Why does Chytridiomycosis drive some frog populations to extinction and not others? The effects of interspecific variation in host behaviour.

A thesis submitted by
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STATEMENT OF THE CONTRIBUTION OF OTHERS

This thesis was co-supervised by Ross Alford and Lin Schwarzkopf, but also received valuable input from a number of people. Ross Alford contributed in the form of advice on ideas, experimental design, statistical support, editorial assistance, and funded the majority of project costs. Lin Schwarzkopf provided useful comments and editorial assistance on the thesis. Richard Speare, Robert Puschendorf, Robert Jehle, Jérôme Pellet, Lee Skerratt, Andrea Phillott, Bryan Windmiller and Ruth Campbell provided editorial assistance for individual chapters. PCR diagnostic tests for *Batrachochytrium dendrobatidis* were performed by Ruth Campbell at the School of Veterinary and Biomedical Sciences, and Alex Hyatt at the Australian Animal Health Laboratory at CSIRO.

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ABSTRACT

Infectious diseases currently pose a great threat to global biodiversity. One of the most alarming wildlife disease to date is chytridiomycosis, a fatal disease of amphibians caused by the pathogen *Batrachochytrium dendrobatidis.* Chytridiomycosis has been implicated in mass mortalities, population declines, and local and global extinctions of many species of amphibians around the world. However, while some species have been severely affected by the disease, other, sympatric species remain unaffected. One reason why some species decline from chytridiomycosis and others do not may be interspecific differences in behaviour, which may affect the probabilities of acquiring and succumbing to infections. Host behaviour can either facilitate or hinder pathogen transmission, and transmission rates in the field are likely to vary among species according the frequency of factors such as physical contact between frogs, contact with infected water, and contact with environmental substrates that may serve as reservoirs. Similarly, the thermal and hydric environments experienced by frogs can strongly affect their susceptibility to chytridiomycosis, so some interspecific differences in the effects of the disease may also be caused by differences in microenvironment use among species.

I examined the potential effects of behaviour on the susceptibility of different host species to declines caused by chytridiomycosis by tracking three species of stream-breeding frogs in northern Queensland, Australia. The species historically co-occurred at many sites in the Wet Tropics, but high elevation (> 400 m) populations of two species declined to differing degrees in association with outbreaks of chytridiomycosis in recent decades, while low elevation populations remained apparently unaffected. The waterfall frog *Litoria nannotis,* declined to local extinction at all known high elevation sites. All studied populations of the green-eyed tree frog *Litoria genimaculata* at high elevation sites declined to low numbers and then recovered. The third species, the stoney creek frog *Litoria lesueuri,* is not known to have experienced population declines even at high elevations.

I used radio telemetry and harmonic direction finding to track frogs at five sites. Surveys lasted 16 days and were conducted in both the cool/dry season and the warm/wet season. The location of each frog was determined once during the day and once at night over the duration of the survey period. At each location, I recorded contact with other frogs, stream water, and other environmental substrates, its three-dimensional position, movement, habitat type, and body temperature. Retreat sites of *L. lesueuri* and *L. nannotis* were also sampled for *B. dendrobatidis*. Harmonic direction finding obtained fewer fixes on frogs but measures of movement and habitat use did not differ significantly between techniques. In total, 117 frogs were tracked: 28 *L. nannotis,* 27 *L. genimaculata* and 62 *L. lesueuri*. Frequency of contact with other frogs and with water was highest in *L. nannotis,* intermediate in *L. genimaculata*, and lowest in *L. lesueuri.* Environmental substrate use differed among species, and *B. dendrobatidis* was not detected at retreat sites. Movement and habitat use also

differed significantly among species. *Litoria lesueuri* moved more frequently and greater distances and was often located away from streams, moving between intact rainforest and highly disturbed environments. *Litoria genimaculata* moved less frequently and shorter distances, and was more restricted to stream environments, occasionally moved large distances along and between streams, but was never located outside of intact rainforest. *Litoria nannotis* remained in streams during the day, did not move large distances along or move between streams, and was always located within intact rainforest.

In addition to tracking data, I designed, tested, and deployed novel physical models to record the thermal conditions experienced by frogs, regardless of cutaneous resistance to water-loss. These models were placed in species-specific diurnal retreat sites; providing profiles integrated over time of the thermal and hydric regimes of the microenvironments experienced by each species.

Microenvironmental conditions experienced by frogs differed markedly among species and seasons. Retreat sites of the most susceptible species, *L. nannotis*, were almost always within the thermal optimum and never above the thermal tolerance of *B. dendrobatidis*, while retreat sites of the least susceptible species, *L. lesueuri*, were commonly above the thermal optimum and thermal tolerance of *B. dendrobatidis*. Hydric conditions were most suitable for *B. dendrobatidis* growth at *L. nannotis* retreat sites.

Species-specific differences in behaviour are therefore likely to have large implications for the susceptibility of species to decline due to chytridiomycosis. This thesis provides the first empirical confirmation that species-specific differences in behaviour are likely to affect the susceptibility in nature of amphibians to chytridiomycosis. The behaviour of the species most susceptible to *B. dendrobatidis* related declines was the most favourable for the transmission, growth and development of *B. dendrobatidis,* while the behaviour of the species least susceptible to *B. dendrobatidis* related declines had the least favourable for its transmission, growth and development. Species-specific differences in the behaviour of frogs in the field may also explain why infected individuals of some species experience rapid mortality in the laboratory, yet are able to carry infections for extended periods in the field. Temporal and spatial variation in microenvironments available to and used by frogs may also explain variation in infection prevalence and host mortality. Information on amphibian behaviour and microenvironmental use may be useful in evaluating the susceptibility to declines caused by chytridiomycosis in species that presently occur in areas without *B. dendrobatidis.*

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CHAPTER ONE: GENERAL INTRODUCTION

In recent decades, a number of pathogenic infectious diseases that represent a substantial global threat to human health have emerged, including acquired immunodeficiency syndrome, severe acute respiratory syndrome, and avian influenza. In addition to those that are direct threats to human health, infectious diseases affecting wildlife and plant populations are emerging at unusually high rates and currently pose a great threat to global biodiversity (Harvell et al. 1999; Ward & Lafferty 2004). Species that are thought to have declined or been eliminated due to disease are taxonomically diverse and include island land birds (van Riper III et al. 1986; Warner 1968), grassland mammals (Ginsberg et al. 1995; Thorne & Williams 1988) snails (Daszak & Cunningham 1999), and rainforest frogs (Berger et al. 1998; Bosch et al. 2001; Bradley et al. 2002; Lips et al. 2006; Rachowicz et al. 2006; Weldon & Du Preez 2004). In addition to causing declines, pathogens may prevent the recovery of a species by persisting within the host population, or within environmental reservoirs or alternate hosts (Lafferty & Gerber 2002; Retallick 2002). Furthermore, if the disease itself does not eliminate the host species, reduced host abundance after a disease outbreak will increase susceptibility to extinction by other factors, such as random stochastic events (Lafferty & Gerber 2002).

In addition to causing the dramatic increases in host morbidity and mortality typical of disease outbreaks, pathogens are capable of affecting wildlife populations in more subtle ways. Pathogens can have detrimental effects of the survival and fecundity of host individuals and therefore of host populations (Tompkins & Begon 1999), and it has recently been shown that pathogens are actually capable of regulating wildlife populations (Hudson et al. 1998). Pathogens may also have large impacts on host population structure, altering population characteristics such as age structure (van Rensburg et al. 1987), and the outcome of interspecific interactions such as competition (Hudson & Greenman 1998; Parris & Cornelius 2004; Tompkins et al. 2003), or predation (Webber et al. 1987). Pathogens can also lower host reproductive output (Saumier et al. 1986) and alter host movement patterns (Anderson & Trewhella 1985; Steck & Wandeler 1980). In addition, the generally lower abundance and diversity of pathogens affecting introduced organisms may be responsible for their success outside of their natural range (Hatcher et al. 2006; Torchin & Mitchell 2004).

Pathogens may also change communities in a more indirect manner, with reductions in the populations of affected species drastically affecting the entire community. For instance, a reduction in rabbit abundance due to myxoma virus in Britain caused altered vegetation structure, increases in populations of voles and ants, and declines in stoats and buzzards (Ross 1982). Similarly, the dramatic declines in amphibian populations that have occurred at some sites are likely to cause long-term reductions in the abundance of predators that prey on amphibians, and alterations to primary production and algal community structure (Whiles et al. 2006).

Pathogens can also act as strong selective forces shaping the species richness and structure of entire communities, particularly when the pathogen is not host specific. By causing the mortality of a range of species, pathogens may convert species rich ecosystems into depauperate communities dominated by a few resistant species (Burdon 1991). This phenomenon has been observed in rainforest frogs (Lips et al. 2006), island birds (van Riper III et al. 1986; Warner 1968) and terrestrial flora (Burdon 1991; Weste et al. 2002). In each instance, there is a gradient in susceptibility to decline, with some species in the community experiencing severe population declines while other species decline less dramatically or not at all. One reason for this may be interspecific variation in host behaviour, and how it influences opportunities for transmission, and the progress and outcome of infection.

Transmission is often considered the driving force in the dynamics of infectious diseases (Begon et al. 2002), and the long-term spread and persistence of many diseases depends largely on the contact rate between susceptible hosts and infectious pathogens in a population (Swinton 1998). Traditional models of direct transmission have assumed that contact rate and hence transmission is directly proportional to host population size or density (Anderson & May 1979; Anderson & May 1981; Watanabe 1987). However, opportunities for transmission between individuals may be almost entirely independent of population size simply due to host behaviour (Ezenwa 2004; Loehle 1995; McCallum et al. 2001). For example, the formation of aggregations by host populations promotes contact between individuals, and in many host-pathogen systems it is positively correlated with both the prevalence and intensity of contact-transmitted parasites (Anderson & May 1979; Brown & Brown 1986; Coté & Poulin 1995; Ezenwa 2004; Hoogland 1979). Outbreaks of disease are also most commonly observed in aggregations of individuals (Vermeer 1969; Wobester et al. 1979). In contrast, predominantly solitary or non-social species will come into direct contact almost exclusively for reproduction, and it is likely that, for these species, the majority of direct transmission occurs at this time (Loehle 1995). Differences among species in opportunities for pathogen transmission may be particularly important in determining their relative susceptibilities to decline.

Disease dynamics may also be affected by species-specific movement patterns (Altizer et al. 2000; Ezenwa 2004) and habitat use (Brooks et al. 2006; Grutter 1998; Krasnov et al. 1998). As pathogens and parasites often accumulate in the hosts' environment over time, less mobile hosts tend to have higher contagious parasite burdens (Altizer et al. 2000; Ezenwa 2004). Additionally, as the survival of parasites and pathogens in the environment varies spatially with respect to habitat features, topography and microenvironmental conditions (Arthurs et al. 2001; Blanford & Thomas 2000; Carruthers & Haynes 1986; Dinnik & Dinnik 1961; Hochberg 1989; Levine 1963; Roland & Kaupp 1995), host habitat use will influence pathogen transmission rates among species (Holt et al. 2003). Opportunities for transmission are likely to be higher in host species that spend the greatest amount of time in habitats that are conducive to the survival of a pathogen (Burdon et al. 1989). As

a result, the abundance and species composition of parasites affecting host species differs between closely located habitats (Grutter 1998; Krasnov et al. 1998) and with frequency of contact with environmental reservoirs (Brooks et al. 2006). For example, platyhelminth parasites species richness in amphibian species is highly correlated with the amount of time the host spends in association with aquatic habitats (Brooks et al. 2006). The movement and migration of host organisms may also serve to disperse or contain pathogen spread (Watanabe 1987).

An understanding of the movement patterns and habitat use of a species can also be used to identify species at most risk of extinction in human-modified landscapes (Hanski & Zhang 1993; Travis & Dytham 1999), or under future climate scenarios, and is necessary for effective habitat protection. Species with high dispersal rates and broad habitat tolerances are likely to rapidly recolonise sites after local extinctions (Waldman & Tocher 1998). In contrast, species with low dispersal rates or narrow habitat tolerances may be unable to recolonise sites, are more likely to become isolated in suitable habitat patches surrounded by disturbed habitat, and experience little or no gene flow between populations (Bohonak 1999; Waldman & Tocher 1998). Protection from habitat modification and the maintenance of habitat connectivity is likely to be extremely important for the long-term conservation of species with low dispersal ability.

Host behaviour may also influence the outcome of infection. Once a host becomes infected with a pathogen, the development of disease is not guaranteed. Disease is the result not only of an interaction between a pathogen and a host, but also between the environment and the host-pathogen system. As a result, a pathogen will only cause disease in a host if environmental conditions are conducive to do so, causing the common phenomena of seasonally or climatically fluctuating disease prevalence in a population. Seasonal patterns of disease outbreaks have been particularly well documented in aquaculture species (Bruno et al. 1995; Enger et al. 1991) and phytopathogens of important crop species (Cowling & Gilchrist 1982). In particular, temperature can strongly affect the occurrence and development of many diseases (Colhoun 1973) and especially in non-endothermic hosts, may actually determine the outcome of infection (Blanford & Thomas 1999a; Blanford & Thomas 1999b; Blanford & Thomas 2000; Blanford et al. 1998; Carruthers et al. 1992; Inglis et al. 1996; Woodhams et al. 2003). Other environmental factors such as humidity and insolation may also be important, especially in infections by fungal pathogens (Benz 1987). For example, low humidity may completely prevent fungal pathogens from infecting potential hosts, regardless of how many spores are in contact with a potential host (Hajek & St Leger 1994).

A further indication of the importance of the environment on host-pathogen interactions is the disparity between host mortality in laboratory versus in field experiments. Laboratory studies examining the effect of specific pathogens on their hosts often result in the rapid mortality of all host organisms, but when similar experiments are conducted in the field, results tend to be much more variable and the pathogen is often unsuccessful in causing host mortality or even morbidity

(ie. Blanford & Thomas 1999a; Blanford & Thomas 1999b; Inglis et al. 1996; Johnson & Goettel 1993; Lisanksy 1997; Lobo Lima et al. 1992). For example, after the application of entomopathogens to control grasshoppers in the field, no reduction in host population was observed, despite substantial mycosis of grasshoppers in greenhouse cages (Inglis et al. 1996). The application of the same entomopathogens on field populations of grasshoppers at other times and at other sites has yielded extremely variable results (Inglis et al. 1996).

While correlations can be made with disease susceptibility and macro-scale environmental conditions such as mean ambient temperature, such broad scale measurements of the environment are unlikely to reflect the true environment of host-pathogen interactions (Thomas & Blanford 2003). Although ectothermic body temperature is related to environmental temperature, its exact relationship reflects a complex interplay between the behaviour, physiology, morphology, ambient conditions and microenvironment of an animal (Carey 1978). As a result, the actual microenvironments experienced by hosts and their pathogens may differ greatly from macroclimatic conditions (Brattstrom 1963). For instance, basking behaviour in the frog *Rana mucosa* caused an average of 14.4ºC elevation of body temperature compared to frogs in the shade (Bradford 1984). Consequently, body temperatures in ectotherms may exceed the upper limits for pathogen survival, even when ambient temperatures do not (Carruthers et al. 1992), and behavioural thermoregulation, or simply microhabitat selection, may control or eliminate infection. Even relatively small differences in temperature may be critical in determining disease susceptibility, for example an increase of just 2ºC in the diurnal maximum temperature experienced by the variegated grasshopper *Zonocerus variegates* leads to recovery from a fungal disease that otherwise causes high mortality rates (Blanford et al. 2000). In addition, many species of ectotherm exhibit a fever response following infection, whereby thermoregulatory behaviour rather than physiology is modified to enable hosts to attain a new preferred body temperature (Thomas & Blanford 2003). Behavioural fever has been shown to increase host survival in a number of host-pathogen associations, including those with reptile (Kluger et al. 1975), fish (Covert & Reynolds 1977) and insect hosts (Adamo 1998; Bronstein & Conner 1984; Inglis et al. 1996; Karban 1998)

Species-specific differences in the susceptibility of organisms to disease may be at least partially explained by species-specific differences in both normal (non-infected) thermoregulatory behaviour and the ability to display behavioural fever in response to infection. Despite the potential importance of host behaviour, there is little known of the effects of host behaviour on susceptibility to disease in wildlife populations.

Chytridiomycosis

Perhaps the most alarming wildlife disease known at present is chytridiomycosis, a potentially fatal disease of amphibians caused by the pathogen *Batrachochytrium dendrobatidis* (Berger et al. 1998;

Lips et al. 2003a). Chytridiomycosis has been implicated in mass mortalities, population declines, and extinctions of amphibian populations and species around the world (Berger et al. 1998; Bosch et al. 2001; Bradley et al. 2002; Lips 1999; Lips et al. 2006; Longcore et al. 1999; Pessier et al. 1999; Rachowicz et al. 2006; Weldon & Du Preez 2004). The pathogen spreads within and among individual amphibians via the release of motile, waterborne zoospores (Nichols et al. 2001).

In many cases, frog population declines attributed to *B. dendrobatidis* have been dramatic, resulting in the rapid extinction of 50% or more of the anuran species at particular sites (Lips 1999; Lips et al. 2006) and large reductions in the abundance of remaining species (Lips et al. 2006; Lips & Donnelly 2002). However, in almost all cases, amphibian species that have disappeared or declined due to chytridiomycosis coexist with non-declining species (Lips et al. 2006; Lips & Donnelly 2002; Puschendorf et al. 2006; Retallick et al. 2004). Many of these non-declining species are highly susceptible *B. dendrobatidis* in the laboratory, but are able to persist with infections in the wild (Berger et al. 1999; McDonald & Alford 1999; Woodhams et al. 2003). In addition, infected individuals of a wide range of other amphibian species may survive for years in the wild with no clinical signs of disease (Hanselmann et al. 2004; Hopkins & Channing 2003; Kriger & Hero 2006; McDonald et al. 2005; Retallick et al. 2004). Differences in the susceptibility of amphibian species in the field must, therefore, not be entirely due to innate properties of the species that confer resistance to the disease, but due to some external factor or factors that are present in the field but absent from laboratory experiments.

Interspecific variation in behaviour may explain why some frog species decline when other, co-occurring species do not. More susceptible species may simply behave in a manner favourable to the transmission, growth and survival of *B. dendrobatidis,* while the least susceptible species may behave in a manner least conducive to *B. dendrobatidis* transmission, growth and survival.

Behaviour may affect the probablity of a species coming into contact with *B. dendrobatidis.* Transmission rates are therefore likely to vary among species, and frog species that do not appear to be susceptible to chytridiomycosis related declines in the wild may simply not be coming into contact with *B. dendrobatidis* zoospores. Differences among species in opportunities for the transmission of a pathogen may be particularly important in determining their relative susceptibilities to decline. Transmission rates in the field are likely to vary among species according the frequency of behaviours such as physical contact between frogs or with environmental reservoirs, which are likely to be high-risk in terms of *B. dendrobatidis* transmission. There is presently little information on the extent of physical contact between frog species, particularly with respect to contact away from breeding sites.

Disease dynamics in other host pathogen systems are highly influenced by species-specific movement patterns (Altizer et al. 2000; Ezenwa 2004) and habitat use (Brooks et al. 2006; Grutter 1998; Krasnov et al. 1998), and these factors may be particularly important in determining the

susceptibility a species to decline (Bohonak 1999; Waldman & Tocher 1998). The same trends should hold true for amphibians infected with *B. dendrobatidis*, however we currently have a limited understanding of the movement and habitat use of amphibians. To date, our knowledge of these factors has been derived almost exclusively from studies on salamanders and on pond-breeding frogs in temperate regions. However, declines have been most frequent and severe in tropical, stream-breeding species (Lips et al. 2003b; Stuart et al. 2004; Williams & Hero 1998). The movements of tropical stream-breeding frogs are likely to differ from temperate, pond-breeding species, with reproduction occurring within their non-breeding home range (Duellman & Trueb 1986). The limited information we have on the ecology and behaviour of these species is derived primarily from nocturnal stream surveys (ie. Richards & Alford 2005), and there is little information on the diurnal habits, movement, and use of off-stream habitat of the vast majority of tropical species. This lack of information is largely due to the complex, densely vegetated habitats and the cryptic appearance and habits of the majority of tropical, stream-breeding amphibians.

Host behaviour may also influence the outcome of chytridiomycosis, and species-specific differences in the microenvironments selected by amphibian species may be a major reason why chytridiomycosis affects some species more than others. In particular, the thermal and hydric environments chosen by an amphibian may have a large influence on disease development. The growth and survival of *B. dendrobatidis* is highly influenced by temperature in culture (Longcore et al. 1999; Piotrowski et al. 2004), and the progress and outcome of chytridiomycosis in infected amphibians in the laboratory is influenced by thermal conditions (Berger et al. 2004; Carey et al. 2006; Woodhams et al. 2003). Elevated body temperatures are also capable of clearing frogs of infection (Woodhams et al. 2003). Hydric conditions are also important for *B. dendrobatidis* in culture (Berger 2001; Johnson et al. 2003), and *in vivo*, with disease progression in the laboratory is more rapid in constant saturated humidity or mist than in constant rain or in dry air (Alford and Woodhams, unpublished data). At present little is known of the microenvironmental conditions selected by declining and non-declining frog species, particularly away from nocturnal breeding sites.

The general aim of this study was, therefore, to study the potential affects of host behaviour on the susceptibility of different host species to decline due to the disease chytridiomycosis. In order to achieve this, I tracked three species of stream-breeding frogs in northern Queensland, Australia. These species co-occur but have declined to varying degrees in recent decades; the waterfall frog *Litoria nannotis,* which has experienced large and long-lasting population declines (IUCN Endangered), the green-eyed tree frog *Litoria genimaculata,* which declined and then recovered (IUCN Least Concern, however Australian populations of this species are considered to be Near Threatened) and the stoney creek frog *Litoria lesueuri* which has not experienced population declines (IUCN Least Concern). Specifically, my aims were to determine if the susceptibility of amphibian species to chytridiomycosis-associated declines is related to:

- Opportunities for the transmission of *B. dendrobatidis*
- Movement patterns and habitat use
- Microenvironment selection

In order to accomplish these goals, I first set out to compare the effectiveness of two different methods of tracking frogs: radio-telemetry and harmonic direction finding, and develop a method of characterizing the thermal and hydric conditions of amphibians in the field using models.

This thesis is constructed as a series of stand-alone, but interrelated manuscripts. CHAPTER TWO and CHAPTER THREE develop and assess the techniques used in the study; CHAPTER TWO compares the use of the two different tracking techniques, radio telemetry and harmonic direction finding, for the study of rainforest frogs in a complex, three-dimensional environment. CHAPTER THREE examines a novel technique for determining the microenvironment of frogs with variable cutaneous resistance to water-loss. The remaining chapters investigate interspecific variation in host behaviour and how this may influence disease susceptibility. CHAPTER FOUR examines the behaviour of these species in terms of opportunities for transmission of *B. dendrobatidis*. CHAPTER FIVE examines the role of retreat sites in the transmission of *B. dendrobatidis*. CHAPTER SIX details the movement patterns and habitat use of the species. CHAPTER SEVEN examines the microenvironmental profiles of the species in terms of implications for disease development. CHAPTER EIGHT summarises my findings, outlines conservation implications and recommends future research directions.

CHAPTER TWO: TECHNIQUES FOR TRACKING AMPHIBIANS: HARMONIC DIRECTION FINDING VERSUS RADIO TELEMETRY*

* Modified version of this chapter: Rowley, J. J. L. and Alford, R. A. (In press) Techniques for tracking amphibians: The effects of tag attachment, and harmonic direction finding versus radio telemetry. *Amphibia-Reptilia*

Abstract

To gain information on the microhabitat use, home range and movement of a species, it is often necessary to remotely track individuals in the field. Radio telemetry is commonly used to track amphibians, but can only be used on relatively large individuals. Harmonic direction finding can be used to track smaller animals, but its effectiveness has not been fully evaluated. I compared harmonic direction finding and radio-telemetry using data collected in the field. I fitted rainforest stream frogs of three species with tags of either type, located them diurnally and nocturnally for approximately two weeks, and compared movement parameters between techniques. In the field, I obtained fewer fixes on frogs using harmonic direction finding, but measures of movement and habitat use did not differ significantly between techniques. Because radio telemetry makes it possible to locate animals more consistently, it should be preferred for animals large enough to carry radio tags. If harmonic direction finding is necessary, it can produce reliable data, particularly for relatively sedentary species.

Introduction

Information on the spatial behaviour and movement patterns of individuals is necessary for a complete understanding of species' ecology. For most amphibian species, patterns of movement and habitat use are poorly known, especially in habitat away from breeding sites. One of the most valuable techniques for gaining information on the microhabitat use, home range and movement of a species is remotely tracking individuals in the field.

The most commonly used method to track amphibians is radio telemetry, but this technique suffers two major drawbacks. First, while the size and weight of radio transmitters have decreased considerably over time, they are still too heavy to attach to small species or individuals. The lightest commercially available transmitters weigh approximately 0.7 g when packaged and ready for attachment. If the rule that animals should carry no more than 10% of their body mass (Richards et al. 1994) is followed, the smallest frog that can be radio tracked will weigh 7 g. Other authors have suggested a more conservative 5% limit (Kenward 1987; White & Garrott 1990), which would preclude tracking animals weighing less than 14 g. Second, battery lifetime limits the duration of tracking; this is particularly true for the smallest transmitters (ie. <1g), in which batteries last for several weeks at most.

Another method for remotely tracking small animals is the harmonic direction finder. The harmonic direction finder is a hand-held device that emits a continuous, unmodulated signal that nonlinear conductors, such as diodes, reflect at twice the original wavelength. The harmonic

direction finder detects the reflected signal and transforms it to an audible signal. Therefore, if a harmonic tag is attached to a study animal, its location can be determined using a similar technique to that used in radio telemetry. To date, harmonic direction finding has been used on carabid beetles (Hockmann et al. 1989; Lövei et al. 1997; Mascanzoni & Wallin 1986; Wallin & Ekbom 1988), snails (Janßen & Plachter 1998; Lövei et al. 1997; Stringer & Montefiore 2000; Stringer et al. 2004), insects (Roland et al. 1996; Williams et al. 2004), snakes (Engelstoft et al. 1999; Webb & Shine 1997), juvenile toads (Leskovar & Sinsch 2002; Leskovar & Sinsch 2005) and small hylid frogs (Pellet et al. 2006).

