et al. 2013).

Overall, using pairs of physiological models to estimate amphibian body temperatures has many useful applications for ecological studies. Model pairs are relatively inexpensive, easy to construct in large numbers, and accurately measure the ranges of temperatures available to individual amphibians in the field. Our results demonstrate that comparing thermal parameters (e.g., mean, standard deviation, minimum, maximum) estimated for each type of model with the same parameters estimated using measurements taken from an individual in the same location can make it possible to calibrate estimates and gain an idea of the average degree of resistance to evaporative water loss a species exhibits in the field. The model that is permeable to water loss can also be used to measure relative desiccation rates experienced by amphibians in different microhabitats by weighing the model before and after placement in the field (Spotila and Berman 1976, Schwarzkopf and Alford 1996, Rowley and Alford 2010, Chapters 4-6). Our study demonstrates that by deploying model pairs at frog activity sites, large amounts of accurate thermal data can

be collected without investing in expensive automated telemetry systems, or even radiotransmitters. These models also can be used to collect thermal data on amphibians that are too small to carry radiotransmitters; individuals could be located using other methods, such as harmonic direction finding (Rowley and Alford 2007d, Chapters 4-6), fluorescent powder tracking (Rittenhouse et al. 2006), or visual observations during mark-recapture studies or other field surveys, and models could be placed at these locations. Using this method to collect data on the thermal and hydric conditions experienced by amphibians will advance many aspects of amphibian biology, and combining this information with detailed data on patterns of microhabitat use and movement can be used to address many urgent ecological questions.

Table 2.1. Results from reduced major axis regressions between body temperature parameters of green-eyed treefrogs (*Litoria serrata*) measured using temperature-sensitive radiotransmitters and a non-contact infrared thermometer, and between transmitter temperatures and temperatures measured using two types of thermal models that are either impermeable or permeable to evaporative water loss. Shown are the R^2 values, slopes, and Y-intercepts for regressions involving the mean, minimum, maximum, and standard deviation of temperatures recorded for 61 frogs that were radiotracked at four field sites over a two-week period at each site. Each regression estimates the temperature parameter obtained for each individual using the second measurement method as a function of the parameter obtained using temperature-sensitive radiotransmitters. All relationships are statistically significant ($P < 0.001$). Slopes that are significantly different from one are denoted by [a], and Y-intercepts that are significantly different from zero are denoted by [b].

Temperature parameter	Transmitters and infrared thermometer			Transmitters and impermeable models			Transmitters and permeable models		
	R^2	Slope	Y-intercept	R^2	Slope	Y-intercept	R^2	Slope	Y-intercept
Mean	0.731	1.059	-1.179	0.946	0.994	-0.162	0.914	1.253[a]	-3.518[b]
Minimum	0.263	0.706[a]	2.733[b]	0.519	0.679[a]	3.897[b]	0.490	0.733[a]	3.382[b]
Maximum	0.488	1.405[a]	-4.168	0.627	0.929	2.232	0.311	1.491[a]	-7.316
Standard deviation	0.458	1.090	-0.034	0.884	1.142[a]	-0.153	0.675	2.118[a]	-1.386[b]

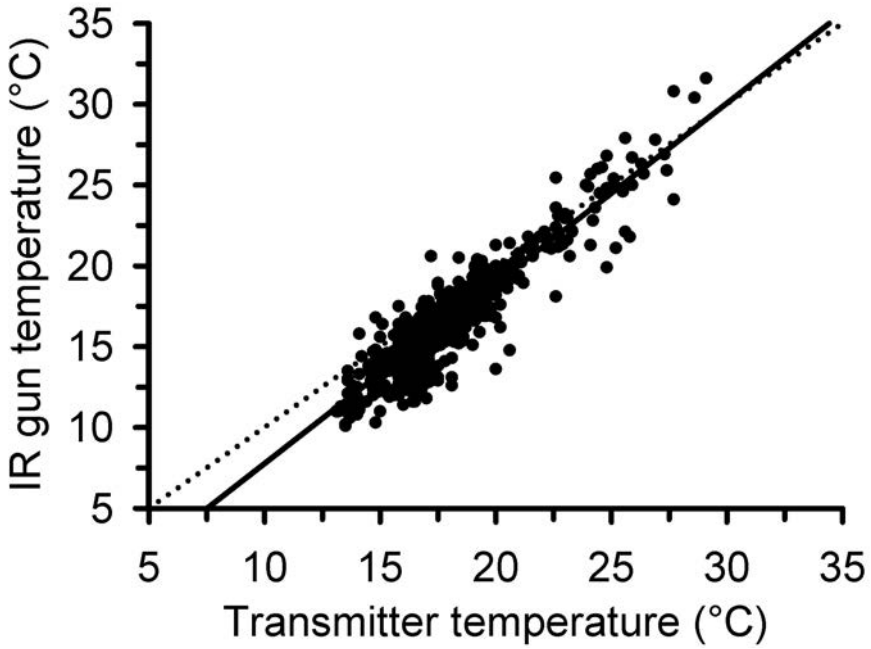

Figure 2.1. Relationship between body temperatures of green-eyed treefrogs (*Litoria serrata*) measured using a non-contact infrared thermometer ("IR gun") and temperatures measured simultaneously by temperature-sensitive radiotransmitters carried by the frogs. The solid line indicates the regression line, and the dotted line indicates y = x.

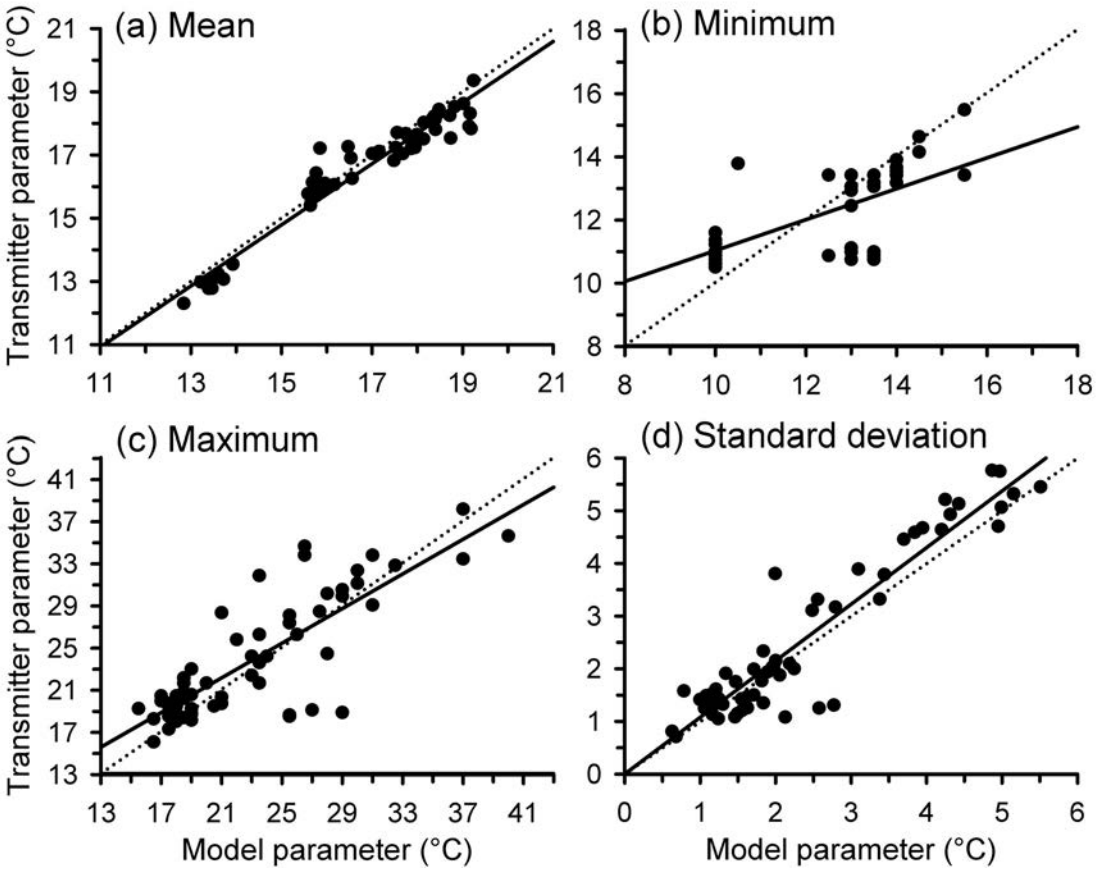

Figure 2.2. Relationships between body temperature parameters estimated for green-eyed treefrogs *Litoria serrata* measured semi-continuously using temperature-sensitive radiotransmitters, and thermal models that were impermeable to water loss and placed at locations used by the same frogs. Shown are relationships for the (a) mean, (b) minimum, (c) maximum, and (d) standard deviation of temperatures experienced by frogs over a two-week period. For each relationship, a solid line indicates the regression line, and a dotted line indicates y = x.

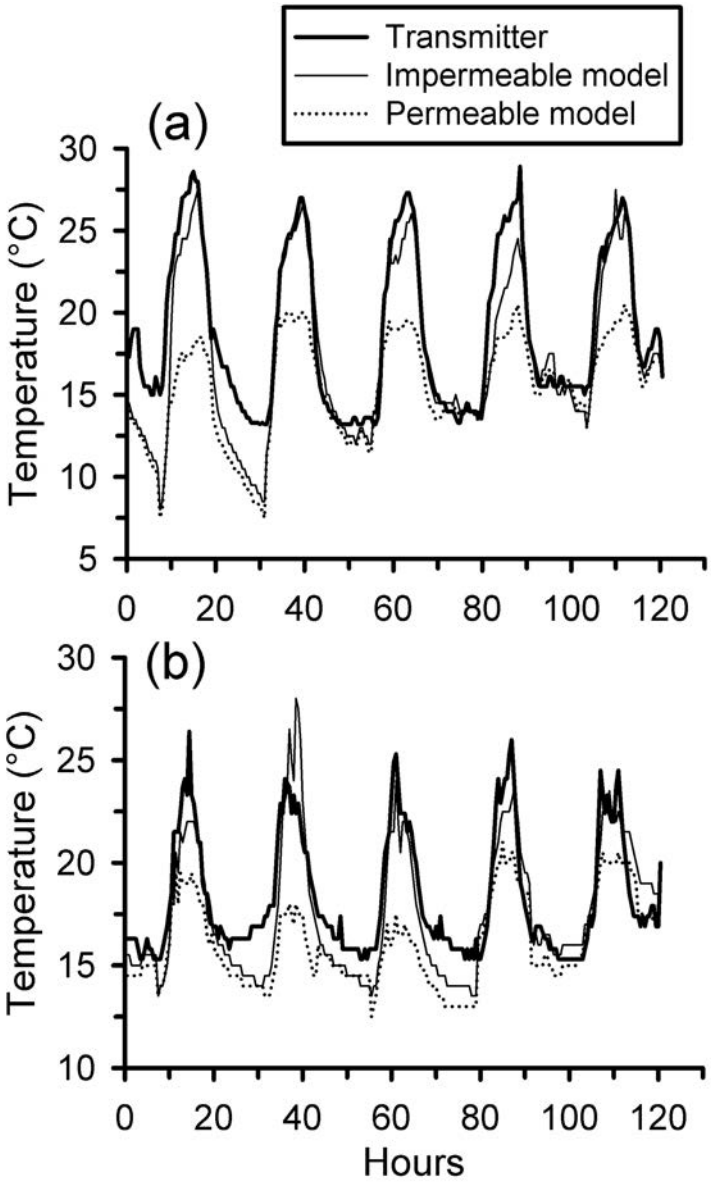

Figure 2.3. Representative examples of body temperatures over five days for two green-eyed treefrogs (*Litoria serrata*) at two different field sites. Body temperatures were measured semi-continuously by temperature-sensitive radiotransmitters and by two types of thermal models that were either impermeable or permeable to water loss.

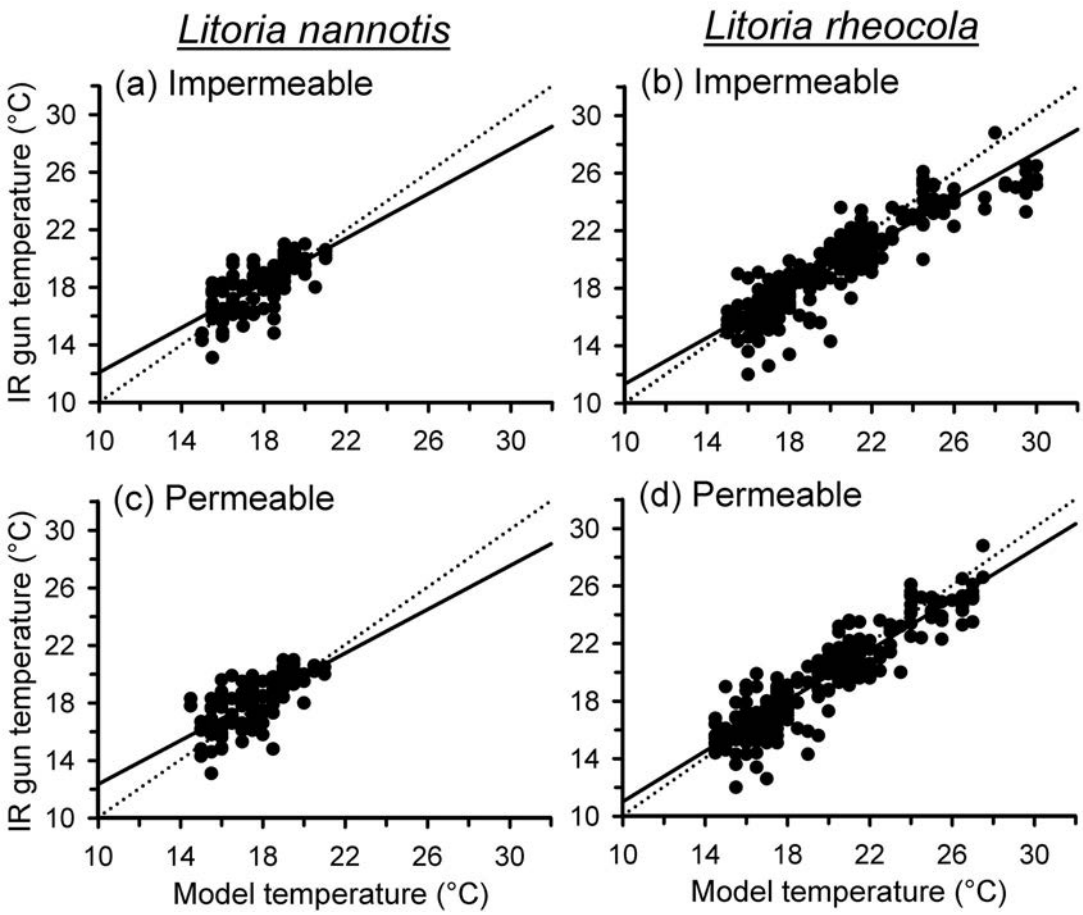

Figure 2.4. Relationships between body temperatures of waterfall frogs (*Litoria nannotis*) and common mistfrogs (*Litoria rheocola*) measured using a non-contact infrared thermometer ("IR gun") and thermal models that were either impermeable or permeable to water loss. For each relationship, a solid line indicates the regression line, and a dotted line indicates y = x.

Chapter 3: Does waterproofing Thermochron iButton dataloggers influence temperature readings?

Elizabeth A. Roznik and Ross A. Alford

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Abstract

Miniature Thermochron iButton dataloggers have transformed the ways in which researchers collect thermal data. However, one important limitation is that these dataloggers are not waterproof, which can lead to device failure and loss of data under field conditions. Several methods have been used to increase their water resistance, but no study to date has investigated whether any of these techniques affects the accuracy of temperature readings. Waterproofing potentially could affect the accuracy of iButtons by biasing temperatures or altering rates of warming and cooling. We compared temperature profiles of unmodified Thermochron iButtons (model DS1921G) to iButtons that we coated with a clear plastic dip (designed to coat tool handles) to determine whether this waterproof coating affects the accuracy of temperatures they record. We also compared temperatures recorded by uncoated and coated iButtons that were embedded within physical models that mimic frog body temperatures. Finally, we used our field data to test whether coating iButtons with plastic prevents

failure of dataloggers during fieldwork. Although we found statistically significant differences between the temperatures recorded by uncoated and coated iButtons, and also between uncoated and coated iButtons embedded in frog models, these effects were relatively small (0-1.3°C). We also found that coating iButtons with plastic reduced the likelihood of device failure under field conditions (from 8.3% to 0%). We conclude that coating Thermochron iButtons with plastic is an affordable and reliable method of waterproofing dataloggers that prevents device failure and data loss with minimal influence on temperature readings.

Introduction

Miniature temperature dataloggers have become valuable tools for the study of thermal ecology in a diversity of ectotherms and heterothermic endotherms, including mammals (Warnecke et al. 2007), birds (Laurila and Hohtola 2005), reptiles (Downs et al. 2008), amphibians (Rowley and Alford 2010), fishes (Donaldson et al. 2009), and invertebrates (Jones et al. 2004). They have been used to record body temperatures of animals of all sizes, ranging from elephants (Kinahan et al. 2007) to elephant shrews (Mzilikazi et al. 2002), through surgical implantation (Taylor et al. 2004), insertion into the gastrointestinal tract (Kinahan et al. 2007) or ear canal (Munn et al. 2009), or attachment to the animal's outer surface (Grayson and Dorcas 2004). Miniature thermal dataloggers also have been incorporated into physical models used to predict the body temperatures of frogs (Rowley and Alford 2010) and mussels (Jost and Helmuth 2007). Additionally, they have been used to measure microhabitat temperatures of nests (Guillemette et al. 2009, Angilletta et al. 2009), burrows (Woodman 2008), roosts (Dechmann et al. 2004), tree hollows (Isaac et al. 2008), and rock crevices (Pike et al. 2010), as well as environmental temperatures of air (Lundquist and Huggett 2008), soil (Penman and Towerton 2008), and water (Johnson et al. 2005). Some researchers even have placed miniature dataloggers in bird nests to study incubation patterns and nest status (Hartman and Oring 2006, Zangmeister et

al. 2009), and others have reconstructed miniature dataloggers to further reduce size and weight, allowing implantation or attachment to even smaller animals (Robert and Thompson 2003, Lovegrove 2009).

The Thermochron iButton, manufactured by Dallas Semiconductor (a subsidiary of Maxim Integrated Products, Sunnyvale, California, USA) is currently the most popular and inexpensive miniature temperature datalogger commercially available (Angilletta and Krochmal 2003, Davidson et al. 2003, Hubbart et al. 2005). Although there are wide applications for Thermochron iButtons, one important limitation is that they are not waterproof. This is especially problematic in studies of thermal ecology; these often involve placing dataloggers in moist locations, which can lead to device failure and data loss. For example, device failure has occurred with dataloggers submerged underwater (3 of 7 failed, Wolaver and Sharp 2007; 40 of 500 failed, Johnson et al. 2005) and those implanted into animals (3 of 6 failed, Lovegrove and Génin 2008). Although Dallas Semiconductor has developed a waterproof iButton Capsule (model DS9107) to hold an iButton, this increases the volume of the device by 586% (from 17 x 6 mm to 28 x 25 mm) and the weight of the device by 558% (from 3.3 g to 21.7 g), making this housing too large and heavy for many applications. Additionally, the capsule costs as much as the iButton itself, increasing the total cost per datalogger from $25 to $50 USD, which may not be economically feasible for studies involving the use of large numbers of dataloggers. Even if it is possible to replace failed devices, the lost data may not be replaceable. As an alternative solution, many researchers have waterproofed iButtons by sealing them with materials such as plastic tool dip (designed to coat tool handles; e.g., Grayson and Dorcas 2004, Taylor et al. 2004, Donaldson et al. 2009), surgical wax (e.g., Downs et al. 2008, Bieber and Ruf 2009, Gür et al. 2009), parafilm (Schwanz et al. 2010), silicone sealant (Lautz et al. 2010), and balloons (Lutterschmidt et al. 2006, Kearney et al. 2011).

The temperatures recorded by iButtons are accurate and precise (Angilletta and Krochmal 2003, Davidson et al. 2003, Hubbart et al. 2005). However,

waterproofing could potentially affect the accuracy of iButtons by biasing temperatures or altering rates of warming and cooling. No previous study has evaluated the effects of any type of waterproofing on the accuracy of thermal measurements taken using iButtons. Our study evaluated whether, and to what extent, waterproofing Thermochron iButtons using plastic coating affects the accuracy of temperature readings taken using iButtons alone and iButtons embedded in frog thermal models (Chapter 2) under a variety of environmental conditions. We used plastic coating because it is completely waterproof and very durable, which is necessary when using dataloggers in the environment.

Methods

General plastic coating methods

Our experimental design is summarised in Table 3.1. In all experiments, we used factory-calibrated iButtons (model DS1921G, precision: 0.5°C, accuracy: ± 1.0°C). We first programmed 180 iButtons to record temperatures every 10 min. After these iButtons had recorded ambient temperatures for three hours in a shaded location, we selected 90 of them at random and waterproofed them with clear plastic tool dip (Plasti Dip, Plasti Dip International, Blaine, MN, USA; Figure 3.1). To do this, we tied a length of cotton thread around the datalogger, and dipped the datalogger into a can of plastic tool dip. We then suspended the datalogger until the plastic has set, and then cut the thread and sealing the hole with plastic (Figure 3.1).

Dataloggers alone

When an iButton is used alone to record air temperatures, its heat exchange with the environment should be affected by conduction, convection, and radiation. Any coating is likely to alter one or more of these processes and thus change an iButton's thermal properties, which could affect its accuracy. We investigated whether waterproofing iButtons using plastic coating influences temperature readings taken

using iButtons alone in both full sunlight and full shade. This allowed us to understand the influence of waterproofing on datalogger accuracy under a variety of conditions of exposure to solar radiation. All iButtons were placed on a grassy lawn in Townsville, Queensland, Australia on 12 May 2010 at 22:00, which was on the night preceding a day when the weather was predicted to be mostly sunny. We placed 30 iButtons (15 coated, 15 uncoated) in an area that received direct sunlight throughout the day, and we placed 30 iButtons (15 coated, 15 uncoated) nearby, but shaded by a tarpaulin elevated 1 m above the ground. We left the dataloggers to record temperatures for 24 h, beginning at 00:00. After the experiment, we extracted coated iButtons from the plastic coating using a scalpel and downloaded all data.

To understand whether plastic coating had an overall effect on iButton readings, and whether there was an interaction between coating and degree of exposure to solar radiation, we analysed our data using a two-way ANOVA with iButton type (uncoated or coated) and exposure type (sun or shade) as factors, and the mean temperature averaged over the 24-h period as the dependent variable. To further understand how coating may have influenced temperatures over time, we also analysed data from each exposure group separately using a repeated measures ANOVA with iButton type (uncoated or coated) as the factor, temperature as the dependent variable, and each 10-min temperature reading as the repeated measure. Our goal was to understand how waterproofing and time of day influenced temperatures within each treatment, and not whether temperatures differed among treatments (i.e., sun vs. shade).

Dataloggers embedded in physical models

When an iButton is embedded in a frog physical model, heat exchange between the iButton and its environment is primarily by conduction; therefore, we hypothesized that any effects of waterproofing on iButton accuracy should be minimal. To understand whether coating iButtons with plastic influences temperature readings

of physical models mimicking the thermal properties of frogs (Rowley and Alford 2010, Chapter 2), we embedded 30 coated and 30 uncoated iButtons in 60 “impermeable” frog models, and 30 coated and 30 uncoated iButtons in 60 “permeable” frog models following the methods of Rowley and Alford (2010; Figure 3.1). These two model types are made of 3% agar and are either impermeable or permeable to water loss; when used together they define the upper and lower boundaries of possible amphibian body temperatures for the locations in which they are placed (Rowley and Alford 2010).

Treatment groups of physical models were replicated in full sunlight and full shade to allow us to determine the influence, if any, of differences in exposure to solar radiation on any effects of iButton waterproofing. Physical models were placed in the same sunny or shady locations as used for iButtons alone. In total, we placed 30 impermeable models (15 coated, 15 uncoated dataloggers) and 30 permeable models (15 coated, 15 uncoated dataloggers) in an area that received direct sunlight throughout the day, and we placed 30 impermeable models (15 coated, 15 uncoated dataloggers) and 30 permeable models (15 coated, 15 uncoated dataloggers) in a nearby location, but shaded with a tarp elevated 1 m above the ground. We left dataloggers to record temperatures for 24 h, beginning at 00:00. After the experiment, we extracted iButtons from the models, and if necessary from the plastic coating using a scalpel, and downloaded all data.

To understand whether plastic coating had an overall effect on iButtons embedded in physical models, and whether there was an interaction between coating and degree of exposure to solar radiation, we analysed data from each model type separately using a two-way ANOVA with iButton type (uncoated or coated) and exposure type (sun or shade) as factors, and the mean temperature averaged over the 24-h period as the dependent variable. To further understand how coating may have influenced temperatures over time, we also analysed data from each treatment group using a repeated measures ANOVA with iButton type (uncoated or coated) as the factor, temperature as the dependent variable, and each 10-min temperature reading

as the repeated measure.

Effects of plastic coating on datalogger failure rates

In addition to studying the effects of plastic coating on the accuracy of iButton temperature readings, we also tested whether waterproofing prevents failure of dataloggers under field conditions. To do this, we recorded the numbers of iButtons deployed and the numbers that failed during three separate field trips. We defined failed iButtons as those from which we could not download data, and which could not be re-programmed. During our fieldwork, all iButtons were embedded in frog physical models (Rowley and Alford 2010) and placed in diurnal and nocturnal microhabitats used by treefrogs in riparian areas. The iButtons were left uncoated during two separate three-week trips to tropical rainforests in northern Queensland, Australia during the dry season (when it rained very little). After experiencing failure of iButtons, we coated iButtons with plastic during one six-week trip to the same field sites during the wet season (when it rained frequently). We used a contingency table analysis to examine the numbers of failed iButtons on these field trips. For this analysis, we used iButton type (uncoated or coated) and status of iButtons after fieldwork (functioning or failed) as the factors, and the number of iButtons in each category as the dependent variable. Although these data confound season and coating status of iButtons, we believe that coating status is almost certainly the cause of any significant differences in failure rates because all were embedded in agar, providing identical local moisture environments, and mean temperatures only differ by about 10°C between the seasons.

Results

Effects of plastic coating on datalogger temperatures

Prior to waterproofing iButtons, but after assigning them to treatment groups, we compared temperature readings among all treatment groups at one randomly selected time and found that their mean temperatures were not significantly different

($F_{10,168}$ = 1.202, P = 0.289) and their variances were not significantly different (F = 0.724, P = 0.716). Thus, any subsequent differences in temperature means among treatments should be due to the effects of waterproofing on accuracy, rather than being caused by differences in bias among the iButtons allocated to each treatment. During our experiment, four of 180 (2%) uncoated iButtons failed: two in impermeable models placed in sun, and two in permeable models in shade (Table 3.1). Additionally, one iButton failed to program properly, and therefore did not record any temperature data.

During our experiment, iButtons recorded temperatures ranging from 16 to 44°C. We did not find significant differences in the overall mean temperatures averaged over the 24-h period for iButtons alone ($F_{1,55}$ = 0.067, P = 0.797), impermeable models ($F_{1,54}$ = 0.651, P = 0.423), or permeable models ($F_{1,54}$ = 0.022, P = 0.882). Coated and uncoated dataloggers did not have significantly different responses to degree of exposure to solar radiation (sun or shade) for iButtons alone ($F_{1,55}$ = 0.978, P = 0.327), impermeable models ($F_{1,54}$ = 1.164, P = 0.286), and permeable models ($F_{1,54}$ = 0.633, P = 0.430).

When testing for differences in uncoated and coated iButtons over time, we did not find any significant differences in the mean temperatures recorded by uncoated and coated dataloggers for iButtons alone, iButtons embedded in impermeable models, or iButtons embedded in permeable models placed in sun or shade (Table 3.1, Figures 3.2-3.3). However, there were significant interactions between the effects of treatment (coated or uncoated) and time on temperature for iButtons alone in sun, iButtons alone in shade, and iButtons embedded in permeable models in shade (Table 3.1, Figures 3.2-3.3). For these comparisons, the largest differences were during the warmest part of the day (08:00-16:00); coated dataloggers were warmer than uncoated dataloggers, and mean differences in temperatures during these times ranged up to 1.3°C (Table 3.1, Figures 3.2-3.3). For all comparisons, the differences during the coolest part of the day (16:00-08:00) were much smaller and all were within

the accuracy range of the dataloggers (±1.0°C).

There were no detectable differences in the rates of warming and cooling for coated and uncoated iButtons alone or embedded in physical models (Figures 3.2-3.3). Additionally, there were no significant differences between the ranges of temperatures defined by uncoated and coated dataloggers embedded in pairs of physical models (one impermeable and one permeable, each member of each pair containing either coated or uncoated loggers) in sun ($F_{1,26} = 1.126$, $P = 0.298$; non-significant interaction: $F_{143, 3718} = 0.557$, $P = 1.000$) or shade ($F_{1,26} = 0.028$, $P = 0.867$; non-significant interaction: $F_{143, 3718} = 0.722$, $P = 0.994$).

Effects of plastic coating on datalogger failure rates

We found that coating iButtons with plastic significantly reduced the probability of device failure and data loss ($\chi^2 = 16.107$, df = 1, $P < 0.0001$). During the two field trips when dataloggers were left uncoated, we observed an average failure rate of 8.3% (N = 32 of 387); 7.7% (N = 15 of 194) failed on our first trip, and 8.8% (N = 17 of 193) failed on our second trip. By contrast, when dataloggers were coated during our third trip (N = 200), we did not experience any iButton failure and could download data from all dataloggers.

Discussion

We found that coating Thermochron iButtons with plastic is an affordable ($0.10 USD/datalogger) and reliable method to protect them from moisture damage. This method of waterproofing changes the size and weight of the datalogger only slightly; our technique increased iButton mass by an average of 0.1 g and volume by 16 mm^3 (Figure 3.1). This is substantially less than the additional mass, volume, and cost (18.4 g, 598 mm^3, $25 USD) of the waterproof DS1907 iButton Capsule produced by Dallas Semiconductor. However, unlike the iButton Capsule, plastic tool dip does not protect dataloggers against solvents and pressure, which may be necessary in

some studies.

Waterproofing iButtons using plastic could potentially affect the accuracy of iButtons by biasing temperatures or altering rates of warming and cooling. Although we found that the plastic coating had statistically significant effects on the accuracy of temperature readings of iButtons, these effects were relatively small (Table 3.1, Figure 3.2); the majority of average temperature differences we found were ≤ 0.7°C (Table 3.1), which is less than the manufacturer-specified accuracy of 1.0°C. We did not find any indication that coating iButtons altered rates of warming and cooling under field conditions (Figure 3.2). We found that the greatest differences between uncoated and coated dataloggers were for iButtons alone (i.e., not in physical models) placed in direct sunlight; here we found that the average difference during the warmest part of the day was 1.3°C (Table 3.1, Figure 3.2). This is likely explained by small effects of the coating on rates of radiation and conduction of heat. This has implications for studies in which dataloggers may be exposed to direct sunlight, such as those investigating the thermal ecology of basking turtles in which dataloggers are attached to turtle carapaces (e.g., Grayson and Dorcas 2004, Greaves and Litzgus 2007). Researchers should be aware that temperatures may be artificially elevated in these situations; however, a 1.3°C difference may be acceptable depending upon the objectives of the study. For example, the data may still be useful in determining relative patterns of thermoregulation and activity.

We also found that coating iButtons with plastic is an acceptable method of waterproofing when dataloggers are embedded in agar physical models (Schwarzkopf and Alford 1996), such as those used to estimate amphibian body temperatures (Rowley and Alford 2010). We compared the accuracy of uncoated and coated dataloggers within two types of models (i.e., permeable and impermeable to water loss) that define the upper and lower boundaries of possible body temperatures for amphibians (Rowley and Alford 2010). Nearly all temperature differences averaged during the warmest part of the day (08:00-16:00) and averaged over the entire 24-h

period were within the level of accuracy of the dataloggers (i.e., 1.0°C; Figure 3.3). We also did not find any indication that coated iButtons in physical models had significantly different rates of warming and cooling (Figure 3.3) or ranges of temperatures (i.e., impermeable temperature minus permeable temperature) defined by uncoated and coated dataloggers within pairs of physical models placed in sun or shade.

We found that coating iButtons with plastic successfully reduced the likelihood of device failure and data loss under field conditions. We experienced a total failure rate of 8.3% of uncoated dataloggers during our fieldwork, which is within the range of failure rates reported in other studies (Johnson et al. 2005, Wolaver and Sharp 2007, Lovegrove and Génin 2008). However, after waterproofing, we did not experience any iButton failure during our fieldwork and we were able to download data from all dataloggers. We used the same iButtons during all trips, which could mean that iButtons that were more prone to water damage failed during the first two trips, when iButtons were left uncoated; however, approximately the same percentage of iButtons failed during the first and second trip (each lasting three weeks), suggesting that this does not explain our results. Although most studies that reported failed iButtons have involved placing them in moist areas (Johnson et al. 2005, Wolaver and Sharp 2007, Lovegrove and Génin 2008), failure also occurs in areas that are usually dry, presumably during periods of heavy rainfall (e.g., 9.2% failure rate for iButtons [N = 94 of 1020] placed beneath rocks for 100 days, D. A. Pike, University of Sydney, unpublished).

We conclude from our experiments and field trials that coating Thermochron iButtons with plastic is an affordable and reliable method of waterproofing dataloggers to prevent device failure and loss of thermal data. When iButtons are not placed alone in direct sunlight, the effects of the plastic coating on temperature readings should be minimal. However, using coloured plastic dip (e.g., Robert and Thompson 2003, Grayson and Dorcas 2004) or applying multiple coats of plastic may magnify these effects. Our results apply only to Thermochron iButtons and Plasti Dip or similar plastic

coatings; other temperature dataloggers and waterproofing materials should be tested prior to use to assess possible effects on the accuracy of thermal measurements and effectiveness against moisture damage.

Table 3.1. Results from separate repeated-measures ANOVAs comparing uncoated and coated (with plastic tool dip) Thermochron iButtons under sunny and shady conditions; this includes iButtons alone and iButtons that were embedded in two types of physical models (impermeable and permeable to water loss) used together to estimate amphibian body temperatures (see methods for details). Also shown are the average differences in temperature during the entire 24 h and only during the warmest part of the day (08:00-16:00) for both types of dataloggers within each treatment group. Mean signed differences (coated – uncoated) indicate the average temperature differences between coated and uncoated iButtons, and mean unsigned differences (i.e., absolute; |coated – uncoated|) show the average magnitude of the differences between iButton types. Note that sample sizes differ because four iButtons in physical models failed, and one iButton did not program properly. Bold typeface indicates significant results.

Comparison	Total N	Main effect (coated/uncoated)		Interaction (time × temperature)		Mean difference during 24 h (°C) ± SD		Mean difference during warmest part of day (°C) ± SD	
		F	P	F	P	Signed	Unsigned	Signed	Unsigned
Sun									
iButtons alone	30	0.577	0.454	5.154	**<0.0001**	0.2 ± 0.97	0.7 ± 0.64	1.3 ± 0.85	1.3 ± 0.78
Impermeable models	28	0.035	0.853	0.505	1.000	0.0 ± 0.43	0.3 ± 0.33	0.0 ± 0.62	0.5 ± 0.34
Permeable models	30	0.369	0.548	1.183	0.070	0.1 ± 0.34	0.3 ± 0.25	0.5 ± 0.23	0.5 ± 0.20
Shade									
iButtons alone	29	0.345	0.562	8.246	**<0.0001**	-0.1 ± 0.68	0.6 ± 0.39	0.7 ± 0.62	0.7 ± 0.59
Impermeable models	30	3.134	0.088	0.185	1.000	0.2 ± 0.11	0.2 ± 0.10	0.2 ± 0.17	0.3 ± 0.15
Permeable models	28	0.216	0.646	1.622	**<0.0001**	-0.1 ± 0.49	0.4 ± 0.34	0.0 ± 0.77	0.7 ± 0.31

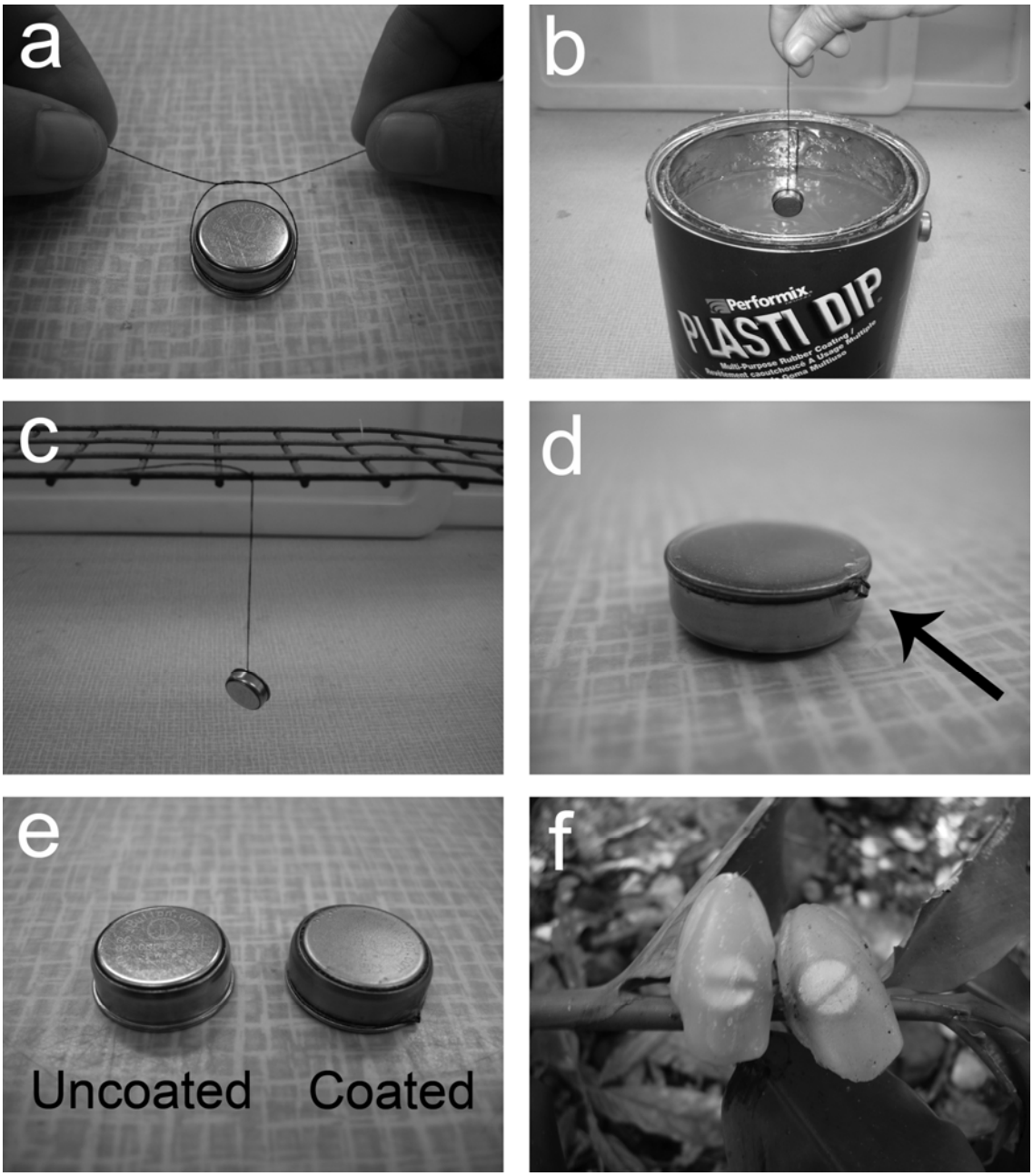

Figure 3.1. The process of coating a Thermochron iButton with plastic tool dip: (a) tying a length of cotton thread around the datalogger, (b) dipping the datalogger into a can of plastic tool dip, (c) suspending the datalogger until the plastic has set, and (d) cutting the cotton thread and sealing the hole with plastic. Also shown are (e) a comparison of an uncoated datalogger and one coated with clear plastic, and (f) the frog physical models used during the field trials.