Harmonic direction finding has three main advantages over radio telemetry. First, because the harmonic tag needs no battery, it can be extremely lightweight (Riley et al. 1998) and can be used on animals as small as bees and moths (Roland et al. 1996). Second, it has relatively long functional life because it does not rely on a battery for power. Tags can therefore function for as long as they remain attached to the animal. Third, harmonic tags can be made extremely inexpensively (ie. for several dollars versus several hundreds of dollars; Langkilde & Alford 2002), which is useful when there is a high rate of tag loss. There are, however, several disadvantages to the technique. Perhaps the most obvious of these is that the reflected signal from all tags is the same, so that individual tagged animals must be identified in another way once they are found. This is a particular problem when frogs are detected using the harmonic direction finder, but cannot be visually located. Further, detection range is typically much shorter than for radio telemetry (ie. tens of meters versus hundreds of meters; Langkilde & Alford 2002). This may be especially true in field sites that are rugged or densely vegetated, because rocky terrain can reflect either or both of the signals emitted by the detector or the tags, and vegetation absorbs radio waves at the frequencies used (Sizun 2005).

My study aimed to compare radio telemetry and harmonic direction finding in terms of both proportion of fixes obtained and measures of movement and habitat use by collecting data in the field using both techniques on different individuals of three species of rainforest stream frogs.

Methods

The study was conducted at five sites, all in tropical rainforests in northern Queensland, Australia: Birthday Creek, Paluma State Forest (146º10'02" E 18º58'54" S), Python Creek (145º35'E 17º46'S), an unnamed creek ("lower Tully", 145º41'E 17º48'S) in Tully Falls Forest Reserve, an unnamed creek in Kirrama State Forest (145º52 E 18º11 S; 'Kirrama') and Frenchman Creek, Wooroonooran National Park (145º55' E 17º20' S). All sites are relatively undisturbed rainforest streams. The creek beds are composed of rocks, ranging from small pebbles to large boulders (of over 10 m in diameter). All streams contained pools and riffles, and in addition, most sites contained a number of small (approximately 1-3 m high) waterfalls. Two surveys, one in the cool/dry season (May-September) and one in the warm/wet season (October-April), were carried

out at each site except Birthday Creek, where only a warm/wet season survey was performed. Surveys lasted approximately 16 days.

During surveys, frogs of three species were tracked: the Stoney Creek Frog *Litoria lesueuri* (see below), the Green Eyed Tree Frog *Litoria genimaculata,* and the Waterfall Frog *Litoria nannotis*. All three species are large to medium sized hylid frogs (tracked frogs ranged from 42 to 64 mm snout-vent-length and 6 to 29 g in weight), and exhibit sexual size dimorphism, with females larger then males. Recently, the taxonomy of the *L. lesueuri* group has been revised (Donnellan & Mahony 2004). Two species, *L. jungguy* and *L. wilcoxii*, occur in sympatry in the study sites, hybridise and are indistinguishable on the basis of morphology (Donnellan & Mahony 2004). Population declines have not been observed in the region for either species (McDonald & Alford 1999; McDonald et al. 2005). I therefore continue to refer to the study population as *L. lesueuri* while recognising that the population contains two morphologically indistinguishable species**.** Frogs were tracked using radio telemetry or harmonic direction finding. Only frogs weighing more than 11g were tracked via radio telemetry. Radio transmitters were models BD-2N and BD-2NT, (Holohil Systems Ltd., Ontario, Canada); they weighed approximately 0.67 g including harness, and had a battery life of approximately 3 weeks. The harness was a waistband made of silicone rubber capillary tubing, with an outside diameter of 1 mm. Upon attachment, the tubing was trimmed so that it encircled the waist of the frog, and a fine cotton thread was passed through the tubing and tied to secure the package to the frog, with excess thread then trimmed. This attachment approach minimizes restrictions on frog movement, and does not produce localized irritation in frogs when fitted correctly. If the frog cannot be relocated at the end of the study, the cotton thread will eventually break, allowing the package to fall off. Frogs that were too small to be radio tracked, and a number of larger frogs, were tracked using harmonic direction finding. Harmonic tags were constructed in the same manner as in the laboratory study, but were attached using silicone waistbands. The assembly weighed approximately 0.27 g. Antennas were painted with two bands of colour to allow individual identification when I could visually locate frogs. Both types of tracking devices were fitted in situ and frogs were released at their points of capture after less than five minutes. Frogs wearing either tracking device did not carry harnesses and tags that weighed more than 6% of their total body weight.

Frogs fitted with radio transmitters were tracked using Telonics TR-4 Tracking Receivers (warm/wet season 2004 only) or Habit Research HR2500 Osprey VHF Receivers, with a three-element folding Yagi antennae (A.F. Antronics, White Heath, Illinois, USA). Frogs fitted with harmonic tags were tracked using a RECCO R5 transmitter-receiver (Recco Rescue Systems, Lidingö, Sweden). This system consists of a hand-held aerial and associated electronics that act as both the transmitter and receiver. Maximum detection range for the harmonic tags was 40 m (Langkilde & Alford 2002).

I attempted to determine the location of each frog once during each day (0700-1900) and once each night (1900-0700) during each survey period. When using radio telemetry, I began tracking at the location where each frog was last found. If no signal could be detected at this point, I walked 300-500 m in each direction away from that site, trying to reach elevated positions for maximum detection range. If no signal could be picked up after this (15-45 mins depending on terrain and vegetation type), I began searching for the next frog. When using harmonic direction finding, I walked slowly in parallel lines up and down each side of the stream, and approximately 10 m into the forest on each side of the stream, moving the harmonic direction finding machine at all times, making sure to cover all directions (maximising my chances of detecting frogs located both in the stream and in the canopy). For any frogs I had not yet found (after approximately 1-3 h), I then searched at and around (20m radius) the location where it was last found. If no signal could be picked up after this (5-10 mins), I moved on to searching for the next frog. There were a number of instances where I detected a frog using harmonic direction finding but could not visually locate and therefore identify the individual (ie. when the frog was in the canopy). On these instances, it was often possible to tentatively identify the individual by making the assumption that, of the frogs not located during that survey, it was the individual previously located nearest to this location. I confirmed all such tentative identifications via later sightings. In the process of searching for individual frogs, I never noticeably disturbed other tracked frogs, primarily due to the large size of each study site and scattered spatial distribution of most individuals. Although search effort was highly variable from day to day, I spent approximately the same total number of person-hours using each technique during each survey period.

When frogs were located using either technique, I recorded their location as distance along the stream (m), horizontal distance from the stream (m), and height above stream (m), allowing me to calculate their distance moved per day. I used these variables because they are important aspects of the habitat use of amphibians, and they can be compared to determine whether they are differentially biased by the tracking technique used. I excluded data from the night following tag attachment due to the potential short-term behavioural effects of handling (Langkilde & Alford 2002). To avoid pseudoreplication and biasing my results to frogs that were located more often, I used individuals as replicates and analysed means or proportions for each animal. Due to well-documented differences in behaviour between sexes (eg. Bellis 1965; Dole & Durant 1974; Miaud et al. 2000), and my bias towards tracking males via harmonic direction finding (due to their smaller body size), I performed Mann Whitney tests separately for each sex and species combination. I also examined whether frog body weight differed with tracking device for each sex and species combination. I used Bonferroni adjustments to control Type I error rates. Boxplots were used to visualise the results. In boxplots, bold horizontal lines represent the median, boxes indicate the locations of upper and lower quartiles, bars encompass values up to 1.5 interquartile

range, open circles represent values more than 1.5 interquartile ranges from the nearest quartile and stars indicate data values more than 3 inter-quartile ranges from the nearest quartile.

Results

In total, 117 frogs were tracked during the study (Table 2.1). Although a greater number of males and other smaller individuals were tracked via harmonic direction finding, there was no significant difference in the weight of frogs tracked via either method when each species and sex category was considered separately (Table 2.1). The proportion of attempts upon which fixes were obtained differed significantly between tracking devices for some classes of individuals. I obtained fewer fixes using harmonic direction finding for *L. lesueuri* and *L. nannotis* females, but not for *L. genimaculata* or *L. nannotis* males (Table 2.1, Figure 2.1). Overall, while frogs tracked using radio telemetry were found, on average, on 97.4% of attempts, frogs tracked via harmonic direction finding were located on only 66.8% of attempts. This varied among species; *L. genimaculata* were found 85.4% of the time using harmonic direction finding, compared to 58.2% and 57.7% of the time for *L. lesueuri* and *L. nannotis* respectively. In contrast, the proportion of fixes for each species during radio telemetry varied less, between 95.2 and 99.5%. Compared to radio telemetry, harmonic direction finding typically resulted in a lower average distance moved per day, lower average height above the stream and lower average horizontal distance from the stream for the majority of species and sex combinations, however these differences were not significant (Table 2.1, Figs. 2.2-2.4).

Table 2.1. Mann Whitney tests for differences between harmonic direction finding and radio telemetry in proportions of fixes obtained, average distance moved per day (m), average height above the stream (m) and average horizontal distance from the stream (m). * indicates significance at the Bonferroni-adjusted 0.05 level.

		Number of individuals with each tag type		Tests for difference between radio telemetry and harmonic direction finding in:									
				Frog body weight (g)		Proportion of fixes		Average distance moved per day (m)		Average height above stream (m)		Average horizontal distance from stream (m)	
Species	**Sex**	**Radio telemetry**	**Harmonic direction finding**	**Mann-Whitney *Z***	***P***	**Mann-Whitney *Z***	***P***	**Mann-Whitney *Z***	***P***	**Mann-Whitney *Z***	***P***	**Mann-Whitney *Z***	***P***
L. genimaculata	Female	9	3	-0.463	0.710	-2.97	0.09	-1.202	0.282	-1.017	0.373	-0.832	0.482
	Male	0	15	--	--	--	--	--	--	-	-		-
L. lesueuri	Female	24	6	-0.318	0.765	-3.308	0.002*	-0.985	0.347	-1.192	0.251	-1.867	0.065
	Male	7	25	-2.209	0.025	-3.711	<0.001*	-2.484	0.011	-2.302	0.020	-1.277	0.207
L. nannotis	Female	19	3	-1.128	0.301	-2.795	0.001*	-1.961	0.053	-0.526	0.651	-0.861	0.408
	Male	2	4	-1.97	0.440	-1.174	0.267	-0.926	0.533	-0.939	0.533	-0.939	0.533

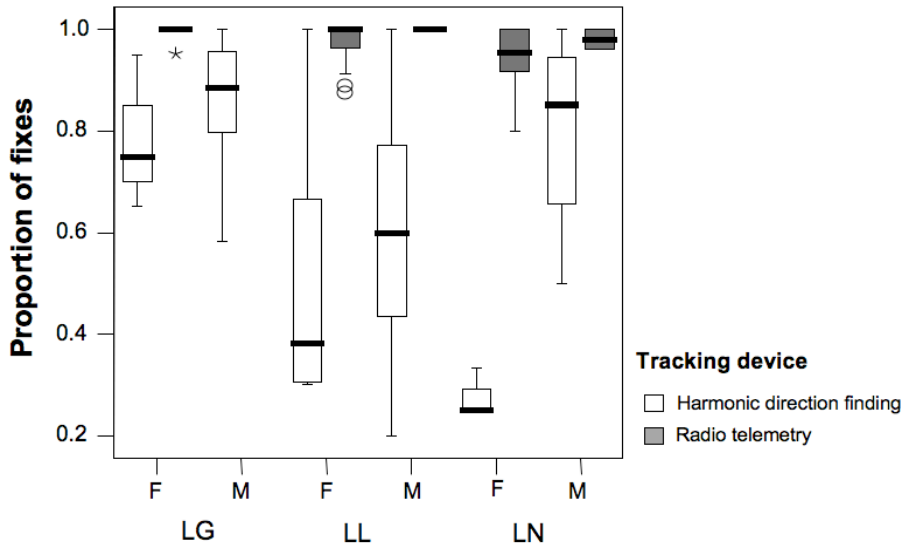

Figure 2.1. Mean proportion of fixes obtained (number of fixes divided by total possible number of fixes for each individual) for *L. genimaculata* (LG), *L. lesueuri* and *L. nannotis* (LN) tracked using harmonic direction finding and radio telemetry.

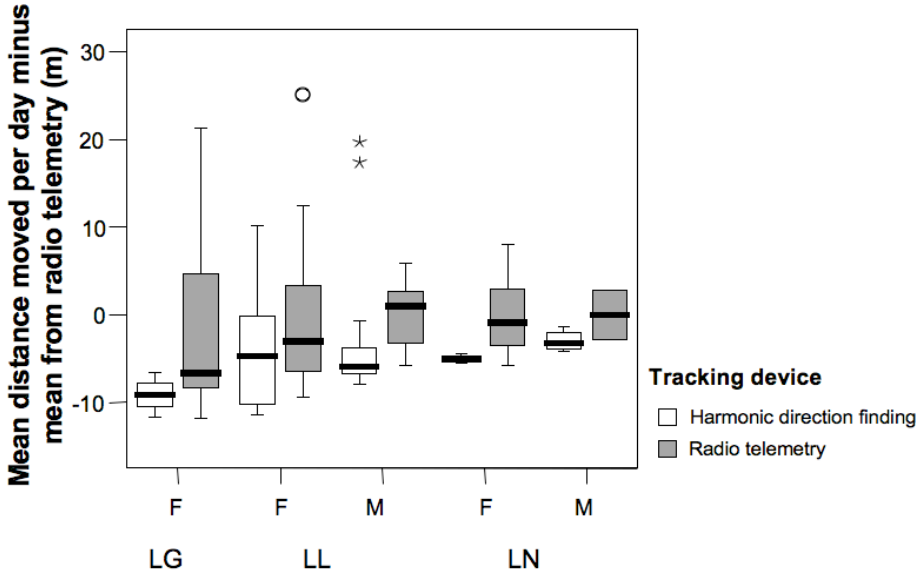

Figure 2.2. Mean distance moved per day for female (F) and male (M) *L. genimaculata* (LG), *L. lesueuri* and *L. nannotis* (LN) tracked using harmonic direction finding and radio telemetry. In order to illustrate differences between techniques, data for each individual were adjusted by subtracting the mean for that species and sex combination obtained via radio telemetry.

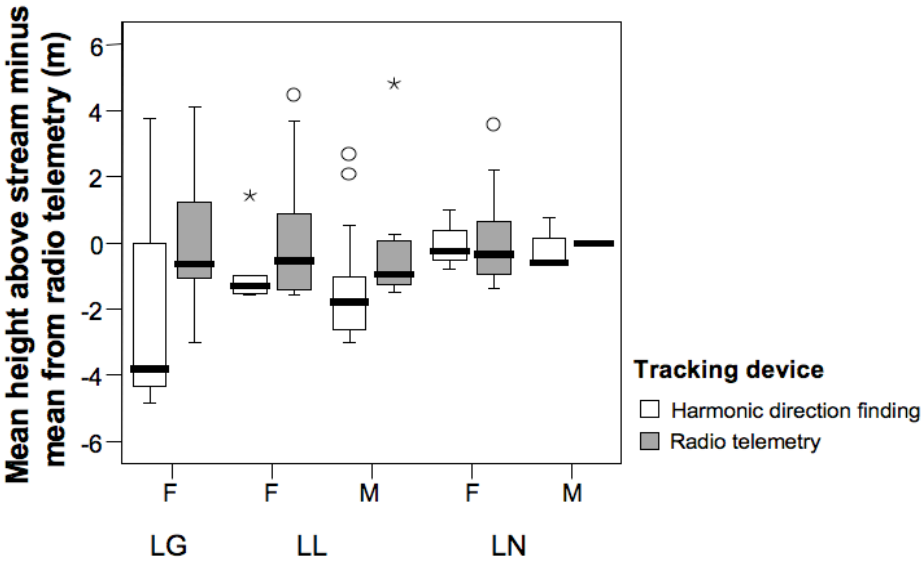

Figure 2.3. Mean height above stream (m) for female (F) and male (M) *L. genimaculata* (LG), *L. lesueuri* and *L. nannotis* (LN) tracked using harmonic direction finding and radio telemetry. In order to illustrate differences between techniques, data for each individual were adjusted by subtracting the mean for that species and sex combination obtained via radio telemetry.

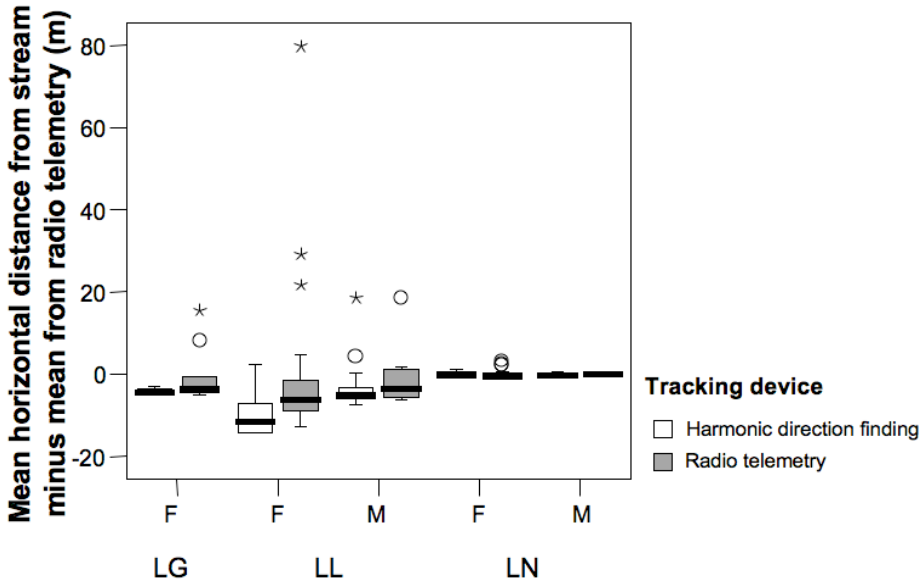

Figure 2.4. Mean horizontal distance from stream for female (F) and male (M) *L. genimaculata* (LG), *L. lesueuri* and *L. nannotis* (LN) tracked using harmonic direction finding and radio telemetry. In order to illustrate differences between techniques, data for each individual were adjusted by subtracting the mean for that species and sex combination obtained via radio telemetry.

Discussion

This study indicates that when harmonic direction finding is used, it is important to consider the limitations of the method. Its principal limitation is that it often produces a relatively low number of fixes for each animal. The proportions of fixes I obtained are comparable to those found in other studies using harmonic direction finding (Leskovar & Sinsch 2005; Webb & Shine 1997; Williams et al. 2004). Although my study revealed no significant effect of tracking device on the average distance moved by frogs, data obtained using harmonic direction finding may be biased towards animals that move relatively short distances, since animals that move large distances often cannot be located (eg., Leskovar & Sinsch 2002; Leskovar & Sinsch 2005; Webb & Shine 1997; Williams et al. 2004). It also may be biased against obtaining fixes in highly concealed retreat sites, since its signal strength is greatly reduced by thick vegetation and rocks. As I spent approximately the same amount of person-hours using each technique, harmonic direction finding was typically much less efficient than radio telemetry. Although it is theoretically possible to intensively search large areas using this technique, factors such as difficult terrain and time constrains often make this impractical.

As in several other studies, I found that in the field, detection range with harmonic direction finding was considerably lower than that reported in optimal (flat, open) study sites (Engelstoft et al. 1999). In fact, while the maximum detection distance for the diodes used in this study is approximately 40 m (Langkilde & Alford 2002), in the field, the maximum detection distance recorded was 12 m, and this was with direct line of sight between the harmonic direction finder and the frog, which was located in the canopy. Detection distance was often as low as 0.5 m when frogs were under rocks within the stream bank, thereby making it necessary to scan around every boulder and in every crevice in order to locate an animal. Therefore, while the distance between parallel scanning paths when using harmonic direction finding was recommended to be 4-8 m for toads in relatively open habitat (Leskovar & Sinsch 2005), the results of this study indicate that in densely vegetated or rocky environments, the distance between parallel scan paths may need to be reduced to as little as 1-2 m. This makes harmonic direction finding a laborious and time consuming activity in such environments, particularly because frogs occur in complex three-dimensional environments, requiring scanning under rocks and up trees.

That tagged animals must be identified in another way once they are found using harmonic direction finding was a problem with species that spent time in inaccessible places such as under boulders or in the canopy. While I was often able to identify the individual by a process of elimination and the location of individuals on previous and subsequent surveys, in studies with longer time intervals between locating frogs, this may not be possible.

This study indicates that realistic and useful information on the behaviour and microhabitat selection of several species of frogs can be obtained using either harmonic direction finding or radio telemetry. It also suggests that the choice of tracking device should depend on both the behaviour of the study species and the nature of the terrain. Harmonic direction finding is more suitable for animals that are relatively sedentary, or have small or otherwise well-defined home ranges. In my study, the type of tracking device did not affect the proportion of fixes obtained for *L. genimaculata*, a species which tends to remain close to the stream (Chapter 6), but had a large impact on the proportion of fixes obtained for the more mobile *L. lesueuri.* The rocky nature of my study sites and the tendency of *L. nannotis* to shelter under large boulders meant that fixes often could not be obtained for this species using harmonic direction finding. The choice of tracking technique should also be influenced by study duration, with the long functional life of harmonic tags making harmonic direction finding more appealing in long-term studies. Although radio telemetry was a more reliable and accurate method of obtaining information on the spatial ecology of my study species, for species that are too small to track using radio telemetry, harmonic direction finding may often be a viable method for obtaining detailed information on their spatial ecology.

CHAPTER THREE: CHARACTERIZING OPERATIVE ENVIRONMENTAL TEMPERATURE IN AMPHIBIANS WITH VARIABLE RATES OF EVAPORATIVE WATER LOSS

Abstract

In ectotherms, body temperature directly influences rates of physiological processes, thereby affecting many ecological interactions. However, body temperatures of terrestrial ectotherms can differ considerably from environmental temperature. Therefore, to understand the biology, ecology, and species interactions of terrestrial ectotherms it is necessary to understand how microenvironmental conditions and behaviour affect their body temperatures in the field. The ranges of body temperatures available to ectotherms with variable rates of water loss, such as amphibians, have been difficult to characterize. I have developed and tested a method for using physical models to measure the range of body temperatures available to amphibians with any degree of cutaneous resistance to water-loss under a variety of environmental conditions. In a laboratory environment, physical models produced an accurate outline of the thermal envelope in which the body temperatures of thermally equilibrated frogs fell. In the field, physical models placed in species-specific microhabitats accurately estimated the ranges of diurnal body temperatures of frogs. My method therefore makes it possible to characterize the effects on body temperature of the microenvironmental conditions available to frogs with levels of detail similar to those produced by the techniques usually used for reptiles. This knowledge is likely to be increasingly useful in understanding and predicting the effects of habitat change or global warming on amphibians. It is also likely to be of use in developing more sophisticated models of energy use and growth within individuals, and thus of population dynamics, and in understanding the factors affecting interspecific interactions, including host-pathogen interactions, in nature.

Introduction

In ectothermic organisms, variation in body temperature directly affects factors such as rates of energy acquisition, growth and reproduction (Bennett et al. 1992). Temperature also exerts strong influence on ecological interactions such as predator-prey and host-pathogen interactions. Temperature changes can completely reverse the outcome of such interactions at both the individual and population levels (Elliot et al. 2002; Inglis et al. 1996; Sanford 1999; Thompson 1978; Woodhams et al. 2003). In many host-pathogen systems, temperature is one of the most important factors influencing the occurrence and development of disease (Colhoun 1973), and small and realistic changes in temperature can dramatically alter both pathogen virulence and host susceptibility (Blanford et al. 2000).

Recent declines in amphibian populations around the world have been attributed to the epidemic disease chytridiomycosis (Berger et al. 1998; Lips et al. 2006). Correlations between the timing of amphibian declines and macroclimatic conditions have suggested that small changes in thermoregulatory opportunities available to rainforest frogs may have contributed to their widespread declines in association with the disease (Pounds et al. 2006). Laboratory experiments have supported the temperature-dependent nature of chytridiomycosis, showing that elevated body temperatures can cure amphibians of the disease (Woodhams et al. 2003). Global warming is also likely to have a large impact on many species, with macroenvironmental modelling suggesting that the distributions of many ectothermic species will be dramatically altered by even small amounts of global warming (Thomas et al. 2004; Williams et al. 2003). However, our ability to extrapolate from such large-scale correlations or from experiments performed under laboratory conditions is limited due our poor understanding of amphibian body temperatures under field conditions, and how these vary over time and space.

Although the body temperature of terrestrial ectotherms is broadly correlated with environmental temperature, the actual body temperatures of many species can differ considerably from macroenvironmental temperatures due to species-specific differences in behaviour, physiology, morphology and microenvironment use. As a result, ectotherms exposed to identical macroenvironmental conditions can experience vastly different body temperatures (Helmuth 2002). The relevance of macroenvironmental temperature to the body temperatures of terrestrial ectotherms is therefore highly questionable (Bryant et al. 2002; Kennedy 1997), making it imperative to understand how microenvironmental conditions are experienced by ectotherms in the field and how they affect body temperature.

The thermal environment of ectotherms is often characterized using physical models. Physical models mimic the thermal properties of a particular animal in steady-state, thereby producing an approximation of body temperature, which is often referred to as the operative environmental temperature (Bakken 1976; Bakken & Gates 1975). Relatively simple physical models made of substances such as hollow copper tubes can be used to mimic the thermal properties of many reptiles (Anderson et al. 2005; Blouin-Demers & Weatherhead 2001; Shine & Kearney 2001), and their use has led to a detailed understanding of many aspects of reptile thermal biology (e.g., Bakken 1989; Bakken 1992; Beaupre 1995; Kearney & Predavec 2000; O'Connor 1999; Peterson 1987).

While such models may be suitable for species with low and constant rates of evaporative water loss (EWL), many species, including some reptiles (e.g., Krakauer et al. 1968) and many amphibians (Shoemaker & Nagy 1977) have relatively permeable skin, resulting in high and variable EWL and concomitant evaporative cooling. The degree of evaporative water loss varies considerably within and between species (Buttemer et al. 1996;

Shoemaker & Nagy 1977; Wygoda 1984). In addition, individuals of many species are able to adjust their rates of water loss over relatively short periods of time (Barbeau & Lillywhite 2005; Withers 1995; Withers et al. 1982). This variability complicates the design of physical models for such animals and the interpretation of data collected using them.

As a consequence of variable EWL, the use of physical models to investigate amphibian thermal biology has been much more limited than in reptiles. To allow EWL to occur, physical models of amphibians for use in the field have been constructed as hollow copper tubes covered in wet fabric (Bartelt & Peterson 2005; Bradford 1984), periodically re-wetted plaster (O'Connor & Tracy 1987), dead or immobilised amphibians (Seebacher & Alford 2002; Wygoda 1989a; Wygoda 1989b), or agar casts of animals (Navas & Araujo 2000; Schwarzkopf & Alford 1996). However, all of these physical models lose water as free water surfaces, and they can therefore only be used to model the body temperatures of amphibians with zero cutaneous resistance to water loss. Although this may be true of many amphibians (Buttemer 1990), an increasing number of anurans, particularly arboreal frogs, have higher levels of cutaneous resistance to water loss (Buttemer et al. 1996; Wygoda 1984). This higher resistance is caused by physical barriers of dermal iridophores, or by properties of mucus or lipid secretions (Christian & Parry 1997; McClanahan & Shoemaker 1987; Shoemaker & McClanahan 1975). In such species, cutaneous resistance to EWL may at times be over 100 times greater than that of 'typical' ranid and bufonid frogs (Buttemer 1990; Buttemer & Thomas 2003), with the skin of some frogs being as resistant to water loss as that of terrestrial reptiles (McClanahan & Shoemaker 1987; Shoemaker et al. 1987). In many species, levels of skin resistance are variable, depending on the current physiological state and behaviour of the individual (Barbeau & Lillywhite 2005; Withers 1995; Withers et al. 1982). Because of this, physical models that simulate zero cutaneous resistance to EWL significantly underestimate body temperature for many amphibians at many times, and such models cannot be used alone to accurately characterize operative environmental temperature.

I suggest that the complications imposed by variation of rates of EWL among and within many amphibian species do not make it impossible to characterize operative environmental temperatures for these species; they simply make it more complex. Instead of a single temperature for any combination of environmental temperature, relative humidity, wind speed, and solar irradiance, the operative environmental temperature available to an amphibian with variable cutaneous resistance can be characterized as falling within an envelope whose upper and lower boundaries are formed by the temperatures experienced by animals with zero and very high skin resistance. I have developed a method for characterizing this envelope using physical models. In this chapter I describe my method and report on experiments examining the thermal properties of the models developed and testing their

predictions against the realized body temperatures of frogs in a laboratory thermal gradient and in the field.

Methods

Design of models

In order to define the upper and lower boundaries of body temperatures available to amphibians occupying any microenvironment, I needed two types of models that were identical except that one had zero resistance to evaporative water loss and the other had near-perfect resistance. I solved this problem by constructing agar models of frogs in the water-conserving posture. Agar is assumed to lose water as a free surface (i.e. a cutaneous resistance of 0; Spotila & Berman 1976). To prevent water loss by conduction to the substrate, I coated the ventral surfaces of all models with an impermeable plastic (PLASTI DIP®, clear, PLASTI DIP International Inc., Blaine, Minnesota USA), designed for coating tool handles and other surfaces. In this condition, models should simulate the thermal performance of frogs with zero resistance to EWL in their dorsal skin (Spotila & Berman 1976), and with their ventral surfaces in contact with a dry, impermeable substrate, as was the case in this experiment. I designated these to be "permeable models". It would also be possible to leave the ventral surface uncoated, allowing water exchange with the substrate, but this would be unlikely to replicate patterns of water exchange experienced by frogs in this experiment, and would not contribute to evaporative cooling. To simulate the thermal performance of frogs with very high skin resistance, I simply coated the remainder of the surface of half of the models with PLASTI DIP®, creating fully-coated "impermeable models".

Model construction

Physical models (Figure 3.1) were made from 3% agar, using moulds in the shape of *Litoria caerulea* (Australian Common Green Tree Frogs) in their water conserving posture (Heatwole et al. 1969). The snout-vent-length (SVL) and weight of the models were approximately 75mm and 44g. The models used in the primary experiment were coloured green using 4 drops per 100ml of green food colouring made by mixing yellow, blue and rose pink food colouring (Queen Fine Foods Pty. Ltd. Alderley, Queensland, Australia) at a ratio of 10:4:1. Small thermal data loggers (Thermochron iButtons, Dallas Semicondictor, Dallas, Texas USA; diameter 15 mm, height 6 mm) were embedded in each model, and were programmed to record the temperature every 30 minutes. Each data logger was tied into a knot in a piece of twine, which was pegged at each end of the mould as the agar set, allowing the data logger to be embedded in the centre of the model. The free twine remaining at each end of the models can be used to attach models to surfaces in the field either by using tape over the twine or by

tying the twine around features such as branches and roots. Half the models I deployed were permeable models, and half were impermeable models.

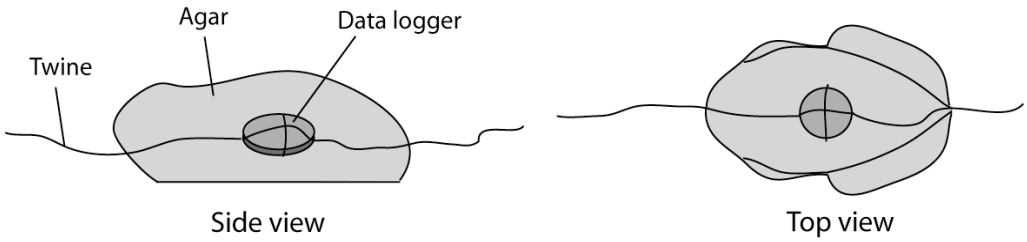

Figure 3.1. Construction of physical models.

Experimental design

I performed an initial series of measurements to compare the spectral absorbences of the models with those of frogs and to determine the extent to which changes in spectral absorbence affect the performance of models exposed to natural sunlight. For spectral absorbence measurements I constructed three permeable and three impermeable green models as described above, and also created three permeable and three impermeable models of uncoloured agar, and three of each type using agar with 3 grams/100 ml of carbon black added, to create models with extremely high absorbence across the spectrum. I measured the absorbence of all of the models and of two *L. caerulea* between 330 and 850 nm using an Ocean Optics S2000 spectrometer and a LiCor halogen light source. Absorbence between 850 and 3000 nm may also be important in determining thermal relations of animals (Tracy 1982), and although the available equipment precluded taking precise measurements in this region of the spectrum, I obtained qualitative information on integrated reflectance in the region above 850 nm by digitally photographing the frogs and model using a B+W 093 filter, which passes only light with wavelengths above 800 nm, and reaches maximum transmission only above 850 nm. I calculated the relative total energy absorption that should occur between 330 and 850 nm in full sunlight and under standard incandescent light for models of each type and for frogs by multiplying my measured absorbence curves by standard curves of energy available at ground level for sunlight and of energy output for incandescent lamps.

In order to determine empirically how model colour affected the temperatures of physical models, I made impermeable and permeable models in three colours as described above. I placed three models of each type in haphazard order on a wire mesh platform elevated approximately 1m above a concrete surface, and situated outdoors in direct sunlight. Model temperature was recorded every 30 minutes from 1030 h on day one until 1000 h on day two for permeable models and until 0800 h on day three for impermeable models.

Measurement ceased at 1000 h on day two for the permeable models because they were rapidly dehydrating and had reached less than 50% of their original weight. There was no cloud-cover during the period of measurement, and air temperatures ranged from 24-36ºC. Relative humidity at the site averaged 70% (range: 18-100 percent).

My main experiment was designed to allow me to measure the body temperatures of frogs exposed to a range of thermal environments in a laboratory gradient, and to determine whether those temperatures fell within the envelope defined by the temperatures of permeable and impermeable models. I set up three opaque, white, plastic containers (60x40x40cm), each with a small water bowl in the center and a metal fly-screen lid. The containers were housed in a constant temperature room, which maintained ambient temperature between 19.5 and 21.5º C. Relative humidity fluctuated between 64 and 96 percent (mean 74 percent). A 150-watt heat lamp was provided at one end of each container and was illuminated between 0930 and 2130 h to create a temperature gradient simulating the normal range of temperatures available to this species. I ran four temporal replicates of the experiment, creating a total of twelve sets of measurements of frog and model temperatures for comparison. Each replicate ran for three days.

At the start of each replicate, models were placed in each container in pairs, each including one permeable and one impermeable model. The pairs of models were located so they spanned the range of thermal and light environments available in the containers. Each model was attached by taping the twine at each end of it onto the container. In the first run of the experiment, the heat source was directed down, towards the centre of one end of the container, and there were four pairs of models per container: top near, bottom near, bottom middle, top far. In the remaining three runs, the light was repositioned to a corner of the container, because frogs in the first temporal replicate appeared to preferentially sit in the corners of the containers. To account for this change, an additional pair of models was added. Instead of one pair located in the middle of the near top of the container, two pairs were placed in the near top; one pair per corner. This adjustment resulted in five pairs of thermal models in each container for the last three experimental periods. The relative locations of the permeable and impermeable models in each pair (right/left) were chosen haphazardly in each container for each temporal replicate.

Twelve adult *L. caerulea* (11 males and one female) were captured near Townsville, Queensland, Australia. They ranged from 74.1-91.8mm SVL and 26-65 g body mass. Prior to experiments, they were maintained in the constant temperature room in which the experiments were carried out, but in smaller containers with no access to heat lamps. Frogs were fed crickets *ad libitum*. Each frog was used in a single run of the experiment.

I recorded the body temperatures of frogs five times per day (0900, 1100, 1300, 1500, and 1700 h) over the three days of each temporal replicate, producing fifteen measurements

for each of the twelve frogs. The first time (0900) was chosen because at that time the frogs had not had access to any source of extra heat for almost 12 h, and their body temperatures should have been similar to those that would be measured during nocturnal readings in the field. Each temporal replicate was set up at least 60 minutes before the first temperature reading was taken, allowing models and frogs to reach a thermal steady-state. At each reading, both skin and cloacal temperatures were measured using a small, chromel-alumel "K" type thermocouple (diameter approx. 1 mm) with the tip coated in plastic, attached to a digital thermometer type 90000. To measure skin temperature, the thermocouple was held firmly against the skin on the lower dorsal area near the thigh while the frog remained in its original position in the container. During body temperature measurement, each frog was held by a single leg, whilst still in the container, and the thermocouple was inserted 10-20mm inside the cloaca until the temperature reading stablised.

Frogs were usually in the water conserving-posture immediately prior to temperature measurement, with most exceptions occurring at 0900 h. When frog temperatures were taken, their location in the tank was noted; this information was later used to determine whether individuals had recently changed location, and to select the pair of models that should represent the upper and lower boundaries of the thermal envelope available to the frogs at each time. All data loggers and the thermocouple were calibrated using a high precision mercury thermometer and a magnetically stirred water bath.

I also examined data collected in a separate study to determine whether data from my physical models delineate the range of body temperatures available to frogs in the field. Adult *Litoria lesueuri* were tracked using radio-telemetry and harmonic radar between 22nd February 2005 and 8th March 2005 in Tully Falls Forest Reserve, Queensland, Australia (145º41'E 17º48'S). Each day (between 1200-1600h), amphibian body temperature was recorded by holding a Raytek ST80 Pro-Plus Non-contact thermometer (RAYST80; "IR thermometer") approximately 5 cm away from the frog and aiming at the lower dorsal area, between the thigh and point of radio-transmitter attachment. Temperatures measured using this technique accurately reflect the skin and cloacal temperatures of frogs (Rowley & Alford in press-a; Appendix 1). During 55 hours of the tracking period, I recorded temperature, using pairs of permeable (without any plastic coating) and impermeable models placed in the field in five sites previously used as diurnal retreat sites by *L. lesueuri*. These data were recorded from 1230h on 25th February 2005 until 1930h on 27th February 2005, when the permeable models had dehydrated by up to 50% of their original weight. During field data collection, the embedded iButtons recorded temperatures of the models every 30 minutes. Frog body temperatures and model temperatures recorded between 1200-1600h were compared graphically to determine the accuracy of models at estimating amphibian body temperatures in the field.

Results

The absorbence spectra of permeable and impermeable models were indistinguishable, indicating that coating models with PlastiDip® does not affect their light absorbence. Integrating the products of mean absorbence measured for frogs and for each type of model with available energy for sunlight at ground level and typical incandescent light from 330 to 850 nm indicated that the green models should absorb a total amount of energy that fell between the amounts calculated for the two *L. caerulea* I measured, and that clear agar models should absorb very slightly less energy than the frog with the lower absorbence, while black models should absorb substantially more than either frog. Infrared photographs indicated that white and green models and *L. caerulea* all had relatively high reflectance in the far infrared, appearing as very similar shades of light grey in photographs taken through the B+W 093 filter with identical exposures under the same lighting conditions.

When models of each type were exposed to full sunlight (Figure 3.2), permeable models equilibrated to very similar temperatures, regardless of their colour. Colour had greater effects on the equilibrium temperatures of impermeable models during daylight hours. During full daylight hours, variation in temperature among models was higher for green models than for clear agar models. The temperatures of green models were usually warmer than those of clear models, with an average difference between 0800 h and 1800 h between the means for green and clear models of 0.8^{o}C (std. dev = 0.8, range = 0 to 2.3, n = 20). Impermeable black models were substantially warmer during daylight hours than impermeable clear or green models, with the daytime mean for black models averaging 3.6 and 4.4 ^{o}C warmer than those of green and clear impermeable models, respectively. Colour had no significant effects on the temperatures of models at night, while permeability continued to affect temperature, with evaporative cooling maintaining the temperatures of permeable models below those of impermeable models (Figure 3.2).

In the laboratory gradient, frog body temperatures fell within the envelope defined by the temperatures of the nearest permeable and impermeable models on 144 of the 180 occasions on which I measured temperatures (Figure 3.3). As expected, permeable models were cooler than frog body temperatures, while impermeable models were warmer than frog body temperatures. When 111 of my observations were made, frogs had changed their location since the last observation, or were being observed for the first time, while in the remainder 69 other observations, frogs were in approximately the same location they occupied at the previous observation. Twenty-eight of the 36 (78%) occasions when frog

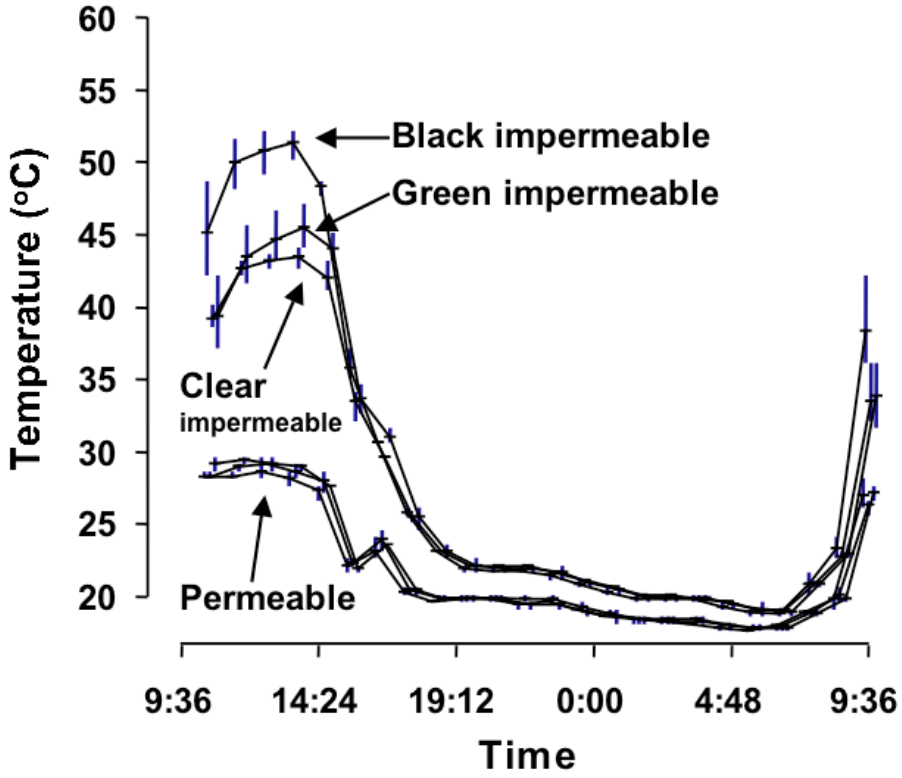

Figure 3.2. Temperatures at hourly intervals for models in full sunlight. Vertical bars indicate ranges for the three replicate models of each colour and permeability, lines connect the means for each colour and type to aid in visualization of patterns. Because all permeable models performed almost identically while well hydrated, I have not attempted to make it possible to distinguish their lines, although the black models were slightly hotter.

cloacal temperatures were outside the envelope occurred when frogs had changed their location since the time of the last observation or were being observed for the first time, and therefore might not have equilibrated thermally. The association between changing location and being outside the thermal envelope is statistically significant (Fisher's Exact Test, two-tailed P=0.034). No cloacal temperature was more than 2.6°C below or 1.2°C above the boundaries of the envelope as defined by the nearest pair of models.

Models were successful in delineating the ranges of body temperatures available to amphibians in the field. All 99 *L. lesueuri* body temperatures obtained during the study period fell between permeable and impermeable model temperatures (n=541 for each model type; Figure 3.4).

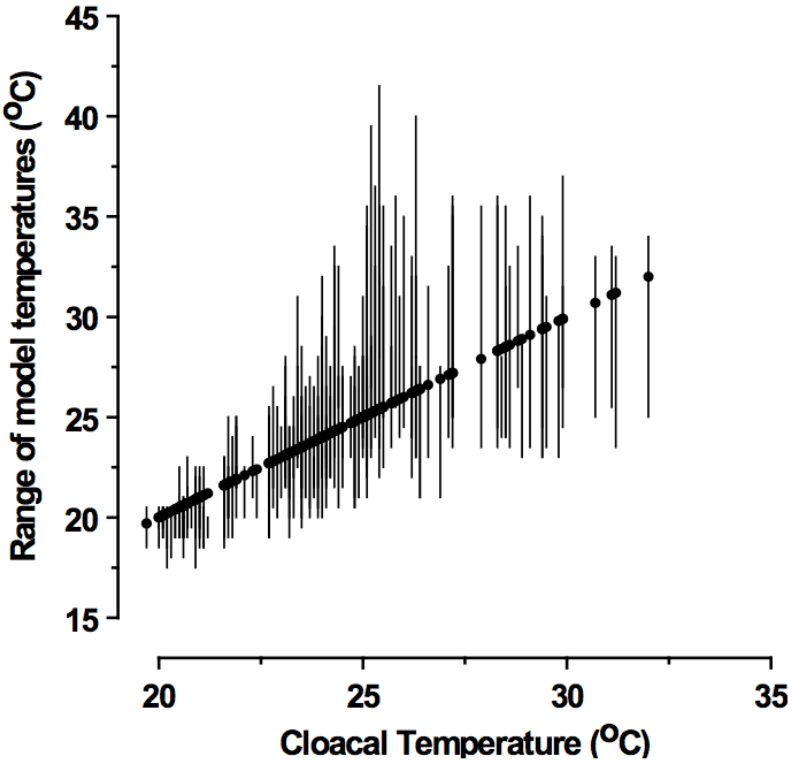

Figure 3.3. Representation of the thermal envelope available to each frog if it was at thermal equilibrium at the time of each measurement of cloacal temperature, based on its location in the experimental chamber. Filled circles indicate the location on both axes of the measured cloacal temperature of each frog. Lines connect the temperatures of the impermeable (upper terminus) and permeable (lower terminus) models nearest each frog at the time its temperature was measured.

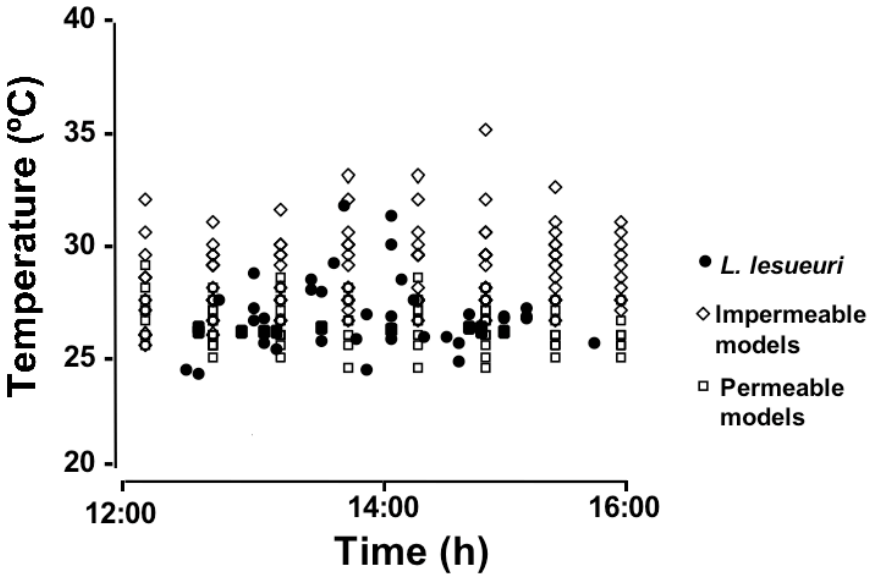

Figure 3.4. Temperatures of permeable and impermeable models placed in known frog retreat sites in the field, and temperatures measured for radio-tracked *L. lesueuri* at the same field site during the same range of dates.

Discussion

The physical models used in this study produced an accurate outline of the thermal envelope into which the body temperatures of thermally equilibrated frogs fell. This should hold true for similar models of any species of amphibian, regardless of species-specific differences or within-species variation in resistance to evaporative water-loss. The body temperatures of amphibians with little or no cutaneous resistance to evaporative water loss (ie. many *Bufo* species; Wygoda 1984) will be close to the temperatures of permeable models, whereas the temperatures of amphibians with high cutaneous resistance to evaporative water loss, (ie. the "water-proof" frogs *Phyllomedusa*) will be close to those of impermeable models. The remainder of species, with intermediate and often variable levels of resistance, will fall within the envelope defined by these extremes.

The species used in this study, *L. caerulea,* can be described as having a moderate cutaneous resistance to water-loss (approx 10 s cm $^{-1}$; Buttemer 1990; Christian & Parry 1997). This value is about an order of magnitude more than most North American hylids, but an order of magnitude lower than those of the 'water-proof" frogs (Buttemer 1990; Withers et al. 1984; Wygoda 1984), which may have cutaneous resistance values as high as 200-300 s cm^{-1}, a value well within the range of many reptile species (Christian & Parry 1997; Geise & Linsenmair 1986; McClanahan & Shoemaker 1987; Shoemaker et al. 1987).

My technique does not attempt to predict the exact body temperatures of specific individual frogs. This would require modelling the precise rate of EWL for individuals in the

field, which is futile for a number of reasons. Firstly, rates of EWL can fluctuate considerably over time in individual frogs due in part to differences in activity and posture (Barbeau & Lillywhite 2005; Withers 1995; Withers et al. 1982), and to temperature-dependent changes in the properties of cutaneous lipids (McClanahan 1978) or mucous gland discharge (Withers et al. 1982). Although many frogs alter EWL with respect to temperature in a moderately predictable manner (Buttemer & Thomas 2003; Withers et al. 1982), in others, EWL fluctuates unpredictably. For instance, Barbeau and Lillywhite (2005) examined six species of treefrogs over a period of ten days, and found that EWL rates varied considerably over time with no discernable trend. In addition, there may also be large seasonal differences in EWL in some species (Loveridge 1976). EWL may also vary among individuals of the same species, with some individuals exhibiting extremely high EWL and succumbing rapidly to dehydration, and others showing a marked decline in EWL over time (Withers et al. 1982). Because rates of EWL are so variable, I suggest that measuring the limits of the envelope of body temperatures available to a species in a given microenvironment is more ecologically relevant for most species than precise measurements for particular individuals can be.

I also suggest that attempting precise matching of absorbence spectra between models and individual frogs is not necessary. Colour and therefore absorbence of a number of different species can alter when basking, or with thermal stress (Kobelt & Linsenmair 1986; Shoemaker et al. 1989; Snyder & Hammerson 1993; Withers 1995). For instance, dark coloured *Litoria rubella* have a mean reflectance of approximately 19%, compared to 32% for light coloured individuals (Withers 1995). I observed a substantial range in total absorbence between the two *Litoria caerulea* I measured for this study, such that the integrated absorbence of green models fell between those of the two individuals. This variation among and within individuals means that precisely modelling even a particular individual of many species is not possible using a static model. However, I found that the relatively large difference in colour between green and clear agar models had only small effects on the daytime temperatures of impermeable models; only the extreme of completely black models had substantial effects on temperature. Even extreme differences in colour had little effect on midday temperatures of permeable models. Thermal envelopes estimated using models are therefore likely to be reasonable approximations of the range of body temperatures available to frogs, even if the absorbence spectra of models are not closely matched to those of individuals of the species being investigated, as long as obviously extreme colours such as black are avoided.