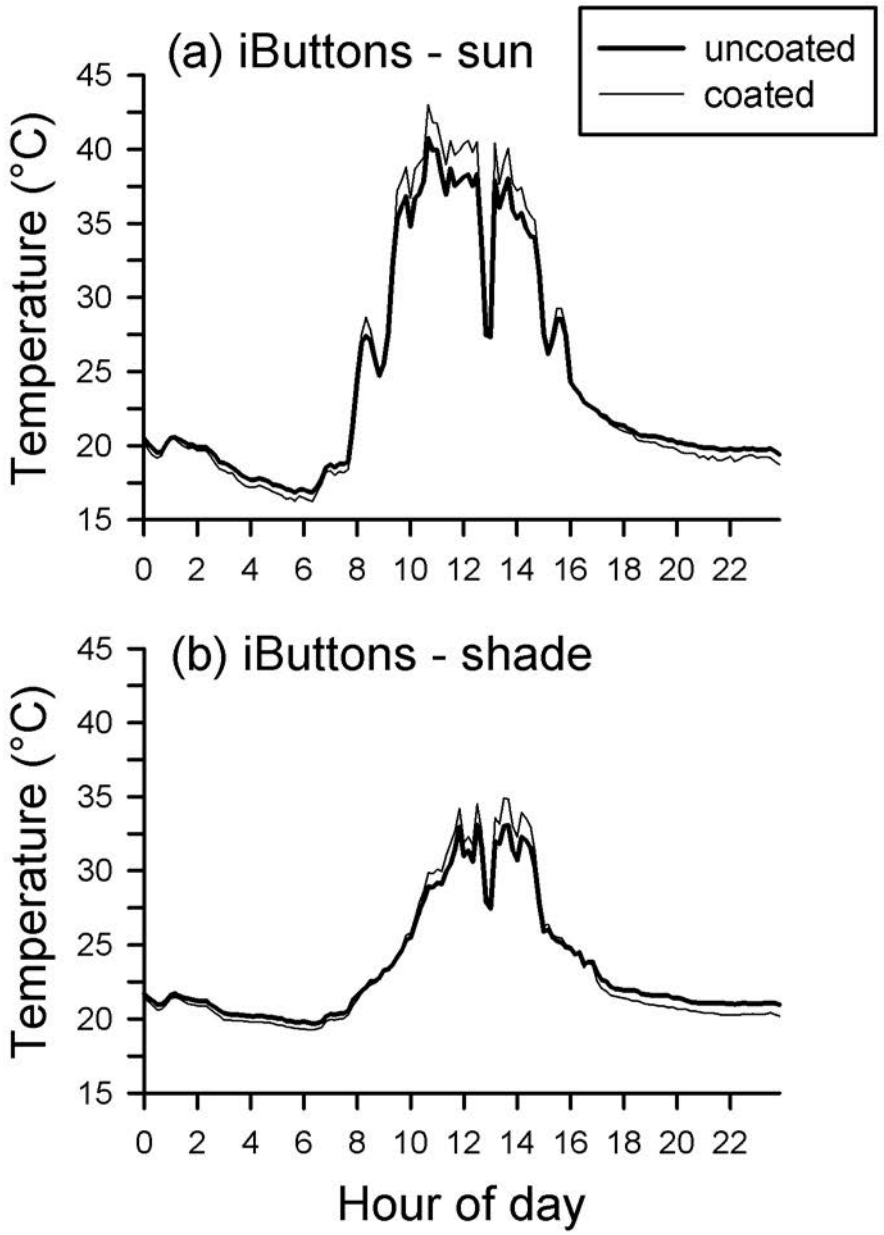

Figure 3.2. Mean temperatures of uncoated and coated (with plastic tool dip) Thermochron iButtons (N = approximately 15 per group) that were placed in sunny and shady conditions for 24 h. For clarity, error bars are not shown.

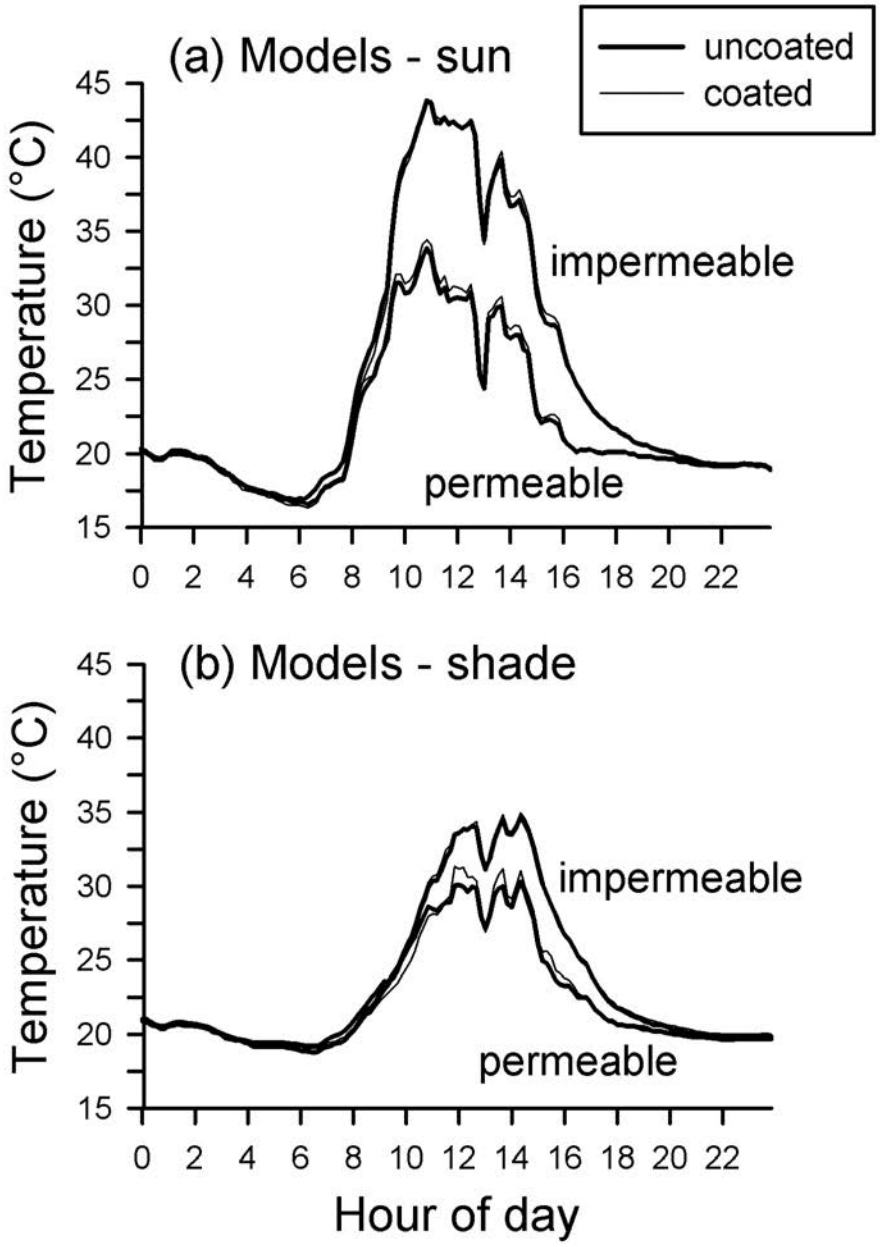

Figure 3.3. Mean temperatures of uncoated and coated (with plastic tool dip) Thermochron iButtons (N = approximately 15 per group) that were embedded in two types of physical models (impermeable and permeable to water loss) used together to estimate amphibian body temperatures (see methods for details) and placed in sunny and shady conditions for 24 h. For clarity, error bars are not shown. Note that not all curves are visible due to the small differences between them.

Chapter 4: Seasonal ecology and behaviour of an endangered rainforest frog threatened by disease

Elizabeth A. Roznik and Ross A. Alford

Abstract

One of the most devastating wildlife diseases ever recorded is chytridiomycosis, a recently emerged disease of amphibians that is caused by the chytrid fungus *Batrachochytrium dendrobatidis*, and has caused severe amphibian declines in many regions of the world. Because this pathogen is transmitted through water and its growth rates are thermally sensitive, environmental conditions and amphibian behaviour are two major factors that influence the vulnerability of amphibians to this pathogen. Therefore, detailed information on the ecology and behaviour of a species is necessary to understand, predict, and manage the impacts of chytridiomycosis. Little is known about the ecology and behaviour of the common mistfrog (*Litoria rheocola*), an endangered rainforest stream frog that occurs in tropical Australia and has declined due to chytridiomycosis. We tracked *Litoria rheocola* to increase our understanding of the behaviour of this species, and how it varies by season and elevation. We found that seasonal differences in environmental temperatures and frog behaviour render this species most vulnerable to *B. dendrobatidis* during cooler months and at higher elevations. During the winter (cool/dry season), frogs moved shorter distances than during the summer (warm/wet season), and they spent less time in vegetation, and more time in the stream, which should increase exposure to aquatic *B. dendrobatidis* zoospores. Frog body temperatures also varied seasonally and by elevation. Low-elevation frogs (40 m ASL) had body temperatures within the optimal range for *B. dendrobatidis* growth (15-25°C) most of the time during winter, but they reached

temperatures above this threshold frequently in summer. At higher elevations (750 m ASL), frogs were within temperatures favourable for *B. dendrobatidis* year-round, and they did not reach body temperatures above 25°C during either season. Our study provides the first detailed information on the ecology and behaviour of *Litoria rheocola* and suggests ecological mechanisms for the patterns of decline and infection dynamics that have been observed in this endangered species.

Introduction

Amphibians have experienced rapid population declines and species extinctions in recent decades, and one-third of extant amphibians are classified as threatened (Stuart et al. 2004). Although there are numerous causes for these losses, including land use change, contaminants, overexploitation, introduced species, and climate change (Collins and Crump 2009, Alford 2010), emerging infectious diseases pose a great threat to global amphibian diversity (Lips et al. 2006, Collins and Crump 2009, Alford 2010). One of the most significant wildlife diseases ever recorded is chytridiomycosis, a recently emerged disease that is caused by the chytrid fungus *Batrachochytrium dendrobatidis* and has caused severe amphibian declines and extinctions in many regions of the world (Kilpatrick et al. 2009). This parasitic fungus attacks the skin cells of amphibians and disrupts their osmoregulatory and transport functions, altering electrolyte concentrations in the blood, which can ultimately cause cardiac arrest if the fungal population on the host reaches a high density (Voyles et al. 2009).

In many regions, amphibians are infected by *B. dendrobatidis* year-round, but they are most vulnerable during cooler months and at higher elevations (Woodhams and Alford 2005, Kriger and Hero 2007, Phillott et al. 2013, Sapsford et al. 2013). This reflects the strong dependence of *B. dendrobatidis* on environmental conditions; this pathogen is highly sensitive to desiccation (Johnson et al. 2003) and its growth and survival rates are strongly influenced by temperature (15-25°C is optimal, >28°C is

lethal; Piotrowski al. 2004, Stevenson et al. 2013). Because of these environmental constraints, the prevalence and intensity of infections in amphibians, as well as mortality rates due to chytridiomycosis, often vary seasonally (Berger et al. 2004, Woodhams and Alford 2005, Kriger and Hero 2007, Sapsford et al. 2013). The behaviour of amphibians can also influence their vulnerability to *B. dendrobatidis* by affecting rates of transmission and the buildup of infections on their skin. Even closely related frog species that occur at the same rainforest streams can have very different patterns of movement, habitat use, and social behaviour, all of which can influence susceptibility to *B. dendrobatidis* (Rowley and Alford 2007a,b). Because aquatic fungal zoospores are transmitted by contact with infected individuals or with contaminated water (Rachowicz and Vredenburg 2004), species that form aggregations or spend more time in water are more likely to be exposed to *B. dendrobatidis* (Rowley and Alford 2007a, Venesky et al. 2011). Additionally, species and individuals that use microclimates that are cooler (<25°C), and thus more favourable to *B. dendrobatidis* growth and survival, are more likely to develop and maintain infections once they are exposed to the fungus (Rowley and Alford 2013).

Although amphibians are one of the most threatened groups of vertebrates, their conservation is often hindered by a lack of basic ecological knowledge. This is particularly true for tropical stream-breeding species, which have experienced more numerous and severe declines than any other amphibian taxa (Williams and Hero 1998, Lips et al. 2003, Stuart et al. 2004). Many of these species occur in remote, montane areas that are difficult to access, and consequently, little is known about their ecology and behaviour. One such species is the common mistfrog (*Litoria rheocola*), an Endangered species (IUCN 2013) that occurs near rocky, fast-flowing rainforest streams in northeastern Queensland, Australia (Hoskin and Hero 2008, Dennis 2012). *Litoria rheocola* is a small treefrog (average male body size: 2.0 g, 31 mm; average female body size: 3.1 g, 36 mm; McDonald and Alford 1999). By the mid-1990s, chytridiomycosis had extirpated this species at higher elevations (>400 m ASL)

throughout its geographic range (Richards et al.1993, McDonald and Alford 1999); however, many populations have subsequently recovered or recolonised these areas (McDonald et al. 2005) and are currently persisting with the pathogen (Sapsford 2012). Habitat modification and fragmentation also threaten *Litoria rheocola* (Hoskin and Goosem 2010, Dennis 2012). Approximately 20% of historical tropical rainforest in northeastern Queensland was cleared by 1983; this was most extensive at low elevations (<80 m ASL), where over 50% was cleared (Winter et al. 1987). Although most remaining rainforest is now protected, small-scale clearing still occurs in non-protected areas (Department of Environment and Resource Management 2010).

Very little is known about the ecology and behaviour of *Litoria rheocola*. Current knowledge is based only on observations of individuals during nocturnal stream surveys (Hodgkison and Hero 2002) and in field enclosures (Retallick 2002). We used harmonic direction finding (Langkilde and Alford 2002, Rowley and Alford 2007d) to track individual *L. rheocola* and study patterns of movement, microhabitat use, and body temperatures. The goal of our study was to understand the behaviour of *L. rheocola*, and how it is affected by season and elevation. This study provides the first detailed information on the ecology and behaviour of *L. rheocola*, and provides background for the formulation of hypotheses on how the environment and behaviour of this endangered species may affect its vulnerability to chytridiomycosis.

Methods

Study sites

We conducted our study at two rainforest streams that differed substantially in elevation; Frenchman Creek (40 m ASL; 17.307°S, 145.922°E) and Windin Creek (750 m ASL; 17.365°S, 145.717°E) are both located in Wooroonooran National Park, Queensland, Australia. Both streams are surrounded by tropical rainforest, characterised by dense vegetation, including large trees (10 m in height), vines, epiphytes, shrubs, and herbaceous plants. These streams vary in width (5-10 m),

contain pools, runs, riffles, and waterfalls, and the streambeds are made of rocks that range in size from small pebbles to large boulders (10 m in diameter). At each site, we captured and tracked frogs along a 400-m section of stream over a three-week period during the winter (cool/dry season) in 2009 (Frenchman Creek: 13 July – 6 August; Windin Creek: 18 August – 9 September), and the summer (warm/wet season) in 2010 (Frenchman Creek: 20 January – 9 February; Windin Creek: 11 February – 3 March).

Tracking

As part of a separate study, we tracked frogs that were infected and uninfected by *B. dendrobatidis*, but the present study focuses on the natural behaviour of uninfected frogs only because infection by *B. dendrobatidis* may alter amphibian behaviour (Parris et al. 2006, Venesky et al. 2009, Han et al. 2011, Chapters 5-7). To prevent disease transmission between frogs during handling, each frog was captured in an unused plastic bag worn as a glove, and was handled only while wearing disposable gloves. Each frog was tested for the presence of *B. dendrobatidis* at first capture, and a second sample was taken at the end of the study period if the frog was recaptured at that time. We swabbed the ventral surface and all four feet of each frog with a sterile rayon swab, covering these areas twice. These samples were analysed using real-time quantitative PCR assays (Boyle et al. 2004). Any frogs that tested positive for the pathogen at the start and/or end of our study period were omitted from all analyses.

We tracked a total of 76 frogs that were uninfected by *B. dendrobatidis*; we tracked 42 during winter (Frenchman Creek: N = 30, Windin Creek: N = 12) and 34 during summer (Frenchman Creek: N = 20, Windin Creek: N = 14). Following capture, we recorded each frog's sex (using presence/absence of distinct nuptial pads), body mass, and snout-urostyle length. We included both males and females in all analyses because females only represented a small proportion of the individuals that we tracked (24%, N = 7), and were not overrepresented in any tracking period (maximum of N = 3

per tracking period); therefore, any gender effects should not be confounded with treatment effects.

After each frog was processed following capture, it was immediately fitted with an external tracking device. Because *L. rheocola* are too small to carry radiotransmitters, we tracked frogs using harmonic direction finding (Langkilde and Alford 2002, Rowley and Alford 2007d). We built tracking devices using SOT-323 surface-mount zero-bias Schottky detector diodes (Agilent Technologies, Forest Hill, VIC, Australia) attached to a belt made of silicone tubing (Gourret et al. 2011). The tubing was cut to length so it just encircled the frog's inguinal region (waist), and a length of cotton thread was passed through the tubing and tied to secure the tubing. The combined mass of the tracking device and belt never exceeded 8% of the frog's body mass, which is below the recommended maximum 10% transmitter-to-body-mass ratio for amphibians (Richards et al. 1994). We excluded all data collected during the 24-hr period following attachment of tracking devices due to potential short-term behavioural effects of handling, which are unlikely to persist after the first night of tag attachment (Langkilde and Alford 2002, Rowley and Alford 2007d).

We tracked frogs using RECCO detectors (models R4 and R8, RECCO Avalanche Rescue System, Lidingö, Sweden). These hand-held devices act as both transmitters and receivers; they emit a continuous signal that is absorbed and re-emitted at a higher frequency by the diodes. We attempted to locate frogs once during each day (1000 hr -1700 hr) and once each night (2000 hr - 0300 hr) throughout the tracking period. To do this, we walked slowly along the centre and edges of the stream and used the detector to scan all areas potentially used by frogs, including rocks in the stream and vegetation along the stream edge. At the end of the tracking period, we removed the tracking devices from all recaptured frogs. Using harmonic direction finding to locate animals is not as effective as radiotelemetry; the tracking detector typically has a maximum detection range of 15 m at rainforest streams and cannot penetrate rock (Rowley and Alford 2007d). However, this was unlikely to cause a bias

toward shorter movements in our study; *Litoria rheocola* has strong site fidelity and when a frog was not found on a particular survey (or surveys), it was almost always found less than 2 m from its most recent known location. This suggests that frogs were moving short distances and sheltering beneath rocks when we could not detect them. Because we were not able to locate all frogs on all surveys, sample sizes vary among analyses, based on available data.

Movements

We examined frog movements at two scales: daily displacement of frogs, and total displacement of frogs over the three-week study period. We marked all frog locations using flagging tape, and recorded their distances along our 400-m stream transect. Each time we located a frog, we measured the frog's height above the stream, and its horizontal distance from the stream edge. To investigate the daily movements of frogs, we measured the distances moved by frogs between consecutive locations; this includes movement from each nocturnal perch site to the subsequent diurnal shelter site, and from each diurnal shelter site to the subsequent nocturnal perch site. *Litoria rheocola* is a treefrog, and individuals move along and at right angles to the stream and also climb up and down vegetation; therefore, they use all three dimensions of space, with their directions of movement largely unconstrained in the horizontal plane but largely restricted to movements up and down individual plants in the vertical direction. Because of these movement patterns, we recorded the horizontal and vertical displacement from the previous location separately (to the nearest 0.10 m). Movement distances were calculated only when individuals were located on consecutive surveys (i.e., day to night, or night to day); when frogs were not located on consecutive surveys, movement distances for the time interval concerned were recorded as missing values and were not included in any analyses. We also determined the probability of movement from day or night locations by calculating the movement probability for each frog (number of times each individual frog moved

between consecutive locations divided by the total number of times the frog was located). We used a two-way MANOVA to analyse the distances moved by individual frogs between day and night locations, and the probability of moving from these locations. We used season (winter or summer) and site (Frenchman Creek or Windin Creek) as independent variables, and the median horizontal distance between locations, median vertical distance between locations, and movement probability as dependent variables.

We examined longer-term movements of frogs by determining their displacement along the stream during our three-week study period. For each frog, we calculated the difference between the minimum and maximum distances along our stream transect at which the frog was observed. We analysed our data using a two-way ANOVA with season and site as independent variables, and total displacement of each frog as the dependent variable.

We also studied the position of frogs in relation to the stream to determine whether their proximity to the stream varied by season and site. We used a two-way MANOVA to analyse the positions of frogs in relation to the stream. We used season and site as independent variables, and median height above stream, and median horizontal distance from the stream edge as dependent variables. Separate analyses were performed for day and night locations.

Body temperatures

We used physical models (Rowley and Alford 2010, Chapter 2) to estimate frog body temperatures over time. Models were placed in each unique location used by each frog. Frogs typically spent the entire day or night in the same microhabitat; therefore, placing models in the locations in which we observed frogs allowed us to accurately measure temperatures experienced by frogs. We placed models in diurnal locations used by frogs to measure temperatures at 30-min intervals from 07:00 to

18:30, and we placed models in nocturnal locations used by frogs to measure temperatures from 19:00 to 06:30. Thermal models consisted of paired frog models made of 3% agar, each embedded with a Thermochron iButton temperature datalogger (Maxim Integrated Products, California, USA; factory-calibrated and accurate to ±0.5°C). Each datalogger was programmed to record temperatures at 30-min intervals, and was waterproofed with a plastic coating during summer tracking to prevent failure from moisture damage (Chapter 3, Roznik and Alford 2012). Model pairs comprised one model that was permeable to water loss, with the other impermeable (i.e., coated with plastic to prevent water loss), which together can be used to define the upper and lower boundaries of possible amphibian body temperatures at the locations used by frogs (Rowley and Alford 2010). We have shown previously that *L. rheocola* body temperatures are highly correlated with temperatures of permeable models (Chapter 2), so we used temperatures from this one model type only.

We used all thermal data collected for each frog to calculate the proportion of body temperatures that occurred in temperature categories that are relevant to the growth of *B. dendrobatidis*: <15°C, 15-25°C, and >25°C (Piotrowski et al. 2004, Stevenson et al. 2013). Growth of *B. dendrobatidis* in northeastern Queensland is fastest between 15°C and 25°C, and slower outside of this temperature range (Stevenson et al. 2013). Although temperatures above 28°C are lethal to *B. dendrobatidis* (Stevenson et al. 2013), we do not distinguish these readings because very few temperature readings were above 28°C. We used a two-way MANOVA to examine frog body temperatures; we used season (winter or summer) and site (Frenchman Creek or Windin Creek) as independent variables, and the proportion of temperatures in each temperature category as the dependent variables.

Microhabitat use

Each time we located a frog, we recorded the substrate that the ventral surface

of the frog was contacting. We defined four substrate categories: vegetation, rock, leaf litter, and soil/coarse woody debris. We also recorded whether the substrate was wet or dry, and whether the frog was in a sheltered or exposed position. For each frog, we calculated the proportion of locations in each substrate category, the proportion of locations that were wet, and the proportion that were sheltered. We performed separate calculations for day and night locations. To analyse microhabitat data, we used multi-response permutation procedures (MRPP) and Monte Carlo re-sampling with 10,000 iterations (using Blossom statistical software; Cade and Richards 2005). We performed the analysis in a stepwise manner, testing for differences between seasons (winter and summer), time of day (day and night), and sites (Frenchman Creek and Windin Creek), in that order. If a difference was detected between groups, the next analysis was performed on each of those groups separately. For all analyses, the dependent variables were the proportion of locations on a wet substrate, proportion of locations in a sheltered position, and the proportion of locations in each of the four substrate categories described above.

We also measured the relative moisture levels of each microhabitat used by each frog by weighing the permeable physical model (described above) to the nearest 0.1 g immediately before and after placement in each frog location, and calculating the proportion of mass lost due to evaporative water loss (Schwarzkopf and Alford 1996, Rowley and Alford 2010). For each frog, we calculated the median proportion of model mass lost, using all of the models placed in locations used by that frog. Separate calculations were performed for day and night models. We analysed our data using a two-way ANOVA with season (winter or summer) and site (Frenchman Creek or Windin Creek) as independent variables, and the median proportion of model mass lost as the dependent variable. Separate analyses were performed for models corresponding to day and night locations.

Perch-site selection

We studied nocturnal perch-site selection by frogs during winter and summer. Because of obvious seasonal differences in perch sites, we used different approaches for each season. During winter, we measured the following characteristics of vegetation used by frogs at each perch site: distance from the stream edge, distance to the nearest riffle, plant height, area covered by the plant's canopy (average length × width), stream depth, and number of rocks within 1 m. We also measured these same characteristics of vegetation that was available to frogs, but was not used by frogs during our study; we selected the nearest plant to the stream every 10 m along both sides of a 200-m section of stream for measurement. To determine whether frogs selected perch sites that were different from available perch sites, we analysed our data using a two-way MANOVA. We used perch type (used or available) and site (Frenchman Creek or Windin Creek) as independent variables, and the six characteristics described above as dependent variables.

During summer, frogs were often located in taller vegetation, especially trees, than during winter. To determine whether this seasonal difference was statistically significant, we compared the height of vegetation used by frogs during the two seasons. We used a two-way ANOVA with season and site as independent variables, and vegetation height as the dependent variable. To understand whether frogs selected trees that were significantly different from available trees during summer, we measured the following characteristics of all trees used by frogs: tree height, height of the lowest branch, and diameter at breast height (DBH). We measured these same characteristics of trees that were available to frogs, but not used during our study; we selected all trees within 3 m of the stream along one side of a 200-m section of stream for measurement. To determine whether frogs selected trees that were different from available trees, we analysed our data using a two-way MANOVA. We used tree type (used or available) and site as independent variables, and tree height, height of the lowest branch, and DBH as dependent variables.

Results

Movements

Daily movement patterns differed significantly by season (MANOVA, $F_{3,27}$ = 3.192, P = 0.039), but not by site (MANOVA: $F_{3,27}$ = 0.351, P = 0.789), and there was a marginally significant interaction between season and site (MANOVA: $F_{3,27}$ = 3.036, P = 0.046). However, after examining the one-way ANOVAs, the only significant result was that frogs showed a seasonal difference in horizontal movements between day and night locations ($F_{1,29}$ = 6.980, P = 0.013), although the seasonal difference in vertical movements between day and night locations was nearly significant ($F_{1,29}$ = 3.619, P = 0.067). The overall pattern was that frogs moved significantly longer horizontal distances between diurnal shelter sites and nocturnal perch sites during summer than in winter at both sites (Figure 4.1). During summer, frogs moved longer vertical distances at Frenchman Creek, and made slightly shorter vertical movements at Windin Creek (Figure 4.1). The probability of movement did not differ significantly in any of these analyses (all P ≥ 0.129); on average, frogs moved from day or night locations 84% of the time.

The length of stream used by frogs during our three-week study period was influenced significantly by the interaction of season and site ($F_{1,60}$ = 4.919, P = 0.320). At Windin Creek, the length of stream used by frogs during summer was 78.9% longer than that used during winter, but at Frenchman Creek, the length of stream used did not differ significantly between seasons. The average stream length (and range) used by frogs at Windin Creek was 2.0 m (0-16) during winter, and 9.5 m (0-57) during summer, and the mean and range across seasons were 4.0 (0-50) at Frenchman Creek.

The position of frogs in relation to the stream during the day differed significantly by season (MANOVA: $F_{2,35}$ = 35.504, P < 0.001) and site (MANOVA: $F_{2,35}$ = 9.783, P < 0.001), and there was a significant interaction between season and site (MANOVA: $F_{2,35}$ = 10.068, P < 0.001). Frog position during the night also differed

significantly by season (MANOVA: $F_{2,40} = 5.571$, $P = 0.007$), but not by site (MANOVA: $F_{2,40} = 1.134$, $P = 0.332$), and there was a significant interaction between season and site (MANOVA: $F_{2,40} = 5.312$, $P = 0.009$). Examining the one-way ANOVAs made it clear that frog perch sites were higher above the stream during summer than winter, and that the extent of this difference depended upon site; heights were similar at both sites during winter, but tended to be higher at Frenchman Creek than Windin Creek during summer during both the day (season × site: $F_{1,36} = 18.322$, $P < 0.001$; Figure 4.2) and night (season × site: $F_{1,41} = 3.855$, $P = 0.056$; Figure 4.2). Perch sites were higher at night than during the day during winter, but were similar in height during the day and night during summer (Figure 4.2). The horizontal distance from stream only differed significantly between seasons during the night ($F_{1,41} = 6.957$, $P = 0.012$), although there was a trend during the day ($F_{1,36} = 3.489$, $P = 0.070$); frogs were observed farther from the stream during summer than winter, and this pattern did not differ between sites during the day (season × site: $F_{1,36} = 0.211$, $P = 0.649$) or night (season × site: $F_{1,41} = 0.154$, $P = 0.697$; Figure 4.2). On average, frogs were observed 0.10 m (range: 0-1.80 m) from the stream during winter, and 0.74 m (range: 0-3.75 m) from the stream during summer.

Body temperatures

Frog body temperatures were warmer during summer than in winter, and were warmer at Frenchman Creek than at Windin Creek during both seasons (Figures 4.3-4.4). The distribution of frog body temperatures within categories relevant to *B. dendrobatidis* growth (<15°C, 15-25°C, >25°C) differed significantly by season (MANOVA: $F_{3,44} = 7.852$, $P < 0.001$), and site (MANOVA: $F_{3,44} = 5.203$, $P = 0.004$), and there was a significant interaction between season and site (MANOVA: $F_{3,44} = 5.161$, $P = 0.004$; Figures 4.3-4.4). The season × site interaction was significant for the proportion of temperatures that were 15-25°C ($F_{1,46} = 11.938$, $P = 0.001$), and greater than 25°C ($F_{1,46} = 15.317$, $P < 0.001$), but not <15°C ($F_{1,46} = 0.847$, $P = 0.362$). The

proportion of temperatures below 15°C was higher during winter than summer at both sites (Figure 4.3). At Frenchman Creek, frogs spent more time in temperatures optimal for *B. dendrobatidis* growth (15-25°C) during winter than summer, and they reached temperatures above this threshold (>25°C) rarely during winter, but frequently during summer (Figures 4.3-4.4). At Windin Creek, frogs were within temperatures optimal for *B. dendrobatidis* more often during summer than in winter, and they did not reach temperatures above this threshold during either season (Figures 4.3-4.4).

Microhabitat use

Microhabitats used by frogs differed significantly by season ($\delta = 3.102$, $P < 0.001$) and time of day during winter ($\delta = 2.505$, $P < 0.001$) and summer ($\delta = 2.744$, $P < 0.001$; Table 4.1). Microhabitat use was similar between the two sites except during the day in summer ($\delta = 2.514$, $P < 0.001$; all other $P > 0.079$). During winter, frogs typically sheltered between moist rocks in the stream bed during the day, and perched on dry, exposed vegetation at night (Table 4.1). During summer, all substrates used by frogs were wet due to frequent rainfall; during the day, frogs at Frenchman Creek typically used vegetation, and frogs at Windin Creek used vegetation, leaf litter, and rocks, and during the night, frogs at both sites typically perched on vegetation (Table 4.1).

Desiccation rates of diurnal microhabitats used by frogs differed significantly by season ($F_{1,93} = 30.763$, $P < 0.001$) and site ($F_{1,93} = 6.762$, $P = 0.011$), and there was a significant interaction between season and site ($F_{1,93} = 14.277$, $P < 0.001$). Relative moisture levels of nocturnal microhabitats used by frogs differed significantly by season ($F_{1,93} = 30.763$, $P < 0.001$) and site ($F_{1,93} = 6.762$, $P = 0.011$; non-significant interaction term). Microhabitats used by frogs during the night were significantly drier during winter than summer, but diurnal microhabitats were significantly drier during summer than winter. Overall, microhabitats used by frogs during the day and night at Frenchman Creek were significantly drier than those used at Windin Creek.

Perch-site selection

During winter, frogs selected nocturnal perch sites that differed significantly from our sample of available perch sites (MANOVA: $F_{6,171} = 18.082$, $P < 0.001$; Table 4.2). Perch site use also differed significantly between sites (MANOVA: $F_{6,171} = 6.544$, $P < 0.001$), although this difference appears to be driven only by differences in water depth below perch sites (one-way ANOVA: $F_{1,176} = 36.226$, $P < 0.001$; all other $P \geq 0.450$). Additionally, there was a significant interaction between perch type (used or available) and site (MANOVA: $F_{1,172} = 2.313$, $P = 0.036$). The overall pattern was that, when compared to available vegetation, frogs selected vegetation that was significantly taller, had a significantly fuller canopy (i.e., was bushier), and was located significantly closer to riffles and closer to the stream edge (Table 4.2). Selected vegetation was also located along significantly shallower areas of the stream with more rocks (Table 4.2). These patterns were similar between sites; however, the difference between the average height of used and available vegetation was greater at Frenchman Creek than at Windin Creek (perch type × site: $F_{1,176} = 7.918$, $P = 0.005$), and the difference between the average water depth below perch sites was marginally greater at Windin Creek than at Frenchman Creek (perch type × site: $F_{1,176} = 3.656$, $P = 0.057$; Table 4.2).

When we compared the height of vegetation used by frogs during winter and summer, we found a significant interaction between vegetation height and site ($F_{1,269} = 11.299$, $P = 0.001$). At Frenchman Creek, frogs used vegetation that was 1.3 m taller, on average, during summer than during winter, but at Windin Creek, average vegetation height was similar between the seasons (0.2-m difference). During summer, frogs often used trees as perch sites; the trees used by frogs were significantly different from available trees (MANOVA: $F_{3,318} = 6.234$, $P = 0.0004$). Tree characteristics also differed significantly by site (MANOVA: $F_{3,318} = 12.006$, $P < 0.001$); trees were taller ($F_{1.320} = 19.334$, $P < 0.001$) and larger in DBH ($F_{1.320} = 4.006$, $P < 0.046$) at Frenchman Creek than at Windin Creek. There was also a significant

interaction between tree type (used or available) and site (MANOVA: $F_{3,318} = 6.888$, $P = 0.0002$). Frogs at Frenchman Creek selected trees that were significantly shorter than available trees (means: 5.8 m, and 9.2 m, respectively), whereas frogs at Windin Creek did not select trees that were significantly different in height than available trees (mean: 3.2 m; tree type × site: $F_{1.320} = 5.466$, $P = 0.020$). At both sites, frogs selected trees with significantly lower branches than available trees ($F_{1.320} = 18.633$, $P < 0.001$); the average height of the lowest branch of used and available trees was 2.3 and 5.0 m, respectively, at Frenchman Creek, and 2.1 and 6.9 m, respectively, at Windin Creek. Frogs did not select trees that differed significantly in DBH ($F_{1.320} = 3.084$, $P = 0.080$) from available trees; on average, the DBH of used trees was 9.5 cm at Frenchman Creek, and 3.2 cm at Windin Creek.

Discussion

Our study provides the first detailed information on the ecology and behaviour of the common mistfrog (*Litoria rheocola*), providing background information for the formulation of hypotheses on how the environment and behaviour of this Endangered species (IUCN 2013) may affect its vulnerability to chytridiomycosis. Overall, we found that *L. rheocola* are relatively sedentary frogs that are restricted to the stream environment, and prefer sections of the stream with riffles, numerous rocks, and overhanging vegetation (Table 4.2). Our study sites spanned a relatively wide elevational range, and despite large differences in environmental temperatures that varied with elevation (Figure 4.4), frog behaviour was remarkably similar between low- and high-elevation streams. This suggests that the recovery of high-elevation populations was not facilitated by behavioural changes in frogs, but by other ecological processes. This may include changes in climate or evolutionary changes in the host and/or pathogen, such as increased host immunity or decreased virulence of the pathogen.

Although *Litoria rheocola* was active year-round, its behaviour varied

substantially between seasons. During summer, frogs moved longer distances between diurnal shelter sites and nocturnal perch sites (Figure 4.1) and spent more time away from the stream (Figure 4.2) than during winter. Frogs typically perched on vegetation during the day and night during summer, but in winter, frogs usually sheltered between wet rocks in the stream during the day, and climbed into vegetation above the stream at night (Table 4.1). Retallick (2002) also found that juvenile and adult *L. rheocola* in field enclosures altered their behaviour by season in similar ways; frogs used elevated perches more often in summer, and aquatic microhabitats more often during winter. Additionally, Hodgkison and Hero (2002) observed more *L. rheocola* at the stream during warmer months, suggesting that during that period frogs used perch sites that were more exposed and elevated than those used during cooler months, when frogs were seen less frequently.

Seasonal differences in frog behaviour should cause levels of exposure to *B. dendrobatidis* to be higher in winter than summer because frogs spent more time in the stream in winter. Infectious *B. dendrobatidis* zoospores are aquatic and are transmitted to frogs by contact with water (Rachowicz and Vredenburg 2004); therefore, the high frequency of contact between frogs and stream water during winter should lead to relatively high rates of pathogen transmission if there are similar numbers of zoospores present in the stream throughout the year. By contrast, frogs spent much time in vegetation above the stream during summer, and thus should be less exposed to the pathogen during the summer season. The sedentary behaviour of *L. rheocola* also may increase the vulnerability of this species to chytridiomycosis, particularly during winter, when movements are reduced. Because pathogens can accumulate in an animal's environment over time, less mobile animals are often more likely to be infected and have higher infection levels (Foldstad et al. 1991, Ezenwa 2004, Altizer et al. 2011). Infected frogs and tadpoles release zoospores, which can survive in environmental reservoirs; they can survive in lake water for up to seven weeks (Johnson and Speare 2003), and for up to three months in sterile moist river sand

without nutrients (Johnson and Speare 2005). Zoospores released from an infected frog that remains within a very restricted area could build up in on its skin, or in its microhabitat, and re-infect the frog, thereby maintaining or increasing its fungal load (Briggs et al. 2010).

Once a frog is exposed to *B. dendrobatidis* zoospores, the environmental conditions and thermoregulatory behaviour of the frog play a major role in determining whether it develops and maintains an infection. Growth rates of *B. dendrobatidis* are strongly influenced by temperature and moisture; the fungus is highly sensitive to desiccation (Johnson et al. 2003), and growth rates are optimal within 15-25°C, but slow dramatically above 25°C (Piotrowski al. 2004, Stevenson et al. 2013). We found that seasonal differences in environmental temperatures and *L. rheocola* body temperatures should cause this species to be more likely to develop and maintain *B. dendrobatidis* infections during cooler months and at higher elevations (Figure 4.3-4.4); these differences correlate with observed patterns of infection prevalence in this species (Sapsford 2012, Sapsford et al. 2013). At our low-elevation stream, the body temperatures of frogs should cause them to be more likely to develop infections during winter than summer. Although some low-elevation frogs occasionally attained body temperatures above 25°C during winter, most frogs regularly reached temperatures above this threshold during summer. High-elevation frogs should be more vulnerable to *B. dendrobatidis* than frogs at the low elevation because their temperatures were largely within the optimal range for pathogen growth (15-25°C) year-round.

Detailed ecological studies are necessary to understand and conserve endangered species. Even closely related frog species that occur at the same rainforest streams can have very different patterns of movement and microhabitat use, and can also differ considerably in their vulnerability to disease-related declines (Rowley and Alford 2007a,b, Chapters 5-6). For species threatened by *B. dendrobatidis*, it is important to study the behaviour of uninfected individuals because this pathogen may alter the behaviour of amphibians (Parris et al. 2006, Venesky et al.

2009, Han et al. 2011, Chapters 5-7). Our study provides detailed information on the movements, microhabitat use, and body temperatures of uninfected *L. rheocola*, and reveals how these behaviours differ by season and elevation. Seasonal differences in environmental conditions and frog behaviour cause this species to be most vulnerable to *B. dendrobatidis* during cooler months and at higher elevations, providing an ecological mechanism for observed patterns of infection dynamics (Sapsford 2012, Sapsford et al. 2013). As with many stream-breeding frog species, females and juveniles are rarely observed because they spend more time away from the stream (Rowley and Alford 2007b); therefore, further study is necessary to understand behavioural differences between sexes and life stages, and the implications for disease risk.

Table 4.1. Characteristics of microhabitats used by common mistfrogs (*Litoria rheocola*) during the day and night at two rainforest streams (Frenchman Creek and Windin Creek) during the winter (cool/dry season) and summer (warm/wet season). Shown are the mean percentages (and ranges) of microhabitats that were wet, sheltered, and characterised by vegetation, rock, leaf litter, and soil/coarse woody debris (wood). Means were calculated using the median value for each individual frog. Only categories that are significantly different from each other ($P < 0.05$) are shown.

Characteristic	Winter		Summer		
	Both sites		Frenchman Creek	Windin Creek	Both sites
	Day	Night	Day	Day	Night
Wet	86.4 (0-100)	9.8 (0-80.0)	100 (100-100)	100 (100-100)	100 (100-100)
Sheltered	86.5 (0-100)	11.0 (0-100)	10.0 (0-50.0)	42.1 (0-100)	1.4 (0-25.0)
Vegetation	7.9 (0-66.7)	87.0 (0-100)	89.2 (50.0-100)	35.4 (0-100)	96.0 (75.0-100)
Rock	67.9 (0-100)	11.8 (0-100)	0 (0-0)	25.2 (0-100)	1.7 (0-25.0)
Leaf litter	22.8 (0-100)	1.3 (0-25.0)	9.3 (0-50.0)	34.7 (0-100)	0.6 (0-12.5)
Soil/wood	1.4 (0-33.3)	0 (0-0)	1.5 (0-14.3)	4.6 (0-33.3)	1.7 (0-25.0)

Table 4.2. Results of separate one-way ANOVAs comparing nocturnal perch sites that were available to and used by common mistfrogs (*Litoria rheocola*) at two rainforest streams (Frenchman Creek and Windin Creek) during winter (cool/dry season). The mean value (and range) is shown for each perch site characteristic at each site.