Models produced an accurate outline of the thermal envelope available to frogs in the laboratory thermal gradient. 144 of 180 measured cloacal temperatures fell within the limits defined by the nearest pair of models. I found a statistically significant association between being outside the boundaries of the envelope and having recently moved, suggesting that most

or all of the cloacal temperatures I measured that were outside the range defined by the nearest pair of models were taken from frogs that were not at thermal equilibrium. Because I did not observe frogs continually, recent changes in location may also explain the remaining instances of body temperatures outside the envelope. Some of these may also have been caused by the fact that frogs were not always immediately adjacent to the nearest pair of models, and hence closer to or farther from the heat source.

Models were also successful in delineating the ranges of body temperatures of amphibians in the field. Simply by placing these models in species-specific microhabitats, they provided a good estimate of the ranges of body temperatures experienced by tracked frogs, even though the frogs occurred in a much wider range of physical locations, and their temperatures were measured over a longer period of time. In order to fully understand the thermal dynamics of a species, models should ideally be placed in a much larger number of species-specific microenvironments over a wider spatial and temporal scale.

In addition to characterising the thermal environment, these physical models can be used to evaluate the relative effects of the environmental conditions at sites on water relations by weighing permeable models over time (e.g., Schwarzkopf & Alford 1996). It is possible to characterise the envelope of thermal and humidity conditions available to frogs by placing pairs of models in the field in retreat and activity sites. The physical models I have described are easy to construct in large numbers, and are also relatively inexpensive to produce, features that are highly desirable, particularly if a large number of replicates are needed (Bakken 1992). The models are also compact and self-contained, and can therefore be placed in the exact shelter sites used by the study species without altering their structure, unlike bulkier physical models (e.g., Bartelt & Peterson 2005).

Using data obtained from these models in the field in conjunction with the detailed knowledge of temporal patterns of microhabitat use made possible by advances in tracking technology, it will be possible to develop pictures of temporal patterns of body temperature experienced by frogs that are as detailed as those that have been available for some time for many reptiles. Variation in relative rates of water loss among sites and through time can also be determined.

The knowledge gained using these models is likely to be increasingly useful in understanding the effects of climate change on amphibians. The majority of predictive models forecast a global temperature rise of 1.5-4.5ºC (e.g., Houghton et al. 2001), and such blanket figures have been used in predicting species responses (e.g., Brereton et al. 1995; Thomas et al. 2004; Williams et al. 2003) despite its questionable relevance to micro-environmental conditions or body temperature (Kennedy 1997). Detailed species-specific data such as that obtained using these models will therefore be essential for accurately predicting responses and designing management plans. By altering the size and shape of these physical models, it

will be possible to characterize the thermal environment of other small ectotherms with variable rates of water loss. This approach is also likely to be of use in developing more sophisticated models of energy use and growth within individuals, and thus of population dynamics, and in understanding the factors affecting host-pathogen interactions in nature (e.g., Woodhams et al. 2003).

CHAPTER FOUR: BEHAVIOUR OF AUSTRALIAN RAINFOREST STREAM FROGS MAY AFFECT THE TRANSMISSION OF CHYTRIDIOMYCOSIS

Abstract

The amphibian disease chytridiomycosis, caused by the pathogen *Batrachochytrium dendrobatidis,* has been implicated in mass mortalities, population declines, and extinctions of amphibians around the world. In almost all cases, amphibian species that have disappeared or declined due to chytridiomycosis coexist with non-declining species. One reason why some species decline from chytridiomycosis and others do not may be interspecific differences in behaviour. Host behaviour could either facilitate or hinder pathogen transmission, and transmission rates in the field are likely to vary among species according to the frequency of factors such as physical contact between frogs, contact with infected water, and contact with environmental substrates that may serve as reservoirs. I tracked 117 frogs (28 *L. nannotis,* 27 *L. genimaculata* and 62 *L. lesueuri*) in five sites where *B. dendrobatidis* is endemic in the rainforest of tropical northern Queensland and recorded the frequency of frog-to-frog contact and the frequency of contact with stream water and environmental substrates. Frequency of contact with other frogs and with water were highest in *L. nannotis,* intermediate in *L. genimaculata*, and lowest in *L. lesueuri.* Environmental substrate use also differed among species. These species-specific opportunities for disease transmission were correlated with conservation status: *L. nannotis* is the species most susceptible to chytridiomycosis-related declines, and *L. lesueuri* is the least susceptible. Interspecific variation in transmission probability may therefore play a large role in determining why chytridiomycosis drives some populations to extinction and not others.

Introduction

Approximately one-third of all known amphibian species are currently listed as threatened (Stuart et al. 2004). Declines of many amphibian species are attributed to the disease chytridiomycosis (Berger et al. 1998), which has been implicated in mass mortalities, population declines, and local extinctions in Australia, New Zealand, Central and North America, Europe and Africa (Bell et al. 2004; Berger et al. 1998; Bosch et al. 2001; Bradley et al. 2002; Lips et al. 2006; Lips et al. 2005; Muths et al. 2003; Rachowicz et al. 2006; Weldon & Du Preez 2004). Chytridiomycosis is caused by the fungal pathogen *Batrachochytrium dendrobatidis*, which belongs to the order Chytridiales (Longcore et al. 1999). It is the only chytrid known to cause disease in vertebrate hosts (Berger et al. 1998), and infects keratinized tissues in the epidermis of metamorphosed amphibians (Longcore et al. 1999) and the mouthparts of larval anurans (Fellers et al. 2001; Rachowicz & Vredenburg 2004). Chytridiomycosis can cause rapid mortality (Nichols et al. 2001), with frogs of

susceptible species dying less than three weeks after experimental infection in the laboratory (Berger et al. 2005a; Berger et al. 1998; Berger et al. 2004). The only known mechanism of transmission among hosts, and of intrahost increase in pathogen load, is via infection and reinfection by motile, waterborne zoospores (Longcore et al. 1999).

In almost all cases, amphibian species that have disappeared or declined due to chytridiomycosis coexist with non-declining species (Lips et al. 2006; Lips & Donnelly 2002; McDonald & Alford 1999; Retallick et al. 2004). One reason why some species decline from chytridiomycosis and others do not may be interspecific differences in behaviour. Transmission can be the driving force in the dynamics of infectious diseases (Begon et al. 2002), and the long-term spread and persistence of many diseases depends largely on the contact rate between susceptible hosts and infectious pathogens (Swinton 1998). This suggests that differences among species in opportunities for the transmission of *B. dendrobatidis* may affect their susceptibility to chytridiomycosis. Despite the importance of transmission in the epidemiological process, very little is known about transmission of *B. dendrobatidis*, particularly in the field. The only route of transmission that has been established by controlled experimentation is transmission via contact with water that was previously in contact with infected tadpoles or adults (Berger et al. 1998; Parris & Cornelius 2004; Rachowicz & Vredenburg 2004; Retallick 2002). However, infective zoospores are present on, and can be recovered from, the skin surfaces of infected animals (Berger et al. 1998; Pessier et al. 1999), and *B. dendrobatidis* DNA has been detected on wet rocks at a site during an epidemic (Lips et al. 2006). It therefore seems likely that transmission also occurs via contact with infected individuals or contaminated environmental substrates.

Host behaviour could either facilitate or hinder pathogen transmission, and transmission rates in the field are likely to vary among species depending on the frequency of behaviours such as physical contact between frogs, contact with infected water, and contact with environmental substrates that serve as reservoirs. In this study, I tracked three species of rainforest stream frogs at five sites and recorded their frequency of contact with other frogs, contact with stream water and contact with environmental substrates.

Methods

The study was conducted at five tropical rainforest sites in northern Queensland, Australia: Birthday Creek, Paluma State Forest (146º10'02" E 18º58'54" S, 800 m asl), Python Creek (145º35'E 17º46'S, 200 m asl), an unnamed creek (145º41'E 17º48'S; 70 m asl) in Tully Falls Forest Reserve, an unnamed creek in Kirrama State Forest (145º52 E 18º11 S; 200 m asl) and Frenchman Creek, in Wooroonooran National Park (145º55' E 17º20' S 20-100 m asl). All sites were relatively undisturbed rainforest streams with rocky beds. All streams had pools and riffles, and most sites had a number of waterfalls.

Frogs of three species were tracked; the stoney creek frog *L. lesueuri*, which has not experienced population declines (IUCN Least Concern; see below), the green eyed tree frog *L. genimaculata,* which declined and then recovered (IUCN Least Concern, however Australian populations of this species are considered to be Near Threatened), and the waterfall frog *L. nannotis,* which has experienced large and long-lasting population declines (IUCN Endangered; IUCN 2006; McDonald & Alford 1999; McDonald 2002; McDonald et al. 2005). Recently, the taxonomy of the *L. lesueuri* group has been revised (Donnellan & Mahony 2004). Two species, *L. jungguy* and *L. wilcoxii*, occur in sympatry in the study sites, hybridise, and are indistinguishable on the basis of morphology (Donnellan & Mahony 2004). Population declines have not been observed in the region for either species (McDonald & Alford 1999; McDonald et al. 2005). I therefore continue to refer to the study population as *L. lesueuri* while recognising that the population contains two morphologically indistinguishable species. All species of frogs tracked were large to medium-sized hylids (males 5.4-12.5 g, females 6.5-41.3 g), and were tracked using either radio telemetry or harmonic direction finding.

Only frogs weighing more than 11g were tracked via radio telemetry. Radio transmitters (models BD-2N and BD-2NT; Holohil Systems Ltd., Ontario, Canada; weighing approximately 0.67 g including harness and with a battery life of approximately 3 weeks) were attached to a harness made of silicone tubing, designed to minimise restrictions on movement and avoid causing discomfort to the frog. This harness and transmitter was placed around the waists of frogs. Frogs that were too small to be radio tracked, and a number of larger individuals, were tracked using harmonic direction finding (Langkilde & Alford 2002). This required attachment of a small diode with whip antenna to the same specially designed harness (total weight approximately 0.23g), which was then placed around the waist of a frog. Tracking devices were fitted *in situ* and frogs were released at point of capture after less than five minutes of handling. Frogs wearing either tracking device always had harnesses and associated equipment that weighed less than 6% of their total body weight, just over half the recommended maximum relative weight for an attached tag (10% of the body weight; (Richards et al. 1994).

Frogs fitted with radio transmitters were tracked using a Telonics TR-4 Tracking Receiver (Telonics, Inc., Mesa, AZ, USA; 2004 warm/wet season only) and a HABIT Research HR2500 Osprey VHF Receiver (HABIT Research, Victoria, B.C., Canada); I used a three-element folding Yagi antennae with both receivers (A.F. Antronics, White Heath, Illinois, USA). Frogs fitted with diodes were tracked using a portable RECCO R5 transmitter-receiver (Recco Rescue Systems, Lidingö, Sweden). The system consists of a hand-held device that acts as both the transmitter and receiver, and a battery and earphones. Tags were

self-built using commercial germanium diodes (see Langkilde & Alford (2002) for a description of methodology).

Surveys lasted 16 days and were conducted in the cool/dry season (May-September) and one in the warm/wet season (October-April) for each species at two sites, except for Paluma, which was only surveyed in the warm/wet season. *Litoria genimaculata* was tracked at Paluma in late 2003, *L. genimaculata* and *L. nannotis* were tracked simultaneously at Kirrama and Tully Gorge during 2004, and *L. lesueuri* were tracked at Lower Tully and Babinda during 2005. During surveys, the location of each frog was determined once during the day (0900-1800 h) and once at night (1900-0400 h).

I recorded instances of frog-to-frog contact when I observed direct skin-to-skin contact between frogs and when aggregations of more than three individuals occupied an area of less than 0.3 m^2. At each observation of contact between frogs, I recorded the species involved and the nature of the contact, (ie. amplexus, sharing a retreat site or a nocturnal perch site). I recorded instances of contact with water when I observed at least part of the frog in contact with stream water, or when the frog had crossed the stream since the last observation, and there was no way for the frog to cross the stream without entering the stream water (ie. the frog could not have crossed the stream using dry rocks or overhead vegetation). This provides an estimate of the minimum rate of contact with water, since frogs may come into contact with water without crossing streams. During all observations, I recorded the substrate type with which the ventral surface of the frog was in contact. Substrate type was classified into six categories: bare ground, dry rock, leaf litter, vegetation, wood (dead wood such as logs or sticks), and wet rock.

I excluded data from the night following tag attachment due to the potential short-term behavioural effects of handling (Langkilde & Alford 2002). Any effects are unlikely to persist after the first night of tag attachment (Rowley & Alford in press-b; Chapter 2). To avoid pseudoreplication or biasing my results towards frogs that were located more frequently, I used individuals as replicates and compared summary statistics calculated for each animal. To analyse frog-to-frog contact and contact with water, I performed Kruskal-Wallis tests to detect differences among species and separate Mann-Whitney U tests for each species to detect differences with respect to season and sex. I used Bonferroni adjustments to control Type I error rates. To analyse substrate use, I examined diurnal and nocturnal data separately due to obvious differences between the diurnal and nocturnal behaviour of all species. I then performed multi-response permutation procedures (MRPP) using Blossom Statistical Software (Cade & Richards 2005). I performed the analysis in a stepwise fashion, testing for differences between species, sexes, seasons, and sites, in that order. If a difference was detected between groups, the next analysis was performed on each of those groups separately. I used Monte Carlo hypothesis testing, with 20,000 iterations.

Results

A total of 117 frogs (28 *L. nannotis,* 27 *L. genimaculata* and 62 *L. lesueuri*) were tracked during the study period. Approximately equal numbers of frogs of each sex were tracked using both tracking techniques; 64 (55%) of individuals were females and 61 (52%) were tracked via radiotelemetry. Throughout the survey periods, a total of 2111 fixes or locations of individual frogs were obtained. On average, each frog was located 17 times (minimum 5, maximum 29).

The frequency of contact between frogs differed significantly among species (Table 4.1; Figure 4.1). Individual *L. nannotis* were in contact with other individuals during an average 28% of observations, while *L. genimaculata* and *L. lesueuri* were only in contact with other individuals during an average of 3% and 2% of observations, respectively. Type of contact also differed among species (Table 4.2), however, in all species, direct contact was always between conspecifics. The frequency of physical contact did not differ between seasons only in *L. nannotis*, which was more often in contact with conspecifics in the warm/wet season (Table 4.1; Figure 4.1). There was no difference in the frequency of physical contact between sexes in *L. nannotis* and *L. genimaculata,* but female *L. lesueuri* were more often in contact than male *L. lesueuri* (Table 4.1).

The frequency of contact with stream water also differed significantly among species, and was highest in *L. nannotis* (Table 4.2; Figure 4.1). Overall, *L. nannotis* were observed in stream water or had crossed the stream since the last observation during 84% of observations. In contrast, *L. genimaculata* and *L. lesueuri* were observed in contact with water on an average of only 16% and 13% of observations respectively. Rates of contact with stream water differed between seasons only in *L. lesueuri*; they were higher in the warm/wet season (Table 4.2; Figure 4.1), and did not differ with sex for any species (Table 4.2).

Table 4.1. Differences in direct and indirect contact among species (Kruskal Wallis tests), between seasons and between sexes (Mann-Whitney U tests). * indicates significance at the Bonferroni-adjusted 0.05 level.

		Frog-to-frog contact		**Contact with stream water**	
Test	**Species**	**Test statistic (X^2 or Z)**	**P**	**Test statistic (X^2 or Z)**	**P**
Species	--	18.573	<0.001*	60.492	<0.001*
Season	*L. lesueuri*	-1.008	0.313	-3.607	<0.001*
	L. genimaculata	-0.789	0.648	-0.960	0.347
	L. nannotis	-2.775	0.010*	-1.004	0.324
Sex	*L. lesueuri*	-2.636	0.008*	-0.993	0.321
	L. genimaculata	-0.395	0.829	-0.541	0.614
	L. nannotis	-0.852	0.460	-1.887	0.059

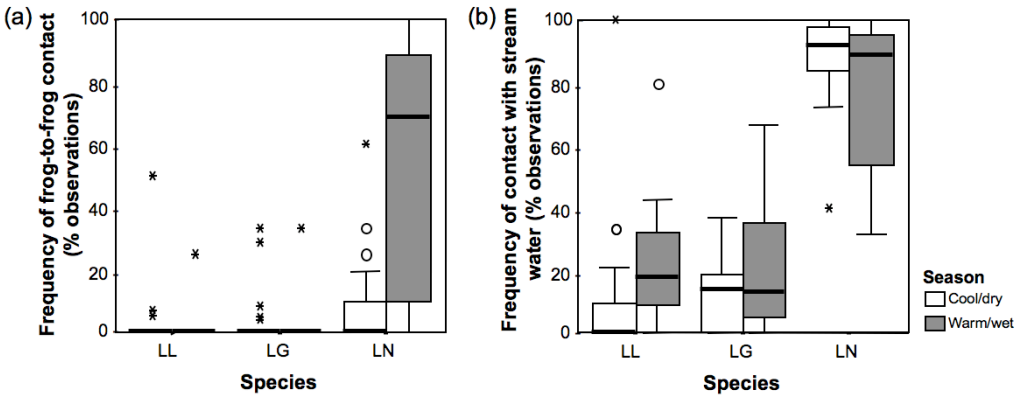

Figure 4.1. Frequency of (a) frog-to-frog contact and (b) contact with stream water (% of observations) in *L. lesueuri* (LL), *L. genimaculata* (LG) and *L. nannotis* (LN) in the cool/dry and warm/wet seasons. In these plots, bold horizontal lines represent the median, boxes indicate the locations of upper and lower quartiles, bars encompass values up to 1.5 interquartile range, open circles represent values more than 1.5 interquartile ranges from the nearest quartile and stars indicate data values more than 3 inter-quartile ranges from the nearest quartile.

Table 4.2. Frequency (and percent) of each type of frog-to-frog contact observed in this study.

	Retreat site	**Amplexus**	**Perch site**
L. lesueuri	2 (29%)	5 (71%)	0
L. genimaculata	0	2 (50%)	2 (50%)
L. nannotis	45 (100%)	0	0

Substrate use differed among species and between diurnal and nocturnal surveys (Tables 4.3 and 4.4). *Litoria lesueuri* were most often on bare ground or leaf litter during diurnal surveys, moving onto a wider range of substrate types at night. *Litoria genimaculata* were most often on vegetation, generally in the forest canopy, during both diurnal and nocturnal surveys. In instances when I could visually locate individual *L. genimaculata* in the canopy, frogs were sitting on leaves at the ends of branches. *Litoria nannotis* was most commonly on wet rock, with frogs typically sheltering under large boulders in the stream during the day and often moving on to terrestrial vegetation and dry rock at night.

Substrate use differed between sexes in *L. lesueuri* and *L. nannotis* (Tables 4.3 and 4.4). In *L. lesueuri,* substrate use differed subtly between sexes, but in *L. nannotis,* males were almost always on wet rock, while females were found more often on other substrates including vegetation and dry rock (Table 4.4). Substrate use differed with season for *L. lesueuri* males, and *L. genimaculata* (Tables 4.3 and 4.4). In the cool/dry season, *L. lesueuri*

males spent more time on bare ground and less time on dry rocks and *L. genimaculata* spent more time on dry rock and less time on leaf litter, vegetation and bare ground (Table 4.4).

Table 4.3. Multi-response permutation procedures (MRPP) for differences between species, sex and season using Monte Carlo hypothesis testing. I performed the analysis in a stepwise fashion. If a difference was detected between groups, the next analysis was performed on each of those groups separately. * indicates significance at 0.05 level.

Test			δ	**P**
Species			4.185	<0.001*
Sex	*L. leseuri*		5.919	0.024*
	L. genimaculata		4.870	0.548
	L. nannotis		4.123	0.003*
Season	*L. leseuri*	Female	5.66	0.792
		Male	4.961	<0.001*
	L. genimaculata	---	4.251	0.011*
	L. nannotis	Female	4.214	0.113
		Male	---	---

Table 4.4. Substrate use of *L. lesueuri*, *L.genimaculata* and *L. nannotis*. Only species, sex and season categories that are significantly different from each other are shown.

		L. lesueuri			***L. genimaculata***		***L. nannotis***	
		Female	**Male**		**---**	**---**	**Female**	**Male**
		---	**Cool/dry season**	**Warm/wet season**	**Cool/dry season**	**Warm/wet season**	**---**	**---**
Bare ground	Diurnal	19.34 (0-66.7)	39.01 (0-100)	9.1 (0-30.8)	0	30.4 (0-100)	0	0
	Nocturnal	8.1 (0-33.3)	26.67 (0-66.7)	10.4 (0-28.6)	0	19.7 (0-66.7)	0	0
Dry rock	Diurnal	3.8 (0-60)	1.8 (0-25)	8.3 (0-33.3)	25.6 (0-100)	3.3 (0-60)	10.5 (0-66.7)	2.8 (0-12)
	Nocturnal	8.2 (0-50)	2.3 (0-33)	20.2 (0-60)	21.4 (0-69.2)	5.6 (0-50)	14.4 (0-62.5)	4.1 (0-15.4)
Leaf-litter	Diurnal	63.6 (0-100)	55.3 (0-100)	52.9 (0-100)	5.9 (0-33.3)	57.8 (0-100)	0.2 (0-5)	0
	Nocturnal	39.9 (13.3-85.7)	16.4 (0-80)	30.8 (0-61.5)	2.8 (0-25)	24.8 (0-85.7)	0	0
Vegetation	Diurnal	5.6 (0-41.7)	3.4 (0-22)	5.9 (0-40)	63.7 (0-100)	3.5 (0-27.3)	20.2 (0-55.6)	7.1 (0-16)
	Nocturnal	30.5 (0-80)	48.8 (0-100)	25.2 (0-50)	72.7 (23.1-100)	41.6 (0-100)	36.4 (0-88.9)	9.0 (0-30.8)
Wood	Diurnal	2.7 (0-33.3)	0.5 (0-11.1)	14.7 (0-100)	0	1.6 (0-33)	2.9 (0-33.3)	0
	Nocturnal	11.4 (0-16.7)	5.8 (0-44.4)	5.2 (0-20)	0	7.9 (0-50)	4.3 (0-40)	0
Wet rock	Diurnal	1.5 (0-33.3)	0	0	4.8 (0-22.2)	0.9 (0-33.3)	66.2 (16.7-96)	90.2 (72-100)
	Nocturnal	1.2 (0-16.7)	0	8.3 (0-60)	3.1 (0-22.2)	0.4 (0-16.7)	45.0 (0-92.3)	88.9 (53.9-100)

Discussion

The frequencies of frog-to-frog contact and contact with stream water and different environmental substrates differed significantly among species. As these factors should affect rates of transmission of *B. dendrobatidis* via known or highly likely routes, transmission rates should also vary among species.

The frequency of contact between frogs was highest in *L. nannotis*, primarily because the species aggregates at retreat sites. The formation of aggregations by host populations promotes contact between individuals, and in many other host-pathogen systems it is positively correlated with both the prevalence and intensity of contact-transmitted parasites (Anderson & May 1979; Brown & Brown 1986; Coté & Poulin 1995; Ezenwa 2004; Hoogland 1979). Outbreaks of disease are also most commonly observed in aggregations of individuals (Vermeer 1969; Wobester et al. 1979). In contrast, predominantly solitary or non-social species such as *L. lesueuri* and *L. genimaculata* will come into direct contact almost exclusively for reproduction, and it is likely that, for these species, the majority of direct transmission occurs at this time (Loehle 1995). This may be especially true for the transmission of *B. dendrobatidis* in *L. lesueuri*, as *L. lesueuri* comes into contact with stream water infrequently. Therefore, while traditional models of direct transmission have assumed that contact rate is directly proportional to host population size or density (Anderson & May 1979; Anderson & May 1981; Watanabe 1987), this study confirms that rates of contact between individuals may be almost entirely independent of population size due to host behaviour (Ezenwa 2004; Loehle 1995; McCallum et al. 2001). As a result, even in the absence of alternative hosts or environmental reservoirs, *B. dendrobatidis* may be able to persist in *L. nannotis* populations containing only a small number of individuals, and thereby be more likely to result in local extinctions, compared to both *L. genimaculata* and *L. nannotis*.

In all three species, contact between frogs was always between conspecifics, providing almost no opportunities for cross-species pathogen transmission. Therefore, contact with stream water or other environmental substrates that serve as reservoirs are likely to be the main source of transmission between species in this system. Contact with stream water was more frequent in *L. nannotis*, which was in contact with the stream during the majority of observations. Increased frequency of contact with stream water is likely to increase the rate of transmission of *B. dendrobatidis* in a species for a number of reasons. First, *B. dendrobatidis* is known to be transmitted via water in the laboratory (Berger et al. 1998; Parris & Cornelius 2004; Rachowicz & Vredenburg 2004) and in field enclosures (Retallick 2002). Second, *B. dendrobatidis* zoospores are aquatic, highly sensitive to desiccation (Johnson et al. 2003), and can survive and remain infective in the laboratory for at least seven weeks in sterile lake water (Johnson & Speare 2003). Additionally, tadpoles often have a high infection

prevalence, may not be susceptible to the pathogen, and can persist in the stream environment for several years (Rachowicz et al. 2006; Woodhams & Alford 2005), thereby providing a continual source of zoospores to the stream. Lastly, amphibian population declines have been greatest in species with strong associations with streams (Hero et al. 2005; Lips et al. 2003b; McDonald & Alford 1999; Williams & Hero 1998), and while purely terrestrial species may be infected with *B. dendrobatidis* (Lips et al. 2006), they do not typically experience population declines, or experience reduced rates of decline, even when in sympatry with rapidly declining species (Hero et al. 2005; Lips et al. 2006; McDonald & Alford 1999; Williams & Hero 1998).