Characteristic	$F_{1,176}$	P	Frenchman Creek		Windin Creek	
			Used	Available	Used	Available
Distance from riffle (m)	36.076	<0.001	0 (0-0)	4.5 (0-30.0)	0.4 (0-10.0)	4.3 (0-20.0)
Distance from stream (m)	7.618	0.006	0.8 (0-4.3)	1.3 (0-3.6)	0.9 (0-3.1)	1.0 (0-2.7)
Plant height (m)	16.361	<0.001	2.6 (0.6-6.0)	1.6 (0.5-3.5)	2.1 (0.5-4.0)	1.9 (0.3-3.3)
Canopy area (m^2)	6.749	0.010	4.0 (0.1-40.0)	1.7 (0.1-11.2)	3.4 (0.1-12.0)	2.4 (0.3-14.3)
Water depth (m)	9.242	0.003	0.2 (0.1-0.3)	0.2 (0.1-0.5)	0.1 (0.1-0.4)	0.1 (0.1-0.4)
Number of rocks	54.579	<0.001	21.0 (0-55)	7.7 (0-32)	19.8 (0-55)	8.5 (0-25)

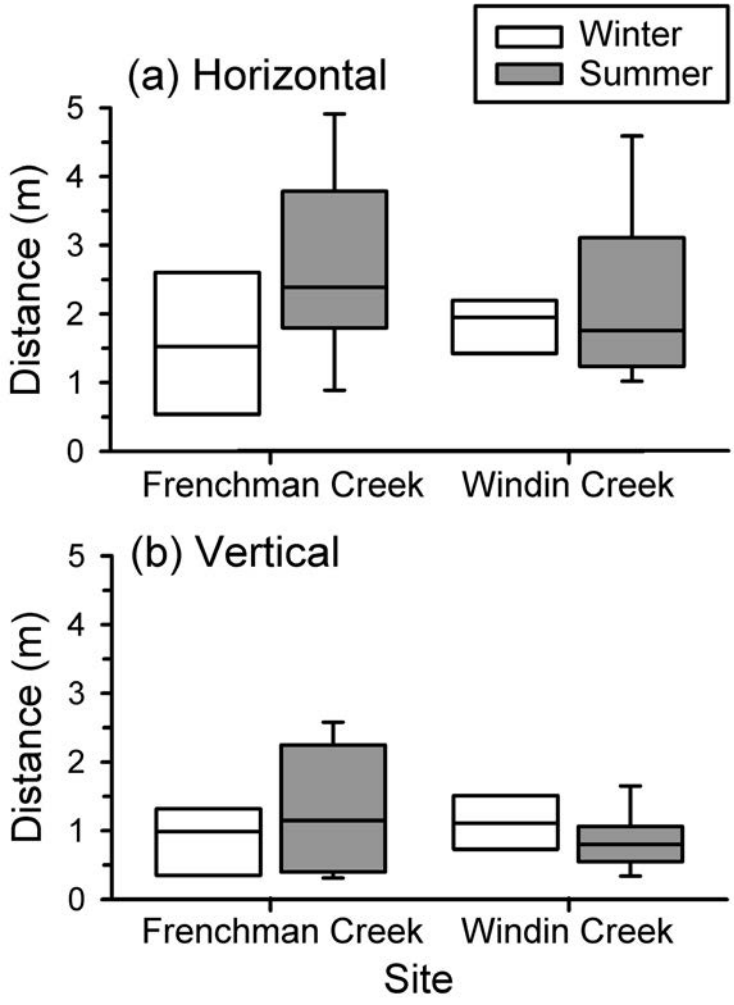

Figure 4.1. Box plots of (a) horizontal and (b) vertical distances moved by common mistfrogs (*Litoria rheocola*) between day and night locations at two rainforest streams (Frenchman Creek and Windin Creek) during winter (cool/dry season) and summer (warm/wet season).

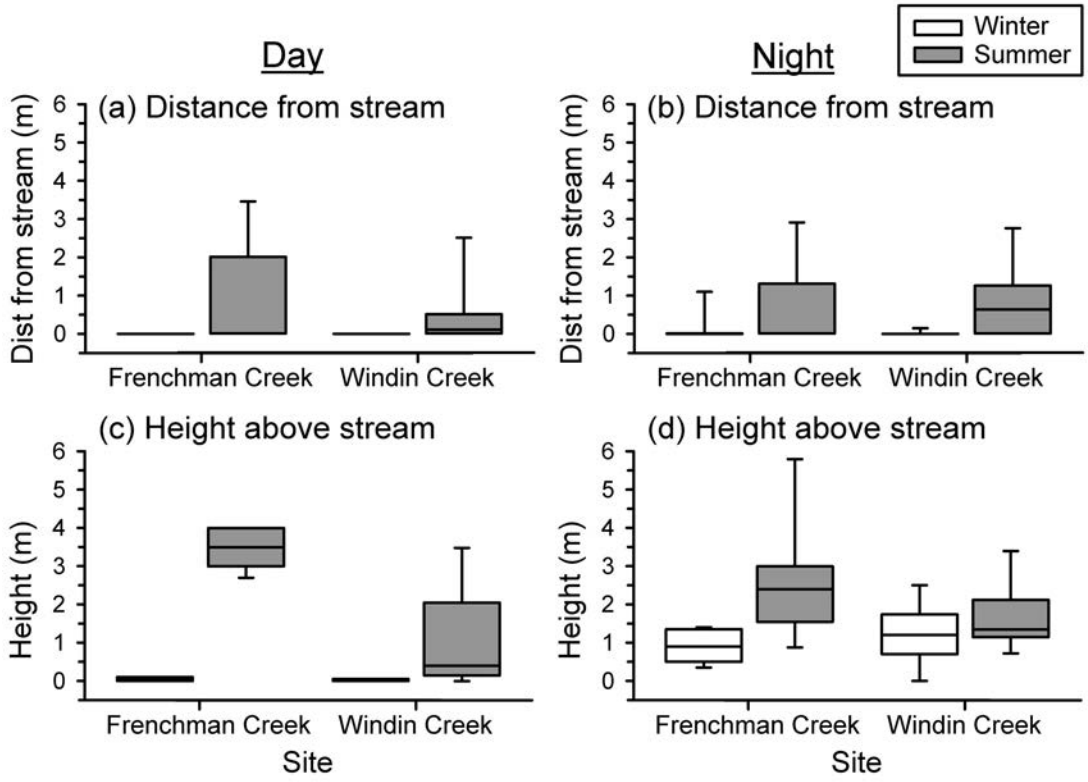

Figure 4.2. Box plots of (a-b) horizontal distances from the stream edge, and (c-d) vertical heights above the stream where common mistfrogs (*Litoria rheocola*) were located during the day and night at two rainforest streams (Frenchman Creek and Windin Creek) during the winter (cool/dry season) and summer (warm/wet season).

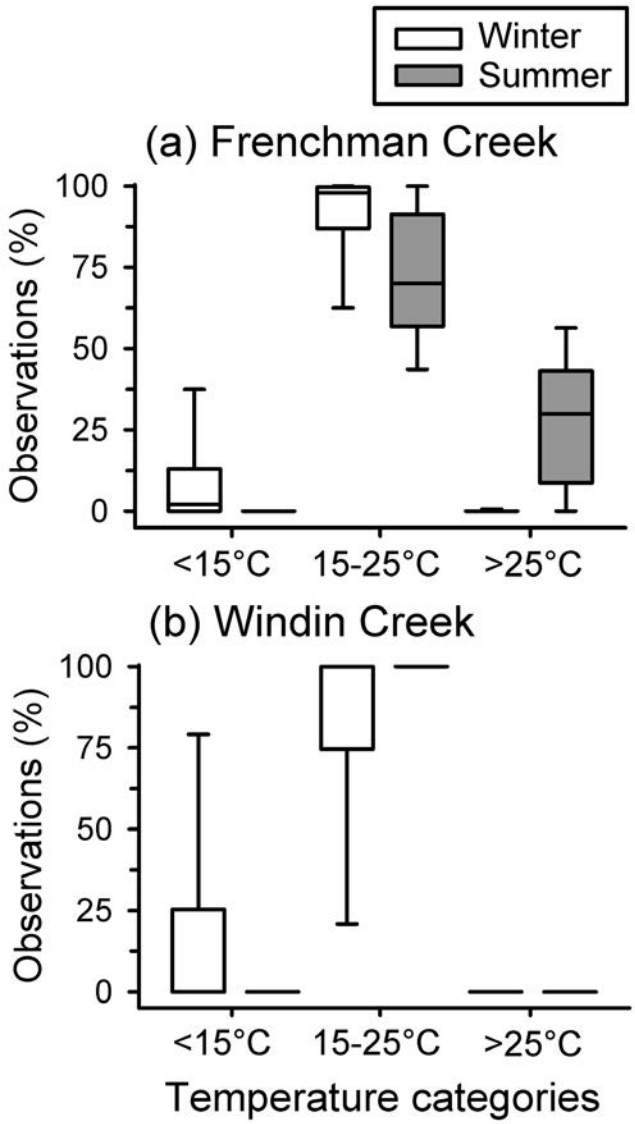

Figure 4.3. Distribution of the body temperatures of common mistfrogs (*Litoria rheocola*) within categories relevant to *Batrachochytrium dendrobatidis* growth (<15°C, 15-25°C, >25°C) at two rainforest streams (Frenchman Creek and Windin Creek) during winter (cool/dry season) and summer (warm/wet season).

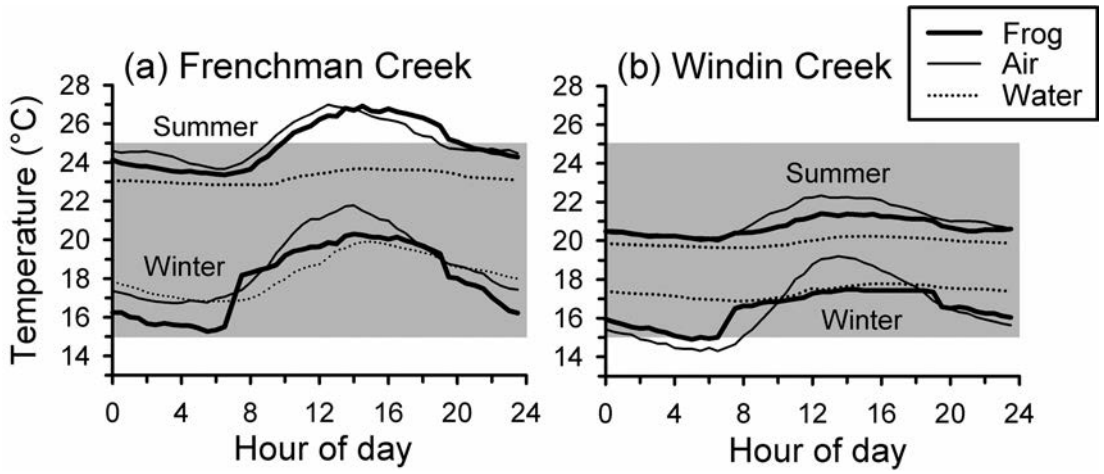

Figure 4.4. Mean body temperatures of common mistfrogs (*Litoria rheocola*) over the 24-hr diel period at two rainforest streams (Frenchman Creek and Windin Creek) during winter (cool/dry season) and summer (warm/wet season). Also shown are the mean ambient air and water temperatures at each site during each season. The optimal thermal range for *Batrachochytrium dendrobatidis* growth (15-25°C) is shaded.

Chapter 5: Individual behaviour influences infection risk: frogs and the chytrid fungus *Batrachochytrium dendrobatidis*

Elizabeth A. Roznik and Ross A. Alford

Abstract

Environmental variation can have significant effects on host-pathogen interactions, but the mechanisms linking environmental conditions to infection dynamics are often poorly understood. The pathogenic fungus *Batrachochytrium dendrobatidis*, which has caused amphibian declines and extinctions in many regions, is highly sensitive to temperature and moisture. However, little is known about how the thermal and hydric conditions selected by individual frogs in nature influence their risk of infection by *B. dendrobatidis*. We tracked infected and uninfected individuals of three species of rainforest stream frogs (*Litoria nannotis, L. rheocola,* and *L. serrata*), recorded their body temperatures semi-continuously, and measured relative desiccation rates at their selected locations. Our study demonstrates that the body temperatures and desiccation rates of individual frogs in nature are related to their infection status. In each of our three study species, the probability of infection increased as rates of desiccation at diurnal and nocturnal locations decreased, indicating that frogs that chose wetter locations were more likely to be infected. Temperature relations were more complex; on average, infected frogs had cooler body temperatures than uninfected frogs in *Litoria rheocola* and *L. serrata*. However, in *L. nannotis*, infected frogs had more stable and moderate body temperatures than uninfected frogs. Infection probability decreased with increasing frequency of body temperatures above

25°C in *L. serrata*, and the proportion of body temperatures below 16°C was greater in infected *L. rheocola* and *L. serrata*, but greater for uninfected *L. nannotis*. Overall, individual frogs that used cooler, moister microenvironments were more likely to be infected than frogs that experienced warmer, drier conditions. These relationships are likely explained by differences in rates of pathogen transmission, survival, and reproduction associated with these microenvironments. Fully understanding the interactions between individual behaviour and pathogen infection can help explain population-level patterns of infection in nature, and is essential for understanding and ultimately managing this important host-pathogen system.

Introduction

Environmental conditions can strongly influence host-pathogen interactions. The seasonal cycles of many infectious diseases are caused directly or indirectly by changes in temperature, precipitation, and humidity (Dowell 2001, Woodhams and Alford 2005, Altizer et al. 2006, Murray et al. 2013). Despite environmental conditions affecting the biology of both hosts and pathogens, the mechanisms linking environmental conditions to infection dynamics are poorly understood in many host-pathogen systems. Many pathogens are sensitive to temperature and moisture, and small changes in these conditions can have important implications for their growth and survival (Harvell et al. 2002, Murray et al. 2013, Stevenson et al. 2013). Environmental variation can also influence host susceptibility by affecting the immune responses of hosts (Wright and Cooper 1981, Zapata et al. 1992, Carey et al. 1999, Raffel et al. 2006) or their exposure to pathogens through changes in behaviour (Dowell 2001, Altizer et al. 2006, Rowley and Alford 2007a, Chapter 4). Effects of environmental conditions on host-pathogen interactions are especially important for ectothermic hosts because their body temperatures are regulated by ambient temperatures, which can vary daily, seasonally, and geographically (Rowley and Alford 2009).

Environmental conditions strongly influence interactions between amphibians

and the pathogenic fungus *Batrachochytrium dendrobatidis*, which causes the disease chytridiomycosis. This pathogen has caused severe amphibian declines and extinctions in many regions of the world (Kilpatrick et al. 2009). The prevalence and intensity of infections in amphibians, as well as mortality rates due to chytridiomycosis, often vary seasonally. These are typically highest during cooler months and at higher elevations (Woodhams and Alford 2005, Kriger and Hero 2007, Phillott et al. 2013), and in cooler habitats with high levels of forest canopy cover (Puschendorf et al. 2011, Becker et al. 2012). These patterns have been attributed to the strong thermal and hydric sensitivity of *B. dendrobatidis*; this fungus requires relatively cool, moist conditions to survive and reproduce (15-25°C optimal; >28°C lethal; Johnson et al. 2003, Piotrowski et al. 2004, Stevenson et al. 2013). Infection dynamics may also be related to the effects of temperature on host immune responses (Carey et al. 1999, Raffel et al. 2006, Ribas et al. 2009, Rollins-Smith et al. 2011), or to seasonal changes in amphibian behaviour that alter transmission rates (Rowley and Alford 2007a, Chapter 4). For example, transmission rates could be reduced during rainy periods, when amphibians spend less time in contact with bodies of water, which harbour infectious *B. dendrobatidis* zoospores (Chapter 4).

Amphibian species vary considerably in their susceptibility to *B. dendrobatidis*. In nearly all communities where amphibian species have disappeared or declined due to chytridiomycosis, other amphibian species have persisted unaffected (McDonald and Alford 1999, Retallick et al. 2004, Lips et al. 2006). Even in areas where *B. dendrobatidis* is endemic, some species consistently have lower prevalence of infection than other species (Rowley and Alford 2009). Species-specific variation in susceptibility can often be explained by differences in behaviour. The most vulnerable species are highly aquatic (Lips et al. 2003, Rowley and Alford 2007a, Bancroft 2011), have a higher frequency of body temperatures below 25°C (Rowley and Alford 2013), and/or form aggregations with high levels of physical contact between individuals (Rowley and Alford 2007a). These ecological characteristics can explain differences in

rates of pathogen transmission, survival, and reproduction.

The thermoregulatory behaviour of individual frogs within a species also can influence their probability of infection. One study of Panamanian frogs found that at the population level, the mean body temperature of populations of infected frogs was higher than in populations of uninfected frogs, which suggests that infected frogs behaviourally elevated their body temperatures in response to the pathogen ("behavioural fever"; Richards-Zawacki 2009). However, a study of three species of Australian rainforest frogs found that both within and across species, individuals with a higher percentage of body temperatures above 25°C were more likely to be uninfected (Rowley and Alford 2013). This suggests that individuals that chose higher body temperatures for reasons other than infection were less prone to acquire or retain infections than those that did not, unless behavioural fever persists long after infections have been lost. These divergent patterns could both be important in the interactions between individual frog behaviour and *B. dendrobatidis*; it is possible that individuals that choose warm, dry microenvironments are less likely to acquire and maintain infections, and that at some stage of infection buildup, individuals alter their behaviour to seek out warmer or drier conditions. There is a clear need to further explore these relationships. In particular, very little is known about the relationship between infection probability and frog body temperatures below the thermal optimum of *B. dendrobatidis* (15°C; Stevenson et al. 2013), or how water use and desiccation rates of individual frogs are related to infection status and intensity. Contact with water can expose amphibians to aquatic *B. dendrobatidis* zoospores and can also influence their body temperatures and the thermal environment experienced by *B. dendrobatidis* inhabiting their skin.

Fully understanding the interactions between individual amphibian behaviour and *B. dendrobatidis* can help explain population-level patterns of infection in nature, and is essential for understanding and ultimately managing this important host-pathogen system. Because of the importance of temperature and moisture in our host-

pathogen system, we examined both the body temperatures and desiccation rates of individual frogs and their relationships with *B. dendrobatidis* infection status and intensity. We tracked infected and uninfected individuals of three species of rainforest stream frogs. Using semi-continuous measures of frog body temperature, we quantified the proportion of body temperatures above, within, and below the optimal temperature range for *B. dendrobatidis* growth (15-25°C; Piotrowski et al. 2004, Stevenson et al. 2013). We also used physical models to measure the relative desiccation rates experienced by frogs during the day and night. Finally, we modelled the effects of thermal and hydric conditions experienced by individual frogs on *B. dendrobatidis* infection probability, and on the infection intensity of infected frogs.

Methods

Study species

The waterfall frog (*Litoria nannotis*), common mistfrog (*L. rheocola*), and green-eyed treefrog (*L. serrata*) are treefrogs that occur near rainforest streams in northeastern Queensland, Australia (Hoskin and Hero 2008). *Litoria nannotis* typically perch on boulders near waterfalls and fast-flowing sections of stream (Hodgkison and Hero 2001, Rowley and Alford 2007b, Puschendorf et al. 2012), *L. rheocola* use rocks and streamside vegetation in faster-flowing sections of stream (Dennis 2012, Chapter 4), and *L. serrata* are more arboreal than the other species and usually perch on vegetation near slower-flowing sections of stream (Rowley and Alford 2007b). *Litoria nannotis* and *L. rheocola* are currently classified as Endangered (IUCN 2013) and were extirpated by chytridiomycosis at higher elevations (>400 m ASL) throughout their range by the mid-1990s (Richards et al.1993, McDonald and Alford 1999); however many populations have subsequently recovered or recolonised areas where they had been extirpated (McDonald et al. 2005). *Litoria serrata* populations >400 m ASL also suffered declines during initial outbreaks of chytridiomycosis, but none were known to be extirpated and all have subsequently recovered to pre-decline

abundances (McDonald and Alford 1999), and this species is currently classified as Least Concern (IUCN 2013). *Batrachochytrium dendrobatidis* is still present in all populations of all three species that have been sampled, sometimes reaching high prevalences (Puschendorf et al 2011, Sapsford 2012).

Study sites

We tracked *L. nannotis* and *L. serrata* at four rainforest streams in northeastern Queensland, Australia; two streams were located at low elevations (<400 m ASL) and two at high elevations (>600 m ASL). At each site, 20 male *L. nannotis* and 15 male *L. serrata* were tracked. We tracked *L. rheocola* at one low- and one high-elevation rainforest stream; at each site, 40 *L. rheocola* (both sexes) were tracked. Field sites were selected at different elevations to ensure that tracked frogs encountered the full range of environmental conditions available throughout their geographic range during the time of sampling. Tropical rainforest surrounded the streams, characterised by dense vegetation composed of large trees (10 m in height), vines, epiphytes, shrubs, and herbaceous plants. Although most sites were in relatively undisturbed rainforest, several sites were damaged by a tropical cyclone in 2011 (Chapter 9). Stream width varied from 5-10 m and streambeds were composed of rocks ranging in size from small pebbles to large boulders (10 m in diameter). All streams contained pools, runs, and riffles, and most had several waterfalls.

Tracking took place over 10-14 days (21 days for *L. rheocola*) at each site during the winter (cool/dry season) in 2009 (*L. rheocola*), 2010 (*L. nannotis*) and 2011 (*L. serrata*). We tracked *L. rheocola* at Frenchman Creek (17.307°S, 145.922°E; 40 m ASL; 13 July – 6 August) and Windin Creek (17.365°S, 145.717°E; 750 m ASL; 18 August – 9 September), which are both in Wooroonooran National Park. We tracked *L. nannotis* at Kirrama Creek #8 (18.196°S, 145.868°E; 170 m ASL; 5-19 June) and Kirrama Creek #11 (18.214°S, 145.798°E; 850 m; 18-29 July), which are both in Girramay National Park, Tully Creek in Tully Gorge National Park (17.773°S,

145.645°E; 150 m ASL, 7-17 July), and Windin Creek (see above; 20 June – July 4). We tracked *L. serrata* at Kirrama Creek #1 in Girramay National Park (18.203°S, 145.886°E; 100 m ASL; 4-18 July), Stoney Creek in Djiru National Park (17.920°S, 146.069°E; 20 m ASL; 12-25 August), Birthday Creek in Paluma Range National Park (18.980°S, 146.168°E; 800 m ASL; 19 July – 1 August), and Windin Creek (see above; 26 August – 8 September).

Infection status and intensity

To prevent disease transmission between frogs during handling, each frog was captured in a previously unused plastic bag worn as a glove, and was handled only while wearing a new pair of disposable gloves. To determine whether frogs were infected by *B. dendrobatidis*, we took a swab sample from each frog at first capture, and a second sample was taken at the end of the study period if the frog was recaptured at that time. We swabbed the ventral surface and all four feet of each frog with a sterile rayon swab, covering these areas twice. These samples were analysed using real-time quantitative PCR assays (Boyle et al. 2004). For all data analysis, a frog was considered infected if either or both of the swab samples tested positive for *B. dendrobatidis*. To determine the infection intensity for frogs that tested positive for *B. dendrobatidis*, we used the maximum number of zoospore equivalents present on either of the swab samples.

Tracking

We used two different methods to track frogs; *Litoria nannotis* and *L. serrata* were tracked using standard radiotelemetry, and *L. rheocola* was tracked using harmonic direction finding (Langkilde and Alford 2002, Rowley and Alford 2007d). For *L. nannotis*, we used model BD-2NT radiotransmitters (0.44 g, Holohil Systems Ltd., Carp, Ontario, Canada), and for *L. serrata*, we used model A2414 radiotransmitters (0.30 g, Advanced Telemetry Systems, Isanti, Minnesota, USA). For *L. rheocola*, we

built tracking devices using SOT-323 surface-mount zero-bias Schottky detector diodes (Agilent Technologies, Forest Hill, Victoria, Australia; Gourret et al. 2011). Each tracking device was attached to frogs externally by a belt made of silicone tubing; a length of cotton thread was passed through the tubing and tied to secure the tubing around the frog's inguinal region (waist). The combined mass of the tracking device and belt never exceeded 8% of the frog's body mass, which is below the recommended maximum 10% transmitter-to-body-mass ratio for amphibians (Richards et al. 1994). Radiotracked frogs were located using a handheld three-element Yagi antenna with a Sika receiver (Biotrack Ltd., Wareham, Dorset, UK), and harmonic-direction tracked frogs were located using RECCO detectors (models R4 and R8, RECCO Avalanche Rescue System, Lidingö, Sweden). We attempted to locate all frogs once each day (10:00-17:00) and once each night (20:00-03:00 hr) throughout the study period. At the end of the study period, we removed tracking devices from all recaptured frogs. We excluded all data collected during the 24-hr period following attachment of tracking devices due to potential short-term behavioural effects of handling, which are unlikely to persist after the first night of tag attachment (Langkilde and Alford 2002, Rowley and Alford 2007d).

Body temperatures

We used two different methods to record frog body temperatures semi-continuously. *Litoria serrata* were fitted with temperature-sensitive radiotransmitters, which provide accurate measures of the body temperatures of free-ranging frogs (Chapter 2). For these transmitters, a change in temperature resulted in a corresponding increase or decrease in transmitter pulse rate. The pulse rate of each transmitter was recorded every 15 min during the study period by an automated datalogging receiver (model SRX400A, Lotek Wireless, Newmarket, Ontario, Canada). Two or three four-element Yagi antennas were mounted temporarily in trees at each field site and used with the receiver to maximize detection of transmitter signals.

Recorded pulse rates were later converted to temperature using calibration curves provided for each transmitter by the manufacturer.

For *L. nannotis* and *L. rheocola*, we collected thermal data using physical models (Rowley and Alford 2010; Chapter 2) that were placed in each unique location used by each frog. We placed models in diurnal locations used by frogs to measure temperatures between 07:00 and 18:30, and we placed models in nocturnal locations used by frogs to measure temperatures between 19:00 and 06:30. Thermal models (Rowley and Alford 2010) consisted of paired frog models made of three percent agar, each embedded with a Thermochron iButton temperature datalogger (Maxim Integrated Products, California, USA; factory-calibrated and accurate to ±0.5°C). Each datalogger was programmed to record temperatures at 30-min intervals, and was waterproofed with a plastic coating to prevent failure from moisture damage (Chapter 3, Roznik and Alford 2012). Model pairs were composed of one model that was permeable to water loss, with the other impermeable (i.e., coated with plastic to prevent water loss), which together can be used to define the upper and lower boundaries of possible amphibian body temperatures at the locations used by frogs (Rowley and Alford 2010). We have shown previously that *L. nannotis* body temperatures are highly correlated with temperatures of impermeable models, and *L. rheocola* body temperatures are highly correlated with temperatures of permeable models (Chapter 2), so we used temperatures from one model type only to estimate thermal parameters for individuals of each species.

We used all thermal data collected for each frog to calculate the proportion of body temperatures that occurred in temperature categories that are relevant to the growth of *B. dendrobatidis*: <16°C, 16-25°C, and >25°C. Growth of *B. dendrobatidis* in northeastern Queensland is fastest between 15°C and 25°C, and slower outside of this temperature range (Stevenson et al. 2013). We used 16°C as our lower threshold to account for device accuracy and to encompass temperature data from all field sites. Temperatures above 28°C are lethal to *B. dendrobatidis* (Stevenson et al. 2013), but

we do not distinguish these readings because very few temperature readings were above 28°C.

Desiccation rates

We also used our physical models to measure the relative desiccation rates experienced by frogs (Schwarzkopf and Alford 1996, Rowley and Alford 2010). We weighed each permeable model to the nearest 0.1 g immediately before placement in each frog location and immediately after retrieval, and we calculated the proportion of mass lost due to evaporative water loss. Models for *L. serrata* and *L. nannotis* were placed in frog microhabitats for 24 hr and models for *L. rheocola* were placed out for 48 hr. For each frog, we separately calculated the mean proportion of mass lost from all models placed in diurnal and nocturnal locations used by that frog.

Data analysis

We used two types of generalised linear mixed-effects models to examine the effects of thermal and hydric conditions experienced by individual frogs on their infection status, and on their infection intensity if they were infected. Infection status was coded as a binomial response variable, so we used generalised linear mixed-effects models with a binomial family and a logit link function. The $\log_{10}$ of infection intensity (zoospore equivalents per swab sample), was used as a linear response variable and modelled using generalised linear mixed-effects models with a Gaussian family and an identity link function. We evaluated sets of candidate models that included combinations of the following fixed effects: proportion of body temperatures below 16°C, proportion of body temperatures above 25°C (only for infection status models for *L. serrata*), mean desiccation rate at diurnal locations, and mean desiccation rate at nocturnal locations. For all models, we included site identity as a random effect to control for any effects specific to particular sites. Although our sites were chosen to span a wide range of elevations, and thereby a wide range of

environmental conditions, elevation was not included as an effect in models because we wanted to evaluate the effects of measured environmental variables, many of which vary with elevation. Any effects of elevation not accounted for by measured environmental variables should be accounted for by site effects. To avoid overfitting models, we did not include interactions between variables.

For each species and type of analysis, we developed a set of candidate models that included models with all combinations of one, two, three, and four variables, and used Akaike's Information Criterion with adjustment for finite sample size (AICc) to determine the strength of evidence for each model relative to the candidate set of models, using the criteria of Burnham and Anderson (2002). We averaged the best-supported models (ΔAICc <2) to obtain final models. We also determined the overall relative importance of each variable in explaining the infection status and intensity of each species by summing the Akaike weights of all models containing that variable. All statistical analyses were performed in program R, version 2.15.2 (R Core Team 2012) using the lme4 (Bates et al. 2012) and MuMIn (Barton 2013) packages.

Results

Infection status

The thermal and hydric conditions experienced by infected frogs influenced their probability of infection (Table 5.1, Figures 5.1-5.5). For *Litoria nannotis*, two models with ΔAICc <2 (maximum Nagelkerke R^2 = 24.8%) were averaged to produce a final model that indicates that the probability of infection increased as the proportion of body temperatures below 16°C decreased, and rates of desiccation at nocturnal locations decreased (Table 5.1, Figures 5.2-5.5). The model containing these two fixed effects was significantly different from a null model containing only the intercept and random effects of sites (χ^2 = 9.122, df = 2, P = 0.010). For *L. rheocola*, we obtained one model with ΔAICc <2 (R^2 = 47.9%) that indicates that the probability of infection increased as rates of desiccation at both diurnal and nocturnal locations decreased

(Table 5.1, Figures 5.4-5.5). The model containing these two fixed effects was significantly different from a null model containing only the intercept and random effects of sites (χ^2 = 22.437, df = 2, P < 0.001). For *L. serrata*, four models with ΔAICc <2 (maximum R^2 = 27.8%) were averaged to produce a final model that indicates that the probability of infection increased when frogs had increasing proportions of temperatures below 16°C, decreasing proportions of body temperatures above 25°C, and decreasing rates of desiccation at diurnal and nocturnal locations (Table 5.1, Figures 5.2-5.5). The model containing these four fixed effects was nearly significantly different from a null model containing only the intercept and random effects of sites (χ^2 = 9.085, df = 4, P = 0.059).

Infection intensity

The thermal and hydric conditions experienced by infected frogs also affected their infection intensity (Table 5.2, Figures 5.1, 5.6-5.7). For *L. nannotis*, two models with ΔAICc <2 (maximum Nagelkerke R^2 value: 6.2%) were averaged to produce a final model that indicates that infection intensity increased as proportions of body temperatures below 16°C increased and rates of desiccation at diurnal and nocturnal locations increased (Table 5.2, Figures 5.6-5.7). However, the model containing these three fixed effects explained relatively little variation in the data (6.2%) and was not significantly different from a null model containing only the intercept and random effects of sites (χ^2 = 2.978, df = 3, P = 0.395). For *L. rheocola*, four models with ΔAICc <2 (maximum R^2 value: 25.3%) were averaged to produce a final model that indicates that infection intensity increased as proportions of body temperatures below 16°C increased and rates of desiccation at diurnal and nocturnal locations increased (Table 5.2, Figures 5.6-5.7). The model containing these three fixed effects was significantly different from a null model containing only the intercept and random effects of sites (χ^2 = 15.039, df = 3, P = 0.002). For *L. serrata*, six models with ΔAICc <2 (maximum R^2 value: 53.3%) were averaged to produce a final model, which indicates that infection

intensity increased when frogs had increasing proportions of body temperatures below 16°C, increasing rates of desiccation at diurnal locations, and decreasing rates of nocturnal desiccation (Table 5.2, Figures 5.5-5.6). The model containing these three fixed effects was significantly different from a null model containing only the intercept and random effects of sites ($\chi^2 = 8.167$, df = 3, P = 0.043).

Discussion

Our study demonstrates that the body temperatures and desiccation rates of individual frogs in nature are significantly related to their *Batrachochytrium dendrobatidis* infection status and intensity. In all three species of frogs that we studied, we found that locations chosen by infected frogs caused lower rates of desiccation, and were therefore wetter than locations used by uninfected frogs (Table 5.1, Figures 5.4-5.5). This indicates that frogs that used moister microenvironments were more likely to become and remain infected than frogs that preferred drier microenvironments. This is consistent with the desiccation tolerance and transmission mode of *B. dendrobatidis*; this pathogen cannot tolerate desiccation (Johnson et al. 2003) and it is primarily transmitted to frogs through contact with water and wet substrates. However, it is also possible that infected frogs may have altered their behaviour by selecting wetter microenvironments. This might represent manipulation of the host by *B. dendrobatidis* because it would accelerate pathogen growth on hosts and facilitate pathogen dispersal. However, such manipulation would ultimately reduce the lifespan of the host and its pathogen population because *B. dendrobatidis* infections disrupt the ability of amphibian skin to control water uptake, which ultimately causes death when high densities of pathogen populations lead to reduced electrolyte concentrations that disrupt cardiac function (Voyles et al. 2009).

Similar relationships between moisture and *B. dendrobatidis* infections have been observed in other field and laboratory studies. Droughts can reduce *B. dendrobatidis* intensity and mortality (Terrell et al. 2014), and amphibians captured in

aquatic environments are more likely to be infected than individuals found in nearby terrestrial environments (Hossack et al. 2013); this likely reflects habitat preferences by individuals that influence pathogen exposure and survival. In the laboratory, infected amphibians that were maintained in wet conditions had an increased probability of mortality as compared to those maintained in drier conditions, presumably because of differences in pathogen growth rates (Bustamante et al. 2010, Murphy et al. 2011). Increased use of wet microenvironments by infected frogs may also affect their body temperatures. In our study, individual frogs that had lower rates of desiccation during the day also had cooler body temperatures, but frogs that had lower desiccation rates at night tended to have warmer body temperatures (Figure 5.8). This pattern is related to air and water temperatures at our rainforest streams; water temperatures are moderate and constant, and they are usually cooler than air temperatures during the day, but warmer than air temperatures at night (Figure 5.2).

The body temperatures of individual frogs were related to their infection status (Table 5.1, Figures 5.2-5.3, 5.5). In *Litoria serrata*, the probability of infection decreased as frogs had increasing proportions of body temperatures above 25°C (Figure 5.3, 5.5). This was likely caused by decreased rates of pathogen growth on frog skin at warmer temperatures because the growth and reproduction of *B. dendrobatidis* cultures slow dramatically under constant laboratory temperatures above 25°C (Piotrowski et al. 2004, Stevenson et al. 2013). Our results could also reflect the positive effects of warmer temperatures on the frogs' immune systems (Carey et al. 1999, Raffel et al. 2006, Ribas et al. 2009, Rollins-Smith et al. 2011). Laboratory studies have shown that infected amphibians maintained at temperatures above 25°C are able to reduce or eliminate their infections (Woodhams et al. 2003, Chatfield and Richards-Zawacki 2011, Geiger et al. 2011). Our results followed a pattern similar as that found by Rowley and Alford (2013) for three species of Australian rainforest frogs (*Litoria "lesueuri*," *L. nannotis*, and *L. serrata*), and do not support the hypothesis that infected frogs behaviourally elevate their body

temperatures in response to infection by *B. dendrobatidis* (Richards-Zawacki 2009). In our study, the probability of infection decreased with an increase in the proportion of body temperatures above 25°C in *L. serrata*, and the average body temperatures of uninfected frogs reached higher temperatures than those of infected frogs for all three species that we studied (Figure 5.2). This suggests that frogs selecting warmer microenvironments for reasons unrelated to their *B. dendrobatidis* infection status are either less likely to become infected, clear infections more rapidly, or both. This relationship between temperature and infection probability can explain changes of population-level patterns of infection prevalence between seasons and habitat types (Woodhams and Alford 2005, Becker et al. 2012, Whitfield et al. 2012, Phillott et al. 2013, Sapsford et al. 2013).

This is the first study to find that the proportion of body temperatures occurring below the thermal optimum of *B. dendrobatidis* is also a predictor of infection status (Table 5.1, Figure 5.3, 5.5). However, we found that patterns differed among species; in *L. nannotis*, uninfected frogs had a greater proportion of body temperatures below 16°C (a similar trend occurred in *L. rheocola*), and in *L. serrata*, uninfected frogs had a smaller proportion of body temperatures below 16°C (Figure 5.3). These patterns could be caused by differences in water use among the species. *Litoria nannotis* spent large portions of time in the stream during the day and night (Chapter 6), and body temperatures of infected frogs closely mimicked water temperatures, whereas body temperatures of uninfected frogs were more variable and reached both warmer and cooler temperatures (Figure 5.2). By contrast, *L. serrata* used drier microenvironments, and infected frogs were typically cooler than uninfected frogs and had greater proportions of body temperatures below 16°C (Figures 5.2-5.3). As already discussed, differences between the body temperatures of infected and uninfected frogs may be caused by individual variation in microenvironment selection, or changes in behaviour once frogs became infected. Regardless of the cause, the cooler body temperatures of infected frogs are beneficial to *B. dendrobatidis* growth, which increases as

temperature decreases within the thermal range of 15-25°C (Piotrowski et al. 2004, Stevenson et al. 2013). Although overall growth is slower below 15°C, *B. dendrobatidis* maintains reproductive fitness at cooler temperatures by producing more zoospores per zoosporangium, and the zoospores also remain infectious for longer periods of time (Woodhams et al. 2008, Stevenson et al. 2013).

As well as affecting probability of infection, our results indicate that the thermal and hydric conditions experienced by frogs of two species affected the intensity of their infections (Table 5.2, Figures 5.6-5.7). Contrary to our initial expectations, in many cases environmental factors affected infection intensity in the opposite direction from their effects on infection probability. For *L. rheocola* and *L. serrata*, intensity was positively related to the proportion of body temperatures below 16°C (Figure 5.6), but the relationships between intensity and desiccation rates differed between the species. For *L. rheocola*, infection intensity increased with increasing rates of desiccation at diurnal and nocturnal locations, but for *L. serrata*, infection intensity increased as diurnal desiccation increased, but decreased as rates of nocturnal desiccation increased (Figure 5.7). These results suggest that there may be threshold relationships, whereby the pathogen begins to influence host behaviour or physiology differently above a certain infection load (Vredenburg et al. 2010). For example, below a threshold intensity increasing desiccation rates may suppress pathogen growth on infected frogs; however, above this threshold intensity, a frog's behaviour or the permeability of its skin may change in ways that facilitate the rapid buildup of infection. Further research is necessary to fully understand the relationships between infection intensity and the environmental conditions experienced by infected frogs.

A first step in understanding the interactions between *B. dendrobatidis* and individual amphibian behaviour is to document the relationships between the microenvironments used by frogs and their infection status and intensity. Our results demonstrate that uninfected frogs used microenvironments that were warmer and drier than those used by infected frogs. This is likely caused by differences in patterns of

microhabitat use between infected and uninfected frogs. Our results do not conclusively establish whether these differences in behaviour are a cause and/or a consequence of infection. It is possible that the patterns we have demonstrated reflect pre-existing behavioural differences among individuals that affect their chances of acquiring infections and remaining infected. It is also possible that the patterns are a result of changes in the behaviour of infected frogs. To disentangle these hypotheses, it will be necessary to conduct laboratory experiments that involve comparisons of frog behaviour before and after frogs are infected. Fully understanding the interactions between individual amphibian behaviour and *B. dendrobatidis* is essential for understanding and ultimately managing this important host-pathogen system.

Table 5.1. Generalised linear mixed-effects models (family: binomial, link function: logit) were used to examine relationships between the thermal and hydric conditions experienced by individual frogs and their infection status (infected or uninfected). For each species, we developed a set of candidate models combining the random effect of site with all combinations of one, two, three, or four fixed effects. Fixed effects were the proportion of body temperatures below 16°C, proportion of body temperatures above 25°C (for *Litoria serrata* only), mean diurnal site desiccation rate, and mean nocturnal site desiccation rate. Models in the candidate set were ranked according to Akaike's Information Criterion with adjustment for finite sample size (AICc). All models that we tested are shown, but only models with ΔAICc <2 were strongly supported by our data and included in final averaged models.

Candidate models

Model effects	AICc	ΔAICc	Weight	R^2
Litoria nannotis				
Night desiccation	71.764	0.000	0.474	0.231
<16°C, Night desiccation	73.116	1.352	0.241	0.248
Day desiccation, Night desiccation	74.019	2.255	0.154	0.231
<16°C, Day desiccation, Night desiccation	75.477	3.714	0.074	0.249
Intercept only	77.739	5.976	0.024	0.064
<16°C	78.711	6.948	0.015	0.091
Day desiccation	79.148	7.384	0.012	0.081
<16°C, Day desiccation	80.285	8.521	0.007	0.106
Litoria rheocola				
Day desiccation, Night desiccation	59.077	0.000	0.668	0.479
<16°C, Day desiccation, Night desiccation	61.235	2.158	0.227	0.485
Day desiccation	63.769	4.693	0.064	0.345
<16°C, Day desiccation	64.766	5.690	0.039	0.371
Night desiccation	71.697	12.620	0.001	0.168
<16°C, Night desiccation	71.933	12.856	0.001	0.212
Intercept only	76.797	17.721	<0.001	<0.001
<16°C	78.244	19.168	<0.001	0.018
Litoria serrata				
<16°C, Day desiccation	72.326	0.000	0.247	0.278
<16°C, Night desiccation	72.927	0.601	0.183	0.267
<16°C	73.631	1.306	0.129	0.210
<16°C, >25°C	73.786	1.460	0.119	0.251
<16°C, Day desiccation, Night desiccation	74.662	2.336	0.077	0.279
<16°C, >25°C, Day desiccation	74.686	2.360	0.076	0.278
<16°C, >25°C, Night desiccation	75.124	2.799	0.061	0.270
Intercept only	76.851	4.525	0.026	0.100
<16°C, >25°C, Day desiccation, Night desiccation	77.115	4.789	0.023	0.279
Day desiccation	77.428	5.103	0.019	0.134
Night desiccation	78.385	6.060	0.012	0.114
>25°C	78.546	6.220	0.011	0.111
Day desiccation, Night desiccation	79.665	7.340	0.006	0.135
>25°C, Day desiccation	79.717	7.391	0.006	0.134
>25°C, Night desiccation	80.531	8.205	0.004	0.117
>25°C, Day desiccation, Night desiccation	82.040	9.714	0.002	0.135

Final averaged models

Model effect	*L. nannotis*	*L. rheocola*	*L. serrata*
Intercept	2.121	1.899	-1.255
<16°C	-0.879	-	2.710
>25°C	-	-	-1.827
Day desiccation	-	-5.692	-1.929
Night desiccation	-49.935	-3.466	-1.305

Table 5.2. Generalised linear mixed-effects models (family: Gaussian, link function: identity) were used to examine relationships between the thermal and hydric conditions experienced by infected frogs and their infection intensity (log10 of zoospore equivalents per sample). For each species, we developed a set of candidate models combining the random effect of site with all combinations of one, two, three, or four fixed effects. Fixed effects were the proportion of body temperatures below 16°C, mean diurnal site desiccation rate, and mean nocturnal site desiccation rate. Models in the candidate set were ranked according to Akaike's Information Criterion with adjustment for finite sample size (AICc). All models that we tested are shown, but only models with ΔAICc <2 were strongly supported by our data and included in final averaged models.