Different species used different environmental substrates, and in some cases substrate use varied between sexes and seasons as well. Because the abundance and composition of other chytrids differ among macrohabitats (ie. vegetation type; Letcher et al. 2004) and microhabitats (ie. with distance from moss; Letcher & Powell 2002), it is highly probable that the abundance of *B. dendrobatidis* zoospores also differs among environmental substrates, and hence exposure to *B. dendrobatidis* probably differs among species, and even between sexes and seasons for some species. However, there are a large number of uncertainties in quantifying the risks associated with contact with stream water or environmental substrates. There is currently no information on the relative abundance of zoospores in the environment, and we do not yet know the relative importance of different areas of the stream or different environmental reservoirs for the persistence and transmission of *B. dendrobatidis* in the field. While *B. dendrobatidis* DNA has been detected on environmental substrate samples during epidemics (Lips et al. 2006), it is not known whether these substrates contain viable zoospores. In addition, *B. dendrobatidis* DNA was not detected on environmental substrate samples taken at *L. lesueuri* retreat sites where *B. dendrobatidis* is endemic (Rowley et al. 2007; Chapter 5). Further work to determine how important environmental substrates may be as reservoirs for infective zoospores is urgently required.

Frequency of contact with other frogs or with water differed between seasons only in *L. lesueuri*, which was more often in contact with water during the warm/wet season. There was little evidence that the frequency of contact with other frogs or with water differed between sexes for any of the species. In other study species, sex may greatly influence opportunities for disease transmission. For example, host behaviour has been implicated in the differential survival of *Bufo boreas* during *B. dendrobatidis* outbreaks (Carey et al. 2006). In this species, adult males spend several weeks in frequent direct contact with other males and in continuous contact with water during the breeding season, while females are thought to spend less than one day at breeding sites (Carey et al. 2006). Perhaps because of this, adult females appear to live longer than adult males during outbreaks of *B. dendrobatidis* (Carey et al. 2006).

In addition to frequency of contact between frogs, with stream water or environmental reservoirs, the duration of contact is likely to be important. In the laboratory, the duration of exposure to *B. dendrobatidis* can influence the probability of successful transmission and the speed of disease progression, with longer exposures to *B. dendrobatidis* resulting in shorter average survival times in *B. boreas* (Carey et al. 2006). In my study species, duration of contact between frogs was highest when sharing communal retreat sites or when in amplexus. The duration of time spent in retreat sites differed among species; while *L. nannotis* and *L. genimaculata* often remained in retreat sites for days at a time, *L. lesueuri* rarely spent more than 12 hours at a single retreat site (Chapter 6). Amplexus duration in this study was always less than 12 hours in *L. lesueuri* and *L. genimaculata*. I never observed *L. nannotis* in amplexus, probably due to the concealed location of their breeding sites (under rocks in fast flowing stream water, often behind inaccessible boulders; Liem 1974; Rowley pers. obs.). The duration of time that frogs spent in contact with stream water also varied among species. The majority of contact with water in *L. lesueuri* and *L. genimaculata* occurred briefly when crossing a stream, while *L. nannotis* spent all day and often all night in contact with stream water. In addition, *L. nannotis* often returned to the same retreat sites, which were often shared with a large number of conspecifics, after nocturnal excursions (Chapter 6). Such behaviour is likely to increase the opportunities for transmission in *L. nannotis*, as parasites may accumulate in the hosts' environment over time (Altizer et al. 2000). Species that rarely return to the same diurnal retreat sites, such as *L. lesueuri* (Rowley, unpubl. data) may reduce their chances of infection. The duration of time in retreat sites with a certain substrate type (ie. wet rock) may also be important.

The dose of *B. dendrobatidis* zoospores that a frog encounters may also influence the probability and outcome of infection in frogs. In *B. boreas,* high doses of *B. dendrobatidis* result in shorter average survival times (Carey et al. 2006). This pattern appears typical of other host-pathogen systems, with higher infective doses leading to higher mortality rates and decreased host survival times (Brunner et al. 2005; Steven & Matthew 2001; van Beek et al. 2000). Because the intensity of infection by *B. dendrobatidis* must reach a particular threshold of zoosporangia before individuals succumb to chytridiomycosis, larger inocula are likely to reach lethal levels sooner (Carey et al. 2006). It is currently not known which behaviours would expose frogs to the highest concentration of zoospores, although direct contact with a highly infected frogs seems likely to expose individuals to the highest zoospore concentrations. Contact with environmental substrates may also expose frogs to high zoospore concentrations if *B. dendrobatidis* is able to grow on these substrates or if long residence times of infected frogs in retreat sites lead to high concentrations of zoospores in the environment.

In this study, it is likely that I underestimated the frequency of contact in all frog species. As frogs were not observed continuously, individuals may have briefly come into contact with other individuals, or with the stream, without this being recorded, particularly at night, when frogs were active. While increasing the frequency of surveys would allow a more accurate estimate of opportunities for transmission, the relative differences between species are unlikely to change. Increasing survey frequency would also increase the probability of disturbing frogs and hence influencing their behaviour.

Despite its importance, transmission is only one part of the entire sequence of host-parasite interactions beginning with pathogen survival in the environment and terminating in either host mortality or pathogen elimination. Even if a pathogen and its host come into contact with each other, successful invasion of the host by the pathogen is not guaranteed. For example, many fungal pathogens require very specific environmental conditions to germinate, and low humidity may completely prevent fungal pathogens from infecting potential hosts, regardless of how many spores are in contact with a potential host (Hajek & St Leger 1994). Other factors such as host microenvironment selection may also eliminate infection once it occurs, since exposure to temperatures of 37ºC for less than 6 hours can eliminate infection from captive frogs (Woodhams et al. 2003). Timing of transmission may therefore be extremely important. In particular, as host mortality due to chytridiomycosis is highest in winter, exposure to *B. dendrobatidis* during the winter months may have greater consequences for host survival.

In summary, due to behavioural differences, *L. lesueuri, L. genimaculata* and *L. nannotis* differ greatly in their rates of contact with other frogs, stream water and different environmental substrate types. Because these rates are likely to affect their levels of exposure to *B. dendrobatidis,* the transmission of *B. dendrobatidis* is likely to also differ between species. Frequency of frog-to-frog contact and contact with water are correlated with the conservation status of the frogs; the highest rates of contact between frogs and with water occurred in *L. nannotis*, the species most susceptible to chytridiomycosis-related declines, intermediate rates occurred in *L. genimaculata*, the species with intermediate susceptibility to decline, and the lowest rates occurred in *L. lesueuri*, the species least susceptible to chytridiomycosis-related declines. Interspecific variation in transmission may therefore play a role in determining why chytridiomycosis drives some populations to extinction and not others. As frog behaviour is likely to vary geographically within single species, further research is required in order to determine whether this association holds true for other species and geographical regions.

CHAPTER FIVE: RETREAT SITES OF RAINFOREST STREAM FROGS ARE NOT A RESERVOIR FOR *BATRACHOCHYTRIUM DENDROBATIDIS* IN NORTHERN QUEENSLAND, AUSTRALIA

*Rowley, J. J. L., Skerratt, L. F., and Alford, R. A. & Campbell, R. (2007) Retreat sites of rain forest frogs are not a reservoir for *Batrachochytrium dendrobatidis* in northern Queensland, Australia. *Diseases of Aquatic Organisms.***74**: 7-12.

Abstract

Chytridiomycosis is a potentially fatal disease of amphibians caused by *Batrachochytrium dendrobatidis*, and is implicated in declines and extinctions of amphibian populations and species around the world. To cause local host extinction, a disease organism must persist at low host densities. One mechanism that could facilitate this is the ability to persist in the environment. In the laboratory, *B. dendrobatidis* spreads by both frog-to-frog and environment-to-frog transmission, and can persist on a number of biotic substrates. In the field, *B. dendrobatidis* has been detected on environmental samples taken during an epidemic, but it is not known if it persists in the environment when endemic. Retreat sites of two species of Australian rainforest stream frogs (*Litoria lesueuri* and *Litoria nannotis*) were sampled 0-3 days after occupation during the wet and cool/dry seasons in northern Queensland where chytridiomycosis has been endemic for at least ten years. The intensity and prevalence of infection in frogs during sampling were comparatively low compared with epidemics. Diagnostic quantitative PCR did not detect *B. dendrobatidis* in any retreat site samples. It thus appears that retreat sites are not a major environmental source of infection when *B. dendrobatidis* occurs at low prevalence and intensity on frogs. This suggests that control efforts may not need to eliminate the organism from the environment, at least when prevalence and intensity of infection are low in frogs. Simply treating hosts may be effective at controlling the disease in the wild.

Introduction

Amphibian species around the world are declining at an alarming rate, many of them to extinction (Alford & Richards 1999; Blaustein & Wake 1990; Kiesecker et al. 2001; Stuart et al. 2004). Currently, approximately 43% of all known amphibian species are considered to be declining (Stuart et al. 2004). Many of these declines are thought to be due to the amphibian disease chytridiomycosis (Berger et al. 1998; Lips et al. 2006), which can be fatal to many species and is implicated in mass mortalities, population declines, and extinctions in Australia, New Zealand, Central and North America, Europe and Africa (Bell et al. 2004; Berger et al. 1998; Bosch et al. 2001; Bradley et al. 2002; Lips 1999; Lips et al. 2006; Lips et al. 2005; Muths et al. 2003; Weldon & Du Preez 2004).

Chytridiomycosis is caused by the pathogen *Batrachochytrium dendrobatidis*, which belongs to the order Chytridiales (Longcore et al. 1999). Members of this order, commonly referred to as chytrids, are ubiquitous fungi found in aquatic habitats and moist soils (Sparrow 1960). They occur as saprobes or parasites on a wide range of substrates including algae, other aquatic fungi, aquatic and terrestrial plants, spores, microscopic animals and their eggs, and chitinous insect exoskeletons (Sparrow 1960), and subsist by degrading cellulose, chitin and keratin (Powell 1993). A number of chytrids are parasitic, infecting plants, algae, protists, invertebrates and vertebrates (Powell 1993). *Batrachochytrium dendrobatidis* is the only chytrid known to cause disease in vertebrate hosts (Berger et al. 1998), breaking down keratin that occurs in the epidermis of adult amphibians and the mouthparts of larval anurans. Chytridiomycosis can cause rapid mortality, with infected frogs of susceptible species dying within three weeks of infection in the laboratory (Berger et al. 1998; Berger et al. 2004; Nichols et al. 2001). In the laboratory, the disease is highly contagious (Nichols et al. 2001), spreading within and among individuals via motile, waterborne zoospores (Longcore et al. 1999).

One of the unusual aspects of chytridiomycosis is that it drives many host species to local extinction during outbreaks (Berger et al. 1998; Lips et al. 2006). For a pathogen to cause local host extinction it must be capable of persisting and infecting new hosts even at very low host population densities (Anderson & May 1986; Dobson & May 1986). One potential mechanism for this is the persistence and growth of *B. dendrobatidis* in tadpoles and adults that do not die from infection (Berger et al. 1998, Berger et. al. 1999; Daszak et al. 1999). The broad host range of *B. dendrobatidis* also provides a reservoir of infection enabling the most susceptible species to chytridiomycosis to be driven to extinction (Berger et al. 2004). An alternative source of infection that could facilitate extinction is the persistence of free-living stages (Daszak & Cunningham 1999; Godfray et al. 1999).

A number of aquatic pathogens are able to persist as viable organisms in the environment by forming biofilms on both abiotic and biotic surfaces (Carli et al. 1993; Hood & Winter 1997; Signoretto et al. 2005). Zoospores of many chytrids can persist in films of water on plants and in soil, and in ponds and rivers (Carlile & Watkinson 1994), and have been detected on mossy rocks (Dewel et al. 1985) and canopy leaves (Longcore 2005). In the laboratory, *B. dendrobatidis* can be cultured on tryptone agar without keratin or keratin derivatives (Longcore et al. 1999; Pessier et al. 1999), will persist in sterilized water for several weeks and will grow for at least one generation on cleaned epidermal keratin or dead amphibians (Longcore et al. 1999). In addition, zoosporangia can attach to and grow on dead algae and insect exoskeletons (Johnson & Speare 2003) and survive for at least three months in sterile sand or bird feathers (Johnson & Speare 2005). Another source of infection that could facilitate extinction is the ability of *B. dendrobatidis* to infect alternative hosts. All

these factors indicate that *B. dendrobatidis* may be able to do some or all of the following: persist for some time in the environment, grow saprophytically, or infect alternate hosts (Longcore et al. 1999).

The only route of transmission that has been established by controlled experimentation is transmission via contact with water that was previously in contact with infected tadpoles or adults (Berger et al. 1998; Parris & Cornelius 2004; Rachowicz & Vredenburg 2004; Retallick 2002). However, infective zoospores are present on, and can be recovered from, the skin surfaces of infected animals (Berger et al. 1998; Pessier et al. 1999), and *B. dendrobatidis* DNA has been detected on wet rocks at a site during an epidemic (Lips et al. 2006). It therefore seems likely that transmission also occurs via contact with infected individuals or contaminated environmental substrates.

During a recent mass mortality event in Panama, Lips et al. (2006) detected *B. dendrobatidis* via PCR on six of seven substrate samples associated with dead frogs and on one of nine stream boulders. However, it is not known if *B. dendrobatidis* is present on substrates only during epidemics, nor is it known whether *B. dendrobatidis* persists on these substrates or if transmission to amphibians occurs.

If *B. dendrobatidis* can persist or grow in the environment, this will be an important source of infection, particularly if environmental persistence occurs in sites where frogs spend large proportions of their time, such as retreat sites. Particularly during daylight hours, nocturnal frogs usually remain motionless in species-specific retreat sites. Most species of Australian rainforest stream frog occupy these sites from 12 hours to five days at a time, and can return to them repeatedly (Rowley, unpublished data). Retreat sites therefore might provide opportunities for reinfection of individuals and transmission between individuals. This could be particularly important because the highest intensities of infection with *B. dendrobatidis* occur on the ventral surfaces of frogs (Berger et al. 2005b), which are most often in contact with substrates. I examined the possibility that reinfection and transmission might occur via infected substrates by using diagnostic PCR to determine if *B. dendrobatidis* persisted in the diurnal retreat sites of two species of rainforest stream frog known to be infected with *B. dendrobatidis* in the field.

Methods

The diurnal retreat sites used by most species of frogs are unknown. I located the diurnal retreat sites used by two species of frogs by tracking individuals at three relatively undisturbed rainforest streams in northern Queensland, Australia; Frenchman Creek, in Wooroonooran National Park (145º55'E 17º20'S, 20-100m asl), and Python Creek (145º35'E 17º46'S, 200m asl) and an unnamed creek ("Lower Tully Creek", 145º41'E 17º48'S, 70m asl) in Tully Falls Forest Reserve. Surveys were conducted in both the warm/wet and the

cool/dry season at each site; each survey was approximately 16 days in duration. Mean air temperature in the warm/wet season was 25ºC, (range= 21-32ºC) and in the cool/dry season was 18ºC (range= 13-25ºC).

During the study, I tracked large to medium sized hylid frogs of two species, *Litoria lesueuri* and *Litoria nannotis*, and swabbed their diurnal retreat sites. Recently, the taxonomy of the *L. lesueuri* group has been revised (Donnellan & Mahony 2004). Two species, *L. jungguy* and *L. wilcoxii*, occur in sympatry in the region, may hybridise, and are indistinguishable on the basis of morphology (Donnellan & Mahony 2004). I therefore continue to refer to them as *L. lesueuri*. I tracked *L. nannotis* at Python Creek from 23 July- 6 August 2004 and 25 March- 8 April 2004, and *L. lesueuri* at Frenchman Creek from 15-29 March and 3-17 August 2005, and at Lower Tully Creek from 22 February- 9 March and 25 August- 9 September 2005. Frogs were tracked using either radio telemetry or harmonic direction finding (Langkilde & Alford 2002). Only frogs weighing more than 11g were tracked via radio telemetry. Radio transmitters (models BD-2N and BD-2NT; Holohil Systems Ltd., Ontario, Canada; weighing approximately 0.67 g including harness and with a battery life of approximately 3 weeks) were attached to a harness made of silicone tubing, designed to minimise restrictions on movement and avoid causing discomfort to the frog. Frogs that were too small to be radio tracked, and a number of larger individuals were tracked using harmonic direction finding. This required attachment of a small diode to the same specially designed harness (weighing approximately 0.27g including harness). Frogs were fitted with tracking devices *in situ* and released at their point of capture in less than five minutes. Tracking devices and harnesses did not weigh more than 6% of a frogs total body weight, which is just over half the recommended maximum relative weight for an attached tag (10% of the body weight; Richards et al. 1994). The weights of frogs tracked did not change over the study period (Wilcoxon Signed Ranks Test; Z= -1.361, p= 0.173, n=70), and frogs with tracking devices attached appeared to use retreat sites similarly to individuals without tracking devices; tracked frogs were commonly observed in close association with, and sharing retreat sites with, frogs without tracking devices.

Frogs fitted with radio transmitters were tracked using a three-element folding Yagi antennae (A.F. Antronics, White Heath, Illinois, USA) and Habit Research HR2500 Osprey VHF receivers. Frogs fitted with diodes were tracked using a portable RECCO R5 transmitter-receiver unit (Recco Rescue Systems, Lidingö, Sweden).

After I located a frog in a diurnal retreat site, I flagged the site, photographed its position, and constructed a detailed diagram allowing us to accurately relocate the site. I returned to retreat sites approximately 24, 48 and 72 hours after initial location and, if the frog had left, I swabbed its exact previous location five times with a sterile cotton swab (Medical Wire & Equipment Co. (Bath) Ltd., Wiltshire, UK).

Diurnal retreat sites for *L. lesueuri* were typically on the ground, on leaf litter, gravel, soil or clay, and it was possible to sample almost every site. In contrast, diurnal retreat sites for *L. nannotis* were typically inaccessible, being under large boulders, in rock fissures or caves. While it was possible to swab individual retreat sites of *L. lesueuri* over time, it was not possible to swab most of the *L. nannotis* retreat sites found during 2004. I therefore focused on two accessible sites under waterfalls at Python Creek where I always found aggregations of *L. nannotis* during the day, and swabbed these sites during 2005, at intervals of approximately four days. Although I attempted to relocate each *L. lesueuri* retreat site every 24 h, there were a number of instances when I was unable to relocate the retreat site of a frog.

Individual *L. lesueuri* were swabbed pre- and post-tracking by swabbing their ventral surface, hands and feet with a sterile cotton swab. These samples and samples taken from retreat sites were evaluated for the presence of *B. dendrobatidis* using Taqman diagnostic quantitative PCR (Boyle et al. 2004). DNA was extracted with PrepMan Ultra, and amplified using primers ITSI-3 Chytr and 5.8S Chytr (Boyle et al. 2004). Each sample was tested in triplicate, and a sample was only recorded as positive if all three replicates indicated the presence of *B. dendrobatidis*. If only one or two replicates were positive for the presence of *B. dendrobatidis*, the sample was regarded as a "suspicious positive" and retested. As a large number of samples (>400) were collected from *L. lesueuri* retreat sites, I only evaluated samples collected from sites used by frogs that tested positive or suspicious positive for *B. dendrobatidis.*

Results

Infection prevalence was low for *L. lesueuri* during the survey periods. At Frenchman Creek, infection prevalence was only 4.5% (n=22) during the warm/wet season surveys and 7.7% (n=26) during the cool/dry season surveys. Similarly, at Lower Tully Creek, infection prevalence was 6.3% (n=16) during surveys in the warm/wet season surveys and 17.4% (n=23) during the cool/dry season surveys. In frogs that tested positive or suspicious positive for *B. dendrobatidis,* infection intensity was also low (Table 5.1).

Of the 81 retreat site swabs from 36 *L. lesueuri* retreat sites, *B. dendrobatidis* was detected using quantitative PCR from only one sample (1.2% of samples tested). Of the 41 swabs taken of *L. nannotis* retreat sites, *B. dendrobatidis* was detected in three samples (7.3% of retreat sites tested). Because all detected levels of *B. dendrobatidis* were very low, and no swabs tested positive on all three replicate tests, I regarded these only as suspicious positives and retested them. On retesting, all samples were negative for *B. dendrobatidis* in all three wells (Tables 5.1 and 5.2).

Table 5.1. Presence of *B. dendrobatidis* at retreat sites 0-3 days after use by *L. lesueuri* that tested positive or suspicious positive for *B. dendrobatidis.*

			Intensity of *B. dendrobatidis* infection (estimated no. of zoospore genome/swab)			**Number of retreat sites testing**	
Season	**Site**	**Frog ID (#)**	**Pre-tracking**	**Post-tracking**	**Days since use**	**Negative**	**Positive**
Warm/wet	Frenchman Creek	1	<1 [a]	<1 [a]	1	1	0
					3	1	0
	Lower Tully Creek	2	6	4 [a]	1	4	0
					2	3	0
Cool/dry	Frenchman Creek	3	57	--	1	3	0
					2	2	0
					3	1	0
					1 [b]	1	0
		4	2327	--	1	2	0
					2	2	0
	Frenchman Creek	5	<1 [a]	--	1	5	0
	Lower Tully Creek				2	4	0
					3	3	0
		6	<1	0	0	1	0
					1	4	0
					2	4	0
					3	2	0[c]
					1 [b]	1	0
		7	0	498	0	0	0
					1	1	0
					2	2	0
					3	2	0
		8	1 [a]	0	0 [b]	1	0
					1	5	0
					2	5	0
					3	2	0
		9	8	41	1	4	0
					2	4	0
					3	4	0
		10	121	--	1	1	0
					2	3	0
					3	3	0
Total						81	0

[a.] suspicious positive.

[b.] Frog present at retreat site for over 48 hours

[c.] Initially one sample tested positive for *B. dendrobatidis* in one of three wells, estimated less than one zoospore genome equivalent present, retested negative in all three wells.

Table 5.2. Presence of *B. dendrobatidis* at retreat sites after use by *L. nannotis* at Python Creek, Tully Gorge.

Season	Location	Date	Number of retreat sites testing	
			Negative	Positive
Warm/wet	1	24/2/05	6	0[a]
		28/2/05	4	0[a]
		04/3/05	4	0[b]
	2	24/2/05	6	0
Cool/dry	1	31/8/05	7	0
		04/9/05	8	0
		08/9/05	6	0
Total			41	0

[a] Initially tested positive for *B. dendrobatidis* in one of three wells, estimated less than one zoospore genome equivalent present. Retested negative in all three wells.

[b] Initially tested positive for *B. dendrobatidis* in one of three wells, estimated four zoospore genome equivalents present. Retested negative in all three wells.

Discussion

The retreat sites tested did not harbour *B. dendrobatidis* within 0-3 days after use, as determined by swabbing and diagnostic PCR. It appears that retreat sites are not a reservoir of infection when *B. dendrobatidis* occurs at low prevalence and intensity on frogs. It is also likely that they are not a major mechanism of transmission within populations where *B. dendrobatidis* occurs at low prevalence and intensity, unless transmission occurs shortly after (<12 h) *B. dendrobatidis* zoospores are shed at a retreat site by an infected frog.

Both the prevalence and intensity of infection were low for *L. lesueuri* when retreat sites were sampled. The prevalence and intensity of infection for *L. nannotis* at the time of the retreat site survey are unknown, however, in a creek adjacent to Python Creek at similar times of the year, they were 50% and 35 zoospore genome equivalents per swab, respectively, in the cool/dry season, and 17% and 5 zoospore genome equivalents per swab in the warm/wet season (McDonald and Skerratt, unpublished data). I suggest that retreat sites may become an important source of infection and reservoir when prevalence and intensity are higher, such as in recent outbreaks in Panama (Lips et al. 2006).

The survival of *B. dendrobatidis* in the environment is likely to vary due to differences in various abiotic and biotic factors. Laboratory studies have demonstrated that *B. dendrobatidis* growth and survival depend on substrate pH (Johnson & Speare 2005; Piotrowski et al. 2004) and the presence and composition of bacteria and oomycetes (Harris et al. 2006; Longcore et al. 1999). It is therefore possible that conditions were not optimal at my study sites, and that variation in such factors may cause differential survival of *B.*

dendrobatidis in the environment between sites. It is also possible that I failed to detect *B. dendrobatidis* DNA due to inhibition of the PCR although I found no widespread evidence of inhibition.

The few low initial positive tests for retreat sites that tested negative upon retesting might be explained by contamination of samples during loading of the PCR machine. This could be due to aerosolisation of DNA created during pipetting. Automation of this procedure and reducing the number of standards in each PCR run might reduce contamination, as might automation of extraction procedures and conducting extractions on one sample at a time. A positive control swab is included in each batch of 24 extractions immediately prior to the negative control swab during extractions and loading of samples into the PCR machine. It is possible that this control may be contributing to contamination, however it is important to maintain this control as it increases the sensitivity of the negative control to test for contamination during extractions and loading. To maximize the chances of detecting contamination of reagents at any step of the PCR, a negative control that contains only reagents is included in each PCR run. If either of the negative controls is positive then all the samples are retested. Regardless of the cause, these findings demonstrate that low-level contamination of PCR tests can occur and that is important to test samples in triplicate to ensure a high specificity for the PCR test. Tests with only a proportion of replicates positive especially when the concentration of detected DNA is low should be regarded as "suspicious positives" and should be retested if one wishes to confirm that they are positive.

Further work is required to determine whether retreat sites are important in the host-pathogen relationships between *B. dendrobatidis* and amphibians, particularly in regions and in species where the prevalence and intensity of *B. dendrobatidis* infection is high. Although *B. dendrobatidis* was not detected at frog retreat sites in the current study, it is still possible that the presence of an environmental reservoir is one factor causing the extremely high transmission rates seen in chytridiomycosis outbreaks in some systems (Berger et al. 1998; Lips et al. 2006). My results, however, suggest that the environment may not provide a continual source of infection for amphibian species when the prevalence and intensity of infection with *B. dendrobatidis* are low. It may be possible to eliminate or greatly reduce the intensity of *B. dendrobatidis* in these situations by treating amphibians, without needing to decontaminate the environment, thereby enabling recolonization or the successful release of captive-bred animals.

CHAPTER SIX: MOVEMENT PATTERNS AND HABITAT USE OF RAINFOREST STREAM FROGS IN NORTHERN QUEENSLAND, AUSTRALIA: IMPLICATIONS FOR EXTINCTION VULNERABILITY

Abstract

Biological diversity is declining around the world at an accelerating rate. In order to understand the causes of extinction, and attempt to prevent future extinctions, species-specific ecological and behavioural information is required. This information is currently lacking for the vast majority of species. Amphibians are one of the most highly threatened groups of animals, but their effective conservation is hampered by a paucity of basic ecological knowledge, particularly for tropical stream-breeding species in which declines have been most common and severe. I examined the movement patterns and habitat use of three stream-breeding frogs at five sites in northern Queensland, Australia. Movement and habitat use differed significantly among species. *Litoria lesueuri* moved more frequently and greater distances and were often located away from streams, moving between intact rainforest and highly disturbed environments. *Litoria genimaculata* moved less frequently and shorter distances, and were more restricted to stream environments, but were often located in the canopy. *Litoria genimaculata* occasionally moved large distances along and between streams, but were never located outside of intact rainforest. *Litoria nannotis* moved almost as frequently as the other species, but remained in streams during the day, only moving away from them at night. *Litoria nannotis* did not move large distances along or move between streams, and were always located within intact rainforest, almost always returning to the same sheltered positions within streams after nocturnal excursions. The differences among species in terms of movement patterns and habitat use may have important implications for the ability of these species to cope with disease, habitat modification or climate change. Because of its sedentary behaviour, narrow habitat tolerance and affinity for stream environments, *L. nannotis* may be more vulnerable to extinction in human-modified landscapes.

Introduction

Biological diversity is declining around the world at an accelerating rate (Balmford et al. 2003; Brook et al. 2003; Thomas et al. 2004), with current extinction rates estimated to be up to a thousand times greater than background (Baillie et al. 2004; Lawton & May 1995). In order to form effective conservation strategies aimed at reducing this rate of loss, it is necessary to understand the causes of extinction and why some species are more vulnerable to extinction than others (Kotiaho et al. 2005). However, in order to do this, basic, species-

specific ecological and behavioural information is required; information which is currently unavailable for the vast majority of species (Greene 1994).

Core aspects of the ecology and population biology of a species are movement patterns and habitat use. An understanding of these factors is the first step in habitat protection, and can be used to identify species at most risk of extinction in human-modified landscapes (Hanski & Zhang 1993; Travis & Dytham 1999), or under future climate scenarios. Other potential threats such as disease may also be affected by species-specific movement patterns (Altizer et al. 2000; Ezenwa 2004) and habitat use (Brooks et al. 2006; Grutter 1998; Krasnov et al. 1998).

Amphibians are one of the most highly threatened groups of animals. Currently, almost one-third of amphibian species are listed as globally threatened and almost half are known to be experiencing population declines, making amphibians more highly threatened and declining faster than either birds or mammals (Stuart et al. 2004). Perhaps more than any other vertebrate group, the effective conservation of amphibians is hampered by a lack of basic ecological information. To date, our knowledge of amphibian movement and habitat use has been derived almost exclusively from studies on salamanders and on pond-breeding frogs in temperate regions. However, declines have been most frequent and severe in tropical, stream-breeding species (Lips et al. 2003b; Stuart et al. 2004; Williams & Hero 1998). While temperate, pond-breeding species typically hibernate in terrestrial areas away from their breeding sites, and migrate to breeding ponds during the summer months (Dole & Durant 1974), the movements of tropical stream-breeding amphibians are likely to differ, with reproduction occurring within their non-breeding home range (Duellman & Trueb 1986).

I examined the movement patterns and habitat use of three stream-breeding frogs in northern Queensland, Australia. These species co-occur but have declined to varying degrees in recent decades; the waterfall frog *Litoria nannotis,* has experienced large and long-lasting population declines (IUCN Endangered), the green eyed tree frog *Litoria genimaculata,* has declined and then recovered (IUCN Least Concern, however Australian populations of this species are considered to be Near Threatened) and the stoney creek frog *Litoria lesueuri* has not experienced population declines (IUCN Least Concern; see below; IUCN et al. 2006; McDonald & Alford 1999; McDonald 2002; McDonald et al. 2005).

The limited information we have on the ecology and behaviour of these species is derived primarily from nocturnal stream surveys (ie. Richards & Alford 2005). There is presently very little information on the diurnal habits, movement, and use of off-stream habitat of these species due to their complex, densely vegetated habitats and their cryptic appearance and habits. In addition, there is almost no information on how these or any other species of amphibians use three-dimensional space. In this study, I tracked frogs of each species and examined differences between species, sexes and seasons.

Methods

Study species

All species are large to medium-sized hylid frogs, with females larger than males (Table 6.1). Recently, the taxonomy of the *L. lesueuri* group has been revised (Donnellan & Mahony 2004). In tropical northern Queensland, the group was split into two species, *L. jungguy* and *L. wilcoxii*, which occur in sympatry, hybridise in the region and are indistinguishable on the basis of morphology (Donnellan & Mahony 2004). Population declines have not been observed in either species. I therefore continue to refer to the study population as *L. lesueuri* while recognising that the population contains two morphologically indistinguishable species.

Table 6.1. Mean snout-vent-length (SVL) and weight of individuals tracked.

Species	Sex	Mean SVL (mm)	Mean Weight (g)
L. genimaculata	Female	60.5	18.6
	Male	41.9	6.0
L. lesueuri	Female	64.5	29.4
	Male	42.0	7.4
L. nannotis	Female	52.0	13.7
	Male	45.4	9.9

Study site

The study was conducted at five sites within the tropical rainforests of northern Queensland, Australia; Birthday Creek, Paluma State Forest ("Paluma", 146º10'02" E 18º58'54" S, 800 m asl), Python Creek ("Tully Gorge", 145º35'E 17º46'S, 200 m asl), an unnamed creek ("Lower Tully", 145º41'E 17º48'S; 70 m asl) in Tully Falls Forest Reserve, an unnamed creek in Kirrama State Forest ("Kirrama", 145º52 E 18º11 S; 200 m asl) and Frenchman Creek, in Wooroonooran National Park ("Babinda", 145º55' E 17º20' S 20-100 m asl). All sites are relatively undisturbed rainforest streams. The creek beds are composed of rocks, ranging from small pebbles, to large boulders (of over 10 m in diameter). All streams contained pools and riffles, and most sites contained a number of waterfalls. A marked transect was established along each stream to serve as a reference for frog locations.

Tracking

Frogs were tracked using either radio telemetry or harmonic direction finding. Only frogs weighing more than 11g were tracked via radio telemetry. Radio transmitters (models BD-2N and BD-2NT; Holohil Systems Ltd., Ontario, Canada; weighing approximately 0.67 g including harness and with a battery life of approximately 3 weeks) were attached to a harness made of silicone tubing, designed to minimise restrictions on movement and avoid

causing discomfort to the frog. Frogs that were too small to be radio tracked and a number of larger individuals were tracked using harmonic direction finding (Langkilde & Alford 2002). This requires attachment of a small diode with a whip antenna to the same specially designed harness (weighing approximately 0.23g including harness), which is then placed around the waist of a frog. Frogs were weighed, tracking devices were fitted *in situ* and frogs were released at point of capture after less than five minutes of handling. Frogs wearing either tracking device did not carry harnesses and associated equipment that weighed more than 6% of their total body weight. This percentage is just over half the recommended maximum relative weight for an attached tag (10% of the body weight; Richards et al. 1994).

Frogs fitted with radio transmitters were tracked using a Telonics TR-4 Tracking Receiver (Telonics, Inc., Mesa, AZ, USA; 2004 warm/wet season only) and a HABIT Research HR2500 Osprey VHF Receiver (HABIT Research, Victoria, B.C., Canada); I used a three-element folding Yagi antenna with both receivers (A.F. Antronics, White Heath, Illinois, USA). Frogs fitted with diodes were tracked using a portable RECCO R5 transmitter-receiver (Recco Rescue Systems, Lidingö, Sweden). The system consists of a hand-held device that acts as both the transmitter and receiver, and a battery and earphones. Tags were self-built using commercial germanium diodes (see Langkilde & Alford (2002) for a description of methodology). In the field, I obtained fewer fixes on frogs using harmonic direction finding, but measures of movement and habitat use did not differ significantly between techniques.

Two surveys, one in the cool/dry season (May-September) and one in the warm/wet season (October-April), were carried out at each site except Birthday Creek, where only a warm/wet season survey was performed. Surveys lasted approximately 16 days. The location of each frog was determined once during the day (0700-1900) and once at night (1900-0700) over the duration of the survey period. At each survey, the position of each frog was recorded as its location along the transect (m), horizontal distance from the stream (m), and approximate elevation above stream (m). Distance moved between surveys was calculated for each individual by summing the distances moved between successive observations and dividing by the total number of surveys I tracked each individual. As movements were assumed to be straight lines between two successive points they were estimates of the minimum distances moved by frogs; actual distances would be greater. At each observation, I recorded whether the frog was in intact rainforest (surrounded by rainforest, with no obvious signs of anthropogenic-modification), in a disturbed, non-forested area less than 5m from intact rainforest (ie. roads), or more than 5m from intact rainforest (ie. pasture or agricultural crops). I also recorded if frogs were in sheltered or exposed positions.

I excluded data from the night following tag attachment due to the potential short-term behavioural effects of handling (Langkilde & Alford 2002). Any effects are unlikely to

persist after the first night of tag attachment (Rowley & Alford in press-b; Chapter 2). To avoid pseudoreplication and biasing my results by including more data on frogs that were located more often, I used individuals as replicates and compared summary statistics calculated for each animal. I used median rather than mean values for each individual as I was interested in how long the average distance moved by each individual was, as opposed to the average of all the distances it moved, which would be distorted due to the infrequent, large movements made by many individuals. I also examined maximum values separately as I wanted to capture such infrequent, but important, long distance movements. Due to obvious differences between the diurnal and nocturnal behaviour of all species, I examined diurnal and nocturnal data separately. In order to determine if there were differences in movement use between groups, I performed multi-response permutation procedures (MRPP) using Blossom Statistical Software (Cade & Richards 2005). I performed the analysis in a stepwise fashion, testing for differences between species, sex season and sites, in that order. If a difference was detected between groups, the next analysis was performed on each of those groups separately. I used Monte Carlo hypothesis testing, with 20,000 iterations, and carried out separate tests for the combination of movement and habitat use variables. Habitat specificity could not be included my analysis as the procedure does not allow variables with constant values as recorded for *L. genimaculata* and *L. nannotis*.

Results

A total of 117 frogs were tracked during the study period; 28 *L. nannotis,* 27 *L. genimaculata* and 62 *L. lesueuri* (Table 6.2). Throughout the survey periods, a total of 2,111 fixes, or locations of individual frogs, were obtained. On average, each frog was located 17 times (with a minimum of 5 fixes and a maximum of 29 fixes). The weight of frogs tracked did not change significantly over the study period (Wilcoxon Signed Ranks Test; Z= -1.361, p= 0.173, n=70). In addition, frogs with attached tags appeared to be uninhibited in their movements, were commonly observed in close association with frogs without tags, and were observed calling and in amplexus as soon as 12 h after tag attachment.

Movement characteristics differed significantly among species (Tables 6.3 and 6.4). *Litoria lesueuri* moved the most often between observations, moving position between 90% of observations, compared to *L. genimaculata* and *L. nannotis*, which moved between only 73% and 71% of observations respectively. *Litoria lesueuri* also moved greater distances compared to the other two species, averaging a median of 5.0 m and a maximum of 47.6 m between surveys. In contrast, *L. nannotis* averaged a median of 2.9 m and maximum of 21.0 m between surveys. Frogs of all species spent several days in the same area, however the median and maximum number of surveys between long-distance movements was highest in *L. nannotis* and lowest in *L. lesueuri* (Table 6.4). The average maximum number of surveys

Table 6.2. Summary of the number of individual frogs tracked.

Species	Season	Site	Sex	Number tracked using	
				Radio-telemetry	Harmonic direction finding
L. genimaculata	Cool/dry	Kirrama	Male	0	2
			Female	4	1
		Python Creek	Male	0	6
			Female	2	0
	Warm/wet	Kirrama	Male	0	0
			Female	2	1
		Python Creek	Male	0	0
			Female	0	0
		Paluma	Male	0	7
			Female	1	1
	Totals		**Male**	**0**	**15**
			Female	**9**	**3**
L. nannotis	Cool/dry	Kirrama	Male	0	3
			Female	3	2
		Python Creek	Male	1	1
			Female	5	1
	Warm/wet	Kirrama	Male	0	0
			Female	3	0
		Python Creek	Male	1	0
			Female	8	0
	Totals		**Male**	**2**	**4**
			Female	**19**	**3**
L. lesueuri	Cool/dry	Babinda	Male	0	6
			Female	10	3
		Lower Tully	Male	2	13
			Female	4	1
	Warm/wet	Babinda	Male	0	3
			Female	6	2
		Lower Tully	Male	5	3
			Female	4	1
	Totals		**Male**	**7**	**25**
			Female	**24**	**6**

Table 6.3. Multi-response permutation procedures (MRPP) for differences between species, sex and season using Monte Carlo hypothesis testing. I performed the analysis in a stepwise fashion. If a difference was detected between groups, the next analysis was performed on each of those groups separately. * indicates significance at 0.05 level.

Test			δ	**P**
Species			4.967	<0.001*
Sex	*L. lesueuri*		5.27	0.037*
	L. genimaculata		5.26	0.017*
	L. nannotis		5.691	0.107
Season	*L. lesueuri*	Female	5.313	0.004*
		Male	5.133	0.125
	L. genimaculata	Female	5.235	0.424
		Male	4.854	0.121
	L. nannotis	---	5.328	<0.001*

where frogs did not move more than five meters was 4.3 in *L. lesueuri,* 6.3 in *L. genimaculata* and 9.6 in *L. nannotis*. Frogs of all species were nocturnal, and no tracked individuals moved during the day.

The degree of association with the stream differed among species, with *Litoria lesueuri* and *L. genimaculata* spending large amounts of time away from the stream (Table 4). *L. lesueuri* was the least associated with the stream, having the highest median and maximum horizontal distances from the stream, but was not often far above the stream. *Litoria lesueuri* often remained away from any stream or water body (up to 260 m) for days at a time. While *L. genimaculata* also moved large horizontal distances from the stream (up to 185 m), and stayed at these distances for days at a time, they were much more arboreal than *L. lesueuri,* and were located in the canopy (up to 20 m above the stream) during most observations. In contrast, *L. nannotis* were almost always restricted to the stream environment during the day, but regularly moved relatively large distances away from the stream at night (up to 35 m horizontally from the stream and 15 m above the stream), returning to the stream by the following diurnal surveys.

In addition to their degree of association with the stream, frogs differed in their degree of habitat specificity. While *L. nannotis* and *L. genimaculata* never moved outside of intact rainforest, *L. lesueuri* were in disturbed, non-forested areas within five meters of intact rainforest during 9.6% of observations. On these occasions, frogs were typically located on dirt roads or mown grass next to sealed roads. However, on 0.7% of observations, *L. lesueuri* were located in non-forested areas further than five meters from intact rainforest. On these

Table 6.4. Movement patterns and habitat use of *L. lesueuri*, *L. genimaculata* and *L. nannotis*. Only categories that are significantly different from each other are shown. Values represent means (and ranges) of data obtained using individual frogs as replicates.

			L. lesueuri			***L. genimaculata***		***L. nannotis***	
			Female		**Male**	**Female**	**Male**	**---**	
			Cool/dry season	**Warm/wet season**	**---**	**---**	**---**	**Cool/dry season**	**Warm/wet season**
Percent of observations individuals moved between surveys			89.4 (67-100)	84.4 (60-100)	92.5 (63-100)	65.6 (29-100)	79.7 (40-100)	60.9 (0-100)	83.8 (38-100)
Distance moved between surveys		Median	5.5 (1-13.2)	6.0 (0.3-11.5)	4.3 (1-14.3)	3.3 (0-11.5)	2.2 (0-4)	0.85 (0-4)	5.7 (0-10)
		Maximum	80.1 (13-306)	31.2 (11-61)	36.9 (5-164)	47.9 (8-170)	13.2 (5-42)	8.25 (0-34)	38 (5-95)
Number of surveys between long distance (>5m) movements		Median	0.1 (0-0.5)	0.2 (0-2.5)	0.5 (0-3)	0.5 (0-4)	6.8 (3-12)	12.3 (4-27)	6 (1-18)
		Maximum	3.6 (1-7)	4.6 (2-10)	4.5 (2-14)	5.6 (2-14)	0.6 (0-2)	7.9 (0-27)	0.1 (0-0.1)
Horizontal distance from stream (m)	Diurnal	Median	22.4 (5-74)	3.3 (0-11)	10.3 (1-57.3)	1.2 (0-5)	0.9 (0-2.5)	0 (0-0)	0.1 (0-0.5)
		Maximum	49.2 (11-260)	12.5 (0-77)	17.2 (2-59)	18.8 (0-150)	3.1 (1-10)	0.6 (0-1)	1.4 (0-7)
	Nocturnal	Median	21.2 (5-71.5)	6.6 (3-10)	9.8 (0-60)	1.5 (0-5)	1.1 (0-2)	0.2 (0-1.5)	1.2 (0-5.5)
		Maximum	50.1 (10-251)	21.3 (8-40)	19.1 (1-75)	23.4 (1-185)	3.1 (1-6)	4 (0-15)	9.7 (1-25)
Elevation above stream (m)	Diurnal	Median	0.8 (0-4.5)	2.1 (0.5-6)	1.7 (0-7)	5.4 (0.3-10)	3.3 (0.2-10.5)	0 (0-0.5)	0.1 (0-0.5)
		Maximum	2.1 (0-10)	5.3 (0.5-29)	4.2 (0-20)	8.3 (0.5-15)	7.4 (0.5-20)	0.9 (0-10)	1.5 (0-15)
	Nocturnal	Median	0.9 (0-4.5)	1.4 (0-6)	1.4 (0-7)	4.3 (0.25-10)	3.5 (1-6)	0.5 (0-4)	2.7 (0-9)
		Maximum	2.9 (0-10)	4.4 (1-15)	4.5 (0-30)	9.6 (1-20)	8.0 (2-20)	2.9 (0-10)	10.5 (2-15)
Percent of observations in sheltered position	Diurnal		42.6 (0-88)	32 (0-61)	51.5 (0-100)	33.8 (0-100)	35 (0-100)	95.8 (33-100)	91.1 (75-100)
	Nocturnal		15.2 (0-50)	6.2 (0-15)	10.8 (0-38)	14.7 (0-50)	9 (1-69)	60 (0-100)	17 (0-58)

occasions, frogs were observed in agricultural land such as sugar cane plantations or pastures up to approximately 100 m from intact rainforest. Individual *L. lesueuri* often moved between forested and disturbed habitat, and were observed crossing sealed roads and using diurnal retreat sites on mown grass, dirt roads and sugar cane plantations, often for days at a time.

Within the forest, both *L. lesueuri* and *L. genimaculata* were observed moving large distances along streams (>100 m) and between streams (>200 m apart) during the survey periods. *Litoria nannotis* did not move great distances along streams (always <50 m) and were never observed moving between streams.

The frequency of observations in which frogs were in sheltered positions differed among species, and between diurnal and nocturnal surveys (Table 6.4). During diurnal observations, *L. nannotis* were in sheltered positions an average of 94% of the time, compared to 45% and 35% for *L. lesueuri* and *L. genimaculata* respectively. Frogs were less frequently in sheltered positions at night. During nocturnal surveys, *L. nannotis* were in sheltered positions an average of only 41% of observations, compared to 11% and 12% for *L. lesueuri* and *L. genimaculata* respectively.

Movement and habitat use characteristics differed with sex in *L. lesueuri* and *L. genimaculata* (Tables 6.3 and 6.4). Females of both species moved greater distances and were less restricted to the stream environment, particularly with respect to horizontal distance, compared to males (Table 6.4). Movement and habitat use did not differ between sexes in *L. nannotis* (Table 6.3).

Movement and habitat use differed with season in *L. lesueuri* females and in *L. nannotis* (Tables 6.3 and 6.4). Compared to the warm/wet season, *L. lesueuri* females moved greater distances, and were less restricted to the stream but remained in sheltered retreat sites more often in the cool/dry season (Table 6.4). *Litoria nannotis* moved less frequently and smaller distances, were more restricted to the stream environment and were more often in sheltered retreat sites in the cool/dry season (Table 6.4).

Discussion

In this study, movement patterns differed significantly among species. *Litoria lesueuri* was the most mobile species, moving the most often and moving the greatest distances and *L. nannotis* was the most sedentary species. The maximum distances moved per day in all my study species were relatively large: 306 m for *L. lesueuri*, 170 m for *L. genimaculata*, and 95 m for *L. nannotis*. In temperate species, the maximum distances moved in this study are typical of migrations to and from breeding sites (Forester et al. 2006; Schroeder 1976; Vasconcelos & Calhoun 2004), whereas they were simply relatively long short-term movements in the activity ranges of my study species. In addition, as these are straight-line

distances, the actual distances moved in my study species were probably much greater (Lemckert & Brassil 2000).

The study species also differed in their degree of association with the stream environment, with *L. lesueuri* and *L. genimaculata* spending large amounts of time at considerable distances from the stream. Although *L. nannotis* did not move away from the stream for extended periods, the species did move relatively large distances away from the stream at night. In previous studies, *L. nannotis* was reported to be more restricted to the stream environment, with fewer fixes obtained away from the stream and no frogs recorded further than 15 m from the stream (Hodgkison & Hero 2001). This disparity may have resulted from differences in transmitters used and proportion of fixes obtained during tracking. Hodgkison and Hero (2001) used transmitters that were substantially heavier than in this study, weighing up to 16% of the frogs' body weight. Further, in that study frogs were located during an average of 46.3% of surveys compared to 97.4% during this study. It seems possible that during many of those failed location attempts, frogs were further from the stream, biasing their data.

Although I did not track frogs long enough to obtain asymptotic estimates of home range size, it is clear that that *L. lesueuri* and, to a lesser extent, *L. genimaculata,* move greater distances than *L. nannotis,* and if any of the species have true home ranges, those of the former species are likely to be larger. Both *L. lesueuri* and *L. genimaculata* made extended journeys, often covering large distances each night, and moving in a single direction for a number of nights. For example, one female *L. lesueuri* moved almost 900 m in straight-line distance in only ten days. In contrast, *L. nannotis* movements were almost exclusively short nocturnal excursions away from the stream, with frogs returning to the stream that same night. The movement patterns I observed in *L. lesueuri* and *L. genimaculata*, with a relatively high incidence of long departures from the starting point, are similar to those observed in *Bufo marinus* by Schwarzkopf and Alford (2002), which they interpreted as meaning that *B. marinus* are nomadic, lacking long-term fixed home ranges. If this is true for the rainforest species I observed, it could explain their relatively low rates of recaptures in long-term mark-recapture studies (e.g., Richards & Alford 2005).

Species differed in their degree of habitat specificity, with *L. lesueuri* the only species observed moving through and using disturbed, non-forested habitats. *L. genimaculata* and *L. nannotis* were never observed outside of intact rainforest, although these species differed in their movements within this habitat, with *L. genimaculata* moving along and between streams, and *L. nannotis* remaining in specific sections of the stream during my survey periods. These findings are consistent with previous descriptions of *L. lesueuri* as a habitat generalist and *L. nannotis* and *L. genimaculata* as associated with wet forests (Williams &

Hero 2001), and may have important implications for population dynamics, dispersal ability and gene flow.

The proportion of time spent in sheltered positions differed considerably among species; it was higher in *L. nannotis* than in *L. lesueuri* and *L. genimaculata*. Frogs in sheltered positions typically experience microenvironmental conditions buffered from temperature extremes, desiccation and direct sunlight, making them less likely to experience physiologically stressful conditions, and it may be that *L. nannotis* is more sensitive to environmental conditions.

In the study species, movement patterns and habitat use differed with sex in *L. lesueuri* and *L. genimaculata*, but not in *L. nannotis*. In amphibians, there appears to be no consistent sexual difference among species with respect to movement patterns. While home range, movements and frequency of movement is higher in females of some species (Bartelt et al. 2004; Bellis 1965; Kramer 1973; Miaud et al. 2000; Muths 2003; Ovaska 1992), they are smaller or do not differ in other species (Dole & Durant 1974; Jameson 1955; Smith & Green 2006). The larger movements I observed for female *L. lesueuri* and *L. genimaculata* may result from high food requirements due to greater reproductive investment (Muths 2003), from larger body size and hence reduced desiccation risk, or from the lack of territoriality or site fidelity observed in males of the same species (Rowley, unpublished data). Females of *L. lesueuri* and *L. genimaculata* are observed much less frequently during nocturnal stream surveys, with 25 times more male than female *L. genimaculata* observed during a seven-year study at one of my sites (Richards & Alford 2005). Rather than a highly skewed sex-ratio, my study reveals that this bias is simply a difference in observability.

Unlike many temperate species, tropical forest frogs are typically active all year and in some species, movement does not differ between seasons (Kam & Chen 2000; Neckel-Oliveira & Gascon 2006). Although all three species in this study were active during both the warm/wet and cool/dry seasons, movement patterns changed seasonally in *L. genimaculata* females and *L. nannotis*. *Litoria nannotis* in particular moved less frequently and smaller distances, and were more often in sheltered positions during the cool/dry season, which may be due to cooler temperatures, lower humidity and less rainfall, or differences in prey availability (Donnelly 1991; Liebermann & Dock 1982). *Litoria genimaculata* females were also more often in sheltered positions in the cool/dry season, but moved greater distances, perhaps due to decreased prey availability causing animals to forage over a greater area.