Candidate models

Model effects	AICc	ΔAICc	Weight	R^2
Litoria nannotis				
Day desiccation, Night desiccation	123.395	0.000	0.477	0.061
<16°C, Day desiccation, Night desiccation	123.886	0.491	0.373	0.062
Day desiccation	127.515	4.119	0.061	0.058
<16°C, Day desiccation	127.971	4.576	0.048	0.058
Night desiccation	129.591	6.196	0.022	0.022
<16°C, Night desiccation	130.102	6.707	0.017	0.022
Intercept only	134.476	11.081	0.002	<0.001
<16°C	134.902	11.506	0.002	0.001
Litoria rheocola				
<16°C, Day desiccation, Night desiccation	116.207	0.000	0.344	0.167
<16°C, Day desiccation	116.682	0.475	0.271	0.128
Day desiccation, Night desiccation	116.868	0.662	0.247	0.253
Day desiccation	118.029	1.822	0.138	0.138
Night desiccation	134.443	18.236	<0.001	0.000
<16°C, Night desiccation	134.443	18.236	<0.001	0.014
<16°C	134.995	18.788	<0.001	0.014
Intercept only	135.067	18.860	<0.001	<0.001
Litoria serrata				
Night desiccation	41.405	0.000	0.183	0.518
Day desiccation, Night desiccation	41.680	0.275	0.160	0.533
<16°C, Night desiccation	41.709	0.304	0.158	0.316
<16°C	42.041	0.636	0.134	0.265
<16°C, Day desiccation, Night desiccation	42.237	0.832	0.121	0.329
Intercept only	42.669	1.264	0.098	0.252
Day desiccation	43.151	1.746	0.077	0.281
<16°C, Day desiccation	43.324	1.919	0.070	0.260

Final averaged models

Model effect	*L. nannotis*	*L. rheocola*	*L. serrata*
Intercept	1.625	1.318	1.318
<16°C	0.185	0.952	0.451
Day desiccation	18.123	8.845	0.246
Night desiccation	4.913	0.563	-1.560

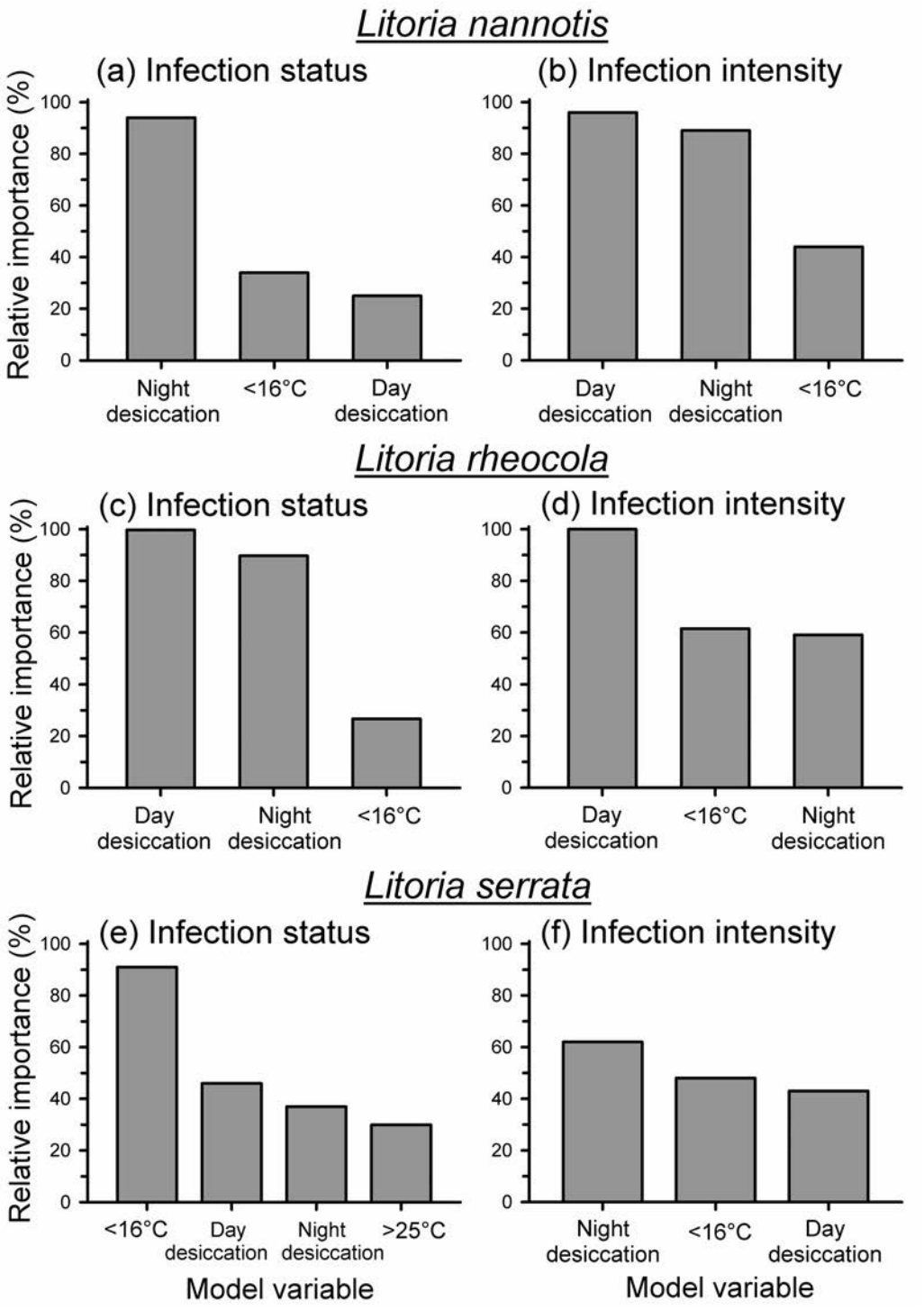

Figure 5.1. Relative importance of thermal and hydric conditions in explaining *Batrachochytrium dendrobatidis* infection status and intensity of individual frogs of three species. These conditions were fixed effects used in generalised linear mixed-effects modelling: the proportion of body temperatures below 16°C, proportion of body temperatures above 25°C (for *Litoria serrata* only), mean diurnal site desiccation rate, and mean nocturnal site desiccation rate. Relative importance values for each fixed effect were calculated by summing the Akaike weight of all models containing that effect. Only model effects that were supported by our analysis are shown.

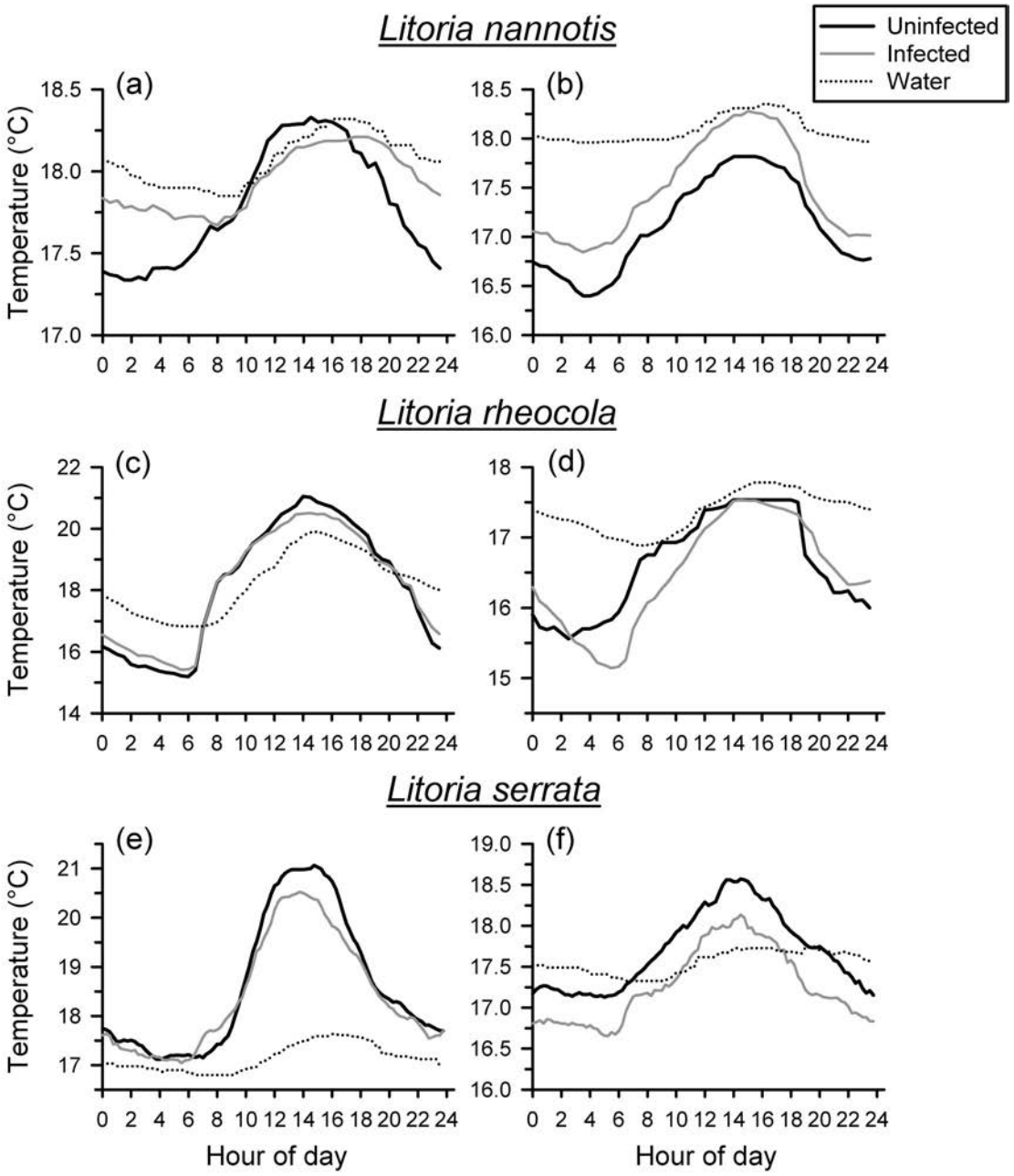

Figure 5.2. Mean body temperatures over the 24-hr diel cycle for frogs of three species that were infected or uninfected by *Batrachochytrium dendrobatidis*. Thermal data are shown at two representative sites for each species. Mean water temperatures at each site are also shown.

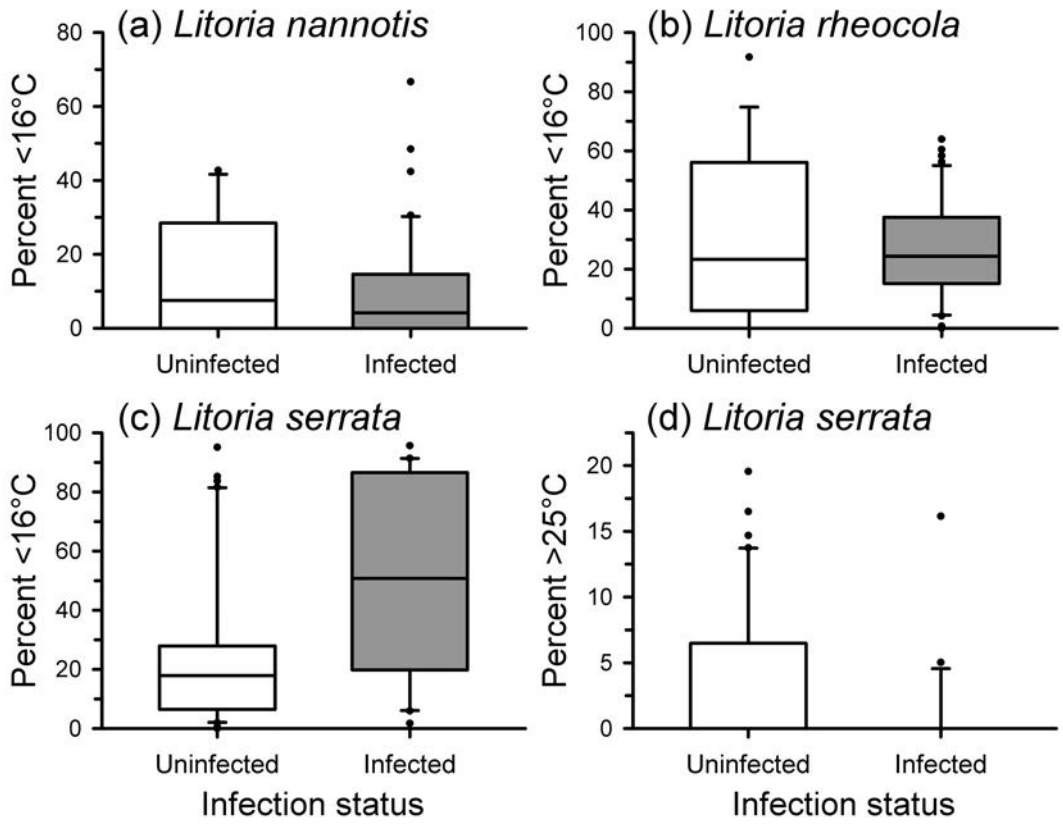

Figure 5.3. Body temperature parameters that were included in generalised linear mixed-effects models explaining *Batrachochytrium dendrobatidis* infection status of individual frogs of three species. These parameters were the percentage of body temperatures of infected and uninfected frogs that were below 16°C for *Litoria nannotis*, *L. rheocola*, and *L. serrata*, and above 25°C for *L. serrata*.

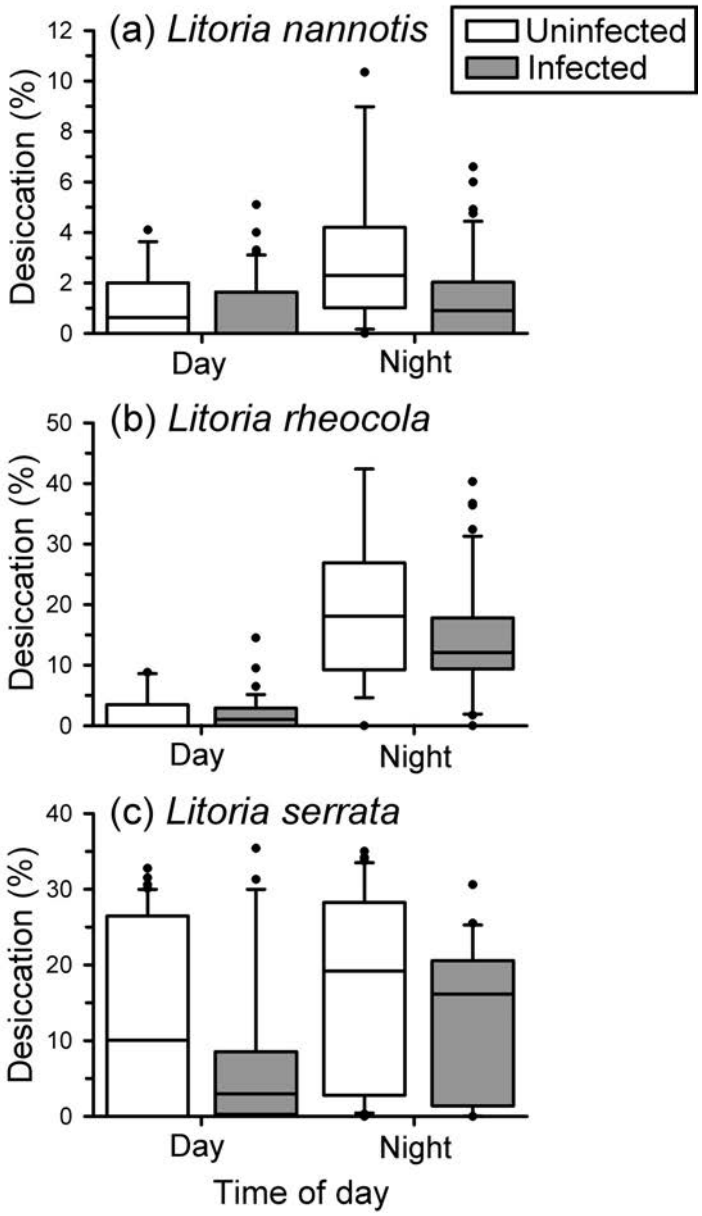

Figure 5.4. Desiccation rate parameters that were included in in generalised linear mixed-effects models explaining *Batrachochytrium dendrobatidis* infection status of individual frogs of three species. These parameters were the mean desiccation rates of physical models over 24 hr (*Litoria nannotis* and *L. serrata*) or 48 hr (for *L. rheocola*) placed in diurnal or nocturnal frog locations.

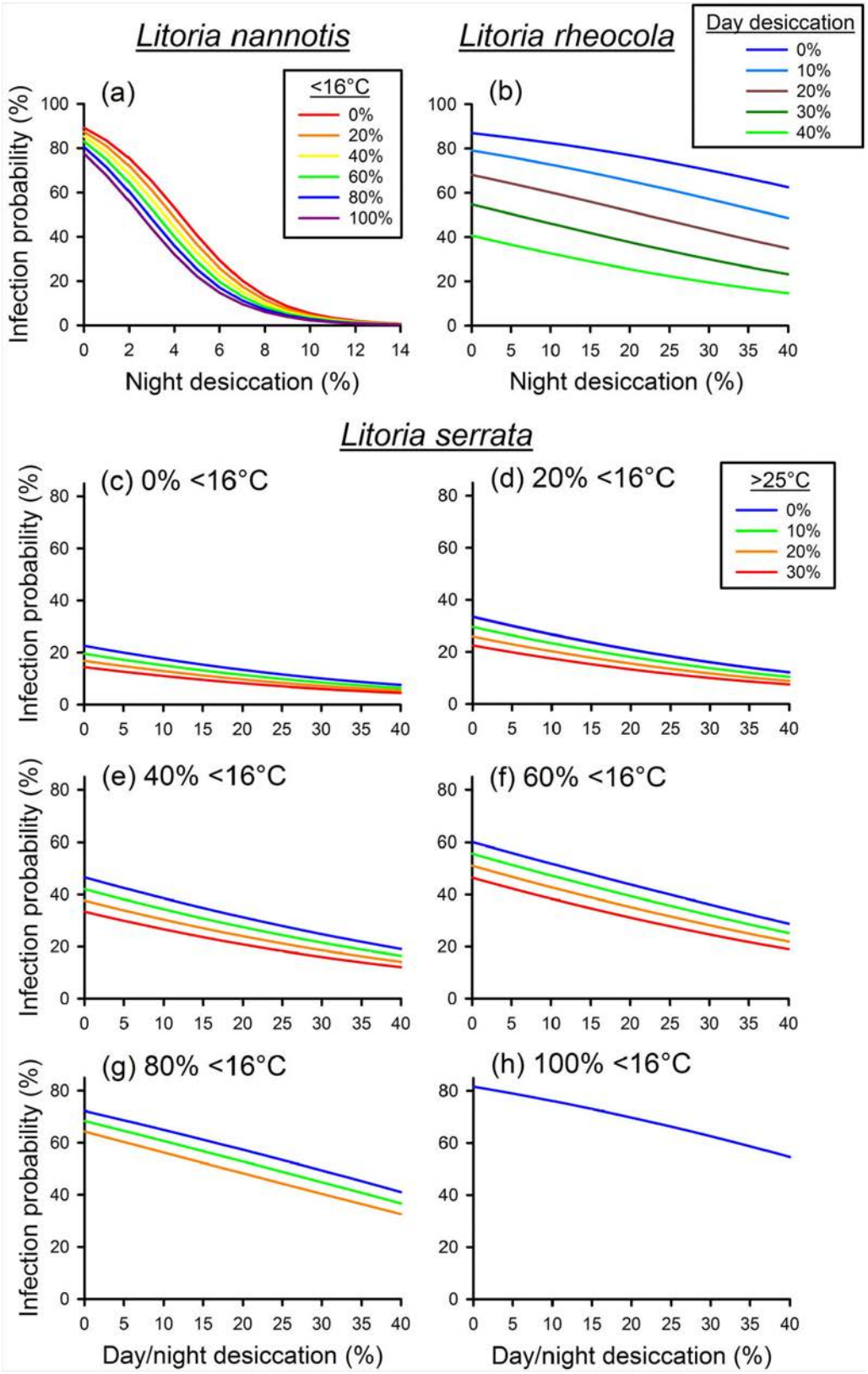

Figure 5.5. Probability of *Batrachochytrium dendrobatidis* infection for individuals of three frog species under various thermal and hydric conditions. These predictions were generated from averaged generalised linear mixed-effects models for each species (see Table 5.1).

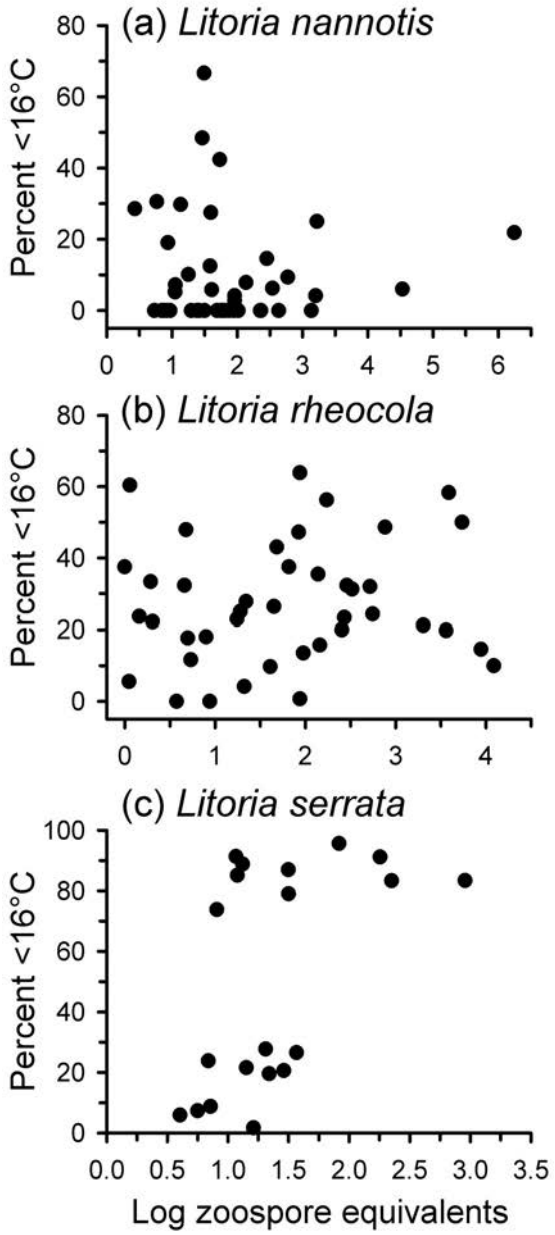

Figure 5.6. Body temperature parameters (percentage of body temperatures below 16°C) that were included in models explaining *Batrachochytrium dendrobatidis* infection intensity (log_{10} of zoospore equivalents) of individuals of three frog species.

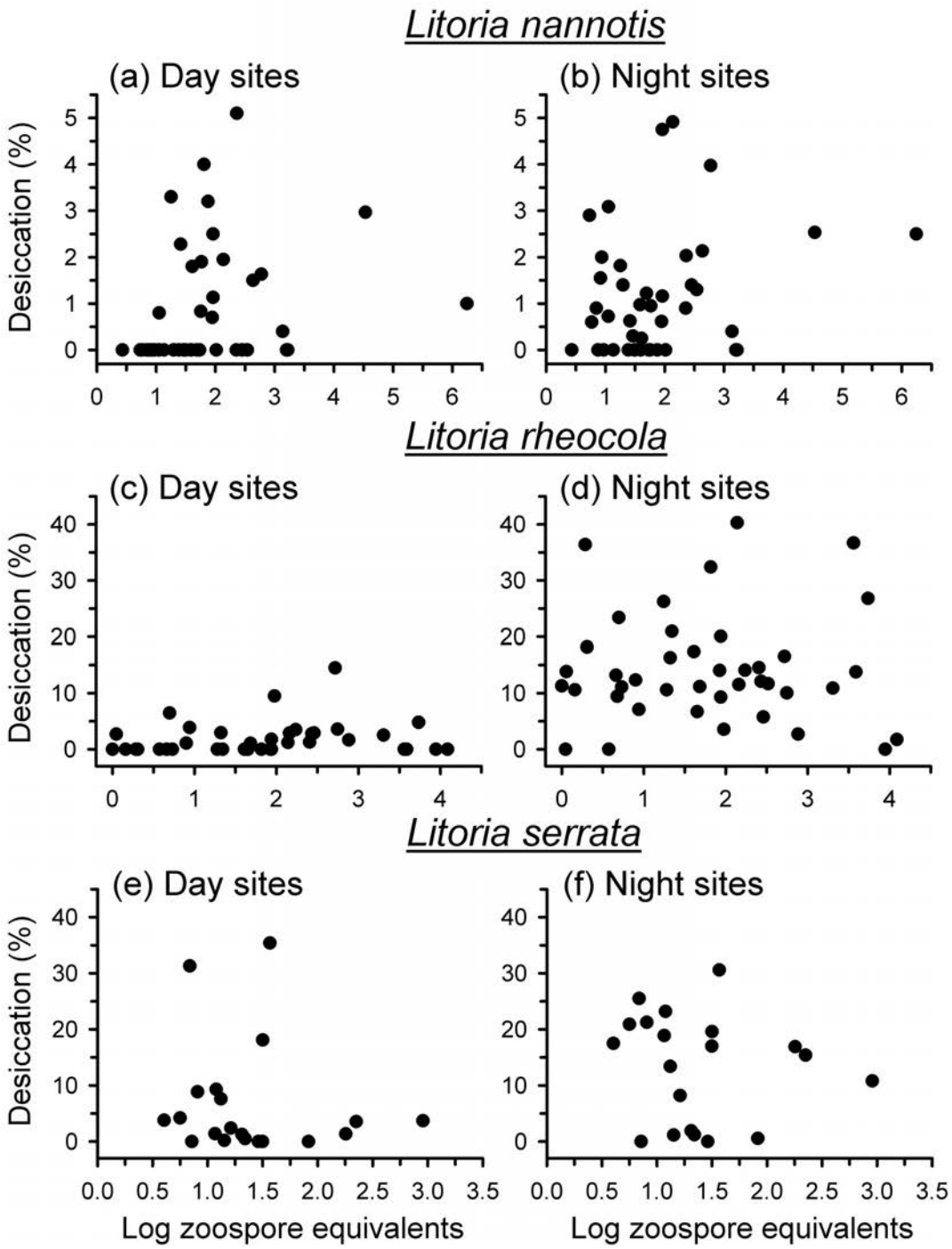

Figure 5.7. Desiccation rate parameters that were included in models explaining *Batrachochytrium dendrobatidis* infection intensity (log_{10} of zoospore equivalents) of individual frogs of three species. These parameters were the mean desiccation rates of physical models over 24 hr (*Litoria nannotis* and *L. serrata*) or 48 hr (for *L. rheocola*) placed in diurnal or nocturnal frog locations.

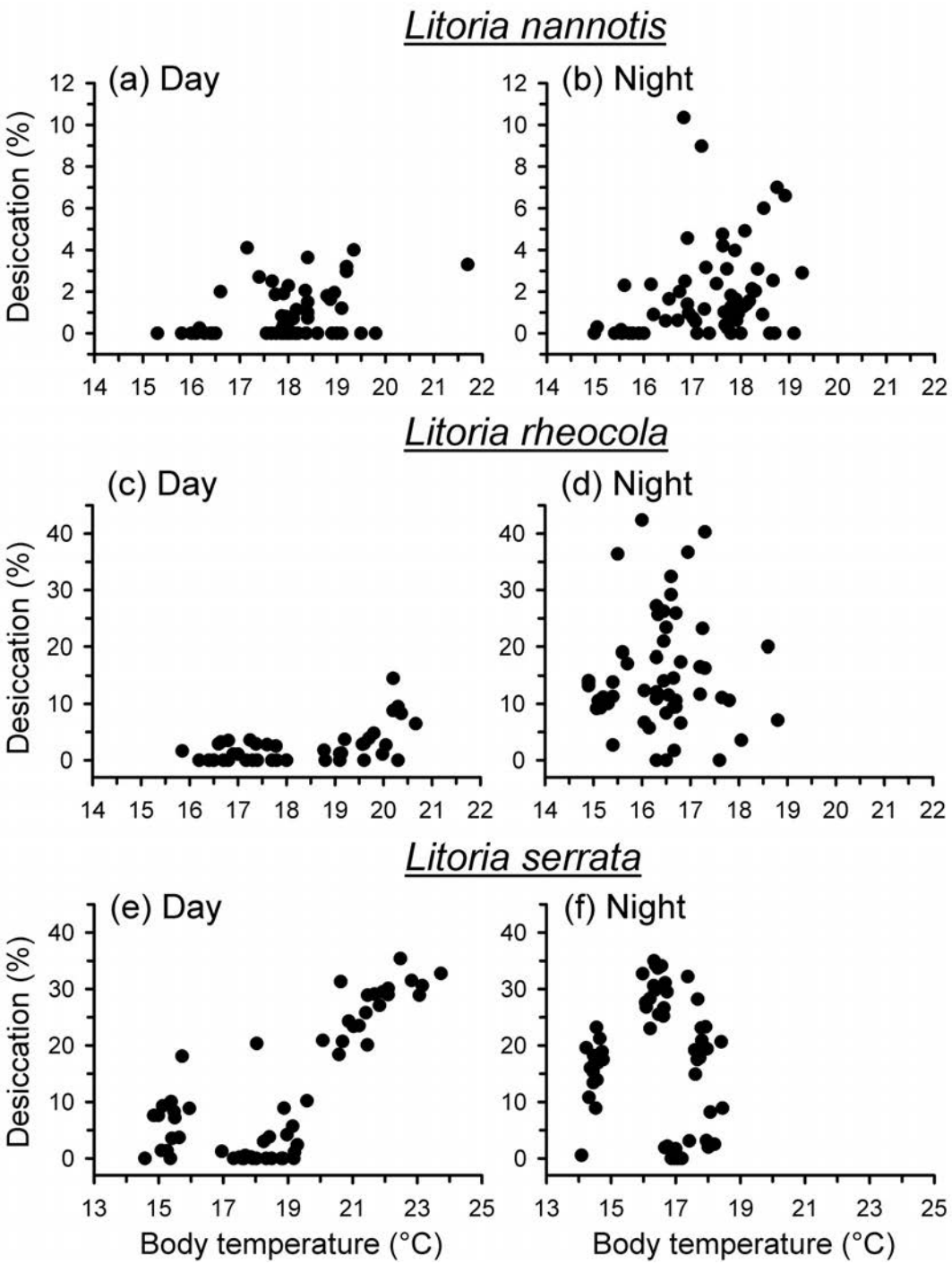

Figure 5.8. Relationships between the mean body temperatures and mean desiccation rates experienced by frogs of three species at diurnal and nocturnal locations.

Chapter 6: Patterns of movement and microhabitat use influence infection risk: individual frogs and the chytrid fungus *Batrachochytrium dendrobatidis*

Elizabeth A. Roznik and Ross A. Alford

Abstract

Diseases are influenced by interactions among the host, pathogen, and environmental conditions. Host behaviour can strongly influence pathogen transmission and the environmental conditions that are experienced by both host and pathogen. The pathogenic fungus *Batrachochytrium dendrobatidis*, which has caused amphibian declines and extinctions in many regions, is transmitted by contact with water and requires cool, moist conditions to survive and reproduce. In some Australian rainforest frog species, infected frogs maintain cooler body temperatures and have lower desiccation rates than uninfected frogs. However, the behavioural mechanisms underlying these differences are unknown. We tracked infected and uninfected individuals of three species of Australian rainforest frogs to understand whether their patterns of movement and microhabitat use were related to their *B. dendrobatidis* infection status and intensity. We found significant relationships between individual frog behaviour and infection probability in two of three species (*Litoria rheocola* and *L. serrata*, but not *L. nannotis*). An increased probability of infection was related to increased use of wet and cool substrates, including rocks and decaying wood, and decreased use of drier and warmer substrates, particularly vegetation. Infected frogs also tended to remain closer to the stream and move less often, but move longer distances when they did move. These movement patterns could be related to the

locations of preferred microhabitats, or could reflect changes in activity that occurred after frogs became infected. We found that the behaviour of *L. rheocola* was also related to infection intensity; frogs with higher infection loads were more likely to use sites on vegetation, and were less likely to use wet, sheltered microhabitats, particularly rocks and leaf litter. Highly infected *L. rheocola* also used microhabitats that were closer to the stream than frogs with lower infection loads. Our results provide the first demonstration that the movements and microhabitats of individual frogs are significantly related to their probability of *B. dendrobatidis* infection, and in one species, to the pathogen loads of individuals. Our results further document how behavioural variation among individuals and among species can affect the interactions of hosts with this important pathogen, which may provide opportunities for natural selection.

Introduction

Diseases are influenced by interactions among the host, pathogen, and environmental conditions. Host behaviour can strongly influence pathogen transmission and the microenvironmental conditions that are experienced by both host and pathogen (Moore 2002). Host social behaviour has long been recognized as an important factor in the transmission of many infectious diseases; social interactions, especially the formation of groups, promote contact between infected and susceptible individuals (Ezenwa 2004, Rowley and Alford 2007a, Dizney and Dearing 2013). The microhabitats selected by hosts can also influence their exposure to pathogens that persist in the environment by either facilitating or hindering pathogen transmission (Moore 2002). In addition, the thermal and hydric conditions of selected microhabitats can influence host immune responses (Carey et al. 1999, Raffel et al. 2006, Ribas et al. 2009, Rollins-Smith et al. 2011) and the fitness of pathogens inhabiting the hosts. Many pathogens are highly sensitive to temperature and moisture, and small changes in these conditions can significantly affect their growth, reproduction, and survival

(Harvell et al. 2002, Stevenson et al. 2013). For ectothermic hosts, microhabitat selection can play an especially important role in their interactions with pathogens because host body temperatures are strongly influenced by the temperatures of the microhabitats they use.

The microhabitats selected by amphibians could have important effects on their interactions with the chytrid fungus *Batrachochytrium dendrobatidis*, which causes the disease chytridiomycosis and is linked to amphibian declines and extinctions in many regions of the world (Kilpatrick et al. 2009). This pathogen attacks the skin cells of amphibians, and infectious zoospores are transmitted through contact with infected individuals or contaminated water or substrates (Rachowicz and Vredenburg 2004). For this reason, host behaviour can play a major role in the transmission of *B. dendrobatidis*; for example, amphibian species that form aggregations and spend more time in contact with water are often more vulnerable to chytridiomycosis (Rowley and Alford 2007a). *Batrachochytrium dendrobatidis* is highly sensitive to temperature and moisture and requires relatively cool, moist conditions to survive and reproduce (15-25°C optimal, >28°C lethal; Johnson et al. 2003, Piotrowski et al. 2004, Stevenson et al. 2013). Because of this sensitivity to environmental conditions, habitats that provide access to warmer and drier microclimates can reduce the effects of disease (Puschendorf et al. 2009, 2011). Local environmental conditions are often driven by habitat type and percentage of forest canopy cover (Puschendorf et al. 2011, Becker et al. 2012, Hossack et al. 2013, Chapter 9). For example, two species of Australian rainforest frogs were able to persist along a section of stream in open-canopy dry forest without clinical signs of chytridiomycosis, but were negatively impacted by the disease at a nearby section of stream surrounded by closed-canopy rainforest (Puschendorf et al. 2011). Direct effects of temperature on the growth rates of *B. dendrobatidis* inhabiting frog skin likely contributed to this pattern (Daskin et al. 2011).

Host movement patterns can also influence rates of pathogen transmission and the buildup of infections within hosts. Because pathogens often accumulate in an

animal's environment over time, more sedentary animals can be more vulnerable to infection (Foldstad et al. 1991, Altizer et al. 2011, Koprivnikar et al. 2012). Individual frogs that change locations infrequently and move short distances may have a higher risk of infection than more active individuals, and once infected, they may be more vulnerable to increases of infection intensity through re-infection (Briggs et al. 2010). An individual's proximity to areas that harbour pathogens can also be related to infection susceptibility. Amphibian species that are closely associated with the stream environment are often more vulnerable to *B. dendrobatidis* than amphibians that use microhabitats located farther away from streams (Lips et al. 2006, Rowley and Alford 2007b). Although movement patterns and other types of behaviours can influence infection probability, they can also change once an animal becomes infected; such changes can reflect defensive responses of the host, manipulation of the host by the pathogen, or incidental side effects of disease (Moore 2002). The three-dimensional use of space by frogs could therefore be related to infection status in complex ways, including changes in microhabitat preferences caused by infection.

Previous research has demonstrated that the thermal and hydric conditions experienced by individual frogs are related to their *B. dendrobatidis* infection status (Richards-Zawacki 2009, Rowley and Alford 2013, Chapter 5). Although one study on Panamanian frogs suggested that infected frogs elevated their body temperatures in response to infection (Richards-Zawacki 2009), studies on three species of Australian rainforest frogs have demonstrated that the infection probability of individual frogs decreased when frogs had higher proportions of body temperatures above the upper thermal limit for *B. dendrobatidis* growth (>25°C; Rowley and Alford 2013, Chapter 5). The frequency of body temperatures below the lower thermal limit for optimal pathogen growth is also related to infection status; for two species, individuals with higher proportions of body temperatures below 16°C had a higher probability of infection in two species, but a lower infection probability in a third species (Chapter 5). Because amphibians use their environment to regulate their body temperatures and desiccation

rates, differences in microhabitat selection and movements between infected and uninfected frogs may explain these patterns. However, we do not know how individuals differ in movement and microhabitat selection, or how these differences may affect the prevalence and intensity of pathogenic infections. This study is the first to examine how variation in these behaviours is related to *B. dendrobatidis* infection status and intensity. We tracked infected and uninfected individuals of three species of Australian rainforest frogs, and examined the types of substrates they used, their positions in relation to the stream, movement distances, and movement probabilities. Our results will increase our understanding of how host behaviour and environment interact to affect this globally important host-pathogen system.

Methods

Study species

The waterfall frog (*Litoria nannotis*), common mistfrog (*L. rheocola*), and green-eyed treefrog (*L. serrata*) are treefrogs that occur near rainforest streams in northeastern Queensland, Australia (Hoskin and Hero 2008). *Litoria nannotis* typically perch on boulders near waterfalls and fast-flowing sections of streams (Hodgkison and Hero 2001, Rowley and Alford 2007b, Puschendorf et al. 2012), *L. rheocola* use rocks and streamside vegetation in faster-flowing sections of streams (Dennis 2012, Chapter 4), and *L. serrata* are more arboreal than the other species and usually perch on vegetation near slower-flowing sections of streams (Rowley and Alford 2007b). *Litoria nannotis* and *L. rheocola* are currently classified as Endangered (IUCN 2013) and were extirpated at higher elevations (>400 m ASL) throughout their range by the mid-1990s due to chytridiomycosis (Richards et al.1993, McDonald and Alford 1999); many populations have subsequently recovered or recolonised areas where they had been extirpated (McDonald et al. 2005). *Litoria serrata* populations above 400 m ASL also suffered declines during initial outbreaks of chytridiomycosis, but none were known to be extirpated and all have subsequently recovered to pre-decline abundances

(McDonald and Alford 1999), and this species is currently classified as Least Concern (IUCN 2013). *Batrachochytrium dendrobatidis* is still present in all populations of all three species that have been sampled, sometimes reaching high prevalences (Puschendorf et al 2011, Sapsford et al. 2013).

Study sites

We tracked *L. nannotis* and *L. serrata* at four rainforest streams in northeastern Queensland, Australia; two streams were located at low elevations (<400 m ASL) and two at high elevations (>600 m ASL). At each site, 20 male *L. nannotis* and 15 male *L. serrata* were tracked. We tracked *L. rheocola* at one low- and one high-elevation rainforest stream; at each site, 40 *L. rheocola* (both sexes) were tracked. Field sites were selected at different elevations to ensure that tracked frogs encountered the full range of environmental conditions available throughout their geographic range during the time of sampling. Tropical rainforest surrounded the streams, characterised by dense vegetation composed of large trees (10 m in height), vines, epiphytes, shrubs, and herbaceous plants. Although most sites were in relatively undisturbed rainforest, several sites were damaged by a tropical cyclone in 2011 (Chapter 9). Stream width varied from 5-10 m and streambeds were composed of rocks ranging in size from small pebbles to large boulders (10 m in diameter). All streams contained pools, runs, and riffles, and most had several waterfalls.

Tracking took place over 10-14 days (21 days for *L. rheocola*) at each site during the winter (cool/dry season) in 2009 (*L. rheocola*), 2010 (*L. nannotis*) and 2011 (*L. serrata*). We tracked *L. rheocola* at Frenchman Creek (17.307°S, 145.922°E; 40 m ASL; 13 July – 6 August) and Windin Creek (17.365°S, 145.717°E; 750 m ASL; 18 August – 9 September), which are both in Wooroonooran National Park. We tracked *L. nannotis* at Kirrama Creek #8 (18.196°S, 145.868°E; 170 m ASL; 5-19 June) and Kirrama Creek #11 (18.214°S, 145.798°E; 850 m; 18-29 July), which are both in Girramay National Park, Tully Creek in Tully Gorge National Park (17.773°S,

145.645°E; 150 m ASL, 7-17 July), and Windin Creek (see above; 20 June – July 4). We tracked *L. serrata* at Kirrama Creek #1 in Girramay National Park (18.203°S, 145.886°E; 100 m ASL; 4-18 July), Stoney Creek in Djiru National Park (17.920°S, 146.069°E; 20 m ASL; 12-25 August), Birthday Creek in Paluma Range National Park (18.980°S, 146.168°E; 800 m ASL; 19 July – 1 August), and Windin Creek (see above; 26 August – 8 September).

Infection status and intensity

To prevent disease transmission between frogs during handling, each frog was captured in a previously unused plastic bag worn as a glove, and was handled only while wearing a new pair of disposable gloves. To determine whether frogs were infected by *B. dendrobatidis*, we took a swab sample from each frog at first capture, and a second sample was taken at the end of the study period if the frog was recaptured at that time. We swabbed the ventral surface and all four feet of each frog with a sterile rayon swab, covering these areas twice. These samples were analysed using real-time quantitative PCR assays (Boyle et al. 2004). For all data analysis, a frog was considered infected if either or both of the swab samples tested positive for *B. dendrobatidis*. To determine the infection intensity for frogs that tested positive for *B. dendrobatidis*, we used the maximum number of zoospore equivalents present on either of the swab samples.

Tracking

We used two different methods to track frogs; *Litoria nannotis* and *L. serrata* were tracked using standard radiotelemetry, and *L. rheocola* was tracked using harmonic direction finding (Langkilde and Alford 2002, Rowley and Alford 2007d). For *L. nannotis*, we used model BD-2NT radiotransmitters (0.44 g, Holohil Systems Ltd., Carp, Ontario, Canada), and for *L. serrata*, we used model A2414 radiotransmitters (0.30 g, Advanced Telemetry Systems, Isanti, Minnesota, USA). For *L. rheocola*, we

built tracking devices using SOT-323 surface-mount zero-bias Schottky detector diodes (Agilent Technologies, Forest Hill, Victoria, Australia; Gourret et al. 2011). Each tracking device was attached to frogs externally by a belt made of silicone tubing; a length of cotton thread was passed through the tubing and tied to secure the tubing around the frog's inguinal region (waist). The combined mass of the tracking device and belt never exceeded 8% of the frog's body mass, which is below the recommended maximum 10% transmitter-to-body-mass ratio for amphibians (Richards et al. 1994). Radiotracked frogs were located using a handheld three-element Yagi antenna with a Sika receiver (Biotrack Ltd., Wareham, Dorset, UK), and harmonic-direction tracked frogs were located using RECCO detectors (models R4 and R8, RECCO Avalanche Rescue System, Lidingö, Sweden). We attempted to locate all frogs once each day (10:00-1700) and once each night (20:00-03:00) throughout the study period. At the end of the study period, we removed tracking devices from all recaptured frogs. We excluded all data collected during the 24-hr period following attachment of tracking devices due to potential short-term behavioural effects of handling, which are unlikely to persist after the first night of tag attachment (Langkilde and Alford 2002, Rowley and Alford 2007d).

Microhabitat use

We defined five substrate categories: vegetation, rock, leaf litter, soil, and decaying wood. Each time we located a frog, we recorded the category of the substrate contacted by its ventral surface. We also recorded whether the substrate was visibly wet or apparently dry, and whether the frog was in a sheltered (i.e., under rocks, leaf litter, or vegetation) or exposed position. For each frog, we calculated the proportion of locations in each substrate category, the proportion of wet locations, and the proportion of sheltered locations.

We also used temperature-sensitive radiotransmitters and physical models (Rowley and Alford 2010, Chapter 2) to measure the average temperatures and

relative desiccation rates experienced by frogs in the microhabitats they used. We used this information to qualitatively describe thermal and hydric characteristics of the substrates used by frogs during our study. Temperature-sensitive transmitters were used for *L. serrata* only; the pulse rate of each transmitter was recorded every 15 min during the study period by an automated datalogging receiver (model SRX400A, Lotek Wireless, Newmarket, Ontario, Canada), which was later converted to temperature using calibration curves provided for each transmitter by the manufacturer (see Chapter 5 for details). Physical models made of agar (Rowley and Alford 2010, Chapter 2) were placed in diurnal and nocturnal locations used by individuals of all three species, and were used to estimate body temperatures of *L. nannotis* and *L. rheocola*, and to measure desiccation rates in the microhabitats used by all species (see Chapter 5 for details). We calculated the average body temperature of each frog in each diurnal and nocturnal microhabitat (day: 7:00-18:30, night: 19:00-6:30), and we weighed each model to the nearest 0.1 g immediately before and after placement in each frog location (over 24 hr for *L. nannotis* and *L. serrata*, and 48 hr for *L. rheocola*), and calculated the percentage of mass lost due to evaporative water loss (Schwarzkopf and Alford 1996, Rowley and Alford 2010).

Movements

At each study site, we used flagging tape to mark a transect along the stream to serve as a reference for frog locations (200-400 m in length), and we also used flagging tape to mark each frog location. Field measurements (to the nearest 0.1 m) were used to calculate the mean value of each of the following five movement parameters for each frog: (1) sum of horizontal and vertical distance between consecutive (day to night, or night to day) locations, (2) horizontal distance between consecutive locations, (3) horizontal distance moved from one day location to the next day location, (4) horizontal distance moved from one night location to the next night location, and (5) total length of stream used during the study. Parameters #3 and #4

were calculated for *L. nannotis* and *L. serrata* only. Movement distances were calculated only when frogs were located on consecutive surveys and changed locations; when frogs were not located on consecutive surveys or did not move from their previous location, movement distances for the time interval concerned were recorded as missing values and were not included in any analyses.

We also calculated the probability of movement (proportion of locations in which the frog changed locations) for each frog (1) between consecutive locations, (2) from one day location to the next day location, and (3) from one night location to the next night location. Parameters #2 and #3 were calculated for *L. nannotis* and *L. serrata* only. We also examined the position of frogs in relation to the stream; we calculated the mean value for each of the following parameters: (1) day height above stream, (2) night height above stream, (3) day horizontal distance from stream, (4) night horizontal distance from stream, (5) day straight-line distance from stream, and (6) night straight-line distance from stream.

Data analysis

Because many of the microhabitat and movement variables were highly correlated, we used principal components analyses (PCAs) to identify correlated variables within microhabitat and movement datasets for each species and combine them into fewer factors. There was little variation in some parameters for some species; therefore, only parameters with variation in at least five individuals were included in analysis (Tables 6.1, 6.2). Separate analyses were performed for data on the microhabitats and movements of each species. These analyses generated factors with scores for each frog and loading values for each variable that designated the importance of the variable on the factor (ranging from -1.0 to +1.0). Factors with eigenvalues >1 were retained for further analysis (Quinn and Keough 2002).

We used two types of generalised linear mixed-effects models to examine patterns of microhabitat use and movement of frogs, and the relationships of these

behaviours with infection status and infection intensity. Infection status was coded as a binomial response variable, so we used generalised linear mixed-effects models with a binomial family and a logit link function. The $\log_{10}$ of infection intensity (zoospore equivalents per swab sample), was used as a linear response variable and modelled using generalised linear mixed-effects models with a Gaussian family and an identity link function. We evaluated sets of candidate models that included all factors from PCAs with eigenvalues >1 as fixed effects. For all models, we included site identity as a random effect to control for any effects specific to particular sites. Although our sites were chosen to span a wide range of elevations, and thereby a wide range of environmental conditions, elevation was not included as an effect in models because we wanted to evaluate the effects of measured environmental variables, many of which vary with elevation. Any effects of elevation not accounted for by measured environmental variables should be accounted for by site effects. To avoid overfitting models, we did not include interactions between variables.

For each species, behaviour type (microhabitat or movement), and analysis type (infection status and infection intensity), we developed a set of candidate models that tested all combinations of fixed effects, in addition to the random effect of site. We used Akaike's Information Criterion with adjustment for finite sample size (AICc) to determine the strength of evidence for each model relative to the candidate set of models, using the criteria of Burnham and Anderson (2002). We averaged the best-supported models (ΔAICc <2) to obtain final models. All statistical analyses were performed in program R, version 2.15.2 (R Core Team 2012) using the lme4 (Bates et al. 2012) and MuMIn (Barton 2013) packages.

Results

Microhabitat use

Principal components analyses

For *Litoria nannotis*, a PCA incorporating seven microhabitat variables that met

the criteria for inclusion in the PCA resulted in three factors that together explained 78.2% of the variation among microhabitat characteristics (Table 6.1). Factor 1 was positively associated with sheltered microhabitats at night, and with diurnal and nocturnal sites on rock, and was negatively associated with sites on vegetation at night, and on leaf litter during the day. Factor 2 was positively associated with sheltered microhabitats during the day and night, and with wet microhabitats at night. Factor 3 was negatively associated with nocturnal sites on wood.

A PCA for *Litoria rheocola* that incorporated nine microhabitat variables that met the criteria for inclusion in the PCA resulted in three factors that together explained 84.3% of the variation among micohabitat characteristics (Table 6.1). Factor 1 was positively associated with nocturnal microhabitats that were wet and sheltered, particularly sites on rock, and this factor was negatively associated with sites on vegetation at night. Factor 2 was positively associated with diurnal sites on vegetation, and negatively associated with diurnal microhabitats that were wet and sheltered, particularly rock. Factor 3 was positively associated with litter used during the day.

For *Litoria serrata*, a PCA incorporating 13 microhabitat variables that met the criteria for inclusion in the PCA resulted in five factors that together explained 78.2% of the variation among microhabitat characteristics (Table 6.1). Factor 1 was positively associated with wet microhabitats during the day and night, and with nocturnal microhabitats that were sheltered; it was also positively associated with diurnal and nocturnal sites on rock, and with sites on leaf litter at night. In addition, Factor 1 was negatively associated with vegetation used during the day and night. Factor 2 was positively associated with diurnal and nocturnal sites on leaf litter, and negatively associated with diurnal and nocturnal sites on rock. Factor 3 was positively associated with wet microhabitats during the day, particularly soil; this factor was also negatively associated with sheltered microhabitats at night, and diurnal and nocturnal sites on wood. Factor 4 was positively associated with sites on wood during the day and night. Factor 5 was positively associated with sheltered microhabitats during the day, and

with sites on wood at night.

Infection status modelling

The microhabitats used by frogs were related to their infection status in *Litoria rheocola* and *L. serrata*, but not *L. nannotis* (Table 6.3, Figure 6.1). For *L. rheocola*, one model with ΔAICc <2 (Nagelkerke R^2: 46.7%) suggests that infection probability increased as values in Factor 1 increased ($\chi^2 = 24.236$, df = 1, $P < 0.001$; Table 6.3, Figure 6.1). This indicates that infected frogs used wet and sheltered microhabitats more often at night than uninfected frogs, particularly rocks, and infected frogs avoided nocturnal microhabitats that were drier and more exposed, especially vegetation (Table 6.1, Figure 6.1). For *L. serrata*, five models with ΔAICc <2 (maximum R^2: 30.4%) were averaged to produce a final model that suggests that infection probability increased as values in Factors 2 decreased, and values in Factors 4 and 5 increased ($\chi^2 = 10.500$ df = 3, $P = 0.015$; Table 6.3, Figure 6.1). This indicates that infected frogs were more likely to use sheltered microhabitats during the day than uninfected frogs, and during both day and night, infected frogs used sites on rock and wood more often than uninfected frogs, and tended to avoid using leaf litter (Table 6.1, Figure 6.1). For *L. nannotis*, the intercept-only model had the second lowest ΔAICc value (0.270), which was substantially <2 (Table 6.3). This suggests that models incorporating microhabitat variables did not improve the fit of the null model; we therefore did not produce an averaged model for this species.

Infection intensity modelling

The microhabitats used by infected frogs were related to their infection intensity in *Litoria rheocola*, but not in *L. nannotis* or *L. serrata* (Table 6.4, Figure 6.2). In *L. rheocola*, three models with ΔAICc <2 (maximum Nagelkerke R^2: 11.2%) were averaged to produce a final model that suggests that infection intensity increased as values in Factors 1 and 3 decreased, and values in Factor 2 increased ($\chi^2 = 25.772$ df

= 3, P < 0.001; Table 6.4; Figure 6.2). This indicates that infected frogs with higher infection loads were more likely to use microhabitats that were drier and more exposed, especially vegetation during the night, and they were likely to avoid using wet and sheltered microhabitats, especially sites on rock and leaf litter (Table 6.1, Figure 6.2). In our model sets for *L. nannotis* and *L. serrata*, only the intercept-only models had ΔAICc <2 (Table 6.4), indicating that there was no relationship between our measures of microhabitat use and infection intensity in these species.

Temperatures and desiccation rates

We measured the average temperatures and relative desiccation rates of microhabitats used by frogs to qualitatively describe thermal and hydric characteristics of the substrates used by frogs during our study. For all three species, substrates used at night were typically cooler and more desiccating than substrates used during the day (Figure 6.3). Notable exceptions to this pattern are vegetation and leaf litter used by *L. serrata*, which were more desiccating during the day than night. Overall, vegetation was the most desiccating substrate, and it was also the warmest substrate used during the day, but the coolest substrate at night. Temperatures of microhabitats on rock were moderate with little variation between day and night; rock was also usually the wettest substrate. Frog microhabitats that consisted of leaf litter were cool and moist for *L. nannotis* and *L. rheocola*, but warmer and drier for *L. serrata*. Wood substrates were moist, but variable in temperature for *L. nannotis*, whereas wood used by *L. serrata* was moist and cool.

Movements

Principal components analysis

For *Litoria nannotis*, a PCA incorporating 14 microhabitat variables that met the criteria for inclusion in the PCA resulted in three factors that together explained 78.7% of the variation among microhabitat characteristics (Table 6.2). Factor 1 was positively

related to the position of frogs in relation to the stream during the day and night, which includes the horizontal distance from stream, straight-line distance from stream, and the height above stream. Factor 2 was negatively related to with distances moved by frogs, including the total (sum of horizontal and vertical) distance moved between day and night locations, the horizontal distance moved between day and night locations, between successive day locations, and between successive night locations, and the total length of stream used during our study. Factor 3 was negatively related to movement probablilty, which includes the probability of movement between day and night locations, between successive day locations, and between successive night locations.

For *Litoria rheocola*, a PCA incorporating 10 microhabitat variables that met the criteria for inclusion in the PCA resulted in three factors that together explained 73.7% of the variation among microhabitat characteristics (Table 6.2). Factor 1 was positively related to the total distance moved between day and night locations, the horizontal distance moved between day and night locations, and the horizontal distance frogs were positioned from the stream at night. Factor 2 was positively related to the straight-line distance from the stream during the day, and the height above stream during the day. Factor 3 was negatively related to the straight-line distance from the stream at night, and the height above stream at night.

A PCA for *Litoria serrata* incorporating 14 microhabitat variables that met the criteria for inclusion in the PCA resulted in four factors that together explained 77.4% of the variation among microhabitat characteristics (Table 6.2). Factor 1 was positively related to the total distance moved between day and night locations, and the position of frogs in relation to the stream during the day and night, which includes the horizontal distance from stream, straight-line distance from stream, and the height above stream. Factor 2 was positively related to the probability of changing nocturnal locations; Factor 3 was positively related to the probability of changing locations between day and night; Factor 4 was positively related to the probability of changing diurnal

locations.

Infection status modelling

The movement patterns of frogs were related to their infection status for *Litoria rheocola* and *L. serrata*, but not *L. nannotis* (Table 6.5, Figure 6.1). For *L. rheocola*, three models with ΔAICc <2 (maximum Nagelkerke R^2: 74.4%) were averaged to produce a final model that suggests that infection probability increased as values in Factors 1 and 3 increased, and values in Factor 2 decreased ($\chi^2 = 39.181$, df = 3, P < 0.001; Table 6.5; Figure 6.1). This indicates that infected frogs were more likely to move longer distances between day and night locations, but use microhabitats that were closer to the stream during the day and night than uninfected frogs (Table 6.2, Figure 6.1). For *L. serrata*, two models with ΔAICc <2 (maximum R^2: 28.2%) were averaged to produce a final model that suggests that infection probability increased as values in Factors 4 increased, and values in Factor 3 decreased ($\chi^2 = 8.810$, df = 2, P < 0.012; Table 6.5; Figure 6.1). This indicates that infected frogs were more likely to move longer distances between successive day locations than uninfected frogs, but change locations between day and night less often (Table 6.2, Figure 6.1). For *L. nannotis*, the intercept-only model had the second lowest ΔAICc value (0.493), which was substantially <2 (Table 6.5). This suggests that models incorporating microhabitat variables did not improve the fit of the null model; we therefore did not produce an averaged model for this species.

Infection intensity modelling

The movement patterns of infected frogs were related to their infection intensity in *Litoria rheocola*, but not in *L. nannotis* or *L. serrata* (Table 6.6, Figure 6.2). In *L. rheocola*, one model with ΔAICc <2 (R^2: 30.9%) was averaged to produce a final model that suggests that infection intensity increased as values in Factor 3 increased ($\chi^2 = 50.400$ df = 2, P < 0.001; Table 6.6, Figure 6.2). This indicates that frogs with

higher infection loads were more likely to use microhabitats that were located closer to the stream (Table 6.2, Figure 6.2). In our model sets for *L. nannotis* and *L. serrata*, only the intercept-only models had ΔAICc <2 (Table 6.6), indicating that there was no relationship between our measures of movement and infection intensity in these species.

Discussion

Variation in the behaviour of amphibians can affect their vulnerability to *B. dendrobatidis* by influencing their exposure to the pathogen and affecting the microenvironmental conditions experienced by both host and pathogen. Behaviour can affect transmission rates of *B. dendrobatidis* in nature (Rowley and Alford 2007a), and the body temperatures of individuals or the mean body temperatures of populations can be related to infection status (Richards-Zawacki 2009, Rowley and Alford 2013, Chapter 5). In three species of Australian rainforest frogs, infected frogs maintain cooler body temperatures and have lower desiccation rates than uninfected frogs (Chapter 5). Our study is the first to demonstrate the behavioural mechanisms underlying these patterns. We found that the microhabitat use and movements of frogs are related to their infection probability and their infection loads.

We found relationships between the microhabitats used by individual frogs and their infection status in two of three species (Figure 6.1). In *Litoria rheocola* and *L. serrata*, infected frogs used microhabitats that were wet and sheltered, particularly those characterised by rock and decaying wood, more frequently than uninfected frogs, and they avoided drier and more exposed microhabitats, especially vegetation (Figures 6.1, 6.3). Although substrates could be wet from rain, most of the wet substrates used by frogs were in contact with stream water. Because *B. dendrobatidis* cannot tolerate desiccation (Johnson et al. 2003) and can be transmitted to frogs through contact with water (Johnson and Speare 2005), our results suggest that frogs that preferred moister microhabitats were more likely to become and remain infected

than frogs that used drier microhabitats. However, it is also possible that infected frogs may have altered their behaviour after becoming infected by selecting wetter microhabitats. If this is the case, it could represent manipulation of the host by *B. dendrobatidis* because it would accelerate pathogen growth on hosts and facilitate pathogen dispersal. It could also reflect behavioural changes in frogs brought about by decreases in skin function and disruptions in skin shedding (Nichols et al. 2001, Voyles et al. 2009). Regardless of the cause, wet microhabitats used by infected frogs are likely to perpetuate their infections and could lead to mortality. In laboratory studies, infected amphibians maintained in wet conditions have an increased probability of mortality as compared to amphibians maintained in drier conditions (Bustamante et al. 2010, Murphy et al. 2011).

The preference of infected frogs for wet microhabitats could influence their body temperatures. Because the water temperatures of our rainforest streams are relatively cool and constant (Chapter 5), frogs that used wet substrates more often were likely to maintain body temperatures within the optimal thermal range for *B. dendrobatidis* growth (15-25°C, Piotrowski et al. 2004, Stevenson et al. 2013). Our previous research has demonstrated that infected individuals of our three study species had cooler body temperatures and lower desiccation rates than uninfected frogs (Chapter 5), so the results we present here provide a mechanism for those relationships for *Litoria rheocola* and *L. serrata*. However, we did not find any differences between the microhabitats used by infected and uninfected *L. nannotis*. This species typically perches on wet boulders near waterfalls and fast-flowing sections of streams during the day and night (Hodgkison and Hero 2001, Rowley and Alford 2007b, Puschendorf et al. 2012), and we found relatively little variation in microhabitat use in comparison to the other species. The differences that have been shown in thermal (Rowley and Alford 2013, Chapter 5) and hydric (Chapter 5) conditions experienced by infected and uninfected *L. nannotis* in nature must arise from subtle differences in behaviour or microhabitat use not captured in our data.

We also found that microhabitat use was related to infection intensity in *L. rheocola*, but not in the other two species (Figure 6.2). In *L. rheocola*, infected frogs with higher infection loads were more likely to use sites on vegetation, and less likely to use wet, sheltered microhabitats, particularly rocks and leaf litter. This pattern is consistent with our results in Chapter 5, which demonstrate that infection intensity in *L. rheocola* increased with increasing desiccation rates and increasing proportion of body temperatures below 16°C. Frogs on vegetation should experience higher rates of desiccation and lose more heat through radiation, and thus experience cooler body temperatures (Figure 6.3). This could represent an adaptive change in behaviour, because both drier environments and temperatures below the optimal thermal range for *B. dendrobatidis* growth should slow pathogen growth rates on infected frogs. Alternatively, use of more exposed locations could be explained by a lower mobility level of frogs with high infection loads.

We found that the infection status of frogs was also related to their movement patterns in *Litoria rheocola* and *L. serrata* (Figure 6.1), but these patterns differed between species. In *L. rheocola*, infected frogs moved longer distances between day and night locations than uninfected frogs, but they were positioned closer to the stream during both day and night. In *L. serrata*, the distances between successive diurnal shelter sites were located farther apart for infected frogs than uninfected frogs, and the probability of changing locations between day and night locations was lower for infected frogs. Some of these movement patterns can be explained by differences in microhabitat use between infected and uninfected frogs. The closer positioning of infected *L. rheocola* to the stream could reflect their preference for wet microhabitats, especially rocks. Infection intensity was negatively related to the distance frogs were positioned from the stream in *L. rheocola*, which indicates that frogs with higher infection loads used microhabitats that were closer to the stream than frogs with lower infection loads. The tendency for infected frogs of both species to move greater distances could be related to the distribution of their preferred microhabitats in the

landscape. Our study streams that had beds composed of smaller rocks, but infected frogs often sheltered in moist crevices formed by larger rocks in the stream, which were not abundant at our study sites (EAR, personal observation). Therefore, it appears that infected frogs may have travelled longer distances to reach those scarce microhabitats.

In *L. serrata*, the lower movement probabilities for infected frogs than for uninfected frogs could reflect causes or consequences of the infection. Because pathogens often accumulate in an animal's environment over time, more sedentary animals can be more vulnerable to infection (Foldstad et al. 1991, Altizer et al. 2011). It is also possible that frogs changed locations less often after they became infected; reductions in levels of movement and activity are common behavioural changes displayed by animals following infection by a pathogen (Moore 2002). Individual *L. nannotis* changed locations much less frequently than individuals of the other species, and we did not find any differences in the movement distances or positions between infected and uninfected *L. nannotis*. The sedentary behaviour of *L. nannotis* may contribute to its high vulnerability to *B. dendrobatidis*.

Our study demonstrates that the patterns of movement and microhabitat use by individual frogs are significantly related to their infection status and intensity. Across species, infected frogs tended to use microhabitats that were wet and sheltered, particularly those characterised by rock and decaying wood, more frequently than uninfected frogs, and they avoided drier and more exposed microhabitats, especially vegetation. This difference could reflect pre-existing differences in individual behaviour, or changes in the behaviour of infected individuals. However, our results suggest that at least in *L. rheocola*, highly infected frogs altered their behaviour to use microenvironments less favourable for *B. dendrobatidis* growth; as infection intensity increased, frogs became more likely to use dry, exposed sites than frogs with lower infection loads, and less likely to use wet, sheltered microhabitats. The infection status of frogs was also related to their movement distances and their positions in relation to

the stream. Infected frogs tended to remain closer to the stream and move less often, but move longer distances when they did move. Our results demonstrate that there can be wide variation among individuals in movement and microhabitat selection, and that this variation both within among species can affect vulnerability to a widespread pathogen, which may provide opportunities for natural selection.

Table 6.1. Eigenvalues and loading values for factors obtained from principal components analyses for characteristics of microhabitats used by frogs of three species. Each frog location was placed into one of five substrate categories: vegetation, rock, leaf litter, soil, and wood; each substrate could also be classified as wet and/or sheltered. For each frog, we calculated the proportion of locations in each substrate category, the proportion of wet locations, and the proportion of sheltered locations, and we used these values in the analyses. Factors obtained from these analyses explained 78.2% of the variation among microhabitat characteristics for *Litoria nannotis*, 84.3% for *L. rheocola*, and 78.2% for *L. serrata*.

Eigenvalues and parameters	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Litoria nannotis					
Eigenvalue	3.491	1.616	1.152	-	-
Variation explained (%)	43.6	20.2	14.4	-	-
Day litter	-0.754	0.289	0.203	-	-
Day rock	0.854	-0.366	0.187	-	-
Day shelter	0.223	0.670	0.448	-	-
Night rock	0.944	-0.145	-0.026	-	-
Night shelter	0.557	0.660	0.087	-	-
Night vegetation	-0.866	-0.018	0.359	-	-
Night wood	0.357	0.615	-0.091	-	-
Litoria rheocola					
Eigenvalue	3.476	2.829	1.280	-	-
Variation explained (%)	38.6	31.4	14.2	-	-
Day litter	-0.350	0.731	0.841	-	-
Day rock	0.394	0.387	-0.434	-	-
Day shelter	0.090	-0.770	0.333	-	-
Day vegetation	-0.323	-0.778	-0.470	-	-
Day wet	0.255	-0.799	0.168	-	-
Night rock	0.906	0.239	0.111	-	-
Night shelter	0.860	0.230	0.008	-	-
Night vegetation	-0.901	-0.312	-0.091	-	-
Night wet	0.805	0.316	0.065	-	-
Litoria serrata					
Eigenvalue	3.405	2.070	1.849	1.763	1.085
Variation explained (%)	26.2	15.9	14.2	13.6	8.3
Day litter	0.116	0.822	0.129	-0.172	0.798
Day rock	0.627	-0.644	0.209	0.074	-0.099
Day shelter	0.360	0.020	0.302	-0.164	-0.072
Day soil	-0.072	0.146	0.470	0.407	-0.112
Day vegetation	-0.607	-0.207	-0.193	-0.487	0.297
Day wet	0.592	0.152	0.526	0.429	0.097
Day wood	-0.211	0.022	-0.599	0.693	-0.135
Night litter	0.538	0.535	-0.307	-0.335	-0.123
Night rock	0.617	-0.649	-0.055	-0.126	-0.152
Night shelter	0.568	0.229	-0.530	-0.176	0.149
Night vegetation	-0.830	0.154	0.379	0.178	0.122
Night wet	0.648	0.326	0.055	0.260	0.054
Night wood	-0.005	-0.038	-0.529	0.613	0.475

Table 6.2. Eigenvalues and loading values for factors obtained from principal components analyses for movements of frogs of three species. The mean values for the following movement measurements were calculated for each frog (except for probabilities) and used in the analyses: (1) sum of horizontal and vertical distance between consecutive locations (Day-night total distance), (2) horizontal distance between consecutive locations (Day-night distance), (3) horizontal distance from one day location to the next (Day-day distance), (4) horizontal distance from one night location to the next (Night-night distance), (5) total length of stream used during the study (Stream length), (6) probability of movement between consecutive locations (Day-night probability), (7) probability of movement from one day location to the next (Day-day probability), (8) probability of movement from one night location to the next (Night-night probability), (9) day height above stream (Day height), (10) night height above stream (Night height), (11) day horizontal distance from stream (Day position), (12) night horizontal distance from stream (Night position), (13) day straight-line distance from stream (Day straight-line position), (14) night horizontal distance from stream (Night straight-line position). Parameters #3, #4, #7, and #8 were included for *Litoria nannotis* and *L. serrata* only. Factors obtained from these analyses explained 78.7% of the variation among microhabitat characteristics for *L. nannotis*, 73.7% for *L. rheocola*, and 77.4% for *L. serrata*.

Eigenvalues and parameters	Factor 1	Factor 2	Factor 3	Factor 4
Litoria nannotis				
Eigenvalue	5.467	3.728	1.824	-
Variation explained (%)	39.1	26.6	13.0	-
Day-night total distance	0.452	-0.708	0.100	-
Day-night distance	0.434	-0.790	0.049	-
Day-day distance	0.239	-0.836	-0.019	-
Night-night distance	0.378	-0.827	0.045	-
Stream length	0.250	-0.885	-0.087	-
Day-night probability	0.408	0.086	-0.719	-
Day-day probability	0.250	0.058	-0.729	-
Night-night probability	0.345	0.080	-0.782	-
Day height	0.823	0.332	0.247	-
Night height	0.885	0.234	0.138	-
Day position	0.779	0.279	0.021	-
Night position	0.876	0.161	0.059	-
Day straight-line position	0.880	0.327	0.193	-
Night straight-line position	0.924	0.208	0.136	-
Litoria rheocola				
Eigenvalue	3.336	2.334	1.700	-
Variation explained (%)	33.4	23.3	17.0	-
Day-night total distance	0.916	-0.182	0.036	-
Day-night distance	0.899	-0.193	0.179	-
Stream length	0.596	-0.235	0.221	-
Day-night probability	0.317	0.376	-0.035	-
Day height	-0.061	0.858	0.394	-
Night height	0.230	0.568	-0.768	-
Day position	0.338	0.191	0.505	-
Night position	0.840	-0.092	0.090	-
Day straight-line position	0.019	0.862	0.473	-
Night straight-line position	0.595	0.470	-0.620	-
Litoria serrata				
Eigenvalue	5.702	2.140	1.882	1.113
Variation explained (%)	40.7	15.3	13.4	7.9
Day-night total distance	0.722	0.120	-0.473	0.060

Day-night distance	0.532	0.511	-0.447	-0.006
Day-day distance	0.211	-0.156	0.119	0.906
Night-night distance	0.624	0.391	-0.438	0.022
Stream length	0.161	0.548	-0.359	0.233
Day-night probability	-0.010	0.490	0.761	0.067
Day-day probability	0.461	0.473	0.348	-0.188
Night-night probability	0.119	0.760	0.349	0.187
Day height	0.808	-0.394	0.105	0.183
Night height	0.841	-0.185	-0.127	-0.095
Day position	0.795	-0.157	0.376	-0.063
Night position	0.795	0.055	0.298	-0.258
Day straight-line position	0.870	-0.339	0.207	0.137
Night straight-line position	0.911	-0.123	-0.001	-0.164

Table 6.3. Generalised linear mixed-effects models (family: binomial, link function: logit) were used to examine relationships between factors obtained from principal components analyses (Table 6.1) for the microhabitats used by frogs of three species and their *Batrachochytrium dendrobatidis* infection status (infected or uninfected). We included site identity as a random effect, and all factors with eigenvalues >1 as fixed effects. For each species, we developed a set of candidate models that examined models combining the random effect of site with all combinations of factors, and we ranked models according to Akaike's Information Criterion with adjustment for finite sample size (AICc). All models with ΔAICc <3 are shown, but only the best-supported models (ΔAICc <2) were averaged to obtain a final model. The estimates for the final models are given below the candidate models. A final model was not produced for *Litoria nannotis* because the ΔAICc value for the intercept-only model was <2, indicating poor model fit.

Candidate models

Model effects	AICc	ΔAICc	Weight	R^2
Litoria nannotis				
Factor 1	91.282	0.000	0.232	0.106
Intercept only	91.553	0.270	0.203	0.063
Factor 1, Factor 3	91.736	0.454	0.185	0.136
Factor 3	92.555	1.273	0.123	0.084
Factor 1, Factor 2	93.482	2.199	0.077	0.106
Factor 2	93.700	2.418	0.069	0.064
Factor 1, Factor 2, Factor 3	93.705	2.423	0.069	0.142
Litoria rheocola				
Factor 1	65.588	0.000	0.442	0.467
Factor 1, Factor 3	67.663	2.076	0.157	0.471
Factor 1, Factor 2	67.907	2.319	0.139	0.467
Litoria serrata				
Factor 2, Factor 5	73.106	0.000	0.130	0.264
Factor 2, Factor 4, Factor 5	73.235	0.129	0.122	0.304
Factor 4, Factor 5	73.965	0.859	0.084	0.248
Factor 2	74.329	1.223	0.070	0.197
Factor 2, Factor 4	74.459	1.353	0.066	0.238
Factor 4	75.130	2.024	0.047	0.181
Factor 2, Factor 3, Factor 4, Factor 5	75.371	2.265	0.042	0.310
Factor 2, Factor 3, Factor 5	75.382	2.276	0.042	0.265
Factor 1, Factor 2, Factor 5	75.462	2.356	0.040	0.264
Intercept only	75.514	2.408	0.039	0.173
Factor 1, Factor 2, Factor 4, Factor 5	75.697	2.591	0.036	0.304

Final averaged models

Model effect	*Litoria rheocola*	*Litoria serrata*
Intercept	1.008	-0.925
Factor 1	0.382	-
Factor 2	-	-0.452
Factor 3	-	-
Factor 4	-	0.320
Factor 5	-	0.461

Table 6.4. Generalised linear mixed-effects models (family: Gaussian, link function: identity) were used to examine relationships between factors obtained from principal components analyses (Table 6.1) for the microhabitats used by frogs of three species infected by *Batrachochytrium dendrobatidis* and their intensity of infection (log_{10} of zoospore equivalents per skin swab). We included site identity as a random effect, and all factors with eigenvalues >1 as fixed effects. For each species, we developed a set of candidate models that examined models combining the random effect of site with all combinations of factors, and we ranked models according to Akaike's Information Criterion with adjustment for finite sample size (AICc). All models with ΔAICc <3 are shown (or the top three models), but only the best-supported models (ΔAICc <2) for each species were averaged to obtain a final model. The estimates for the final model for *Litoria rheocola* are given below the candidate models. Final models were not produced for *L. nannotis* and *L. serrata* because the intercept-only models were the highest-ranking models, indicating poor model fit.

Candidate models

Model effects	AICc	ΔAICc	Weight	R^2
Litoria nannotis				
Intercept only	174.299	0.000	0.785	0.000
Factor 2	178.862	4.563	0.080	0.008
Factor 3	179.018	4.718	0.074	0.002
Litoria rheocola				
Factor 2	123.731	0.000	0.451	0.112
Factor 3	124.732	1.002	0.273	0.000
Factor 1	125.724	1.993	0.167	0.001
Litoria serrata				
Intercept only	42.669	0.000	0.567	0.252
Factor 2	45.493	2.823	0.138	0.305
Factor 5	45.762	3.093	0.121	0.335

Final averaged model

Model effect	*Litoria rheocola*
Intercept	1.868
Factor 1	-0.004
Factor 2	0.083
Factor 3	-0.006

Table 6.5. Generalised linear mixed-effects models (family: binomial, link function: logit) were used to examine relationships between factors obtained from principal components analyses (Table 6.2) for the movements of frogs of three species and their *Batrachochytrium dendrobatidis* infection status (infected or uninfected). We included site identity as a random effect, and all factors with eigenvalues >1 as fixed effects. For each species, we developed a set of candidate models that examined models combining the random effect of site with all combinations of factors, and we ranked models according to Akaike's Information Criterion with adjustment for finite sample size (AICc). All models with ∆AICc <3 are shown, but only the best-supported models (ΔAICc <2) were averaged to obtain a final model. The estimates for the final models are given below the candidate models. A final model was not produced for *L. nannotis* because the ΔAICc value for the intercept-only model was <2, indicating poor model fit.

Candidate models

Model effects	AICc	ΔAICc	Weight	R^2
Litoria nannotis				
Factor 1, Factor 2	83.899	0.000	0.194	0.150
Factor 1	83.967	0.068	0.188	0.107
Intercept only	84.392	0.493	0.152	0.057
Factor 1, Factor 3	84.979	1.080	0.113	0.130
Factor 1, Factor 2, Factor 3	85.067	1.167	0.108	0.171
Factor 3	85.069	1.170	0.108	0.086
Factor 2	85.669	1.769	0.080	0.075
Factor 2, Factor 3	86.396	2.497	0.056	0.104
Litoria rheocola				
Factor 3	46.562	0.000	0.289	0.744
Factor 2	46.975	0.413	0.235	0.740
Factor 1	47.177	0.616	0.212	0.738
Factor 2, Factor, 3	48.921	2.360	0.089	0.746
Factor 1, Factor 3	49.029	2.467	0.084	0.745
Factor 1, Factor 2	49.494	2.933	0.067	0.740
Litoria serrata				
Factor 4	69.489	0.000	0.293	0.252
Factor 3, Factor 4	70.243	0.755	0.201	0.282
Factor 2, Factor 4	71.672	2.183	0.098	0.254
Factor 1, Factor 4	71.711	2.222	0.096	0.254
Factor 1, Factor 3, Factor 4	72.085	2.597	0.080	0.292

Final averaged models

Model effect	*Litoria rheocola*	*Litoria serrata*
Intercept	1.117	-0.941
Factor 1	0.011	-
Factor 2	-0.038	-
Factor 3	0.110	-0.153
Factor 4	-	0.849

Table 6.6. Generalised linear mixed-effects models (family: Gaussian, link function: identity) were used to examine relationships between factors obtained from principal components analyses (Table 6.2) for the movements of frogs of three species infected by *Batrachochytrium dendrobatidis* and their intensity of infection (log_{10} of zoospore equivalents per skin swab). We included site identity as a random effect, and all factors with eigenvalues >1 as fixed effects. For each species, we developed a set of candidate models that examined models combining the random effect of site with all combinations of factors, and we ranked models according to Akaike's Information Criterion with adjustment for finite sample size (AICc). All models with ΔAICc <3 are shown (or the top three models), but only models with ΔAICc <2 are supported by our data. The estimates for the final model for *Litoria rheocola* are given below the candidate models. Final models were not obtained for *Litoria nannotis* and *L. serrata* because the intercept-only models were the highest-ranking models, indicating poor model fit.

Candidate models

Model effects	AICc	ΔAICc	Weight	R^2
Litoria nannotis				
Intercept only	164.446	0.000	0.780	0.000
Factor 3	168.645	4.198	0.096	0.012
Factor 2	169.347	4.900	0.067	0.009
Litoria rheocola				
Factor 3	93.289	0.000	0.759	0.309
Factor 2	98.128	4.838	0.068	0.001
Factor 1	98.414	5.124	0.059	0.045
Litoria serrata				
Intercept only	42.669	0.000	0.837	0.252
Factor 4	48.535	5.865	0.045	0.290
Factor 3	48.561	5.892	0.044	0.230

Final model

Model effect	*Litoria rheocola*
Intercept	1.947
Factor 3	0.363

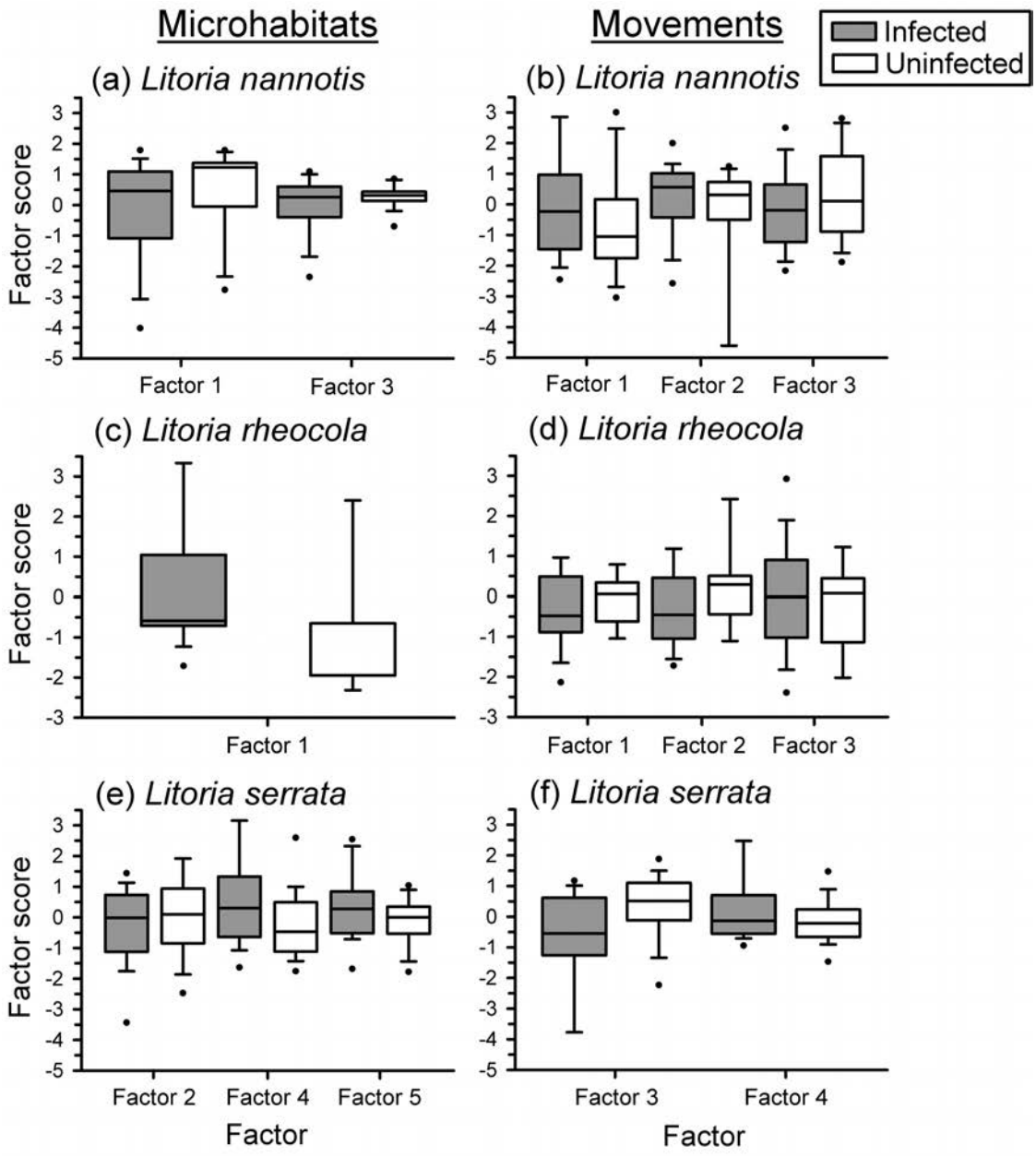

Figure 6.1. Scores from factors derived from principal components analyses for the microhabitat use and movements of frogs of three species that were infected by the chytrid fungus *Batrachochytrium dendrobatidis* or uninfected. All factors supported by our data (ΔAICc <2), as determined by generalised linear mixed-effects models, are shown.