The results of this study have practical implications for visual encounter surveys, the majority of which take place along a stream. As the observability of these species declines with distance from the stream and time spent in sheltered locations, population estimates will vary. At any time, a proportion of the population will be undetectable, and this will vary depending on species, sex and season. For example, during nocturnal surveys during the

cool/dry season, an average of 60% of all *L. nannotis* remained under boulders within the stream, and of the remaining frogs, many moved away from the stream and would go undetected during stream visual encounter surveys. In addition, *L. lesueuri* and *L. genimaculata* spent the majority of their time away from the stream itself, and would generally not be detected. Even when frogs were in or adjacent to the stream during this study, they were often extremely cryptic and difficult to find, and would almost certainly go undetected. If, as my data suggest may be the case for *L. lesueuri* and *L. genimaculata,* at least some species are nomadic, the already difficult problem of conducting censuses in fixed plots becomes more difficult, since even with mark-recapture data it will be difficult to distinguish between emigration and mortality, and population size estimates will be inflated by the immigration of unmarked individuals between surveys.

The movement and habitat use of a species may have important consequences for the disease dynamics of a species. As parasites and pathogens often accumulate in the hosts' environment over time, less mobile hosts tend to have higher contagious parasite burdens (Altizer et al. 2000; Ezenwa 2004). Similarly, the abundance and species composition of parasites in many species differ even between very similar habitats (Grutter 1998; Krasnov et al. 1998). For example, the platyhelminth parasite species richness of a Costa Rican frog assemblage is highly correlated with the amount of time a host spends in association with aquatic habitats (Brooks et al. 2006). In addition, purely terrestrial amphibian species do not typically experience population declines, or experience reduced rates of decline, from the amphibian disease chytridiomycosis (Hero et al. 2005; Lips et al. 2006; McDonald & Alford 1999; Williams & Hero 1998). Therefore, by virtue of its sedentary behaviour and affinity for the stream environment, *L. nannotis* may be more affected by parasites and pathogens than either *L. lesueuri* or *L. genimaculata*. The large movements of *L. lesueuri* within and between streams and different habitat types suggest that it could act as a vector in introducing or reintroducing *B. dendrobatidis* to habitats.

The differences among species in movement patterns and habitat use may have important implications for the ability of these species to cope with habitat modification or climate change. *Litoria lesueuri* appears to have a broad tolerance to varied moisture and thermal regimes, inhabiting highly disturbed environments, frequently moving large distances in excess of 100 m per day and traversing potential barriers such as agricultural land and sealed roads. High dispersal ability and wide habitat tolerance are likely to allow this species to persist in modified landscapes, and be relatively tolerant of habitat modification, habitat fragmentation and climate change. In contrast, the sedentary nature and narrow habitat tolerance of *L. nannotis* are likely to make *L. nannotis* highly vulnerable to disturbance, with the species likely to become isolated in forest fragments and unable to persist in the face of climate change. *Litoria genimaculata* fell between the two species, moving great distances

along and between streams, but not moving outside of the forest. The maintenance of large terrestrial vegetation buffers along streams and connectivity between forest fragments is likely to be extremely important for the long-term conservation of both *L. nannotis* and *L. genimaculata*.

This study demonstrates that similarly sized, closely related species that occur in the same rainforest streams may differ greatly in terms of movement patterns and habitat use. Thus, broad classifications such as 'riparian' may fail to capture important differences between species that can only be gained via detailed ecological studies.

CHAPTER SEVEN: BEHAVIOUR, MICROENVIRONMENT, AND DISEASE VULNERABILITY IN TROPICAL AUSTRALIAN FROGS.

Abstract

The disease chytridiomycosis is implicated in the declines and extinctions of many amphibian populations and species. Some species are severely affected by the disease, while other, sympatric species remain unaffected. Because thermal and hydric environments experienced by frogs can strongly affect their susceptibility to chytridiomycosis, some interspecific differences in the effects of the disease may be caused by differences in microenvironment use. I examined the thermal and hydric microenvironments used by three species that differ in natural susceptibility to decline from the disease. The less susceptible species often reached temperatures above the *in vitro* optimum growth range (17-25ºC) and maximum thermal tolerance (30ºC) of *B. dendrobatidis*, while the most susceptible species was usually within the optimum growth range of *B. dendrobatidis* and never exceeded its thermal tolerance. Interspecific differences in behaviour are therefore likely to affect the susceptibility of amphibians to chytridiomycosis. Temporal and spatial variation in the microenvironments available to frogs may also explain variation in infection prevalence and host mortality, which are usually lower in the warm/wet season, when frog microenvironments are less favourable to *B. dendrobatidis* growth and survival. Relatively subtle changes in the availability of microenvironments due to climate change may be capable of precipitating disease-related amphibian declines and extinctions.

Introduction

The amphibian disease chytridiomycosis (Berger et al. 1998) has been implicated in mass mortalities, population declines, and extinctions of amphibian populations and species around the world (Berger et al. 1998; Bosch et al. 2001; Bradley et al. 2002; Lips 1999; Lips et al. 2006; Rachowicz et al. 2006; Weldon & Du Preez 2004). Chytridiomycosis is caused by the fungus *Batrachochytrium dendrobatidis*, which belongs to the order Chytridiales (Longcore et al. 1999). The disease spreads within and among individual amphibians via the release of motile, waterborne zoospores (Nichols et al. 2001). In many cases, frog population declines attributed to *B. dendrobatidis* have been dramatic, resulting in the rapid local extinction of 50% or more of the amphibian species at particular sites (Lips 1999; Lips et al. 2006) and large reductions in the abundance of remaining species (Lips et al. 2006; Lips & Donnelly 2002). However, in almost all cases, amphibian species that have disappeared or declined due to chytridiomycosis coexist with non-declining species (Lips et al. 2006; Lips & Donnelly 2002; Puschendorf et al. 2006; Retallick et al. 2004). Infected individuals of a wide range of

amphibian species may survive for years in the wild with no clinical signs of disease (Hanselmann et al. 2004; Hopkins & Channing 2003; McDonald et al. 2005; Retallick et al. 2004). Many non-declining species are highly susceptible to *B. dendrobatidis* in the laboratory, succumbing rapidly to chytridiomycosis, but are able to persist with infections in the wild (Berger et al. 1999; Kriger & Hero 2006; McDonald & Alford 1999; Woodhams et al. 2003). Differences in the susceptibility of amphibian species in the field must therefore not be entirely due to innate properties of the species that confer resistance to the disease, but due to some external factor or factors that are present in the field but absent from laboratory experiments.

One factor absent in laboratory experiments but present under field conditions is variation in environmental conditions, and the opportunity to respond behaviourally to such variation. Disease is the result of interactions between a pathogen, its host and the environment; a pathogen will only cause disease in a host if environmental conditions are conducive to do so. Epizootics of infectious diseases are therefore highly influenced by environmental factors, and in some cases, environmental conditions may be the most relevant elements in the epidemiological process (Benz 1987). Temperature can strongly affect the occurrence and development of many diseases (Colhoun 1973) and particularly in non-endothermic hosts, may actually determine the outcome of infection (Blanford & Thomas 1999a; Blanford & Thomas 1999b; Blanford & Thomas 2000; Blanford et al. 1998; Carruthers et al. 1992; Inglis et al. 1996). Other environmental factors such as humidity may also be important, particularly with respect to fungal pathogens (Benz 1987).

In culture, the optimum temperature for *B. dendrobatidis* growth is 17-25ºC (Piotrowski et al. 2004), and while the pathogen can grow at 6-28ºC and can survive at lower temperatures, it dies at temperatures above 29-30°C (Longcore et al. 1999; Piotrowski et al. 2004). In the laboratory, the progress and outcome of chytridiomycosis in infected amphibians is also influenced by thermal conditions. Low and/or fluctuating temperatures can retard the development of the disease in frogs, and elevated body temperatures can clear frogs of infection (Berger et al. 2004; Woodhams et al. 2003). Hydric conditions are also important for *B. dendrobatidis*, with sporangia and zoospores killed by desiccation (Berger 2001; Johnson et al. 2003), and disease progression in the laboratory is more rapid under in mist than in either constant rain or very dry conditions (Alford and Woodhams, unpublished data).

In the field, infection prevalence and host mortality are highly correlated with environmental conditions, and are highest during cooler months (Berger et al. 2004; Bradley et al. 2002; McDonald et al. 2005; Retallick et al. 2004; Woodhams & Alford 2005), and at higher elevations (McDonald et al. 2005; Woodhams & Alford 2005). However, these studies have considered only mean ambient environmental conditions, which are unlikely to reflect the true environment of the host-pathogen interaction (Thomas & Blanford 2003). For

example, while the body temperatures of ectotherms are related to environmental temperature, they also reflect a complex interplay between the behaviour, physiology, morphology, ambient conditions and microenvironment of animals (Carey 1978). As a result, the actual microenvironments experienced by amphibians and their pathogens may differ greatly from macroclimatic conditions (Brattstrom 1963). Recently, a "climate-linked epidemic" hypothesis has been proposed, which rests on the assumption that relatively small shifts in temperature, humidity, and cloud cover may cause local environmental conditions to tip the balance between coexistence with a pathogen and local extinction (Pounds et al. 2006). Although body temperatures of amphibians at night, during cloudy days or in sheltered positions may approach substrate, air or water temperatures, in other conditions many species may attain much higher or lower temperatures than ambient (Brattstrom 1963). For instance, basking behaviour in *Rana mucosa* caused an average of 14.4ºC elevation of body temperature compared to frogs in the shade (Bradford 1984). Consequently, body temperatures in ectotherms may exceed the upper limits for pathogen survival, even when ambient temperatures do not (Carruthers et al. 1992), and behavioural thermoregulation, or simply microhabitat selection, may control or eliminate infection by *B. dendrobatidis*. Even relatively small differences in temperature may be critical in determining disease susceptibility; for example an increase of just 2ºC in the diurnal maximum temperature experienced by the variegated grasshopper *Zonocerus variegates* leads to recovery from a fungal disease that otherwise causes high mortality rates (Blanford et al. 2000).

Amphibians also vary in their association with water, with some species being entirely aquatic and others living almost entirely independently of standing or flowing water (Feder 1992), and therefore experiencing a wide range of hydric environments; this is likely to have large implications for disease progression. To date, there is very little information on the microenvironmental conditions experienced by amphibians, especially when in cryptic retreat sites.

This study examined the microenvironments selected in rainforests of northern Queensland, Australia, by three species of rainforest stream frogs (*Litoria lesueuri, Litoria genimaculata* and *Litoria nannotis*) that have declined to different degrees due to chytridiomycosis. I determined the infection status of frogs for *B. dendrobatidis* using quantitative PCR, then tracked and measured body temperatures of frogs of each species in the field, using radio-telemetry and harmonic direction finding. I placed physical models in species-specific diurnal retreat sites to provide profiles integrated over time of the thermal and moisture regimes of the microenvironments experienced by each species.

Materials and Methods

Study site

The study was conducted at five sites within the tropical rainforests of northern Queensland, Australia: Birthday Creek, Paluma State Forest ("Paluma", 146º10'02" E 18º58'54" S, 800 m asl), Python Creek ("Tully Gorge", 145º35'E 17º46'S, 200 m asl), an unnamed creek ("Lower Tully", 145º41'E 17º48'S; 70 m asl) in Tully Falls Forest Reserve, an unnamed creek in Kirrama State Forest ("Kirrama", 145º52 E 18º11 S; 200 m asl) and Frenchman Creek, in Wooroonooran National Park ("Babinda", 145º55' E 17º20' S 20-100 m asl). All sites were relatively undisturbed rainforest streams. The creek beds were composed of rocks, ranging from small pebbles to large boulders (of over 10 m in diameter). All streams contained pools and riffles, and most sites contained a number of waterfalls. A marked transect was established along each stream to serve as a reference for frog locations.

Tracking

Frogs of three species were tracked: the stoney creek frog *L. lesueuri*, which has not experienced population declines (IUCN Least Concern; see below), the green eyed tree frog *L. genimaculata,* which declined and then recovered (IUCN Least Concern, however Australian populations of this species are Near Threatened), and the waterfall frog *L. nannotis,* which has experienced large and long-lasting population declines (IUCN Endangered; IUCN et al. 2006; McDonald & Alford 1999; McDonald 2002; McDonald et al. 2005). Recently, the taxonomy of the *L. lesueuri* group has been revised (Donnellan & Mahony 2004). Two species, *L. jungguy* and *L. wilcoxii*, occur in sympatry in the study region, hybridise, and are indistinguishable on the basis of morphology (Donnellan & Mahony 2004). Population declines have not been observed in either species. I therefore continue to refer to the study population as *L. lesueuri,* while recognising that the population contains two morphologically indistinguishable species. All species are large to medium sized hylids (males 5.4-12.5 g, females 6.5-41.3 g), and were tracked using either standard radio telemetry protocols or harmonic direction finding.

Only frogs weighing more than 11g were tracked via radio telemetry. Radio transmitters (models BD-2N and BD-2NT; Holohil Systems Ltd., Ontario, Canada; weighing approximately 0.67 g including harness and with a battery life of approximately 3 weeks) were attached to a harness made of silicone tubing, designed to minimise restrictions on movement and avoid causing discomfort to the frog. Frogs that were too small to be radio tracked and a number of larger individuals were tracked using harmonic direction finding (Langkilde & Alford 2002). This required attachment of a small diode with a whip antenna to the same specially designed harness (total weight approximately 0.23g), which was then placed around the waists (posterior abdomen anterior to the hind legs) of frogs. At capture,

frogs were weighed and swabbed for diagnostic PCR on the ventral surface using a sterile cotton swab (Medical Wire & Equipment Co. (Bath) Ltd., Wiltshire, UK). Tracking devices were then fitted *in situ* and frogs were released at point of capture after less than five minutes of handling. Frogs wearing either tracking device did not carry harnesses and associated equipment that weighed more than 6% of their total body weight. This is just over half the recommended maximum relative weight for an attached tag (10% of the body weight; Richards et al. 1994).

Frogs fitted with radio transmitters were tracked using a Telonics TR-4 Tracking Receiver (Telonics, Inc., Mesa, AZ, USA; 2004 warm/wet season only) and a HABIT Research HR2500 Osprey VHF Receiver (HABIT Research, Victoria, B.C., Canada); I used a three-element folding Yagi antennae with both receivers (A.F. Antronics, White Heath, Illinois, USA). Frogs fitted with diodes were tracked using a portable RECCO R5 transmitter-receiver (Recco Rescue Systems, Lidingö, Sweden). The system consists of a hand-held device that acts as both the transmitter and receiver, and a battery and earphones. Tags were self-built using commercial germanium diodes (see Langkilde & Alford (2002) for a description of methodology).

Surveys lasted 16 days and were conducted in July-September in the cool/dry season and February-April in the warm/wet season, at two sites for each species. *Litoria genimaculata* and *L. nannotis* were tracked simultaneously at the same streams during 2004, and *L. lesueuri* were tracked during 2005. In addition, *L. genimaculata* were tracked at Paluma during December in the warm/wet season of late 2003. During surveys, the location of each frog was determined once during the day (0900-1800 h) and once at night (1900-0400 h). Whenever it was possible, the temperatures of located frogs and their substrates were recorded.

I measured temperatures of frogs using two methods. Where it was possible to visually locate individual frogs, and I was able to reach within 0.5 m of the frog, body temperature was measured by holding a Raytek ST80 Pro-Plus Non-contact Thermometer (RAYST80) as close as possible to the frog and aiming at the lower dorsal area, between the thigh and point of radio-transmitter attachment. Emissivity on the thermometer was set at 0.95. I have demonstrated that non-contact infrared thermometers obtain body temperature readings not significantly different from skin or cloacal temperatures measured using a thermal probe (Rowley & Alford in press-a; appendix 1). If I could not visually locate the frog or reach within 0.5m of it, for frogs that were fitted with temperature-sensitive transmitters, I recorded the pulse interval of the telemetry signal by timing 90 pulses with a stopwatch, as transmitters emitted a pulse rate that was proportional to temperature. Prior to use, the transmitters were calibrated at 0.5, 10, 30, 35 and 40°C in a constant-temperature water bath by the manufacturer, and calibration curves were created for each individual

transmitter used. I used these calibration curves to determine transmitter temperature at each reading. Ambient air temperature was recorded in shade approximately 1m above the ground using a calibrated thermometer.

Skin swabs taken from tracked frogs and a number of other frogs at the same stream during surveys were analysed using diagnostic quantitative PCR. DNA was extracted with PrepMan Ultra, and amplified using primers ITS1-3 Chytr and 5.8S Chytr. PCR was performed using a dual-labelled probe and Taqman chemistry in a Corbett Rotorgene machine (Boyle et al. 2004).

I excluded data from the night following tag attachment due to the potential short-term behavioural effects of handling (Langkilde & Alford 2002). Any effects are unlikely to persist after the first night of tag attachment (Rowley & Alford in press-b; Chapter 2). To avoid pseudoreplication or biasing my results to frogs that were located more often, I used individuals as replicates and compared statistics obtained for each animal. In examining frog body temperatures, I looked at three categories of temperature relevant to *B. dendrobatidis* growth *in vitro*; above 25ºC, above 30ºC and below 17ºC. Temperatures above 25ºC and below 17ºC are non-optimal for *B. dendrobatidis* growth *in vitro*, and temperatures above 30ºC are likely to halt the growth and potentially cause the mortality of *B. dendrobatidis* (Longcore et al. 1999; Piotrowski et al. 2004). For each frog, I calculated the average percentage of observations in which body temperatures fell within each temperature category. Infection prevalence was compared among species, seasons and sites using Fisher's exact tests (Uitenbroek 1997).

Physical models

Physical models were used to obtain a detailed understanding of how frog body temperatures vary temporally. Models were constructed by filling rubber moulds in the shape of frogs in the water-conserving posture with 3% agar. Unmodified, these models lose water at rates comparable to frogs with zero skin resistance to evaporative water loss (Spotila & Bergman 1976). I call these 'permeable' models. In order to define the upper and lower boundaries of possible body temperatures for amphibians occupying any microenvironment, I monitored the 'body' temperatures of permeable models and of models coated with impermeable plastic (PLASTI DIP®, clear, PLASTI DIP International Inc., Blaine, Minnesota USA), giving them perfect resistance to evaporative water loss ('impermeable' models). Models of two sizes were used, representing the minimum (50 mm, 15 g) and maximum (75 mm, 44 g) sizes of frogs tracked in the study. Small thermal data loggers (Thermochron iButtons by Dallas Semicondictor, Dallas, Texas USA; diameter 15 mm, height 6 mm) were embedded in all models, and were programmed to record the temperature every 30 minutes. The models were

also used to evaluate the relative moisture conditions experienced by animals (e.g., Schwarzkopf & Alford 1996) by weighing each permeable model *in situ* to the nearest 0.1g every 24 h. I have demonstrated that the temperatures recorded for permeable and impermeable models provide an accurate outline of the envelope of body temperatures available to frogs with variable cutaneous resistance to evaporative water loss over a broad range of environmental conditions (Chapter 3).

Groups of four models (large permeable, small permeable, large impermeable and small impermeable) were placed in previous retreat sites of individuals being tracked. They were placed approximately 5 cm away from each other within the microhabitat, and tied in place using loose twine at the end of each model. The ventral surface of each model was kept firmly in contact with the substrate. Models were collected from the field when at least one permeable model had dehydrated to less than 50% of its original weight. Air temperature was recorded every 30 minutes using an iButton placed permanently in shaded conditions 1m above the ground. To avoid pseudoreplication and biasing my results to models placed in the field for a longer duration, I used individual models as replicates and compared statistics obtained for each retreat site. I calculated the percentage of time spent in each of the three temperature categories defined above. To determine the relative moisture conditions at frog retreat sites, I calculated the average proportional weight change per day for each model. My analyses revealed no differences in the thermal envelopes or water loss rates between large and small models, therefore I report only the results for large models.

Results

Tracking

A total of 117 frogs were tracked during the study period (Table 7.1), producing a total of 2111 fixes or locations of individual frogs. On average, each frog was located 17 times (minimum 5, maximum 29). The weights of the individuals tracked did not change significantly over the study period (Wilcoxon Signed Ranks Test; Z= -1.361, p= 0.173, n=70). Frogs with attached tags appeared to be uninhibited in their movement, were commonly observed in close association with frogs without tags, and were observed calling and in amplexus.

The results of all PCR tests for the presence of *B. dendrobatidis* on frogs of each species are presented in Table 7.2. An initial Fisher's Exact Test on these data, arranged as a 3 X 2 table of species by infection status, showed that infection prevalence differed among species (p<0.001); it was lower in *L. lesueuri* than in either *L. genimaculata* or *L. nannotis* (Table 7.2). Arranging the data in a 2 X 2 table to test for seasonal effects across all species revealed a strong seasonal trend, with higher prevalence during the cool/dry season

Table 7.1. Summary of the numbers of individual frogs of each species and sex tracked at each site in each season.

Species	Season	Site	Sex	Number tracked using Radio-telemetry	Number tracked using Harmonic direction finding
L. genimaculata	Cool/dry	Kirrama	Male	0	2
			Female	4	1
		Python Creek	Male	0	6
			Female	2	0
	Warm/wet	Kirrama	Male	0	0
			Female	2	1
		Python Creek	Male	0	0
			Female	0	0
		Paluma	Male	0	7
			Female	1	1
	Totals		**Male**	**0**	**15**
			Female	**9**	**3**
L. nannotis	Cool/dry	Kirrama	Male	0	3
			Female	3	2
		Python Creek	Male	1	1
			Female	5	1
	Warm/wet	Kirrama	Male	0	0
			Female	3	0
		Python Creek	Male	1	0
			Female	8	0
	Totals		**Male**	**2**	**4**
			Female	**19**	**3**
L. lesueuri	Cool/dry	Babinda	Male	0	6
			Female	10	3
		Lower Tully	Male	2	13
			Female	4	1
	Warm/wet	Babinda	Male	0	3
			Female	6	2
		Lower Tully	Male	5	3
			Female	4	1
	Totals		**Male**	**7**	**25**
			Female	**24**	**6**

Table 7.2. Numbers of individuals testing positive and negative and prevalence of *B. dendrobatidis* infection found at each site during tracking periods.

Species	Season	Site	Number of individuals testing		Infection prevalence (%)
			Negative	Positive	
L. lesueuri	Cool/dry	Babinda	24	2	7.7
		Lower Tully	19	4	17.4
	Warm/wet	Babinda	21	1	4.5
		Lower Tully	15	1	6.3
L. genimaculata	Cool/dry	Kirrama	4	4	50.0
		Tully Gorge	4	5	55.6
	Warm/wet	Kirrama	4	0	0.0
		Tully Gorge	0	0	0.0
L. nannotis	Cool/dry	Kirrama	3	5	62.5
		Tully Gorge	3	5	62.5
	Warm/wet	Kirrama	8	0	0.0
		Tully Gorge	5	4	44.4

(p=0.0040). Because some species were tracked at different sites, I looked for site effects by examining the data for each species as a 2 X 2 table of site against infection status; this indicated that prevalence did not differ significantly among sites within any species (all p>0.05).

Diurnal and nocturnal frog body temperatures often differed considerably from ambient temperature. The maximum difference between body and ambient temperatures was 13.8ºC for *L. genimaculata*, 10ºC for *L. lesueuri*, and 8.3ºC for *L. nannotis*; body temperatures exceeded ambient in each of these cases. Body temperatures of tracked frogs also differed among species, with the highest temperatures recorded in *L. lesueuri* and *L. genimaculata* (Figure 7.1). Both *L. genimaculata* and *L. lesueuri* had body temperatures above 25ºC and 30ºC relatively often (Table 7.3). *Litoria nannotis* body temperatures above 30ºC were never recorded, and were often below 17ºC.

There was a large seasonal effect on body temperature, with frogs of all species attaining higher mean, minimum and maximum body temperatures and spending more time in warmer temperature categories in the warm/wet season than in the cool/dry season (Figure 7.1, Table 7.3). Frog body temperatures also differed among sites (Table 7.3), the most notable difference being at Paluma, the only relatively high elevation site, where, in contrast to the lower elevation sites, *L. genimaculata* body temperatures were always between 17-25ºC. There was also substantial variation among individuals in body temperatures. For example, in the warm/wet season, some *L. lesueuri* were always recorded at temperatures above 25ºC, while others were never recorded above 25ºC.

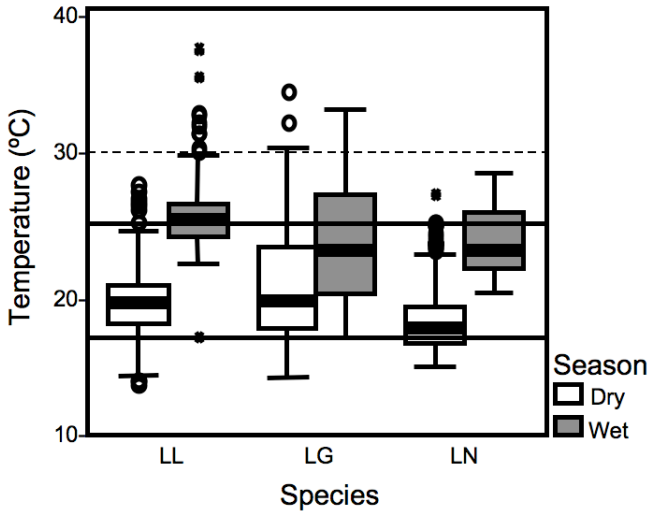

Figure 7.1. Distributions of frog body temperatures in the cool/dry and warm/wet seasons. Data represent all body temperatures recorded during the study. Solid lines indicate *in vitro* thermal optimum for *B. dendrobatidis* and dashed line indicates approximate *in vivo* thermal tolerance of *B. dendrobatidis*.

Table 7.3. Mean (and range) percentage of body temperature measurements taken for each individual that fell within each temperature category.