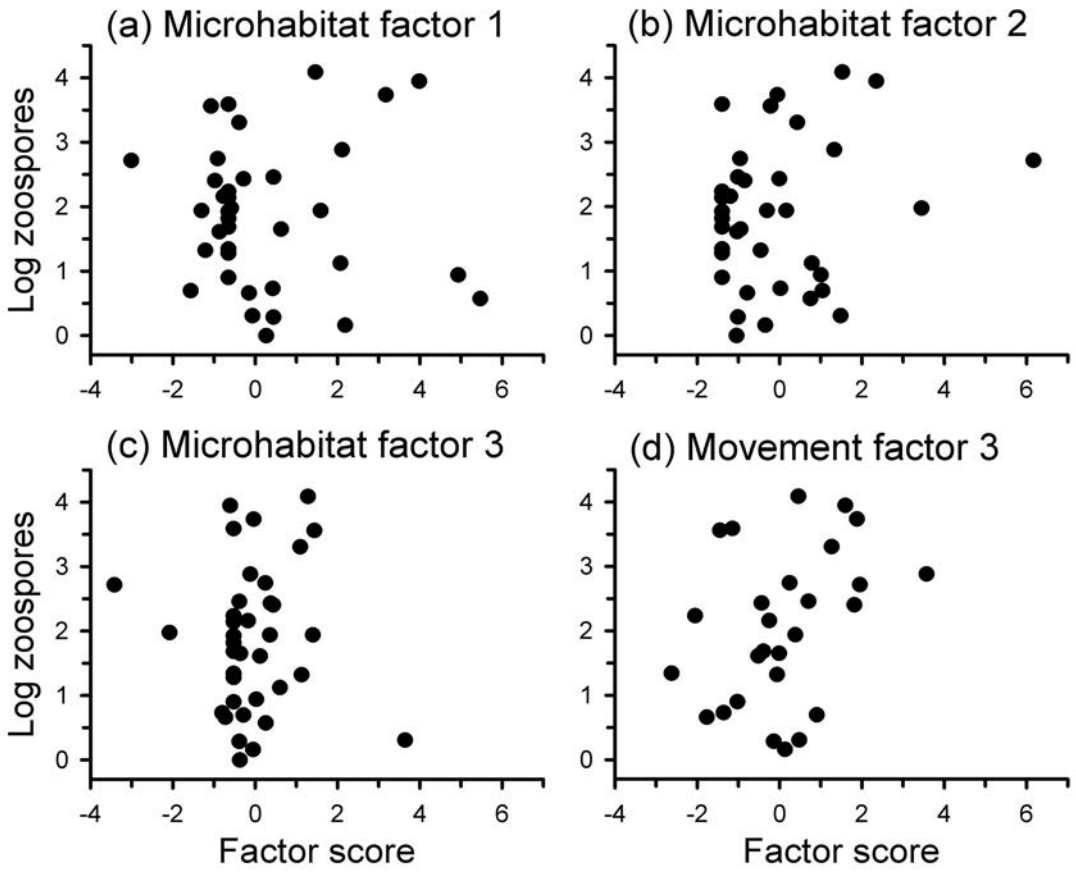

Figure 6.2. Relationships between scores from factors derived from principal components analyses for the microhabitat use and movements of *Litoria rheocola* and the intensity of infection (log_{10} of zoospore equivalents) by the chytrid fungus *Batrachochytrium dendrobatidis*. All factors supported by our data (ΔAICc <2), as determined by generalised linear mixed-effects models, are shown.

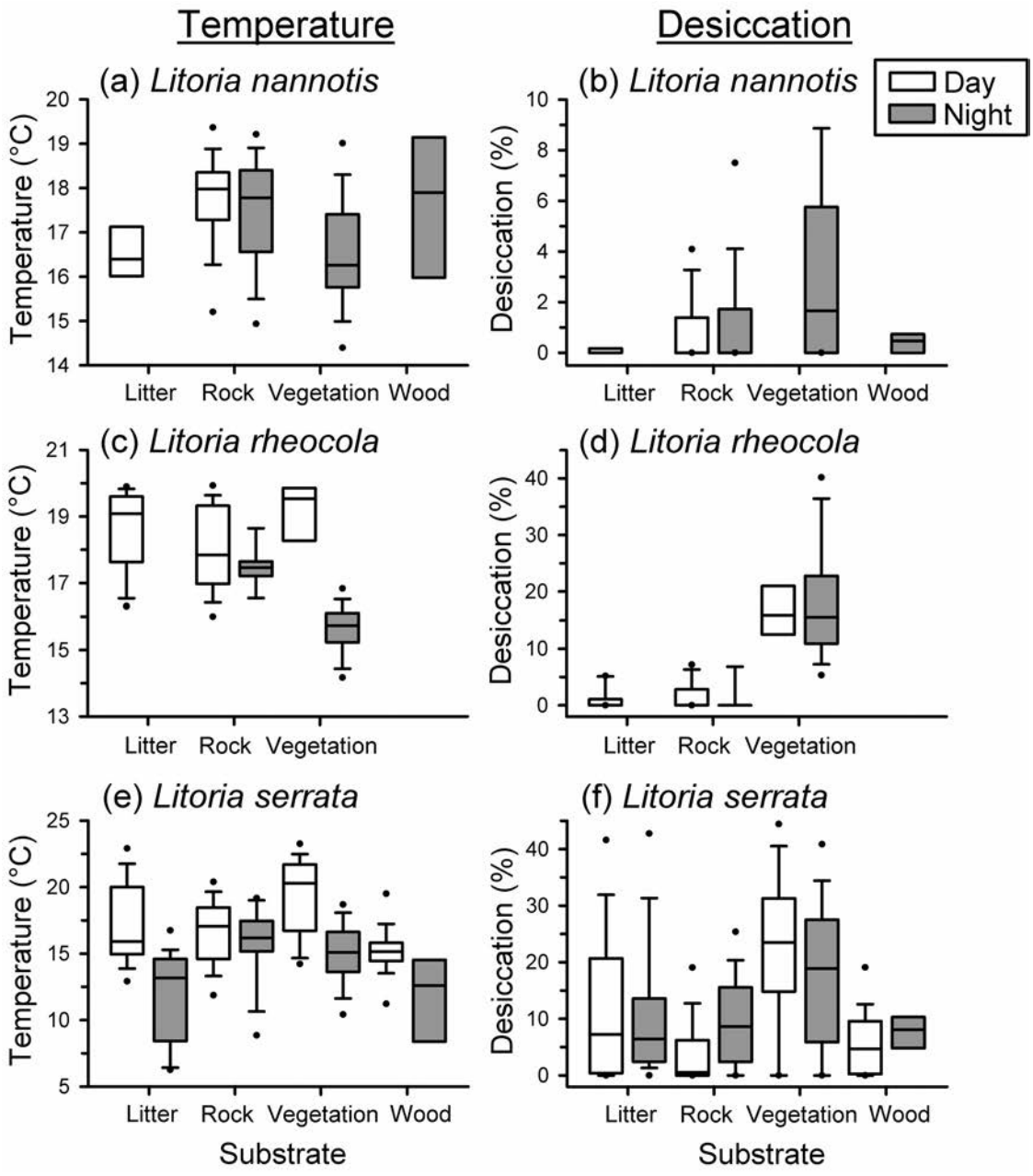

Figure 6.3. Average temperatures and relative desiccation rates experienced by frogs of three species during the day and night during our study when using four types of substrates: leaf litter, rock, vegetation, and wood. Desiccation rates were determined by calculating the mass lost by physical models due to evaporative water loss.

Chapter 7: Behavioural differences between frogs infected and uninfected by the chytrid fungus *Batrachochytrium dendrobatidis*: cause or consequence of infection?

Elizabeth A. Roznik and Ross A. Alford

Abstract

Infections by pathogens can change the behaviour of hosts; however, the behaviour of hosts can also affect the incidence of infections and their course if they are acquired. Australian rainforest frogs infected by the chytrid fungus *Batrachochytrium dendrobatidis* spend more time in wet microhabitats and maintain cooler body temperatures than uninfected individuals. These conditions are favourable for pathogen growth, but it is unknown whether behavioural differences between infected and uninfected frogs reflect effects of innate behaviour on the probability of acquiring or retaining infections, or if they are a result of changes in the behaviour of infected frogs in response to their infection. We performed a laboratory experiment designed to discriminate between these alternatives. We recorded selected body temperatures and water use of naturally infected and uninfected individuals of two frog species (*Litoria nannotis* and *L. serrata*) in thermal gradients, and we re-tested the same individuals after the infected frogs had lost their infections. We found that infection by *B. dendrobatidis* changed the behaviour of *L. nannotis*, but not that of *L. serrata*. Infected *L. nannotis* spent 36% more time in water than uninfected frogs; when previously infected frogs lost their infections, this difference disappeared. Our results suggest that behavioural differences between infected and uninfected frogs can be either a cause (as in *L. serrata*), or a consequence (as in *L. nannotis*) of infection. In *L. nannotis*,

patterns observed in nature may result from changes in the behaviour of infected frogs; once infected, infected frogs prefer wetter microhabitats. However, it is unlikely that changes in behaviour caused by infection are responsible for behavioural differences observed in *L. serrata* in nature; these patterns may reflect variation in individual behaviour that predisposes some frogs to acquiring or maintaining infections. Our results reinforce the importance of individual behaviour in this host-pathogen system, and the complexity of the relationships between *B. dendrobatidis* and different host species. Understanding the nature of relationships between individual amphibian behaviour and *B. dendrobatidis* infection can help explain, predict, and ultimately manage the impacts of chytridiomycosis.

Introduction

The phenotypes of individuals that are infected by a pathogen often differ from those of uninfected individuals (Barber et al. 2000, Moore 2002). These relationships between host phenotype and pathogen infection can reflect causes or consequences of the infection, and these pathways can even co-occur (Blanchet et al. 2009). The behaviour of animals is an example of this; differences in many aspects of the behaviour of infected and uninfected individuals have been observed (Barber et al. 2000, Moore 2002). These differences can reflect effects of behaviour on the probability of acquiring or retaining infections, or they can be a result of changes in the behaviour of infected individuals in response to their infections.

Innate differences in host behaviour can influence pathogen transmission and tolerance, and the buildup of pathogens within hosts (Poulin et al. 1991, Ezenwa 2004, Richards et al. 2010, Sears et al. 2013), especially when individual behavioural traits are consistent over time (Boyer et al. 2010, Koprivnikar et al. 2012, Dizney and Dearing 2013). For example, innate behavioural differences in wood frog *(Lithobates sylvaticus*) tadpoles influence their susceptibility to infection by a parasitic trematode (*Echinoparyphium* sp.); individuals that are consistently more active and exploratory

carry lower parasite loads (Koprivnikar et al. 2012). Pre-existing behavioural differences can contribute to the pattern of distribution of parasites within populations, where most parasites are harboured within few hosts, and the rest of the host population is infected by few or no parasites (Wilson et al. 2001, Poulin 2007).

Infection by pathogens can also change the behaviour of their hosts; modifications to a wide range of behaviours have been reported in many host-pathogen systems (Barber et al. 2000, Moore 2002). These changes may benefit the pathogen, the host, or neither participant in the interaction (Moore 2002, Poulin 2010). Behavioural changes can result from host manipulation by the pathogen, which often facilitates pathogen transmission and decreases host fitness. For example, the trematode *Dicrocoelium dendriticum* must be transmitted from an ant to a sheep; it causes infected ants to climb to the tips of grass blades, where the ants are more likely to be ingested accidentally by grazing sheep (Carney 1969). Some behavioural changes in infected hosts are defensive, benefiting the host by reducing the infection or minimising its negative effects. One defensive response of ectotherms to pathogen infection is “behavioural fever”; many ectotherms elevate their body temperatures by altering their thermoregulatory behaviours, which can enhance host immune responses (Kluger 1979, Wright and Cooper 1981, Boltaña et al. 2013). Finally, changes in host behaviour may simply be side effects of an infection, which may or may not benefit the host or pathogen (Moore 2002, Poulin 2010).

Studying the interactions between a pathogen and its host is important for fully understanding the host-pathogen system, and is vital in attempts to understand, predict, and manage the impacts of emerging diseases. Chytridiomycosis is an amphibian disease that is caused by the chytrid fungus *Batrachochytrium dendrobatidis*; it has recently emerged and caused severe amphibian declines and extinctions in many regions of the world (Kilpatrick et al. 2009). This parasitic fungus attacks the skin cells of amphibians and disrupts their osmoregulatory and transport functions, altering electrolyte concentrations in the blood, which can ultimately cause

cardiac arrest if the fungal population on the host reaches a high density (Voyles et al. 2009). Although *B. dendrobatidis* can influence host survival directly, many individuals carry sublethal infections (e.g., Sapsford 2012), which can affect fitness through changes in host behaviour. Infections can alter the activity levels, foraging performance, and predator-avoidance behaviours of tadpoles (Parris et al. 2006, Venesky et al. 2009, Han et al. 2011, Kleinhenz et al. 2012). However, sublethal effects of *B. dendrobatidis* on the behaviour of adult amphibians are poorly understood.

Previous studies have documented differences between the behaviour of adult frogs that were infected and uninfected by *B. dendrobatidis* (Richards-Zawacki 2009, Rowley and Alford 2013, Chapters 5-6). Studies on Australian rainforest frogs have found that infected individuals have cooler body temperatures and lower desiccation rates, use microhabitats that are cooler and moister, and have movement patterns that differ from uninfected individuals (Rowley and Alford 2013, Chapter 5-6). However, we do not know whether these behavioural differences are a cause or a consequence of infection, or if both pathways may co-occur in this system. The growth, reproduction, and survival of *B. dendrobatidis* are strongly influenced by temperature and moisture (Johnson et al. 2003, Piotrowski et al. 2004, Stevenson 2012); this aquatic fungus thrives under conditions that are moist and cool (15-25°C is optimal, >28°C is lethal) and it is transmitted by contact with water or moist substrates (Rachowicz and Vredenburg 2004). Therefore, it is possible that the behavioural differences documented in Chapters 5 and 6, and by Rowley and Alford (2013) may have determined patterns of infection by *B. dendrobatidis;* individual frogs that preferred cooler, moister microenvironments may have been more likely to become infected than frogs that preferred warmer, drier conditions. Alternatively, frogs may have changed their behaviour after becoming infected by selecting microhabitats that were cooler and moister; this could reflect manipulation of the host by the pathogen or could be a non-adaptive consequence of changes in host physiology. These effects could possibly

interact reciprocally; reciprocal effects occur when a phenotypic trait controls the infection rate, and that trait is then affected by the pathogen (Blanchet et al. 2009). This could occur if frogs that preferred cooler, moister microenvironments are more susceptible to infection, and once they are infected, these preferences become stronger.

Determining whether host behaviour influences infection susceptibility, infection changes host behaviour, or both of these processes interact is very difficult using field data (Blanchet et al. 2009). To enhance our understanding of the causal relationships between amphibian behaviour and infection by *B. dendrobatidis*, we conducted a laboratory experiment. We used two species of frogs with known patterns of behaviour in nature (*Litoria nannotis* and *L. serrata*; Chapters 5-6, Rowley and Alford 2013). We recorded selected body temperatures and water use of naturally infected and uninfected individuals in laboratory thermal gradients, and we re-tested the same individuals after the infected frogs had lost their infections. To gain additional information on sublethal effects of *B. dendrobatidis* infections, we also measured the jumping performance of *L. nannotis* before each thermal gradient trial. Understanding the causal relationships between amphibian behaviour and *B. dendrobatidis* infection is important for understanding and ultimately managing this important host-pathogen system.

Methods

Experimental frogs

We used frogs of two species in our experiment; *Litoria nannotis* and *L. serrata* are both stream-breeding frogs that are endemic to tropical rainforests in northeastern Australia and are susceptible to *Batrachochytrium dendrobatidis* (Hoskin and Hero 2008). These two species behave differently in nature and are differentially susceptible to infection by *B. dendrobatidis* (Rowley and Alford 2013; Chapters 5-6). We collected male frogs from the wild that were either infected or uninfected by *B. dendrobatidis*.

Litoria nannotis were collected from Ethel Creek (18.983°S, 146.211°E) and *L. serrata* were collected from Birthday Creek (18.980°S, 146.168°E); both streams are in Paluma Range National Park in northeastern Queensland, Australia. To prevent disease transmission between frogs during handling, each frog was captured in an unused plastic bag worn as a glove, and was handled while wearing disposable gloves during all aspects of this study. To determine whether frogs were infected by *B. dendrobatidis*, we swabbed the ventral surface and all four feet of each frog with a sterile rayon swab, covering these areas twice. These samples were analysed using real-time quantitative PCR assays (Boyle et al. 2004). All *L. nannotis* used during this study were captured at the same time and from the same site. Of the *L. serrata* captured from the wild, all frogs were infected except for one individual, so we could not obtain enough uninfected frogs from the wild for our experiment. To form a control group of uninfected *L. serrata*, we used the one uninfected frog that was captured with the infected frogs, and added nine additional uninfected frogs that had been collected from the same site six months earlier and maintained in captivity until our experiment. Two *L. serrata* in the infected group died after the completion of Trial 1, so we included two new frogs in Trial 2. These two frogs were collected at the same time as the other infected frogs and were infected at the time of collection, but they had lost their infections before Trial 2 began. After our study was completed, we released all surviving frogs at their exact capture locations.

Thermal gradient experiment

For each species, we tested 10 infected and 10 uninfected control frogs in thermal gradients for 10 days (Trial 1), and all frogs were re-tested for an additional 10 days in the same thermal gradients after the initially infected frogs had lost their infections (Trial 2). Each thermal gradient consisted of a 60 × 15 × 15 cm glass aquarium with a transparent acrylic plastic lid (Figure 7.1). A 4-cm high wall made from transparent acrylic plastic was attached to the bottom of the aquarium using silicone

sealant, creating a 60 × 5 cm section along one side of the aquarium, which was filled with water (Figure 7.1). A heat lamp positioned above one corner of the aquarium created a thermal gradient that allowed frogs to select body temperatures ranging from 20°C at the cool end to 30-50°C at the warm end, depending on their rate of evaporative water loss; thermal models (Rowley and Alford 2010, Chapter 2) were used to measure these temperatures. Small holes in the lids allowed air exchange, but maintained high humidity inside thermal gradients. The laboratory lights were automatically turned on from 7:00 to 19:00 each day, with heat lamps on from 8:00 to 17:00. The laboratory was air-conditioned and maintained at a constant 18°C during the day, but temperatures sometimes became cooler (≥10°C) on cold nights.

Frogs were placed in thermal gradients within two days of collection from the wild, and allowed to adjust to them for 24 hr before each trial began. We recorded body temperatures and behavioural observations of frogs four times each day: 10:00, 12:00, 14:00, and 16:00. We recorded whether each frog was in the water, and we measured its body temperature using a non-contact infrared thermometer (OS425-LS, Omega Engineering Ltd, Irlam, Manchester, UK; Rowley and Alford 2007c). To take a temperature reading, we lifted one end of the lid slightly for <3 seconds, which did not disturb the frog or affect its body temperature. When frogs were completely submerged, we used the water temperature as the frog's body temperature. Water temperatures were measured by waterproofed temperature dataloggers (Chapter 3, Roznik and Alford 2012) placed in each thermal gradient before the experiment began. At the end of Trial 1, all frogs were removed from the thermal gradients and immediately re-tested for infection by *B. dendrobatidis*.

Our previous experience with *L. nannotis* and *L. serrata* in captivity suggested that both species would lose *B. dendrobatidis* infections quickly when maintained under dry conditions in the laboratory. Therefore, to ensure that infected frogs cleared their infections, we simply housed all frogs individually in plastic cages (30 × 20 × 17 cm) with a screened lid and a container of water. *Litoria nannotis* were also provided

with several rocks for shelter because this species frequently uses rock crevices in nature. After 40 days had elapsed since the end of Trial 1, all frogs were tested again for *B. dendrobatidis*; when we confirmed that none were infected, we began Trial 2 of the experiment, which was identical to Trial 1. Frogs were fed crickets immediately before and after each trial, and once during each trial. When frogs were not in thermal gradients, they were fed crickets twice each week, and their cages, water containers, and rocks were cleaned and disinfected once each week using F10 veterinary disinfectant (Webb et al. 2007). Each thermal gradient was also cleaned and disinfected after each trial using F10 disinfectant.

The goal of our study was to understand how frog behaviour is related to *B. dendrobatidis* infection status under laboratory conditions, and whether behaviour changes after frogs lose infections, which would suggest that behavioural differences between infected and uninfected frogs in nature are caused by the infection. Our analysis focused on two behavioural responses of each individual frog that correspond to behavioural traits that are related to infection status in these species in nature (Rowley and Alford 2013, Chapter 5-6); these were the proportion of observations in which the frog was in the water, and the proportion of body temperatures above 25°C (i.e., the threshold at which *B. dendrobatidis* growth slows or ceases; Piotrowski et al. 2004, Stevenson et al. 2013). Both variables were arcsine-square-root transformed prior to analysis. We analysed the data using two repeated-measures ANOVAs, using generalised linear models with individual frog as the random factor, and initial infection status, species, and trial as fixed factors. Initial examination of the data suggested that the variance of the response variables differed between species, so these were allowed to vary in the analyses. These analyses were performed in SPlus, version 4.2, using the menuLme function. Because many of the initially infected frogs had lost their infections by the end of Trial 1 (6 *L. nannotis*, 10 *L. serrata*), we only used data from the first five days of Trial 1 (for both groups of each species) in our analysis, which is when the initially infected frogs were most likely to have remained infected. We used

data from all 10 days of Trial 2 for analysis.

Jumping performance experiment

To gain additional information on sublethal effects of *B. dendrobatidis* infections, we also examined the effects of *B. dendrobatidis* on frog jumping performance by measuring and comparing the performance of the initially infected and uninfected *L. nannotis* that were used in each thermal gradient trial. We did not include *L. serrata* in this experiment because of the different capture histories of initially infected and uninfected control frogs. We measured the jumping distances of initially infected and uninfected *L. nannotis* the night after collection from the wild (just prior to Trial 1), and again after the initially infected frogs had lost their infections (just prior to Trial 2). Both jumping trials took place when this nocturnal species is typically active (20:00 to 23:00), immediately before frogs were placed into thermal gradients at the start of day 0 of each trial, as described above. To measure jumping distance, we lined a corridor with fresh paper for each frog, and placed the frog at one end of the paper. All frogs jumped readily, and left a wet mark on the paper after each jump. We measured the length of each of the first four jumps (between wet marks) to the nearest centimetre using a tape measure, and used these data to calculate the mean, maximum, and standard deviation of jump length for each frog. To examine the effects of infection status on the jumping performance of initially infected and uninfected control frogs, we used three repeated-measures ANCOVAs, using generalised linear models with individual frog as the random factor, initial infection status species and trial as fixed factors, and body mass as a covariate. The responses analysed were mean jump length, maximum jump length, and standard deviation of jump length for each frog.

Results

Thermal gradient experiment

We found significant relationships between frog behaviour and infection by *B. dendrobatidi*s (Table 7.1, Figure 7.2). For proportion of observations in water, the main effect of species was highly significant, indicating that the two species behaved differently in the thermal gradients; overall, *L. serrata* spent much less time in water than *L. nannotis* (Figure 7.2). Several interactions were also significant for water use (Table 7.1). The infection × species interaction was significant, indicating that there were effects of infection on water use that differed between the species. The infection × trial interaction was also significant, indicating that the effects of infection differed between the trials, and the significant species × trial interaction indicated that the overall effect of species changed between trials. The clear pattern was that during Trial 1, infected *L. nannotis* spent a greater proportion of time in the water than uninfected control frogs; the average percentage of observations in water was 72.9% for infected frogs and 36.5% for uninfected control frogs (Figure 7.2). This difference disappeared in Trial 2, when the proportion of time spent in water for previously infected and uninfected control frogs were very similar (Figure 7.2). However, in both trials, *L. serrata* spent little or no time in water, regardless of initial infection status (Figure 7.2).

The only significant effects in the analysis for the proportion of observations above 25°C were the main effect of species and the species × trial interaction (Table 7.1). These indicate that this response differed between the species and that the nature of the difference changed between Trials 1 and 2. Both species changed the proportion of time spent above 25°C between Trials 1 and 2, but in opposite directions; *L. nannotis* spent more time in this temperature range in Trial 2, while *L. serrata* body temperatures were above 25°C more often in Trial 1 (Figure 7.2). The proportion of observations above 25°C did not depend on initial infection status in either species, even in Trial 1, when some frogs were infected (Figure 7.2).

Jumping performance experiment

We found that *B. dendrobatidis* infections significantly affected the jumping performance of frogs; the mean, maximum, and standard deviation of jump length were all affected by infection status (Table 7.2, Figure 7.3). Mean jump length was significantly affected by the initial infection status infection × trial interaction, indicating that there was an effect of infection that differed between Trials 1 and 2 (Table 7.2). The mean jumping distance for infected frogs in Trial 1 was substantially less than their mean jumping distance in Trial 2, when they were no longer infected, whereas the mean jumping distance for uninfected control frogs changed little between trials (Figure 7.3). For maximum jump length, the initial infection status × body mass interaction was significant, indicating that each frog's response was affected by its body mass in a manner that depended on infection status (Table 7.2). The maximum distance jumped was negatively related to body mass in uninfected frogs, but the relationship was slightly positive in infected frogs, and the mean for infected frogs was lower than that for uninfected frogs (Figure 7.3). This suggests that there were weak negative effects of infection on maximum jumping distance that were most important in smaller frogs. When we examined the standard deviation of jump length, we found that there was a significant main effect of trial and a significant interaction between initial infection status × trial (Table 7.2). On average, the distances jumped by frogs were less variable in Trial 2; however, infected frogs were more variable than uninfected frogs during Trial 1, and less variable in Trial 2 (Figure 7.3).

Discussion

Relationships between host behaviour and pathogen infection may exist for different reasons: any host behaviour that varies among individuals may influence the probability of acquiring or retaining an infection; the behaviour of individual hosts can also change in response to their infection (Moore 2002, Poulin 2010). In many cases, host behaviour may drive infection dynamics; an understanding of such processes can

help explain why some populations or species are more vulnerable to disease than others. For example, in many communities where amphibian species have disappeared or declined due to the disease chytridiomycosis, other amphibian species have persisted unaffected (McDonald and Alford 1999, Retallick et al. 2004, Lips et al. 2006). This pattern may be caused by differences among host species in how their behaviour affects, or is affected by, infection by the causative pathogen *Batrachochytrium dendrobatidis* (Rowley and Alford 2007a, 2013, Chapters 5-6). Our study demonstrates that infection by *B. dendrobatidis* can alter the behaviour of some frog species, but not others. Infection by *B. dendrobatidis* changed the microhabitat selection of *Litoria nannotis* in ways that benefit pathogen growth and dispersal and are likely to perpetuate infections in frogs, but we did not find any evidence that this pathogen altered the behaviour of a sympatric species, *L. serrata*.

The proportion of time spent in water differed between species with regard to infection status. On average, infected *L. nannotis* spent 36% more time in water than uninfected control frogs; after initially infected frogs lost their infections, their water use decreased and was similar to that of uninfected control frogs (Table 7.1, Figure 7.2). By contrast, most *L. serrata* spent little or no time in water, regardless of their infection status. It is possible that spending more time in water represents a manipulation of host behaviour by *B. dendrobatidis* because moist conditions increase the survival and facilitate dispersal of aquatic *B. dendrobatidis* zoospores (Johnson et al. 2003, Johnson and Speare 2005); these conditions should also increase recolonisation on the host and thus the buildup of pathogen populations (Carey et al. 2006). These changes could also reflect behavioural changes in frogs brought about by decreases in skin function and disruptions in skin shedding (Nichols et al. 2001, Voyles et al 2009). It seems unlikely that the preference of infected *L. nannotis* for aquatic microhabitats is an adaptive response to infection by *B. dendrobatidis*; spending more time immersed in water is likely to increase water uptake by frogs, which would further aggravate any ionic imbalances experienced by infected frogs (Voyles et al. 2009).

In both species, infection status was unrelated to the proportion of time spent above 25°C (Table 7.1). However, there was a significant difference between species, and the species × trial interaction was also significant. In Trial 1, *L. nannotis* preferred cooler temperatures and *L. serrata* preferred warmer temperatures, whereas the thermal preferences of the species converged in Trial 2. This could reflect acclimation to laboratory temperatures, or the change in *L. nannotis* between trials may reflect the reduction of time spent in water by previously infected frogs because frogs could only attain body temperatures above 25°C in our thermal gradients when they were out of the water. Our results do not support the hypothesis that infected frogs behaviourally elevate their body temperatures in response to infection by *B. dendrobatidis* ("behavioural fever"; Richards-Zawacki 2009). We found that infected frogs have similar or cooler body temperatures than uninfected frogs, which matches the findings of field studies on four species of Australian rainforest frogs, including the two used in this study (Rowley and Alford 2013, Chapters 5-6).

Our thermal gradient results are consistent with patterns in behaviour that have been observed in *L. nannotis* and related species in nature. Field data on *L. nannotis* show that infected frogs experienced lower desiccation rates and used moister microhabitats than uninfected frogs, and their body temperatures more closely mimicked stream temperatures (Chapters 5-6). Because stream temperatures were cool and constant, infected frogs typically had cooler body temperatures than uninfected frogs during the day, but sometimes had warmer body temperatures at night (Chapter 5). Another study also found that individual *L. nannotis* that experienced cooler body temperatures had a higher probability of infection (Rowley and Alford 2013). In *L. nannotis*, our results suggest, at least in part, that these behavioural differences are a result of changes induced by *B. dendrobatidis* infections, rather than differences in the propensity of individual frogs to become or remain infected based on their behavioural patterns.

The behavioural changes that we observed in infected *L. nannotis* are likely to

facilitate the growth of *B. dendrobatidis* populations on their skin, and therefore perpetuate their infections. Growth, reproduction, and survival of *B. dendrobatidis* are strongly influenced by temperature and moisture (Johnson et al. 2003, Piotrowski et al. 2004, Stevenson 2012); this fungus thrives under conditions that are moist and cool (15-25°C is optimal, >28°C is lethal). Therefore, the behavioural changes in infected *L. nannotis* are likely to expose the *B. dendrobatidis* inhabiting their skin to wetter, cooler conditions, which are favourable for fungal growth and could lead to mortality. Infected frogs maintained in warmer and drier conditions survive longer than those in cool and wet conditions (Bustamante et al. 2010, Murphy et al. 2011), and frogs that experience very warm temperatures (>30°C) can also lose *B. dendrobatidis* infections entirely (Woodhams et al. 2003, Chatfield and Richards-Zawacki 2011, Geiger et al. 2011). Our two study species selected very different microenvironments within thermal gradients; *L. nannotis* used locations that were wetter and cooler than *L. serrata* (Figure 7.2). These differences likely contributed to the ability of frogs to lose infections; all of the *L. serrata* (10 of 10) lost their infections after 10 days, but only 60% of the *L. nannotis* (6 of 10) lost their infections after the same time period.

We also found that *B. dendrobatidis* infections had additional sublethal effects on *L. nannotis* by reducing their jumping performance (Table 7.2, Figure 7.3). Mean and maximum jumping distances were shorter in infected frogs than uninfected frogs, and the jumps of infected frogs were also more variable (Figure 7.3). After previously infected frogs lost their infections, their jumping performance was similar to that of uninfected control frogs (Figure 7.3). Another study has found that *B. dendrobatidis* infections influenced the locomotor performance of frogs; peak velocity, but not peak acceleration, was reduced in experimentally infected frogs (Chatfield et al. 2013). Reduced locomotor performance and activity levels are common behavioural changes displayed by animals infected by pathogens (Barber et al. 2000, Moore 2002). These changes can compromise host fitness by decreasing foraging success (Venesky et al. 2009) and increasing the chances of predation (Wassersug and Sperry 1977, Watkins

1996). Changes in the jumping ability of infected frogs also may influence various aspects of their behaviour, such as patterns of movement and microhabitat use (Chapter 6).

Although we found that *B. dendrobatidis* infections altered the behaviour of *L. nannotis*, we did not find any evidence for this in *L. serrata*. Field observations of *L. serrata* have shown that infected frogs had cooler body temperatures, lower desiccation rates, and used cooler, moister microhabitats than uninfected frogs (Rowley and Alford 2013, Chapters 5-6). However, in our experiment infected and uninfected control *L. serrata* had similar patterns of water use and body temperatures, and initially infected frogs did not change their behaviour differently from uninfected control frogs after they lost their infections (Figure 7.2). This result makes it appear unlikely that changes in behaviour caused by infection are responsible for the differences in body temperature and microhabitat use between infected and uninfected frogs observed in this species in nature (Rowley and Alford 2013, Chapters 5-6). Our results are consistent with the alternative hypothesis that behavioural differences between infected and uninfected *L. serrata* in nature may reflect effects of the behaviour of individual frogs on the probability of becoming or remaining infected; frogs that prefer cooler, moister microhabitats may be more susceptible to infection. However, if this were the case, we would expect initially infected frogs and uninfected control frogs to have exhibited consistent behavioural differences throughout our experiment; instead, they behaved similarly. It is difficult to interpret this because our control frogs were not collected at the same time as our experimental frogs. Fully understanding the causal relationships between individual behaviour and *B. dendrobatidis* will require both additional field and experimental data.

Understanding the processes that drive infection dynamics can help explain why some populations or species are more vulnerable to disease than others. We found that *B. dendrobatidis* interacted with our two study species differently, which could explain observed differences in their susceptibility to this pathogen in nature and

their different histories of decline. Infection by *B. dendrobatidis* changed patterns of microhabitat selection by *L. nannotis*, but we did not find any evidence that this pathogen altered the behaviour of *L. serrata*. Changes in the behaviour of infected *L. nannotis* led to increased use of wet microhabitats, which is likely to increase pathogen growth and perpetuate infections in frogs. Therefore, the changes observed in *L. nannotis* could explain why this species often has a higher prevalence of infection than *L. serrata* and has undergone more severe and longer-lasting population declines caused by chytridiomycosis (Richards et al.1993, McDonald and Alford 1999, McDonald et al. 2005). Understanding the causal relationships between individual amphibian behaviour and *B. dendrobatidis* also has important implications for evolution and conservation. If individual variation in behaviour affects susceptibility to infection, natural selection can act on those behavioural traits and species may be able to evolve resistance to *B. dendrobatidis*. This might explain why *L. serrata* populations were able to recover quickly from declines that occurred during the 1990s when chytridiomycosis emerged in northeastern Queensland, Australia, whereas many *L. nannotis* populations still have not recovered (McDonald et al. 2005). Understanding if and how individual variation in behaviour influences infection susceptibility can also be used to inform selective breeding programs for amphibians threatened by chytridiomycosis to increase the success of reintroduction efforts.

Table 7.1. Results of two repeated-measures ANOVAs examining effects on the proportion of observations in water, and proportion of body temperatures above 25°C of initially infected and uninfected frogs immediately before Trial 1 and Trial 2. We used generalised linear models with individual frog as the random factor, and initial infection status, species, and trial as fixed factors (all numerator df = 1, all denominator df = 34). The response variables were arcsine-square-root transformed, and variances were allowed to differ between species. Significant results (α = 0.05) are shown in bold typeface.

Effect	F	P
Water		
Infection	0.516	0.477
Species	67.157	**<0.001**
Trial	1.804	0.188
Infection × Species	4.802	**0.035**
Infection × Trial	5.009	**0.032**
Species × Trial	4.893	**0.034**
Infection × Species × Trial	1.019	0.320
Temperature		
Infection	0.020	0.889
Species	17.797	**0.000**
Trial	0.222	0.641
Infection × Species	0.270	0.607
Infection × Trial	0.229	0.635
Species × Trial	14.856	**0.001**
Infection × Species × Trial	2.598	0.116

Table 7.2. Results of three repeated-measures ANCOVAS examining effects on the jumping performance of initially infected and uninfected frogs immediately before Trial 1 and Trial 2, using generalised linear models with individual frog as the random factor, initial infection status, species, and trial as fixed factors, and body mass as a covariate (all numerator df = 1, all denominator df = 15). The responses analysed were mean jump length, maximum jump length, and standard deviation of jump length for each frog. Significant results (α = 0.05) are shown in bold typeface.

Effect	F	P
Mean		
Infection	3.947	0.066
Trial	0.431	0.521
Mass	3.588	0.078
Infection × Trial	6.047	**0.027**
Infection × Mass	3.079	0.100
Trial × Mass	3.029	0.102
Infection × Trial × Mass	2.832	0.113
Maximum		
Mass	3.584	0.078
Infection	2.942	0.107
Trial	0.848	0.372
Infection × Trial	0.617	0.445
Infection × Mass	4.684	**0.047**
Trial × Mass	1.433	0.250
Infection × Trial × Mass	0.649	0.433
Standard deviation		
Mass	0.918	0.353
Infection	0.061	0.808
Trial	10.902	**0.005**
Infection × Trial	18.489	**0.001**
Infection × Mass	0.186	0.673
Trial × Mass	0.319	0.580
Infection × Trial × Mass	1.743	0.207

Figure 7.1. The thermal gradients used during this experiment. Each thermal gradient consisted of a glass aquarium (60 × 15 × 15 cm) with a heat lamp positioned above one corner, and allowed each frog to select body temperatures ranging from 20°C at the cool end to 30-50°C at the warm end, depending on the frog's rate of evaporative water loss.

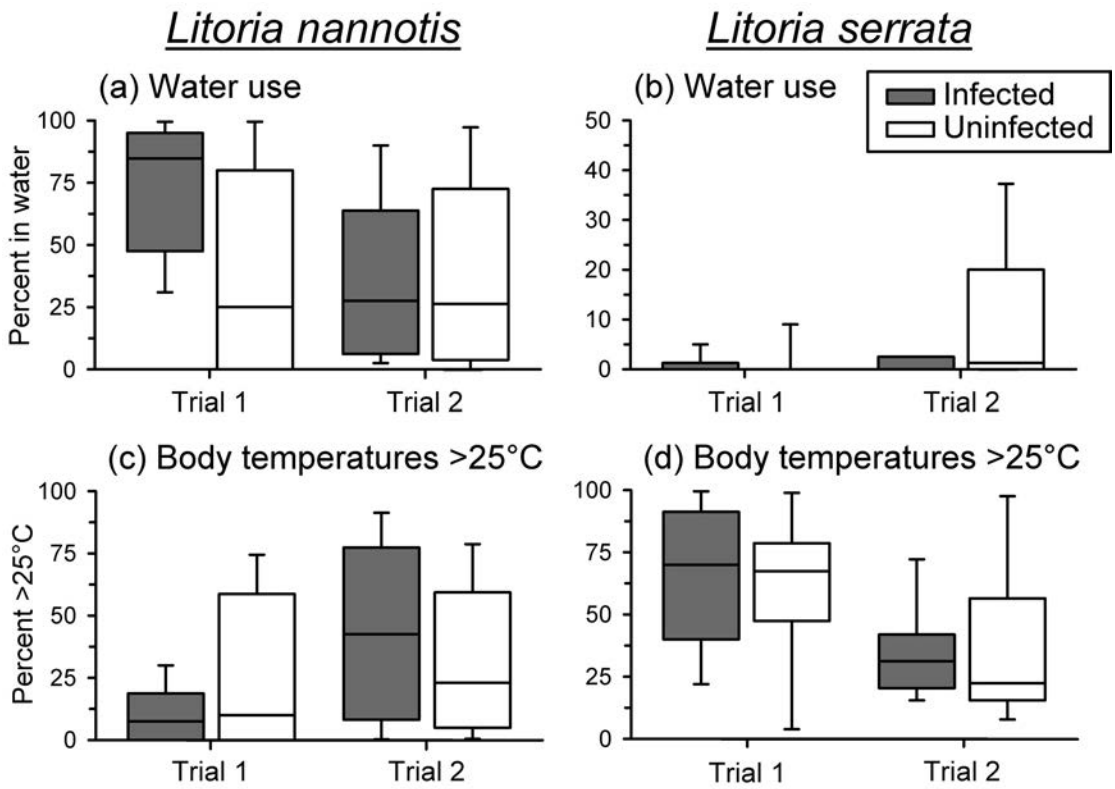

Figure 7.2. Behavioural observations of individual frogs of two species (*Litoria nannotis* and *L. serrata*) that were either infected by the chytrid fungus *Batrachochytrium dendrobatidis* during Trial 1 and uninfected during Trial 2, or uninfected during both trials. Shown are the (a-b) percentage of observations in water, and (c-d) percentage of body temperatures above 25°C. The labels “Infected” and “Uninfected” refer to the initial infection status of frogs during Trial 1.

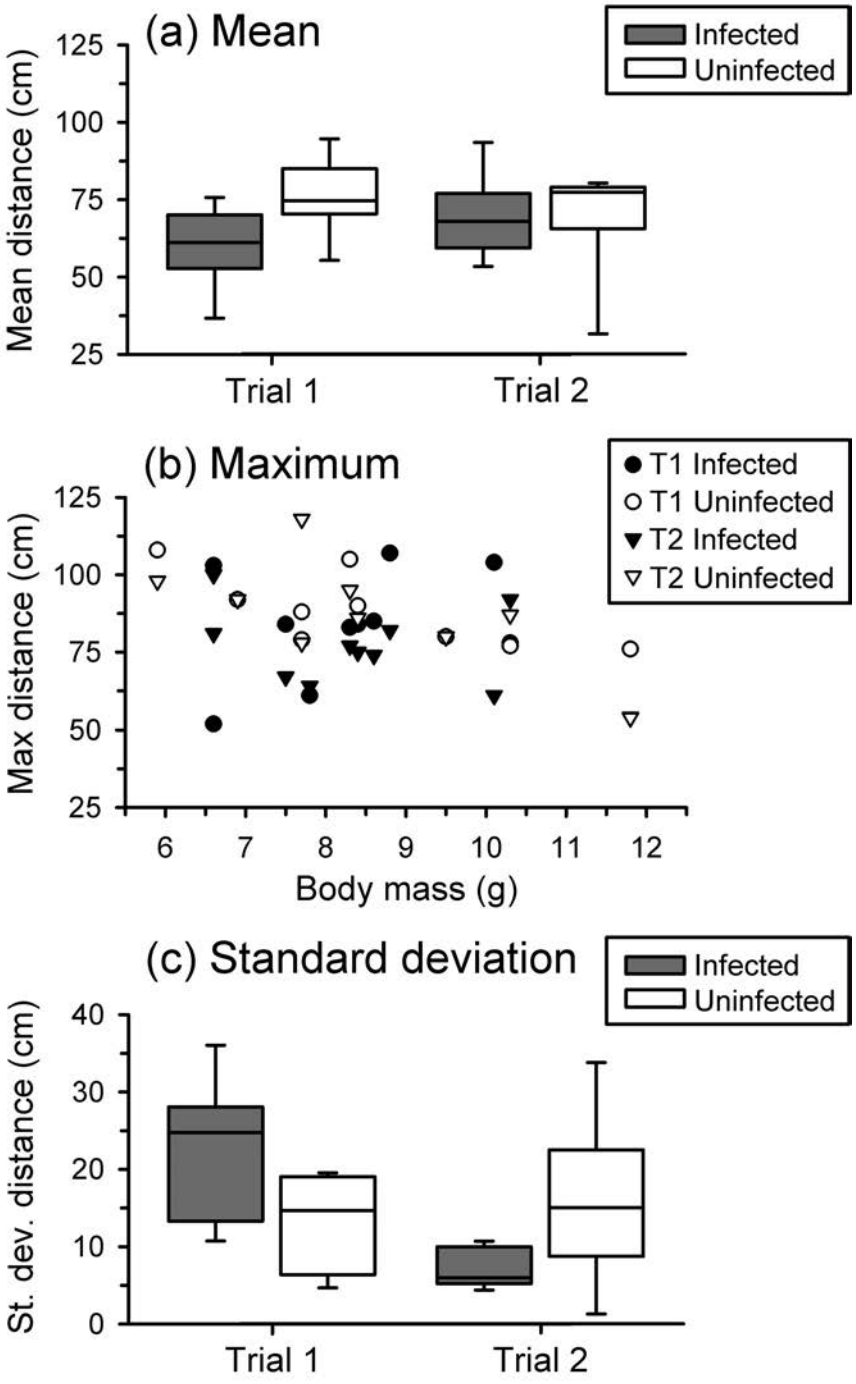

Figure 7.3. The (a) mean, (b) maximum, and (c) standard deviation of distances jumped by individual *Litoria nannotis* that were either infected by the chytrid fungus *Batrachochytrium dendrobatidis* during Trial 1 and uninfected during Trial 2, or uninfected during both trials. The labels “Infected” and “Uninfected” refer to the initial infection status of frogs during Trial 1. The maximum distance (b) is shown for frogs by body mass because of a significant interaction between infection status and body mass.

Chapter 8: Condition-dependent reproductive effort in frogs infected by a widespread pathogen

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Abstract

To minimise the negative effects of an infection on fitness, hosts may respond adaptively by either increasing or decreasing their reproductive effort. In some circumstances, it might be adaptive for a host to decrease current reproductive effort and preferentially allocate resources to other life-history processes, such as immune responses to fight the infection. In other circumstances, however, the optimal life-history strategy may be to increase current reproductive effort to compensate for a potential loss in future reproductive output (e.g., early mortality caused by the infection). We studied effects of the widespread pathogenic fungus *Batrachochytrium dendrobatidis* on the probability of calling in *Litoria rheocola*, a stream-breeding rainforest frog that has declined due to chytridiomycosis, the disease caused by this pathogen. In uninfected frogs, calling probability was unrelated to body condition and relatively constant across seasons, but for infected frogs, calling probability differed among seasons (lowest in winter, highest in summer, intermediate during spring and autumn) and depended strongly on body condition. Infected frogs in poor body condition were up to 40% less likely to call than uninfected frogs in similar condition, but infected frogs in good condition often had higher calling probabilities than uninfected frogs (by up to 30%). We provide the first demonstration that infection by this widespread pathogen can have sublethal effects that interact with body condition to influence calling probability, a major fitness determinant in frogs. These effects likely

have important evolutionary implications for amphibian populations co-existing with this pathogen.

Introduction

Life-history traits of organisms, including growth, reproduction, and longevity, interact to influence fitness. Because these traits are constrained by resource availability, many organisms can adaptively modify their allocation of energy as circumstances change (Stearns 1992, Roff 2002). To maximise fitness, individuals typically maintain moderate levels of current reproductive effort; this results in a longer lifespan and production of more offspring during their lifetime (Partridge and Farquhar 1981, Stearns 1992, Roff 2002). These resource allocation strategies can vary among individuals, with natural selection favouring individuals with resource allocation patterns that enhance lifetime reproductive success (Stearns 1992, Roff 2002).

Diseases can influence host fitness by reducing survival, but they can also have sublethal effects on reproductive success. Sublethal effects occur because pathogens can directly reduce a host's resources, and because the optimal pattern of resource allocation may change when an individual is infected by a pathogen (Sorci et al. 1996, Cressler et al. 2014). In some cases, it might be beneficial for hosts to preferentially allocate resources to immune responses to fight their infections, and to invest less effort in gamete production or reproductive behaviour. For example, in many taxa that rely on vocalising for mate attraction (e.g., birds, frogs, insects), infected males may invest less effort into vocalising, and they may also alter their vocalisations in terms of rate, length, complexity, and frequency (Cade 1984, Garamszegi 2005, Madelaire et al. 2013). Males that vocalise less often should attract fewer mates, mate less often, and produce fewer offspring. Pathogen-induced decreases in sound production may also reduce fitness because calls are subject to sexual selection by females. Infected males that reduce calling effort may be less attractive because sexual selection usually favours males that sustain high levels of

sound production, possibly because this is an honest signal of overall genotypic fitness (Hamilton and Zuk 1982, Welch et al. 1998, Forsman and Hagman 2006).

The optimal life-history strategy for some infected individuals may be to increase investment in current reproductive effort, even at the expense of growth and survival (Clutton-Brock 1984, Forbes 1993, Agnew et al. 2000, Hurd 2009). Life-history theory predicts that current reproductive effort should increase as life expectancy decreases (Clutton-Brock 1984, Stearns 1992, Roff 2002). Both male and female hosts can compensate for an increased risk of mortality posed by a pathogen by breeding at an earlier age or by producing more offspring early in life. For example, among infected females, Tasmanian devils (*Sarcophilus harrisii*) can mature and breed earlier (Jones et al. 2008), and crickets (*Acheta domesticus*) and water fleas (*Daphnia magna*) can lay more eggs (Adamo 1999, Chadwick and Little 2005). Among infected males, frogs (*Lithobates pipiens*) can increase sperm production (Chatfield et al. 2013), flies (*Drosophila nigrospiracula*) and amphipods (*Corophium volutator*) can increase reproductive effort (Polak and Starmer 1998, McCurdy et al. 2000), and beetles (*Tenebrio molitor*) can provide higher-quality nuptial gifts to their mates, which increases their egg production (Hurd and Ardin 2003). Whether hosts increase their reproductive effort in response to an infection is dependent on many factors, including resource availability (van Noordwijk and de Jong 1986). Therefore, hosts in very poor condition may not always be able to increase reproductive effort, either because of the infection or due to other environmental factors (Wilson et al. 2001, Judge et al. 2008).

Understanding how pathogens affect host reproduction, and thus fitness, has direct implications for population demography and evolution. We studied effects of the widespread pathogenic fungus *Batrachochytrium dendrobatidis* on the probability of calling in male frogs. The primary mechanism of attracting and locating mates in most frogs is through male advertisement calls, and populations in many regions of the world are undergoing declines due to chytridiomycosis, the disease caused by *B. dendrobatidis* (Kilpatrick et al. 2009). Although *B. dendrobatidis* can influence host

survival directly, many individuals carry sublethal infections from which they ultimately recover (e.g., Sapsford 2012). Sublethal effects of *B. dendrobatidis* infection are associated with changes in some aspects of frog behaviour (e.g., microhabitat use, movements; Chapters 5-7), and may also affect direct fitness traits, such as energetic investment in mate attraction or gamete production (Chatfield et al. 2013). Because calling requires substantial energy, it is likely that body condition can also mediate calling effort. Overall, infected frogs often have reduced body condition when compared to uninfected frogs, as a result of weight loss (Retallick and Miera 2007, Harris et al. 2009, Murphy et al. 2011), but how body condition interacts with calling effort is unknown. We studied the effects of *B. dendrobatidis* infection and body condition on the calling probability of the common mistfrog, *Litoria rheocola*, a stream-breeding rainforest frog with a history of declines caused by chytridiomycosis (Richards et al.1993, McDonald and Alford 1999). We sampled frog calling behaviour and infection status both spatially (across six sites differing in elevation) and temporally (seasonally), providing a robust test of the hypothesis that infection by *B. dendrobatidis* has sublethal effects that interact with body condition to influence calling probability, and therefore male fitness.

Methods

The common mistfrog (*Litoria rheocola*) is a treefrog that occurs near rainforest streams in northeastern Queensland, Australia (Hoskin and Hero 2008, Dennis 2012). *Litoria rheocola* are typically found on rocks and streamside vegetation in fast-flowing streams (Hoskin and Hero 2008, Dennis 2012, Chapter 4). Males call and females breed year-round, although reproductive behaviour decreases during the coolest weather (Hoskin and Hero 2008, Dennis 2012). By the mid-1990s, chytridiomycosis had extirpated this Endangered species (IUCN 2013) at higher elevations (>400 m) throughout its geographic range (Richards et al.1993, McDonald and Alford 1999). However, many populations have subsequently recovered or recolonised these areas

(McDonald et al. 2005) and now co-exist with the pathogen (Sapsford 2012).

We surveyed frogs at six rainforest streams in northeastern Queensland, Australia (Table 8.1). Tropical rainforest surrounded the streams, which was characterised by dense vegetation composed of large trees (10 m in height), vines, epiphytes, shrubs, and herbaceous plants. Although our sites were in relatively undisturbed rainforest, several sites were damaged by a tropical cyclone in February 2011 (Chapter 9). Stream width varied from 5-10 m, and streambeds were composed of rocks ranging in size from small pebbles to large boulders (10 m in diameter). All streams contained pools, runs, and riffles, and most had several waterfalls.

We captured adult male *L. rheocola* by visually surveying frogs along a 400-m transect at each stream. Surveys were conducted over five nights at each site during each season, from June 2010 through October 2011 (except for spring 2011, when we sampled for one night per site). We conducted two winter (June-July) and two spring (October-November) surveys, and one survey in summer (January-February) and autumn (March-April). We were unable to conduct a summer survey during 2011 at Bobbin Bobbin Creek because this site was inaccessible due to cyclone damage. Because our study focuses on calling behaviour, we only analysed data for male frogs, as determined by the presence of distinct nuptial pads. We recorded whether each male frog was calling prior to capture, and measured its body size (snout-urostyle length to 0.1 mm, and mass to 0.1 g). We used these body size measurements to estimate a body condition index for each frog, using the residual scores from a linear regression of $\log_{10}$ transformed body mass on square-root transformed snout-urostyle length for all frogs sampled (Peig and Green 2009). The resulting positive relationship was strong and highly significant ($r^2 = 0.45$, $F_{1,2486} = 1197.98$, $P < 0.001$). To determine whether frogs were infected by *B. dendrobatidis*, we swabbed the ventral surface and all four feet of each frog with a sterile rayon swab, covering these areas twice. These samples were analysed using real-time quantitative PCR assays (Boyle et al. 2004). We also gave each frog a unique identifying mark using visible implant elastomer

(Nauwelaerts et al. 2000, Sapsford 2012). For analysis, we used the initial capture of each frog and excluded all recaptures, resulting in an independent sample of frogs.

We used generalised linear mixed-effects models to examine potential effects of infection status, body condition, and season on the calling probability of individual frogs. Calling status was coded as a binomial response variable, so we used generalised linear mixed-effects models with a binomial family and a logit link function. We developed a set of candidate models that included models with all combinations of one, two, or three fixed effects, and all two- and three-way interactions. For all models, we included site as a random effect to control for any effects specific to particular sites. We ranked models according to Akaike's Information Criterion with adjustment for finite sample size (AICc) to determine the strength of evidence for each model relative to the set of candidate models, using the criteria of Burnham and Anderson (2002). Statistical analyses were performed in program R, version 2.15.2 (R Core Team 2012) using the lme4 (Bates et al. 2012) and MuMIn (Barton 2013) packages.

Results

We captured a total of 1843 unique male frogs at six study sites during six seasonal samples (Table 8.1). We found that infection status, body condition, and season all influenced the calling probability of individual frogs (Table 8.2, Figure 8.1). A single model was strongly supported by our data (ΔAICc < 2, Nagelkerke $R^2 = 0.163$). This model included infection status, body condition, and season as main effects, and infection status × body condition and infection status × season interactions (Table 8.2), and was significantly different from a null model including only the intercept and the random effects of sites ($\chi^2 = 32.695$, df = 9, $P < 0.001$). Overall, we found that the relationship between body condition and calling probability was strongly influenced by infection status (Figure 8.1). For uninfected frogs, calling probability was relatively constant across seasons; our model suggests a slight decrease with increasing body condition, but the slopes of the lines are near zero in all seasons (Figure 8.1). By

contrast, calling probability for infected frogs differed among seasons (lowest in winter, highest in summer, and intermediate in spring and autumn) and depended strongly on body condition (Figure 8.1). For infected frogs in all seasons, the probability of calling increased strongly as body condition increased; infected frogs with the lowest body conditions were less likely to call than uninfected frogs, and infected frogs with the highest body conditions were more likely to call than uninfected frogs (Figure 8.1).

Discussion

The interactions that we documented among *Batrachochytrium dendrobatidis* infection status, body condition, and season on calling probability in frogs (*Litoria rheocola*) strongly suggest an adaptive, condition-dependent response by hosts. For uninfected frogs, calling probability was unaffected by body condition and relatively constant across seasons (approximately 50%; Figure 8.1), consistent with the observation that *Litoria rheocola* call and breed year-round (Hoskin and Hero 2008, Dennis 2012). However, calling probability of infected frogs differed among seasons and depended strongly on body condition. In each season, the calling probability of infected frogs increased with body condition, such that infected frogs in poor body condition were less likely to call than uninfected frogs in similar condition, but infected frogs in good condition often had a higher calling probability than uninfected frogs (Figure 8.1). This pattern of increased calling probability in infected frogs is consistent with life-history theory, which predicts that reproductive effort should increase as life expectancy decreases (Clutton-Brock 1984, Stearns 1992, Roff 2002). Because *B. dendrobatidis* infections increase the risk of mortality (Berger et al. 1998), it is likely that infected frogs adjusted their reproductive output in response to this risk to maintain lifetime reproductive success. Male frogs (*Lithobates pipiens*) infected by *B. dendrobatidis* have larger testes that contain more sperm than uninfected males (Chatfield et al. 2013), which supports this hypothesis. Empirical studies on other taxa have also shown that reproductive effort can increase as life expectancy decreases

(Forbes 1993, Agnew et al. 2000, Jones et al. 2008, Hurd 2009). We found that infected frogs in poor condition were less likely to call than uninfected frogs, possibly because they were unable to engage in this behaviour due to physiological changes caused by their infections, or because they were adaptively allocating less energy to reproduction, and more to other life-history traits required for immediate survival, such as immune responses to fight their infections.

An alternative hypothesis for increased calling effort in infected frogs is that the pathogen is manipulating the host, potentially to increase pathogen transmission to additional hosts (Moore 2002, Poulin 2007). However, it seems unlikely that changes in calling effort would benefit *B. dendrobatidis* enough to be an adaptive strategy because transmission does not occur solely by physical contact between individual frogs. This pathogen can persist in environmental reservoirs, including water and a wide range of moist substrates (Johnson and Speare 2003, 2005), as well as non-amphibian reservoir hosts (Kilburn et al. 2011, Garmyn et al. 2012). It has also been reported to infect other taxa, including nematodes and crayfish (Shapard et al. 2012, McMahon et al. 2013). Therefore, it seems unlikely that there would be strong selection for host manipulation by *B. dendrobatidis* to increase frog-to-frog contact to increase pathogen transmission rates. Another alternative hypothesis is that changes in the calling probability of infected frogs resulted from side effects of the infection (Moore 2002, Poulin 2007). Incidental side effects of infections are most likely to have negative effects on an energetically expensive activity like calling (e.g., Holmes 1995), and may explain our observations for infected frogs in poor body condition. However, it is unlikely that side effects would increase calling effort, which is an energetically expensive activity (Emerson 2001, Wells 2001). The most plausible explanation for the pattern of increased calling effort by infected frogs in our study is that infected frogs were responding adaptively to *B. dendrobatidis* infection by allocating energy to life-history processes differently from uninfected frogs, especially when they were in good body condition.

The average body condition of frogs changed seasonally; it was lowest in winter, highest in summer, and intermediate during spring and autumn (Figure 8.1). Seasonal changes in body condition were likely caused by associated seasonal changes in energy acquisition or expenditure. During the cooler and drier months, frogs likely acquired less energy than they used for other functions. Reduced energy intake could be associated with low availability of rainforest arthropods during dry months (Janzen 1973, Wolda 1978), which can affect the diets of frogs (Toft 1980). Although seasonal shifts in body condition did not affect the calling probabilities of uninfected frogs, season strongly influenced the calling probabilities of infected frogs (Figure 8.1). Infected frogs were more likely to call during the warmer seasons, when all frogs were in better condition. This led to seasonal differences in the relationships between the calling probability of infected and uninfected frogs. In summer, when frogs were in the best condition, infected frogs were up to 30% more likely to call than uninfected frogs (Figure 8.1). However, in winter, when frogs were in the worst condition, infected frogs were up to 40% less likely to call than uninfected frogs (Figure 8.1). During spring and autumn, infected frogs in poor body condition were less likely to call than uninfected frogs in similar condition, but when infected frogs were in good body condition, their calling probability was often higher than that of uninfected frogs (Figure 8.1).

The effects of *B. dendrobatidis* infection on the probability of calling in our study were presumably related to calling effort. Frogs that we did not observe calling immediately prior to capture may call sometimes, but less often on an hourly or nightly basis than our sample of calling frogs. However, we do not know whether attributes of the calls made by infected frogs differ from those of uninfected frogs, in terms of the rate, pitch, intensity, length, or complexity (Wells 2007). Female frogs typically prefer calls that are louder, longer, and emitted at faster rates because they often indicate genetic superiority of males capable of producing high levels of sound (Welch et al. 1998, Emerson 2001, Wells 2001). Therefore, even if infected *L. rheocola* call as often,

or more often, than uninfected frogs, their calls may not be as attractive to females, which could decrease their mating success. For example, in the frog *Hypsiboas prasinus*, males with higher parasite loads called at slower rates (Madelaire et al. 2013), and are therefore likely to be less attractive to females (Welch et al. 1998, Emerson 2001, Wells 2001). Fully determining the influence of infection on calling and reproductive success is crucial to understanding the complex impacts of disease on life history, as mediated through mate attraction, mating success, and fitness.

An individual's behaviour can be influenced by both body condition and infection by a pathogen, and these factors can interact in complex ways to influence mating opportunities and fitness. We found that the interaction of *B. dendrobatidis* infection status and body condition affected the calling probability of male *L. rheocola*, suggesting that infected frogs in poor body condition will have lower fitness than healthier frogs, but that infected frogs in good body condition may compensate for a potential loss of future reproductive output by increasing their current efforts. However, it is possible that non-calling males may use alternative mating tactics, such as "satellite behaviour," by attempting to intercept females attracted to nearby calling males (Leary et al. 2004, Wells 2007). The effects of *B. dendrobatidis* infections on female reproduction are currently unknown, but understanding whether and how this pathogen alters energetic investment into egg production or female reproductive behaviour (e.g., reproductive frequency, mate choice) is important for fully assessing its impacts on amphibians. *Batrachochytrium dendrobatidis* has devastated amphibian populations in many regions of the world, but many populations are co-existing with the pathogen (Retallick et al. 2004, Sapsford 2012). Elucidating whether populations that are co-existing with the pathogen are experiencing sublethal effects is important for understanding patterns of fitness, and thus potential changes in population demography and evolution.

Table 8.1. Study site details and sample sizes of unique male *Litoria rheocola* (N = 1843) captured at six rainforest streams in northeastern Queensland, Australia. Frogs were ether infected or uninfected by the chytrid fungus *Batrachochytrium dendrobatidis*, and either calling or not calling when encountered during stream surveys.

Site	Coordinates	Elevation (m ASL)	Sample size				
			Calling		Not calling		Total
			Infected	Uninfected	Infected	Uninfected	
Bobbin Bobbin Creek	17.378°S, 145.775°E	700	28	81	37	76	222
Frenchman Creek	17.307°S, 145.922°E	40	10	47	49	270	376
Mena Creek	17.649°S, 145.987°E	60	33	181	38	100	352
Stoney Creek	17.920°S, 146.069°E	20	31	93	36	99	259
Tully Creek	17.773°S, 145.645°E	150	53	241	24	172	490
Windin Creek	17.365°S, 145.717°E	750	14	46	19	65	144

Table 8.2. Generalised linear mixed-effects models (family: binomial, link function: logit) were used to examine relationships between calling by individual *Litoria rheocola* and their *Batrachochytrium dendrobatidis* infection status (infected or uninfected) and body condition index. We included site as a random effect, and infection status, body condition, and season as fixed effects. We developed a set of candidate models combining the random effect of site with all combinations of one, two, or three effects, and all two- and three-way interactions, and we ranked models according to Akaike's Information Criterion with adjustment for finite sample size (AICc). All models that we tested are shown, but only one model (ΔAICc < 2) was strongly supported by our data. The estimates for the final model are given below the candidate models, using winter as the reference season.

Candidate models

Model effects	AICc	ΔAICc	Weight	R^2
Infection, Condition, Season, Infection × Condition, Infection × Season	2327.371	0.000	0.352	0.163
Condition, Season, Condition × Season	2329.675	2.304	0.111	0.159
Infection, Condition, Season, Infection × Condition	2329.869	2.498	0.101	0.157
Infection, Condition, Season, Infection × Condition, Infection × Season, Condition × Season	2330.288	2.917	0.082	0.165
Infection, Condition, Season, Infection × Condition, Infection × Season, Condition × Season, Infection × Condition × Season	2330.719	3.348	0.066	0.169
Infection, Condition, Season, Infection × Season, Condition × Season	2330.765	3.394	0.064	0.163
Infection, Condition, Season, Condition × Season	2331.490	4.119	0.045	0.159
Infection, Condition, Season, Infection × Condition, Condition × Season	2331.849	4.477	0.038	0.160
Infection, Season, Infection × Season	2331.987	4.616	0.035	0.157
Season	2332.170	4.799	0.032	0.152
Infection, Condition, Season, Infection × Season	2332.604	5.233	0.026	0.158
Condition, Season	2332.655	5.284	0.025	0.153
Infection, Season	2334.102	6.731	0.012	0.152
Infection, Condition, Season	2334.547	7.176	0.010	0.153
Infection, Condition, Infection × Condition	2338.938	11.566	0.001	0.148
Condition	2341.858	14.487	0.000	0.143
Intercept only	2341.928	14.557	0.000	0.142
Infection	2343.176	15.805	0.000	0.142
Infection, Condition	2343.307	15.936	0.000	0.144

Final model

Model effect	Estimate
Intercept	-0.413
Infection	-0.357
Condition	-0.276
Season (spring)	0.179
Season (summer)	0.204
Season (autumn)	0.409
Infection × Condition	2.941
Infection × Season (spring)	0.731
Infection × Season (summer)	1.094
Infection × Season (autumn)	0.468

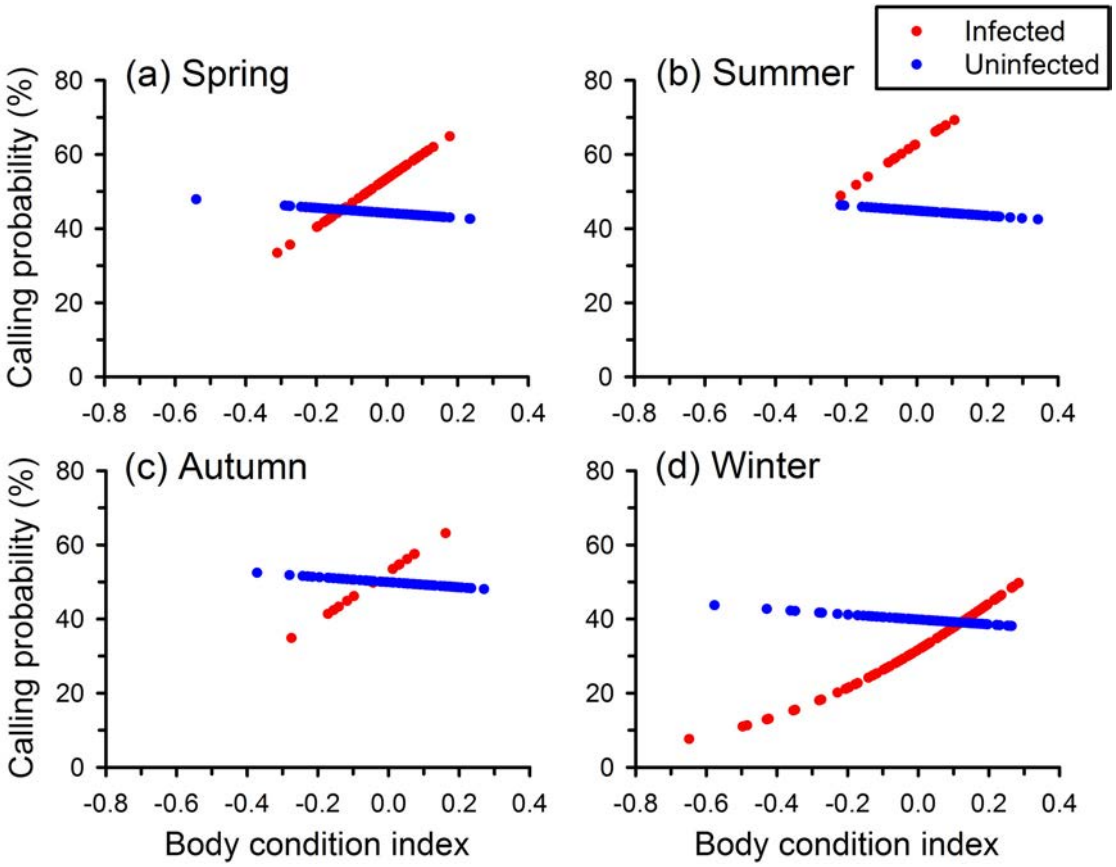

Figure 8.1. Predicted calling probability for each individual male *Litoria rheocola* (N = 1843) sampled during (a) spring, (b) summer, (c) autumn, and (d) winter, based on its body condition and *Batrachochytrium dendrobatidis* infection status. Predictions were generated from the final generalised linear mixed-effects model presented in Table 8.2. Body condition was calculated as the residual for each frog from a regression using data on all male frogs of $\log_{10}$ transformed body mass on square-root transformed snout-urostyle length (Peig and Green 2009).

Chapter 9: Cyclones reduce disease risk in rainforest frogs

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Abstract

Habitat disturbances can influence disease dynamics by affecting the microclimates experienced by hosts and pathogens. Tropical cyclones are fundamental drivers of forest ecosystem dynamics through their impacts on canopy structure, which directly influence microclimates present in the understorey and all layers of the canopy. Therefore, cyclones may be an important driver of disease dynamics, particularly in diseases that are highly sensitive to environmental variation. One such example is the amphibian disease chytridiomycosis, which is caused by the chytrid fungus *Batrachochytrium dendrobatidis*. The impacts of this disease are highest under cool, wet conditions. We investigated how a severe tropical cyclone in northeastern Australia affected rainforest canopy cover, and how these changes influenced microclimatic conditions and *B. dendrobatidis* infection risk in a species of stream-breeding frog (*Litoria rheocola*). We found that the cyclone dramatically reduced rainforest canopy cover at some sites (by up to an average of 28%, and up to 43% at specific locations), and there was a strong positive relationship between canopy cover and infection risk. This association was stronger after the cyclone, when a much greater range in canopy cover was available, and the infection risk for frogs at cyclone-damaged sites was reduced by up to 75%, as compared to frogs at undamaged sites. These patterns emerged because lower levels of canopy cover were associated with higher temperatures and rates of desiccation, which can directly decrease pathogen growth rates and improve host immune responses. Our results contribute to the

growing body of evidence that canopy structure plays an important role in mediating the interactions between amphibians and *B. dendrobatidis* in both tropical and temperate areas worldwide. Many amphibian species that are vulnerable to chytridiomycosis occur in geographic areas prone to natural, stochastic disturbances, suggesting that habitat heterogeneity may help maintain population persistence and recovery. Artificially manipulating shade using targeted vegetation removal could provide a promising strategy to manage chytridiomycosis in amphibian populations on the brink of extinction.

Introduction

Habitat disturbances can strongly influence host-pathogen interactions, but the nature of these effects depends on the ecology, physiology, and behaviour of both hosts and pathogens. Disease transmission rates can increase when habitat disturbances cause hosts to aggregate at high densities (Arneberg et al. 1998, Mbora and McPeek 2008). Habitat changes can also cause stress (Busch and Hayward 2009) or deterioration in body condition (Wilson et al. 2001, Jokela et al. 2005), which can compromise host immune responses and increase susceptibility to disease (Carey et al. 1999). Habitat disturbances can also lead to changes in microclimatic conditions, which can influence host susceptibility by affecting their immune responses (Wright and Cooper 1981, Zapata et al. 1992, Raffel et al. 2006) or their exposure to pathogens through changes in behaviour (Dowell 2001, Altizer et al. 2006, Rowley and Alford 2007a, Chapter 4). Because many pathogens are highly sensitive to temperature and moisture, small changes in these conditions driven by habitat disturbances can have important implications for their growth and survival, either in hosts or environmental reservoirs (Harvell et al. 2002, Murray et al. 2013, Stevenson et al. 2013).

One of the primary drivers of microclimates in forested habitats is canopy cover (Whitmore 1998, Chen et al. 1999, Madigosky 2004). Therefore, any habitat

disturbance that alters canopy structure (e.g., cyclones, droughts, fires, selective logging) may affect the microclimatic conditions available to forest-dwelling organisms. In rainforests, large trees block wind and regulate the amount of solar radiation that penetrates through the canopy, influencing the thermal and hydric conditions in the understorey and all layers of the canopy (Whitmore 1998, Madigosky 2004). Conditions at ground level are dramatically different than above the canopy; temperatures are cooler, humidity is higher, and wind speeds are lower, and these conditions are also much less variable (Whitmore 1998). The size of a canopy gap strongly influences the microclimatic conditions below the canopy; the larger the gap, the more similar it is to conditions outside the forest because the wind speed and amount and intensity of solar radiation are higher than in the forest interior (Whitmore 1998, Madigosky 2004).

Because of this strong relationship between canopy cover and microclimatic conditions, forest canopy structure can be an important driver of the dynamics of diseases that are highly sensitive to environmental variation. One example of this is the amphibian disease chytridiomycosis, which is caused by the chytrid fungus *Batrachochytrium dendrobatidis* and has caused amphibian declines in many regions of the world (Kilpatrick et al. 2009). The effects of this disease are typically strongest during cooler months and at higher elevations (Woodhams and Alford 2005, Kriger and Hero 2007, Phillott et al. 2013, Sapsford et al. 2013). These patterns have been attributed to the strong thermal and hydric sensitivity of *B. dendrobatidis*; this pathogen requires relatively cool, moist conditions to survive and reproduce (15-25°C optimal; >28°C lethal; Johnson et al. 2003, Piotrowski et al. 2004, Stevenson et al. 2013).

Habitat characteristics also play an important role in *B. dendrobatidis* infection dynamics, primarily due to the effects of canopy cover on the microclimates experienced by both hosts and pathogens. Individual frog hosts have a dramatically reduced risk of infection when their body temperatures are above the optimal range for *B. dendrobatidis* growth (Rowley and Alford 2013, Roznik 2013) and when they

experience dry conditions (Roznik 2013). For these reasons, stream-breeding frogs are less susceptible to infection in deforested areas than in natural forest habitats (Van Sluys and Hero 2009, Becker and Zamudio 2011), and some highly vulnerable species persist in open-canopy dry forest, but not in nearby closed-canopy rainforest (Daskin et al. 2011, Puschendorf et al. 2011). The risk of infection is also lower for pond-breeding frogs in anthropogenically disturbed areas than in habitats with natural vegetation (Becker et al. 2012). Natural habitat disturbances can also reduce vegetation density, thereby causing microclimatic conditions to be warmer and drier, which lowers *B. dendrobatidis* infection probability. For example, boreal toads (*Anaxyrus boreas boreas*) captured in recently burned areas were half as likely to be infected by *B. dendrobatidis* as toads in unburned areas (Hossack et al. 2013), presumably because toads in burned areas had warmer body temperatures (Hossack et al. 2009). Together, these studies suggest that any natural or anthropogenic habitat disturbance that alters forest canopy structure can influence *B. dendrobatidis* infection dynamics.

In many tropical and sub-tropical areas, cyclones play an important role in forest ecosystems by influencing their structure, species composition, and functional processes (Turton and Stork 2008). Severe cyclones can cause widespread damage to forest structure by uprooting trees, breaking stems and branches, defoliating trees, and removing vines and epiphytes (Brokaw and Walker 1991, Turton and Stork 2008). These impacts cause dramatic changes in the microclimates present in the forest understorey and in all layers of the canopy (Turton and Siegenthaler 2004, Pohlman et al. 2008, Turton and Stork 2008). These changes could reduce the risk of *B. dendrobatidis* infection in amphibians by exposing them to warmer, drier conditions that are unfavourable for pathogen growth. During a study on seasonal infection dynamics in frog populations at six rainforest streams in northeastern Queensland, Australia, our study sites were impacted to varying degrees by Severe Tropical Cyclone Yasi (Australian Category: 5, Beaufort Scale: 12; wind gusts >285 kph),

presenting us with an opportunity to investigate how the cyclone affected rainforest canopy cover above streams, and how these changes influenced microclimatic conditions and infection risk in a species of stream-breeding frog (*Litoria rheocola*). Tropical stream-breeding amphibians have experienced more numerous and severe declines than other amphibian taxa (Williams and Hero 1998, Lips et al. 2003, Stuart et al. 2004), and many vulnerable species occur in cyclone-prone areas. Therefore, habitat heterogeneity caused by tropical cyclones may help maintain population persistence and recovery. An understanding of these relationships may be useful for identifying amphibian populations most at risk from chytridiomycosis, for locating potential refuges from the disease, and for testing potential habitat manipulation strategies (e.g., Heard et al., 2014).

Methods

Study species

The common mistfrog (*Litoria rheocola*) is a treefrog that occurs near rainforest streams in northeastern Queensland, Australia (Hoskin and Hero 2008, Dennis 2012). *Litoria rheocola* is found near faster-flowing streams; males typically perch on rocks and streamside vegetation at night, and shelter in moist rock crevices or leaf litter during the day (Hoskin and Hero 2008, Dennis 2012, Chapter 4). By the mid-1990s, chytridiomycosis had extirpated this Endangered species (IUCN 2013) at higher elevations (>400 m) throughout its geographic range (Richards et al.1993, McDonald and Alford 1999); however, many populations have subsequently recovered or recolonised these areas (McDonald et al. 2005) and now co-exist with the pathogen (Sapsford 2012).

Study sites

We studied amphibian disease dynamics at six rainforest streams in northeastern Queensland, Australia (Table 9.1, Figure 9.1). Stream width varied from

5-10 m and streambeds were composed of rocks, ranging in size from small pebbles to large boulders (10 m in diameter). All streams contained pools, runs, and riffles, and most had several waterfalls. Our study began in June 2010; all six sites were sampled during winter (June-July) and spring (October-November) in 2010 (sampling methods described in detail below). On 2-3 February 2011, Severe Tropical Cyclone Yasi directly impacted our sites (Australian Category: 5, Beaufort Scale: 12; Figure 9.1). This storm brought wind gusts >285 kph, 5-m tidal storm surges, and 200-300 mm of rain over a 24-hr period (Australian Bureau of Meteorology 2013a). The eye of the cyclone passed directly over two of our study sites (near the cities of Mission Beach and Tully; Australian Bureau of Meteorology 2013a). Prior to the cyclone, streams were surrounded by tropical rainforest characterised by dense vegetation, including large trees (10 m in height), vines, epiphytes, shrubs, and herbaceous plants. After the cyclone, we quantified cyclone damage at all six sites in March-April 2011 and sampled frogs at all sites again during winter (June-July) and spring (October-November) in 2011. We observed that some of our sites were severely damaged by the cyclone, with trees uprooted or snapped off, and severely damaged and defoliated branches (Figure 9.1).

Forest canopy cover

To quantify effects of Cyclone Yasi on rainforest canopy cover, we compared hemispherical photographs of the canopy taken before (October-November 2010) and after (March-April 2011) the cyclone. We took photographs from the centre of the stream at 10-m intervals along a 400-m transect at each of our six study sites, and quantified the percentage of canopy cover using Gap Light Analyzer software (Frazer et al. 2000). Because we wanted to determine whether canopy cover was reduced across each entire site, rather than just at the points we measured it, we paired the canopy cover at each location before and after the cyclone, and performed a one-tailed paired t-test for each site (using all the locations at each site as replicates) that

tested the null hypothesis that there was no site-wide change in canopy cover. Sites at which canopy cover was significantly lower across the site after the cyclone were categorised as damaged sites. We took additional hemispherical photographs of the canopy in June-July 2011 (winter, 4-5 months post cyclone) and October 2011 (spring, 8 months post cyclone) during seasonal frog sampling. These canopy measurements were included in the infection probability modelling (described below) to account for any changes in local canopy cover that may have occurred between samples.

Microenvironmental conditions

We determined whether variation in rainforest canopy cover influenced the microenvironmental conditions experienced by frogs by using physical models that mimic the thermal and hydric properties of frogs (Rowley and Alford 2010, Chapter 2). Models were made of three percent agar and each contained an embedded Thermochron iButton temperature datalogger (Maxim Integrated Products, California, USA; factory-calibrated and accurate to ±0.5°C) that was waterproofed to prevent failure from moisture damage (Chapter 3, Roznik and Alford 2012). Estimating frog body temperatures using these models typically involves model pairs, one of which is permeable to water loss, with the other impermeable (i.e., coated with plastic to prevent water loss), which together can be used to define the upper and lower boundaries of possible amphibian body temperatures at the locations used by frogs (Rowley and Alford 2010). However, in *Litoria rheocola*, frog body temperatures are closely correlated with the permeable model temperatures (Chapter 2), so we only used data from permeable models.

We quantified the thermal and hydric conditions available to frogs under different levels of canopy cover by placing models on rocks in the streambed that are similar to those typically used by *Litoria rheocola* (Roznik 2013). We placed 100 models at haphazard locations along a 400-m section of stream at Frenchman Creek (a site with substantial variation in canopy cover; Figure 9.2) for a 24-hr period in

October 2011. We took a hemispherical photograph above each model, and determined canopy cover (%) using Gap Light Analyzer software (Frazer et al. 2000). Dataloggers recorded temperatures at 15-min intervals, which we used to calculate the mean temperature during the warmest part of the day (10:00-16:00) for each model. We measured desiccation rates for model locations, expressed as the percentage of model mass lost due to water loss over 24 hr, by weighing each model (to 0.1 g) before and after field placement (Schwarzkopf and Alford 1996, Rowley and Alford 2010). We used linear regressions to test for a relationship between canopy cover and mean daytime temperature, and canopy cover and desiccation rate.

Frog infection probability

We sampled adult male *Litoria rheocola* over five nights (one night in spring 2011) at each site during the winter (June-July) and spring (October-November) over a two-year period that included samples before and after Cyclone Yasi (2010-2011). In *L. rheocola*, the prevalence of *Batrachochytrium dendrobatidis* is highest during these cooler months of the year (Sapsford et al. 2013). We visually surveyed for frogs along 400-m transects marked at 10-m intervals using flagging tape. To determine whether frogs were infected by *B. dendrobatidis*, we swabbed the ventral surface and all four feet of each frog with a sterile rayon swab, covering these areas twice. These samples were analysed using real-time quantitative PCR assays (Boyle et al. 2004). We also gave each frog a unique identifying mark using visible implant elastomer (Nauwelaerts et al. 2000, Sapsford 2012), ensuring that our sample of frogs was independent. For analysis, we used the initial capture of each frog (excluding recaptures), and used only data on males (determined by the presence of distinct nuptial pads).

We used generalised linear mixed-effects models to examine the potential effects of canopy cover (arcsine-square root transformed percentage, expressed in degrees, using the nearest measurement along our stream transect for each frog capture), overall level of cyclone damage (whether the site was significantly damaged),

year (2010 or 2011), and season (winter or spring) on the probability of infection of individual frogs. Before Cyclone Yasi, we quantified canopy cover at all sites in October-November 2010, and after the cyclone, we quantified canopy cover at each site each time we sampled frogs. Infection status was coded as a binomial response variable, so we used models with a binomial family and a logit link function. We developed a set of candidate models that included models with all combinations of one, two, three, or four fixed effects, and all two-way, three-way, and four-way interactions. For all models, we also included the random effect of year × season nested within site to control for any effects specific to particular sites. We ranked models according to Akaike's Information Criterion with adjustment for finite sample size (AICc) to determine the strength of evidence for each model relative to the set of candidate models, using the criteria of Burnham and Anderson (2002). These analyses were performed in program R, version 2.15.2 (R Core Team 2012) using the lme4 (Bates et al. 2012) and MuMIn (Barton 2013) packages.

Results

Severe Tropical Cyclone Yasi impacted a large area of the northeastern coast of Queensland, Australia (Figure 9.1), but damage to rainforest canopy cover was spatially heterogeneous. All six of our study sites were in the path of the cyclone, but only two had significant reductions in canopy cover, indicating that damage extended to the entire site; there was very little or no change in canopy cover at our other four sites (Table 9.1, Figure 9.2). We categorised the two sites with statistically significant levels of damage across the site as damaged sites. The eye of the cyclone passed directly over those sites, but the degree of change in the canopy structure differed between them: Stoney Creek decreased much more dramatically (28% average reduction) than did Tully Creek (11% average reduction; Figure 9.2). Canopy cover recovered only minimally during the eight-month period following the cyclone (4% average increase at Stoney Creek, and 3% average decrease at Tully Creek).

Canopy cover strongly and significantly influenced the microclimatic conditions available to frogs on rocks in the streambed, in terms of both body temperatures and water loss, as estimated by data from our physical models (Figure 9.3). Canopy cover was inversely related to both temperature ($F_{1,95} = 41.874$, $R^2 = 0.306$, $P < 0.001$) and desiccation rate ($F_{1,93} = 41.874$, $R^2 = 0.256$, $P < 0.001$), indicating that increased canopy cover lowered temperature and increased moisture retention (Figure 9.3).

We captured a total of 1163 unique male *Litoria rheocola* during four seasonal surveys at each of our six sites (Table 9.1). Our modelling indicated that canopy cover (%), year (2010 or 2011), and season (winter or spring) all influenced the infection probability of individual frogs (Table 9.2, Figure 9.4). Four models with ΔAICc <2 (maximum Nagelkerke $R^2 = 17.9\%$) were averaged to produce a final model that included canopy cover, year, and season as main effects, and canopy cover × year, season × year, year × season, and canopy cover × year × season interactions (Table 9.2). The model containing these effects was significantly different from a null model that included only the random effect of year × season nested within site ($\chi^2 = 20.549$, df = 7, P = 0.004). Overall, frogs were more likely to be infected during winter than in spring, and infection probability was higher during the second year than in the first year (Figure 9.4). Infection probability increased with canopy cover, and this relationship was stronger after the cyclone, when a much greater range in canopy cover was available at our sites overall (Figure 9.4).

Discussion

Changes in habitat structure caused by natural or anthropogenic disturbances can strongly influence host-pathogen interactions as a result of changes in microclimatic conditions. Infection dynamics in the amphibian disease chytridiomycosis, which is caused by the chytrid fungus *Batrachochytrium dendrobatidis*, are highly sensitive to the microclimates used by frogs. Our data from physical frog models indicate that frog microclimates are strongly associated with

canopy cover. As canopy cover decreases, frogs should experience warmer and drier conditions, which are likely to directly slow rates of pathogen growth and reproduction (Piotrowski et al. 2004, Stevenson et al. 2013). We found that Severe Tropical Cyclone Yasi affected rainforest canopy cover, microclimatic conditions, and the probability of infection by the chytrid fungus *Batrachochytrium dendrobatidis* in the stream-breeding frog *Litoria rheocola*. The cyclone dramatically reduced rainforest canopy cover at two of our six streams (by up to an average of 28%, and up to 43% at specific locations), and there was a strong relationship across all of our damaged and undamaged sites between canopy cover and infection risk. The probability of infection increased with canopy cover, and this relationship was stronger after the cyclone, when a greater range of canopy cover was available. Our study demonstrates that changes in microclimatic conditions caused by natural disturbances to forest vegetation can play an important role in influencing host-pathogen interactions.

The probability of infection by *B. dendrobatidis* in frogs depended strongly on canopy cover (Figure 9.4). Prior to the cyclone, there was relatively little variation in canopy cover within our sites, and small differences among our sites (all measured values for canopy cover were 73-93%), and infection probability was relatively constant (Figures 9.2, 9.4). However, after the cyclone, individual measurements of canopy cover were as low as 43%, and most measurements at each of the two sites we categorised as damaged were lower than most measurements at all of the sites we categorised as undamaged (Figure 9.2). These changes in canopy cover strongly influenced infection risk (Figure 9.4). For example, during the winter following the cyclone, frogs at cyclone-damaged sites had a 5-40% chance of infection, whereas infection probability ranged from 40-80% at undamaged sites (Figure 9.4). Our results contribute to a growing body of evidence that canopy structure plays a fundamental role in mediating the interactions between amphibians and *B. dendrobatidis* in both tropical and temperate areas. For pond-breeding frogs, the risk of infection is lower in habitats with lower vegetation density as a result of anthropogenic disturbance or

wildfire (Becker et al. 2012, Hossack et al. 2013). Stream-breeding frogs are also less susceptible to infection in deforested areas than in natural forest habitats (Van Sluys and Hero 2009, Becker and Zamudio 2011), and disease risk is also lower in forest types with naturally lower canopy cover (Puschendorf et al. 2011, 2013).

Canopy cover is an important driver of infection dynamics because large trees influence the microclimatic conditions present below the canopy by slowing air movement and reducing the amount of solar radiation that reaches the forest floor. This causes cooler temperatures, higher humidity, and less variability in these conditions (Whitmore 1998, Madigosky 2004). We found that as canopy cover decreased, air temperature and desiccation rates at frog microhabitats increased (Figure 9.3). The warmer temperatures associated with lower canopy cover can improve the immune responses of frogs (Carey et al. 1999, Raffel et al. 2006, Ribas et al. 2009, Rollins-Smith et al. 2011), and increase frog body temperatures, which can decrease rates of *B. dendrobatidis* growth and survival on hosts (Piotrowski et al. 2004, Stevenson et al. 2013). *Batrachochytrium dendrobatidis* is also sensitive to hydric conditions and cannot tolerate desiccation (Johnson et al. 2003). Therefore, frogs in open-canopy areas should be warmer and drier than frogs in more closed-canopy locations, and thus less likely to acquire and maintain infections. In nature, individual frogs that use warmer, drier microclimates have a lower risk of *B. dendrobatidis* infection (Hossack et al. 2013, Rowley and Alford 2013, Chapters 5-6), and in the laboratory, infected frogs maintained in warmer and drier conditions survive longer than those in cool and wet conditions (Bustamante et al. 2010, Murphy et al. 2011). Frogs that are exposed to very warm temperatures (>30°C) can also lose *B. dendrobatidis* infections entirely (Woodhams et al. 2003, Chatfield and Richards-Zawacki 2011, Geiger et al. 2011).

The benefits of canopy openings in reducing *B. dendrobatidis* infection risk are likely to vary by season, year, and location (e.g., latitude, elevation), and also among species, depending on their preferences and tolerances. Even closely related species

occurring at the same sites can have very different thermal and hydric preferences, and patterns of microhabitat use (Rowley and Alford 2007b, Rowley and Alford 2013, Chapters 5-7). Variation in canopy cover should more strongly affect those species that prefer more exposed microhabitats (particularly basking species), whereas other species could override changes in canopy cover by seeking shelter or spending more time in water. Our study species, *Litoria rheocola*, prefers sheltered diurnal microhabitats, particularly rocks in the streambed (Chapter 4). Despite this, the effects of canopy cover still played a major role in influencing disease risk for this species. Other species and life stages, especially those that are aquatic, could also benefit from canopy disturbance if water temperatures are warmer in habitats with more open canopies (e.g., Forrest and Schlaepfer 2011, Becker et al. 2012).

Even if water temperatures remain cool in open-canopy areas (e.g., streams), semi-aquatic species may still benefit from short periods of exposure to conditions that are warm and dry (Daskin et al. 2011, Puschendorf et al. 2011, Chapter 5). For example, in the semi-aquatic stream frog *Litoria nannotis*, nocturnal desiccation rates strongly influence infection probability (Chapter 5). Furthermore, *L. nannotis* and a closely related species, *L. lorica*, persisted along a section of stream in open-canopy dry forest without clinical signs of chytridiomycosis, but were negatively impacted by the disease at a nearby section of stream surrounded by closed-canopy rainforest (Puschendorf et al. 2011). These semi-aquatic species spend the day in the cool stream, but perch on sun-warmed rocks at the open-canopy site during the early part of the night, so it is likely that direct effects of temperature and desiccation on the growth and reproduction of *B. dendrobatidis* inhabiting frog skin contributed to pattern (Daskin et al. 2011, Puschendorf et al. 2011). Although sunny areas can reduce infection risk for many species, some species or life stages may be highly sensitive to desiccation and warm temperatures and unable to persist in areas with low canopy cover caused by natural or anthropogenic disturbances (Rothermel and Semlitsch 2006, Rittenhouse et al. 2008).

In our frog populations, season and year significantly influenced the probability of infection, likely due to the influences of weather (Table 9.2). The weather was quite different between the two years of our study; during the months of sampling, the mean minimum temperature was lower in 2011 than 2010 (by 3.6°C in winter, and 0.6°C in spring), and the percentage of days above 25°C was also lower in 2011 (by 31% in winter, and 2% in spring; Australian Bureau of Meteorology 2013b). The cooler weather during the second year of our study was conducive to higher infection probabilities (Figure 9.4), likely due to faster pathogen growth rates (Piotrowski et al. 2004, Stevenson et al. 2013). The influence of cooler weather was quite distinct, however, from the influence of canopy cover. Even in a year with relatively high infection risk (2011), the probability of infection was lower at the two cyclone-damaged sites than at the four undamaged sites (Figure 9.4).

We have demonstrated that canopy cover is strongly related to the probability of *B. dendrobatidis* infection in individual frogs, and that tropical cyclones can decrease infection risk by reducing canopy cover. Tropical stream-breeding amphibians have experienced more numerous and severe declines than other amphibian taxa (Williams and Hero 1998, Lips et al. 2003, Stuart et al. 2004), and many vulnerable species occur in cyclone-prone areas. Therefore, habitat heterogeneity caused by tropical cyclones may help maintain population persistence and recovery. An understanding of these relationships can be used to identify amphibian populations most at risk to chytridiomycosis, and also to locate potential refuges from the disease (Puschendorf et al. 2011, 2013). Our results also suggest that it may be possible to reduce the impact of chytridiomycosis by providing canopy openings for populations at risk. This could involve small-scale removal of trees or large branches, targeting those overhanging critical habitat, such as a pond or section of stream (e.g., as has been achieved in other studies of amphibians and reptiles; Pike et al. 2011, Skelly et al. 2014). Even small canopy openings that provide access to warm temperatures for short periods of time (e.g., one hour) can allow populations to

persist that would otherwise be extirpated (Daskin et al. 2011, Puschendorf et al. 2011). Targeted canopy removal could be beneficial for species that are under such severe threat from disease that only a few individuals or populations remain. This could be a promising strategy for *in situ* management of amphibians on the brink of extinction, and could also increase the success of reintroduction efforts for such species.

Table 9.1. Study site details and sample sizes of unique male *Litoria rheocola* (N = 1843) captured at six rainforest streams in northeastern Queensland, Australia, that were impacted by Severe Tropical Cyclone Yasi on 2-3 February 2011. Frogs were captured during seasonal stream surveys before and after the cyclone, and tested for infection by the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* (infected or uninfected). Also shown are statistical results from one-tailed paired t-tests that test whether rainforest canopy cover decreased at each site after the cyclone, with statistically significant results shown in bold typeface.

Site	Coordinates	Elevation (m ASL)	Cyclone damage		Sample sizes				
					Before cyclone		After cyclone		Total
			t (df)	P	Infected	Uninfected	Infected	Uninfected	
Bobbin Bobbin Creek	17.378°S, 145.775°E	700	-1.967 (39)	0.972	35	99	24	13	171
Frenchman Creek	17.307°S, 145.922°E	40	1.073 (37)	0.145	33	152	24	40	249
Mena Creek	17.649°S, 145.987°E	60	0.330 (40)	0.371	59	121	12	34	226
Stoney Creek	17.920°S, 146.069°E	20	25.654 (35)	**<0.001**	50	75	10	26	161
Tully Creek	17.773°S, 145.645°E	150	12.954 (35)	**<0.001**	45	165	16	43	269
Windin Creek	17.365°S, 145.717°E	750	-2.944 (35)	0.997	17	36	6	28	87

Table 9.2. Generalised linear mixed-effects models (family: binomial, link function: logit) were used to examine effects of changes in canopy cover caused by Severe Tropical Cyclone Yasi on the probability of infection by *Batrachochytrium dendrobatidis* in individual *Litoria rheocola*. We included the following variables as fixed effects: canopy cover (%) at each frog's location, cyclone damage (damaged or undamaged site), year (2010 or 2011), and season (winter or spring). We developed a set of candidate models that included models with all combinations of one, two, three, or four fixed effects, and all two- three- and four-way interactions. For all models, we also included the random effect of year × season nested within site. We ranked models according to Akaike's Information Criterion with adjustment for finite sample size (AICc). All models with ΔAICc <3 are shown, but only models with ΔAICc <2 were strongly supported by our data and used to produce a final averaged model.

Candidate models

Model effects	AICc	ΔAICc	Weight	R^2
Canopy, Year, Season, Canopy × Year, Season × Year	1304.142	0.000	0.089	0.168
Canopy, Year, Season, Canopy × Season, Canopy × Year, Season × Year, Canopy × Season × Year	1304.284	0.143	0.083	0.179
Canopy, Year, Season, Canopy × Year	1305.077	0.935	0.056	0.163
Canopy, Year, Season, Canopy × Season, Canopy × Year, Season × Year	1306.118	1.976	0.033	0.168
Canopy, Year, Season, Damage, Canopy × Year, Season × Year	1306.189	2.048	0.032	0.167
Canopy, Year, Canopy × Year	1306.196	2.054	0.032	0.132
Canopy, Season	1306.245	2.104	0.031	0.104
Canopy, Year, Season, Damage, Canopy × Season, Canopy × Year, Season × Year, Canopy × Season × Year	1306.313	2.171	0.030	0.182
Canopy, Year, Season, Season × Year	1306.664	2.523	0.025	0.125
Canopy, Year, Season, Canopy × Season, Canopy × Year	1306.941	2.800	0.022	0.164
Canopy, Year, Season, Damage, Canopy × Year	1307.131	2.989	0.020	0.164

Final averaged model

Model effect	Estimate
Intercept	-2.898
Canopy	0.032
Year	-4.193
Season	1.224
Canopy × Year	0.077
Canopy × Season	-0.060
Year × Season	-6.379
Canopy × Year × Season	0.183

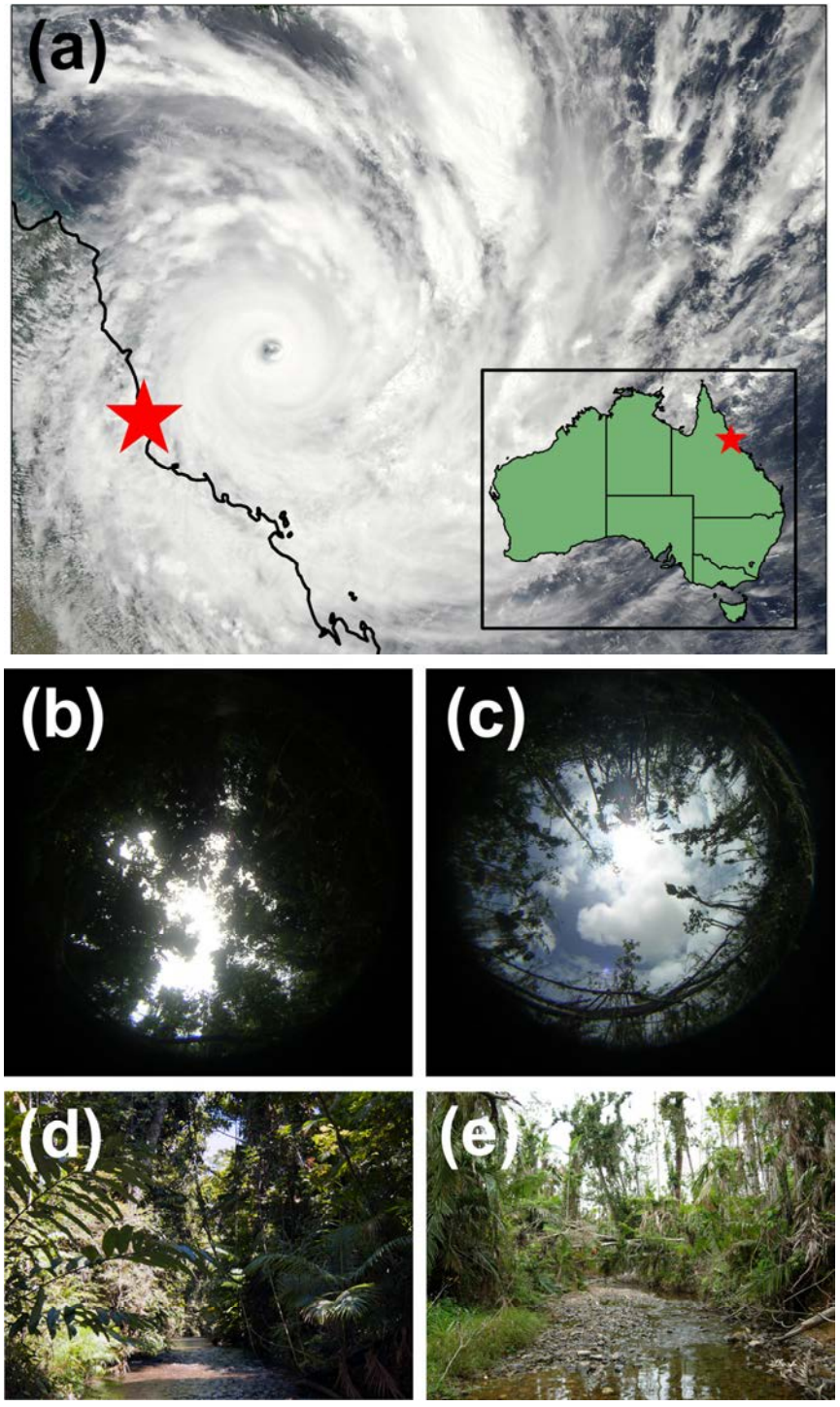

Figure 9.1. Severe Tropical Cyclone Yasi impacted the northeastern coast of Queensland, Australia on 2-3 February 2011. Shown are (a) a satellite image of the cyclone approaching the coast (a star denotes the area encompassed by our study sites, and the inset shows this location within Australia), hemispherical photographs of the rainforest canopy above Stoney Creek that were taken from the same location (80 m along our stream transect) both (b) before and (c) after the cyclone (showing the average canopy cover at that site: 88% and 60%, respectively), and ground-level images of Stoney Creek (taken from different locations) both (d) before and (e) after the cyclone. Images were provided by (a) NASA (Moderate Resolution Imaging Spectroradiometer, Aqua satellite, taken at 13:35 Australian Eastern Standard Time on 2 February 2011), (b-c) Sarah Sapsford, (d) Angus McNab, and (e) Elizabeth Roznik.

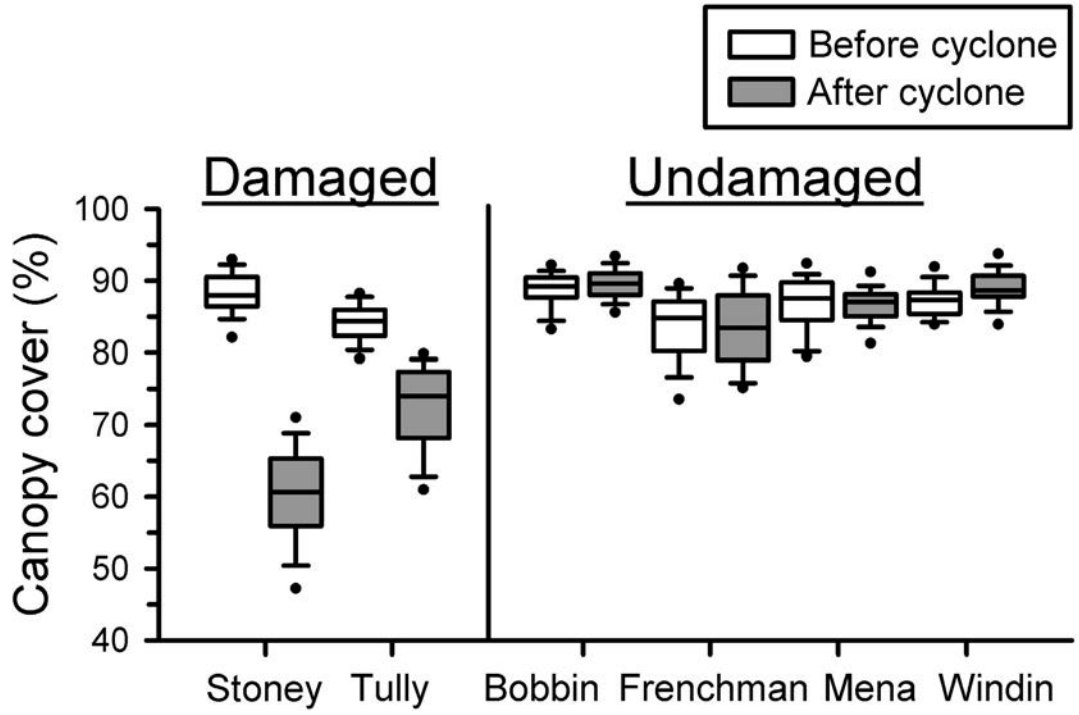

Figure 9.2. Canopy cover (%) before and after Severe Tropical Cyclone Yasi at two sites that were damaged significantly by the cyclone, and four sites that were not damaged significantly (see Table 9.1 for statistical results).

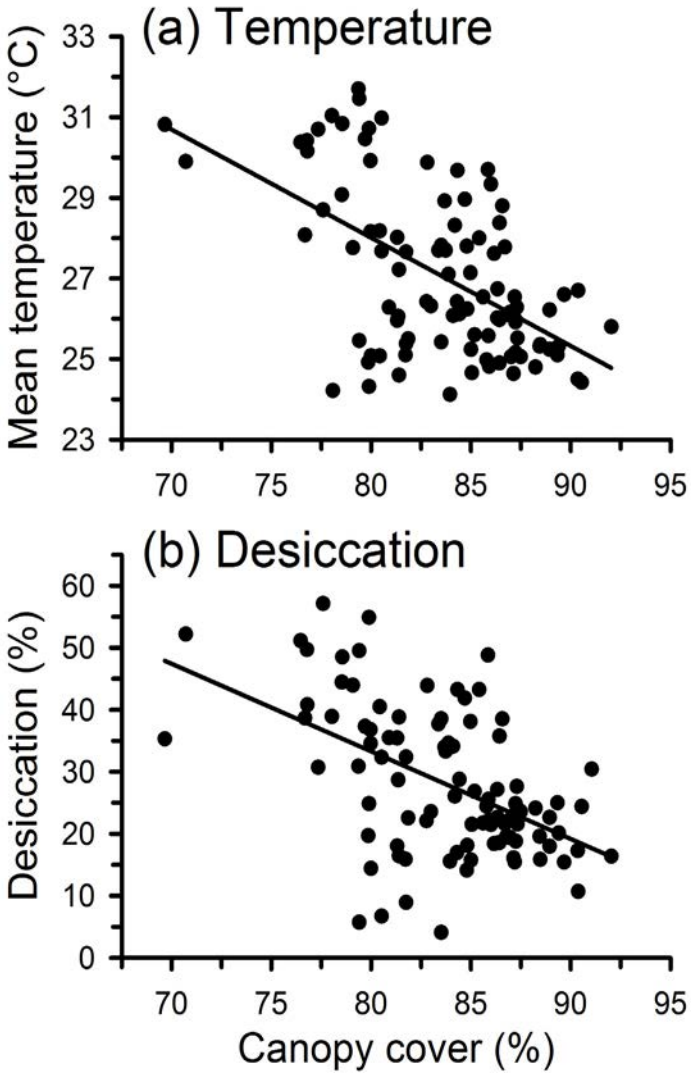

Figure 9.3. Relationships between canopy cover (%) and (a) mean estimated frog body temperature during the warmest part of the day (10:00-16:00), and (b) relative desiccation rate. These responses were estimated using physical models that mimic the thermal and hydric properties of frogs, placed at haphazard locations on rocks in the stream that are similar to those typically used by *Litoria rheocola*. Desiccation rate was calculated as the percentage of model mass lost due to water loss over a 24-hr period.

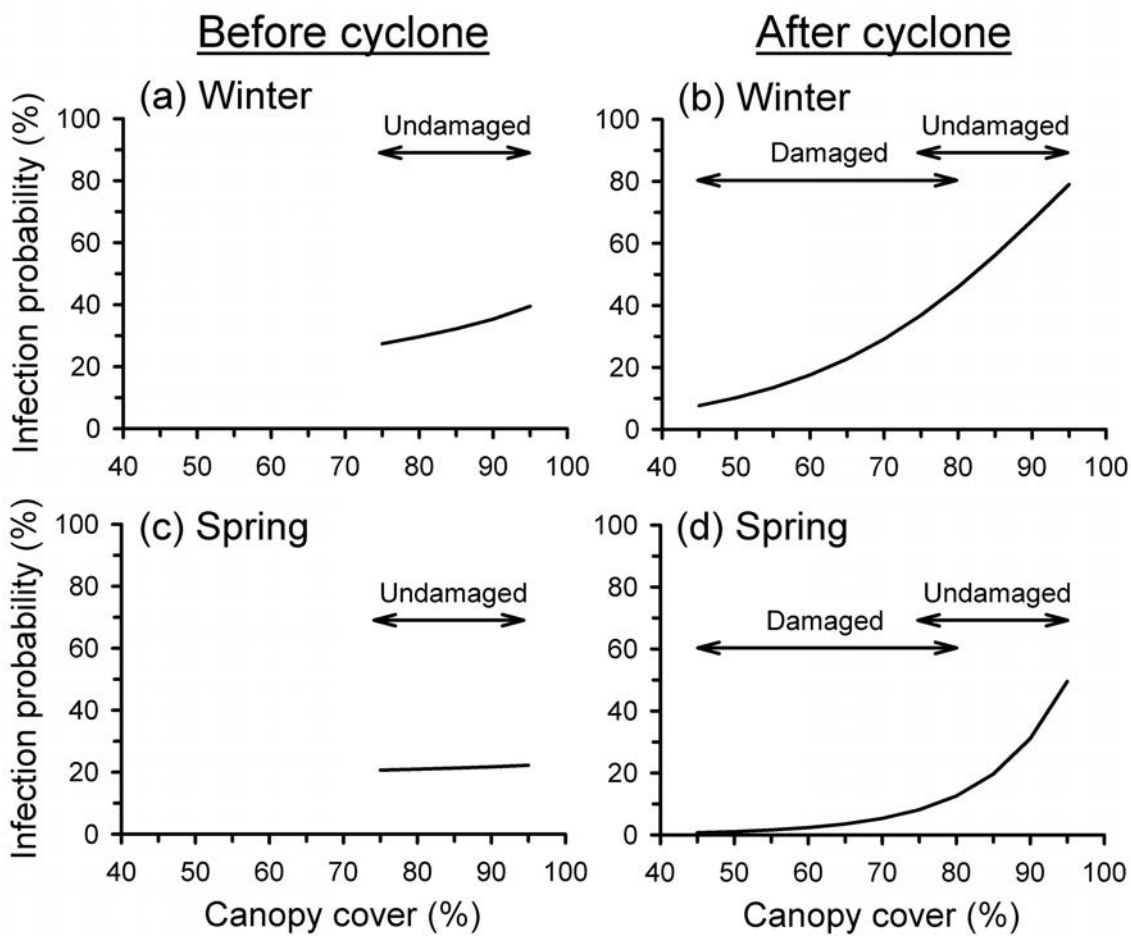

Figure 9.4. Predicted probability of infection by the pathogen *Batrachochytrium dendrobatidis* in the frog *Litoria rheocola* during the winter (a-b) and spring (c-d) before and after Severe Tropical Cyclone Yasi (2010-2011). These predictions were generated from the averaged generalised linear mixed-effects model based on our field data (Table 9.2), and are shown for the range in canopy cover present at our sites before and after the cyclone. Arrows indicate the ranges in canopy cover present at sites that were significantly damaged or were undamaged by the cyclone.

Chapter 10: Summary, implications, and directions for future research

Summary and implications

A major goal of the research presented in this thesis was to increase our understanding of how behaviour affects the interactions of frogs with the pathogen *Batrachochytrium dendrobatidis*. Investigating this topic required a substantial amount of complex equipment and techniques to reliably and repeatedly locate individual frogs and measure their body temperatures. These included an automated radiotelemetry system, which was used to monitor frog body temperatures semi-continuously, specialised gear for tracking frogs manually using radiotelemetry and harmonic direction finding, non-contact infrared thermometers for measuring frog body temperatures, and physical models placed at frog locations to estimate their body temperatures and desiccation rates.

We demonstrate that these techniques can all be used successfully in amphibian fieldwork to provide important ecological information. We tested two techniques for estimating distributions of frog body temperatures: temperature-sensitive radiotransmitters and physical models (Chapter 2). We found that both approaches can provide accurate measurements of the thermal conditions experienced by frogs in nature. We also show that semi-continuous data collection using these techniques produces more accurate thermal profiles than using only data collected at discrete points in time. We improved the previously published physical model technique (Rowley and Alford 2010) by waterproofing the Thermochron iButton dataloggers embedded in these models using a plastic coating (Chapter 3); we show that this coating prevents device failure and data loss with minimal influence on temperature readings. The techniques we developed and tested for locating frogs and

obtaining accurate data on their body temperatures are useful for advancing many aspects of amphibian biology and physiology, and addressing many urgent ecological and conservation questions.

Because the microenvironments used by frogs strongly affect host-pathogen interactions involving frogs and *B. dendrobatidis*, but are constrained by ambient conditions, fine-scale information on the ecology and behaviour of individual species is necessary to understand infection dynamics. We studied the detailed ecology and behaviour of *Litoria rheocola*, an endangered frog that has declined due to chytridiomycosis (Chapter 4). Overall, we found that *L. rheocola* are relatively sedentary frogs that are restricted to the stream environment, and prefer sections of the stream with riffles, numerous rocks, and overhanging vegetation. We also found that frog behaviour differed seasonally, but was similar at low and high elevations. Frogs were most vulnerable to disease during cooler months and at higher elevations, when their body temperatures and frequency of contact with stream water were likely to cause high rates of pathogen transmission and growth. Our study provides the first detailed information on *L. rheocola* behaviour, and suggests ecological mechanisms for the patterns of decline and infection dynamics that have been observed in this endangered species.

We also found that patterns of microenvironment use, microhabitat use, and movement in individual frogs of three species of rainforest frogs (*Litoria nannotis*, *L. rheocola*, *L. serrata*) are related to their infection probability. Individual frogs that used cooler, moister microclimates were more likely to be infected by *B. dendrobatidis* than frogs that experienced warmer, drier conditions (Chapter 5). These relationships are likely explained by differences in rates of pathogen transmission and growth associated with these microclimates. Differences in the microenvironments used by infected and uninfected frogs are caused by their patterns of movement and microhabitat use (Chapter 6). Infected frogs tended to use cooler, moister substrates more often than uninfected frogs (especially rocks and decaying wood, and not

vegetation), and they tended to remain closer to the stream and move less often, but move longer distances when they did move.

The behavioural differences between infected and uninfected frogs that we documented could reflect effects of innate behaviour on the probability of acquiring or retaining infections, or they could be a result of changes in the behaviour of infected frogs in response to their infections. Investigating the nature of these relationships is very difficult using field data. Therefore, to disentangle these hypotheses, we conducted a laboratory experiment on two species (*Litoria nannotis* and *L. serrata*), by comparing the behaviour of individual frogs when they were infected and uninfected (Chapter 7). We found that infection by *B. dendrobatidis* changed the behaviour of *L. nannotis*, increasing their use of aquatic microhabitats and thereby perpetuating infections, but infection did not change *L. serrata* behaviour. These results reinforce the importance of individual behaviour in this host-pathogen system, and the complexity of the relationships between *B. dendrobatidis* and different host species.

We also found that *B. dendrobatidis* infections can have sublethal effects that interact with body condition to influence the calling probability of male *Litoria rheocola*. These effects involve complex, potentially adaptive trade-offs; infected frogs in poor body condition were up to 40% less likely to call than uninfected frogs in similar condition, but infected frogs in good condition often had a higher probability of calling than uninfected frogs (by up to 30%). This pattern of increased calling probability in infected frogs is consistent with life-history theory, which predicts that reproductive effort should increase as life expectancy decreases. Because *B. dendrobatidis* infections increase the risk of mortality, it is likely that infected frogs adjusted their reproductive output in response to this risk to maintain lifetime reproductive success. Investigating sublethal effects of *B. dendrobatidis* infections is important for understanding patterns of fitness, and thus changes in population demography.

Habitat disturbances can influence disease dynamics by altering forest canopy cover, thereby affecting the microclimates experienced by both hosts and pathogens.

We studied the effects of a severe tropical cyclone on our study system and found that the cyclone dramatically reduced rainforest canopy cover at some sites (by up to an average of 28%), which increased temperatures and decreased moisture levels in frog microhabitats. These changes in microclimates reduced infection risk in frogs by up to 75%, as compared to frogs at undamaged sites, presumably by slowing pathogen growth rates. Many species that are vulnerable to *B. dendrobatidis* occur in geographic areas prone to severe tropical storms (e.g., Central and South America, northeastern Australia), and the habitat heterogeneity created by these systems may help maintain population persistence and recovery. The effects of this natural experiment also suggest that artificially manipulating shade using targeted vegetation removal could provide a promising strategy for managing chytridiomycosis in amphibian populations on the brink of extinction.

Directions for future research

Few studies have examined individual variation in amphibian behaviour, and how it affects and is affected by *Batrachochytrium dendrobatidis* infections. The research presented in this thesis demonstrates that the behaviour of individual frogs plays an important role in infection dynamics in this host-pathogen system. Interactions between individual frogs and *B. dendrobatidis* ultimately drive disease dynamics at larger scales (e.g., populations, communities, ecosystems); therefore, an individual-based approach in *B. dendrobatidis* research may be useful for explaining large-scale patterns and addressing new questions. We found that most aspects of behaviour that we studied were highly variable among individuals, even within populations and among individuals with the same infection status. This variability may help explain some of the inconsistent results that have been reported in *B. dendrobatidis* research (e.g., Venesky et al. 2013). If individuals differ consistently in aspects of behaviour that affect susceptibility or tolerance of infections, natural selection may cause some species or populations to evolve tolerance or resistance to *B. dendrobatidis* through changes in

behaviour over time. Researchers may also be able to artificially select for behavioural traits that promote co-existence with this pathogen, as has been suggested for innate immune defences (Venesky et al. 2012). Captive breeding programs are currently being used to raise amphibians for reintroduction into the wild; however, these efforts may not be successful if the individuals that are released cannot co-exist with *B. dendrobatidis*. By conducting behavioural assays (e.g., in thermal gradients), researchers may be able to selectively breed individuals that prefer warmer, drier conditions and are likely to have a low risk of infection and maintain low (nonlethal) infection loads if they become infected. Selecting for host defences that prevent infections or limit the negative effects of infections may increase the success of reintroduction efforts (Venesky et al. 2012).

Other non-behavioural traits of individual amphibians also influence their risk of *B. dendrobatidis* infections. These include antimicrobial peptides and commensal skin bacteria, which are both innate immune defences of amphibian skin (Rollins-Smith and Conlon 2005, Harris et al. 2006). The relationships between individual behaviour and the innate immune system are unknown, however, and future studies should address if and how behaviour is correlated with these traits. For example, frogs acquire commensal bacteria from their environment, so frogs that select different microhabitats may have different assemblages of bacteria living on their skin, which could differentially influence their infection risk. Understanding how the combined effects of behaviour and components of the innate immune system interact with *B. dendrobatidis* is also important, and will provide a more realistic understanding of how these factors influence *B. dendrobatidis* risk in nature.

A more comprehensive understanding of how the behaviour of individuals of different species affects their interactions with *B. dendrobatidis* is needed. Studies on Australian rainforest frogs found that individuals with cooler body temperatures were more likely to be infected (Rowley and Alford 2013, Chapters 5, 7), but in Panamanian frogs, the mean body temperature of frogs in infected populations was warmer than in

populations of uninfected frogs, which suggests that infected frogs behaviourally elevated their body temperatures in response to the pathogen ("behavioural fever"; Richards-Zawacki 2009). These divergent patterns could both be important in different species or at different times. For example, it is possible that individuals that choose warm, dry microclimates are less likely to acquire and maintain infections, but at some stage of infection buildup, individuals alter their behaviour to seek out warmer or drier conditions. Elucidating these relationships, including if and when individuals initiate behavioural fever, is essential for understanding and managing the impacts of disease. This will require studies on a variety of species that occur in different habitats (e.g., ponds) and in different regions of the world.

A realistic understanding of the effects of environmental conditions on *B. dendrobatidis* growth rates is urgently needed. Laboratory experiments have been instrumental in determining the effects of temperature on rates of *B. dendrobatidis* growth, reproduction, and survival *in vitro* (Piotrowski et al. 2004, Stevenson et al 2013), but most of these experiments have been conducted under constant temperatures. Understanding how *B. dendrobatidis* responds to realistic, fluctuating temperatures simulating those experienced by frogs in nature is essential for understanding the impacts of this pathogen on amphibians (e.g., Stevenson 2012, Raffel et al. 2013). This includes experiments that examine *B. dendrobatidis* growth rates *in vitro*, together with *in vivo* experiments on infected frogs that examine the development and outcome of infection. Our study demonstrates the importance of desiccation in mitigating infection risk, even when temperatures are optimal for *B. dendrobatidis* growth (Chapter 5). Therefore, incorporating the effects of moisture and humidity into these experiments will also increase their relevance to natural conditions.

Additional studies will also be necessary to understand the relationships between individual behaviour and *B. dendrobatidis* infection intensity. We found conflicting results in our study; in some cases, environmental factors affected infection intensity in the opposite direction from their effects on infection probability. For

example, in our three study species, the probability of infection was negatively associated with desiccation rate, but in most cases, infection intensity was positively associated with desiccation rate (Chapters 5). This indicates that frogs that were wetter were more like to be infected, but among infected frogs, those that were drier had higher infection loads. These results suggest that there may be important threshold relationships, whereby the pathogen begins to influence host behaviour or physiology differently above a certain infection load (Vredenburg et al. 2010). For example, it is possible that increasing desiccation rates may suppress pathogen growth on infected frogs; however, above a threshold infection load, a frog's behaviour or the permeability of its skin may change in ways that facilitate rapid increases in infection loads. Alternatively, use of drier, more exposed locations could be explained by a lower mobility level of frogs with high infection loads. Further research on frogs spanning a wide range of infection intensities is necessary to fully understand the complex relationships between infection intensity and the environmental conditions experienced by infected frogs.

How females and juveniles interact with *B. dendrobatidis* is poorly known, especially in Australian rainforest frogs. This thesis focuses primarily on adult male frogs because females and juveniles of our study species are infrequently observed along streams. However, females often behave much differently than males (Rowley and Alford 2007b), which could affect their interactions with *B. dendrobatidis*. Juveniles of these species are rarely encountered, and almost nothing is known about their behaviour or ecology from the time that tadpoles metamorphose until sub-adulthood. Further study is necessary to understand behavioural differences between sexes and between life stages, and the implications for infection risk.

Although many amphibians can carry *B. dendrobatidis* infections from which they ultimately recover, the sublethal effects of infections on amphibians are poorly understood, particularly effects on reproduction. We show that *B. dendrobatidis* infections can have sublethal effects that interact with host body condition to influence

calling probability in male frogs (Chapter 8). These results suggest possible effects of infections on fitness, but we did not measure fitness directly. It is possible that non-calling males may compensate for reduced calling effort, such as by using alternative mating tactics (e.g., satellite behaviour), or that increased calling effort in frogs may not actually lead to increased reproductive success. Future studies should address these relationships, as well as effects of infections on female reproductive trade-offs. Understanding whether and how this pathogen alters energetic investment into egg production or female reproductive behaviour (e.g., reproductive frequency, mate choice) is important for fully assessing its impacts on amphibians.

It is becoming increasingly clear that *B. dendrobatidis* infection dynamics are strongly driven by environmental conditions. Our study demonstrates that forest canopy structure plays an important role in mediating the interactions between rainforest stream frogs and *B. dendrobatidis* (Chapter 9). This suggests that it may be possible to reduce the impact of chytridiomycosis by providing canopy openings for populations at risk. This could involve small-scale removal of trees or large branches, targeting those overhanging critical habitat, such as a pond or section of stream. There is an urgent need to test potential habitat manipulation strategies, particularly for species that are under such severe threat from disease that only a few individuals or populations remain. Even small canopy openings that provide access to warm temperatures for short periods of time may have significant effects on disease mitigation and population persistence (e.g., Daskin et al. 2011). Species that prefer higher body temperatures may benefit from the effects of canopy openings by basking, whereas species that prefer lower temperatures may buffer these effects behaviourally by seeking shelter. A better understanding of thermoregulation in species at risk from disease will help determine the effectiveness of such strategies. Manipulating canopy cover could be a promising strategy for *in situ* management of amphibians on the brink of extinction, and could also increase the success of reintroduction efforts for such species.

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