Species	Season	Site	Number of temperature records	% Above 25ºC	% Above 30ºC	% Below 17ºC
L. lesueuri	Cool/dry	Babinda	280	1.3 (0-14.3)	0	13.3 (0-42.9)
		Lower Tully	239	0.2 (0-10)	0	6.2 (0-66.7)
	Warm/wet	Babinda	161	49.8 (0-100)	0	0
		Lower Tully	241	55.6 (0-100)	3.07 (0-25)	0
L. genimaculata	Cool/dry	Kirrama	131	25.8 (0-77.8)	1.59 (0-22.22)	21 (0-54.4)
		Tully Gorge	108	6.6 (0-58.3)	0.52 (0-8.33)	2.6 (0-25)
	Warm/wet	Kirrama	46	86.1 (60-100)	11.67 (0-60)	0
		Tully Gorge	---	---	---	---
		Paluma	58	0	0	0
L. nannotis	Cool/dry	Kirrama	127	0	0	47.3 (0-100)
		Tully Gorge	109	0.7 (0-10.0)	0	31.1 (0-100)
	Warm/wet	Kirrama	57	63.7 (50.0-87.5)	0	0
		Tully Gorge	98	10.6 (0-55.6)	0	0

Physical models

Models placed in species-specific retreat sites showed trends similar to those of frog body temperatures obtained through tracking (Figure 7.2). The permeable and impermeable models indicate the lower and upper limits, respectively, of body temperature that could be attained by a frog occupying a particular retreat site at any time.

Models in *L. lesueuri* retreat sites recorded the highest temperatures and spent the longest average durations of time above both 25ºC and 30ºC (Figure 7.2). At Tully in the warm/wet season, *L. lesueuri* retreat sites were greater than 25ºC for an average of 38-51% of the time, or 9-12 hours a day, depending on model type. *Litoria genimaculata* retreat sites were typically above optimum for less time, and *L. nannotis* retreat sites were almost never above 25ºC. The percentage of time that models were above 30ºC was typically low, averaging at most 1.2 hours per day at *L. lesueuri* retreat sites.

Time that retreat sites were in each temperature category varied seasonally, with higher temperatures attained for longer periods in the warm/wet season (Figure 7.2). Retreat site temperatures for all species often dropped below optimum for *B. dendrobatidis* growth in the cool/dry season, but not in the warm/wet season. As with tracked frogs, there was a large amount of variation in temperatures among sites, and among individual retreat sites. For example, models in *L. lesueuri* retreat sites at Lower Tully during the warm/wet season spent between 0-16.4% of the time (0-4 hours per day) above 30ºC.

Hydric conditions at retreat sites differed among species (Figure 7.3). While *L. nannotis* retreat sites were often saturated, causing many models to gain weight over time, *L. genimaculata* sites typically had low moisture levels, with models rapidly desiccating. *L. lesueuri* retreat sites had intermediate moisture levels. Hydric conditions varied among site, season, and individual retreat site (Figure 7.2).

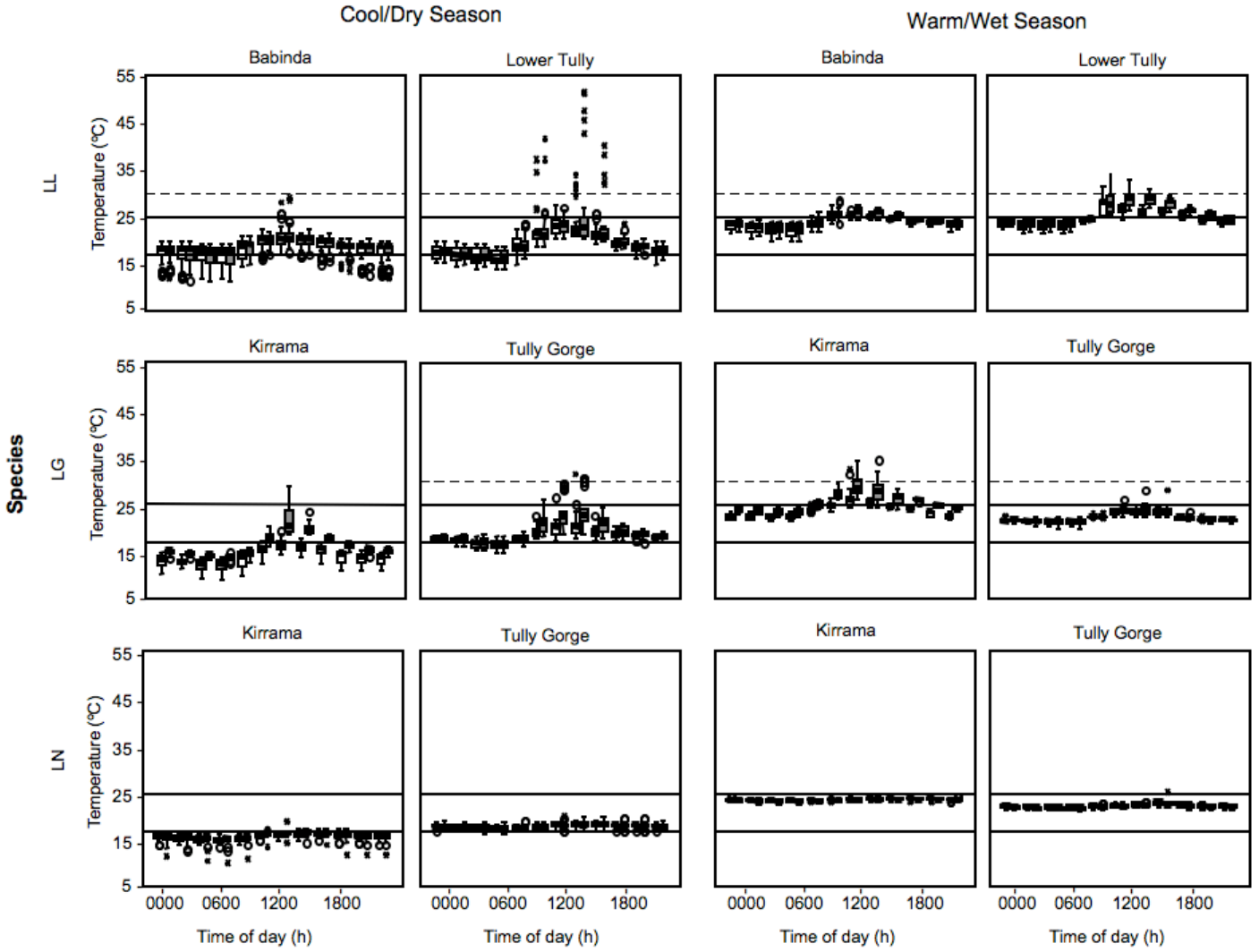

Figure 7.2. Temperatures at species-specific retreat sites. Boxplots show temperatures every 2 hours for permeable (white boxes) and impermeable (grey boxes) models in retreat sites of *Litoria lesueuri* (LL), *L. genimaculata* (LG) and *L. nannotis* (LN). Solid lines indicate in vitro thermal optimum for *B. dendrobatidis* and dashed line indicates approximate in vivo thermal tolerance of *B. dendrobatidis*.

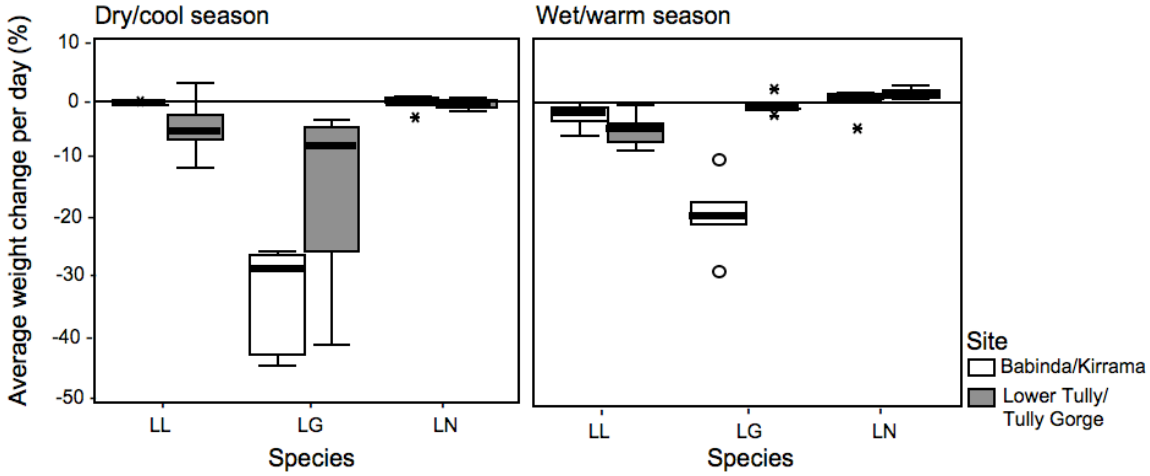

Figure 7.3. Average weight change per day (g) for models in species-specific retreat sites. Models were placed in retreat sites of *L. lesueuri* (LL), *L. genimaculata* (LG) and *L. nannotis* (LN) in the cool/dry and warm/wet seasons.

Discussion

The body temperatures of frogs and the thermal and hydric microenvironments at retreat sites differed from ambient conditions and among species. In particular, the duration of time outside the optimum thermal range and above the thermal tolerance of *B. dendrobatidis* differed markedly among species and seasons. The species least susceptible to decline due to chytridiomycosis in the wild, *L. lesueuri*, spent the largest proportion of time above the thermal optimum and tolerance of *B. dendrobatidis*. In the warm/wet season, *L. lesueuri* retreat sites were above the optimal temperature for *B. dendrobatidis* for an average of 3-12 hours per day, with a maximum of 13 hours, and above 30ºC for an average of 1.2 hours per day. It is likely that this amount of time at non-optimal conditions is sufficient to slow the development of chytridiomycosis in infected individuals, and in some cases, to eliminate infection entirely, as it is capable of doing for fungal diseases in insect hosts (Carruthers et al. 1992). In addition, individual *L. lesueuri* were recorded at very high diurnal temperatures (up to 37.2ºC), which is likely to halt or reverse disease progression much more rapidly. For example, in culture, *B. dendrobatidis* zoospores are capable of surviving four hours at 37ºC and only 30 minutes at 47ºC (Johnson et al. 2003), and 16 hours of exposure to environmental temperatures of 37 ºC can eliminate the organism from infected *Litoria chloris* (Woodhams et al. 2003). Models placed in *L. lesueuri* retreat sites reached well over 37ºC on a number of occasions, and maintained these temperatures for several hours at a time. *Litoria genimaculata* microenvironments were also often above the optimal temperature range for *B. dendrobatidis,* but for less time than *L. lesueuri* microenvironments. *Litoria nannotis* microenvironments were almost never above the thermal optimum for *B. dendrobatidis*,

although they were often below the thermal optimum in the cool/dry season. While low temperatures may slow the development of *B. dendrobatidis*, temperatures only slightly below the thermal optimum of *B. dendrobatidis*, as observed in this study, are unlikely to limit disease development to the same extent as high temperatures. For example, there was no difference in the survival times of infected *Bufo boreas* maintained at 12ºC or 23ºC (Carey et al. 2006) and short-term exposures to 8ºC only slightly delayed mortality in experimental *Litoria chloris* (Woodhams et al. 2003).

The hydric conditions at frog retreat sites also varied among species. *Litoria genimaculata* retreat sites were the driest, with models desiccating rapidly. It is likely that these conditions would halt or even reverse the development of chytridiomycosis in infected frogs as only 1-3 hours of exposure to desiccation kills cultures of *B. dendrobatidis* (Johnson et al. 2003). Microenvironments experienced by *L. lesueuri* were of intermediate humidity, and *L. nannotis* microenvironments were continually saturated or near saturated. *Litoria nannotis* microenvironments are therefore likely to provide optimal hydric conditions for the development of chytridiomycosis.

The environmental conditions experienced by amphibians depend not only on species-specific microhabitat selection, but also on climatic variables, which vary seasonally. Higher infection prevalence and amphibian mortality in the cool/dry season (Berger et al. 2004; Bradley et al. 2002; McDonald et al. 2005; Retallick et al. 2004; Woodhams & Alford 2005) are likely to be linked to effective changes in pathogenicity and host recovery rate, as influenced by seasonal changes in host body temperature observed in this study. Similarly, the ability of frogs to reach high body temperatures, and thereby control *B. dendrobatidis* infection, may change spatially due to factors such as habitat structure, aspect or elevation. In the present study, *L. genimaculata* at the higher elevation site, Paluma, were never recorded above 25ºC, in contrast to the other two sites. This is likely due to both the cooler ambient temperatures and frequent cloud-cover typical of the site. It has been suggested that differences among sites in levels of *B. dendrobatidis* infection in *Taudactylus eungellensis* are correlated with the degree of sunlight and warmth that reaches the streams (Retallick et al. 2004). In addition, habitat structure at sites where *Rana muscosa* has persisted with *B. dendrobatidis* appears to allow higher and more variable temperatures compared to sites where frog die-offs have occurred (Briggs et al. 2005).

Variation in weather conditions within and among my surveys may have also been important, influencing the degree to which frog microclimates differed from ambient. On days with high cloud-cover, frog body temperature was unlikely to be elevated above ambient. In contrast, on clear, sunny days, frog temperatures were above ambient for extended periods of time, potentially long enough to clear infection. It was overcast and intermittently raining for over 35% the time *L. lesueuri* models were in the field, compared to 19% for *L. genimaculata* and *L. nannotis* models. Microenvironmental

conditions were therefore likely to be even warmer and drier for *L. lesueuri* when experiencing the same weather conditions as the other two species.

The temperature categories used in this study, while based upon current knowledge of the growth and survival of *B. dendrobatidis,* are likely to be approximate values and not universally applicable for a number of reasons. Firstly, temperature requirements or optima may differ between strains of the same fungal species (Doberski 1981; Thomas & Jenkins 1997). This appears to be the case for *B. dendrobatidis*; temperature responses of the fungus *in vitro* (Piotrowski et al. 2004) and time to death of infected animals (Berger et al. 2005a) differ among strains. In addition, growth rates and thermal optima estimated from *in vitro* studies may not correspond with field conditions, and may therefore under- or over-estimate the effects of temperature on host-pathogen dynamics (Milner et al. 1980; Thomas & Jenkins 1997). Lastly, although environmental conditions tend to act more strongly on fungi than on their hosts (Benz 1987) the effects of temperature on disease progression are likely to be caused by both its direct effects on pathogen growth rate and survival, and its indirect effects on host immune response. The immune systems of ectothermic vertebrates are temperature dependent, with cold temperatures reducing or eliminating immune system activity (Ainsworth et al. 1991; Carey 2000; Carey et al. 1996; Le Morvan et al. 1998), and even small increases in temperature can have strong positive effects on host immune response (Cohen 1966; Hildemann & Haas 1959; Kluger 1978). Thus, the optimum temperature range for infection may vary among host species even when using the same strain of pathogen (Colhoun 1973). In order to clarify the effects of the environment on the development of chytridiomycosis, there is an urgent need for studies examining the growth and development of different strains of *B. dendrobatidis* under realistic, fluctuating environmental conditions both *in vitro* and *in vivo*.

In conclusion, my findings provide the first evidence of that microenvironmental conditions are likely to affect the susceptibility in nature of amphibians to chytridiomycosis-related declines, with susceptible species using microenvironments in ways that cause them to provide more favourable microenvironments for *B. dendrobatidis* growth and survival. Species-specific differences in microenvironment use may also explain why infected individuals of some species experience rapid mortality in the laboratory, yet are able to carry infections for extended periods in the field. Differences in the microenvironments available to frogs may also explain temporal and spatial variation in infection prevalence and host mortality. In addition, relatively subtle changes in the availability of microenvironments due to climate change may be capable of precipitating disease-related amphibian population declines and extinctions (Pounds et al. 2006).

This study also highlights the need to use fine-scale microenvironmental conditions rather than macroclimatic or ambient conditions in predicting the effects of habitat change or global warming on organisms. The majority of predictive models forecast a global temperature rise of 1.5-

4.5ºC (e.g., Houghton et al. 2001), and such blanket figures have been used in predicting species responses (e.g., Brereton et al. 1995; Thomas et al. 2004; Williams et al. 2003). However, as this study reveals, microenvironmental conditions experienced by organisms may differ greatly from ambient, and from other species in the same habitat. Therefore, macroenvironmental shifts in climate and weather will be filtered by the microenvironmental relationships of species to produce changes in species interactions. Due to microenvironmental selection, certain amphibian species may be capable of buffering themselves against environmental change. Information on amphibian behaviour and microenvironment use may be useful in evaluating the susceptibility to declines caused by chytridiomycosis in species that presently occur in areas without *B. dendrobatidis,* and under future climatic conditions.

CHAPTER EIGHT: GENERAL DISCUSSION

Project background and justification

In recent decades, a number of pathogenic infectious wildlife diseases have emerged that present substantial threats to biodiversity (Harvell et al. 1999; Ward & Lafferty 2004). Many species from a wide range of taxa are thought to have declined or become extinct due to disease (Berger et al. 1998; Bosch et al. 2001; Daszak & Cunningham 1999; Ginsberg et al. 1995; Lips et al. 2006; Thorne & Williams 1988; van Riper III et al. 1986; Warner 1968). One factor common to the majority of threatening wildlife diseases is that they affect multiple host species, and can therefore convert species rich ecosystems into depauperate communities (Burdon 1991; Lips et al. 2006; van Riper III et al. 1986; Warner 1968). For most diseases, there are gradients in susceptibility to decline, with some species in affected communities experiencing severe population declines and others declining less dramatically or not at all.

One cause of these gradients may be interspecific variation in host behaviour, which can influence both the transmission of pathogens among individuals (Ezenwa 2004; Loehle 1995; McCallum et al. 2001), and the progress and outcome of infection within individuals (Altizer et al. 2000; Brooks et al. 2006; Colhoun 1973; Ezenwa 2004; Grutter 1998; Krasnov et al. 1998). Despite the potential importance of host behaviour, little is known of the effects of host behaviour on susceptibility to disease in wildlife populations.

Chytridiomycosis

The amphibian disease chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis,* has been implicated in mass mortalities, population declines, and extinctions of amphibian populations and species around the world (Berger et al. 1998; Bosch et al. 2001; Bradley et al. 2002; Lips 1999; Lips et al. 2006; Longcore et al. 1999; Pessier et al. 1999; Rachowicz et al. 2006; Weldon & Du Preez 2004). In many cases, frog population declines attributed to *B. dendrobatidis* have been dramatic, resulting in the rapid extinction of 50% or more of the anuran species at particular sites (Lips 1999; Lips et al. 2006) and large reductions in the abundance of remaining species (Lips et al. 2006; Lips & Donnelly 2002). However, in almost all cases, amphibian species that have disappeared or declined due to chytridiomycosis coexist with non-declining species (Lips et al. 2006; Lips & Donnelly 2002; Puschendorf et al. 2006; Retallick et al. 2004). Many of these non-declining species are highly susceptible *B. dendrobatidis* in the laboratory, but are able to persist with infections in the wild (Berger et al. 1999; McDonald & Alford 1999; Woodhams et al. 2003). Therefore, differences in the susceptibility of amphibian species in the field must not be entirely due to innate properties of the

species that confer resistance to the disease, but due to some external factor or factors that are present in the field but absent from laboratory experiments.

As is true of other diseases, interspecific variation in behaviour may explain why some frog species decline during outbreaks of chytridiomycosis while other, co-occurring species do not. Differences among species in opportunities for the transmission of a *B. dendrobatidis* may be particularly important in determining their relative susceptibilities to decline, with transmission rates in the field likely to vary among species according the frequency of behaviours such as physical contact between frogs or with environmental reservoirs. However, there is presently little information on the extent of physical contact between frogs of different species. Species-specific movement patterns and habitat use may also be important in determining the susceptibility of species to decline (Altizer et al. 2000; Bohonak 1999; Brooks et al. 2006; Ezenwa 2004; Grutter 1998; Krasnov et al. 1998; Waldman & Tocher 1998), however we currently have a limited understanding of the movement and habitat use of amphibians, particularly for tropical stream breeding specie, where declines have been most frequent and severe (Lips et al. 2003b; Stuart et al. 2004; Williams & Hero 1998). Microenvironments selected by amphibian species may also be a major reason why chytridiomycosis affects some species more than others, as the growth and survival of *B. dendrobatidis* in culture and the progress and outcome of chytridiomycosis in infected amphibians in the laboratory are highly influenced by thermal and hydric conditions (Berger 2001; Berger et al. 2004; Carey et al. 2006; Johnson et al. 2003; Longcore et al. 1999; Piotrowski et al. 2004; Woodhams 2003; Woodhams et al. 2003). Elevated body temperatures are also capable of clearing frogs of infection (Woodhams et al. 2003). At present little is known of the microenvironmental conditions selected by declining and non-declining frog species, particularly away from nocturnal breeding sites.

Aims and approach

The general aim of this study was to examine the potential effects of host behaviour on the susceptibility of different host species to decline due to the disease chytridiomycosis. In order to achieve this, I tracked three species of stream-breeding frogs in northern Queensland, Australia. These species co-occur but have declined to varying degrees in recent decades; the waterfall frog, *L. nannotis,* has experienced large and long-lasting population declines, the green-eyed tree frog *L. genimaculata,* declined and then recovered, and the stoney creek frog *L. lesueuri* has not experienced population declines.

Specifically, my aims were to determine if the susceptibility of amphibian species to chytridiomycosis-associated declines is related to:

- Opportunities for the transmission of *B. dendrobatidis*
- Movement patterns and habitat use

- Microenvironment selection

Development of techniques

To examine the potential effects of host behaviour on the susceptibility of amphibian species to chytridiomycosis-related declines, I first needed to compare the effectiveness of two different methods of tracking frogs: radio telemetry and harmonic direction finding, and develop a method of characterizing the thermal and hydric conditions of amphibians in the field using models. I found that harmonic direction finding resulted in a lower number of fixes for each animal and was less efficient compared to radio telemetry, but that there was no significant effect of tracking device on the values of movement and habitat use variables (Chapter 2), making the technique suitable for use in this thesis. I then developed a novel technique for using physical models to create profiles of the thermal and hydric microenvironments experienced by amphibians in the field, regardless of their cutaneous resistance to water loss. I place models with zero and very high resistance to evaporative water loss in species-specific diurnal retreat sites, and use data loggers to record their temperatures. This provides profiles integrated over time of the thermal and hydric regimes of the microenvironments experienced by each species. I successfully tested and validated this technique in the laboratory and the field (Chapter 3).

Opportunities for transmission of *B. dendrobatidis*

Rates of contact with stream water and with other frogs are likely to affect levels of exposure to *B. dendrobatidis*. The three species differed greatly in their rates of contact with other frogs, stream water and different environmental substrate types (Chapter 4). The frequency of intraspecific contact between frogs was highest in *L. nannotis*, and lowest in *L. lesueuri* and *L. genimaculata*. In all three species, contact between frogs was always between conspecifics, providing no opportunities for cross-species pathogen transmission. Contact with stream water or other environmental substrates that may serve as reservoirs is therefore likely to be the main mechanism of transmission between species in this system. Contact with stream water was more frequent in *L. nannotis*, which was in contact with the stream during 84% of observations. *Litoria lesueuri* and *L. genimaculata* were rarely in contact with stream water. As well as limiting rates of transmission across species, the differences observed in rates of contact with water may have strong effects on intraspecific transmission rates.

Frequency of contact with environmental substrates also varied among species. At present, little is known regarding the persistence of *B. dendrobatidis* on environmental substrates, but the abundance and species composition of other chytrids differ strongly among environmental macro- and microhabitats (Letcher et al. 2004; Letcher & Powell 2002). It is therefore highly probable that the abundance of *B. dendrobatidis* zoospores also differs among environmental substrates, and hence

frequency of contact with different environmental substrates may have important implications for *B. dendrobatidis* transmission and disease progression. All 122 swabs taken from retreat sites in this study tested negative for *B. dendrobatidis* (Chapter 5). Retreat sites may therefore not be a reservoir of infection when *B. dendrobatidis* occurs at low prevalence and intensity on frogs, but may become important reservoirs of infection during epidemics (ie. Lips et al. 2006).

Movement patterns and habitat use

Movement patterns and habitat use differed significantly among species (Chapter 6). *Litoria lesueuri* was the most mobile species, moving the most often and the greatest distances, and was not highly associated with the stream, spending large amounts of time at considerable distances from the stream. Compared to *L. lesueuri, L. genimaculata* moved less frequently and smaller distances. *Litoria genimaculata* was also more restricted to the stream environment in terms of horizontal distance, but was frequently in the canopy above the stream. *Litoria nannotis* was the most sedentary species, and was highly restricted to the stream environment both in terms of horizontal distance and elevation.

Species also differed in their degree of habitat specificity and movement within and between different habitat types. *Litoria lesueuri* was the only species observed in disturbed, non-forested habitats. *Litoria lesueuri* moved great distances along and between streams and within and between intact rainforest and non-forested areas. *Litoria genimaculata* and *L. nannotis* were never observed outside of intact rainforest, although these species differed in their movements within this habitat. During tracking, *L. genimaculata* moved along and between streams, while *L. nannotis* remained in specific sections of the stream, typically returning to the same small section of the stream after nocturnal excursions. The movement patterns and habitat use of a species may have important consequences for both disease dynamics and the ability to cope with habitat modification or climate change (Altizer et al. 2000; Brooks et al. 2006; Ezenwa 2004; Hanski & Zhang 1993; Travis & Dytham 1999). By virtue of its sedentary behaviour and affinity for the stream environment, *L. nannotis* may be more affected by parasites and pathogens and more vulnerable to disturbance than either *L. lesueuri* or *L. genimaculata*.

Microenvironment use

Although all three species co-occurred and therefore experienced similar macroenvironmental conditions, the microenvironmental conditions they experienced differed substantially (Chapter 7). Microenvironments of the species least susceptible to chytridiomycosis related declines, *L. lesueuri*, were most often above the thermal optimum and tolerance of *B. dendrobatidis*, while microenvironments of the species most susceptible to chytridiomycosis related declines, *L. nannotis,* were almost never above the thermal optimum for *B. dendrobatidis*. Season also influenced the

temperatures of amphibian microenvironments; *L. lesueuri* and *L. genimaculata* microenvironments were above the thermal optimum and tolerance of *B. dendrobatidis* for longer durations in the warm/wet season. The hydric conditions at frog retreat sites also varied among species; *L. genimaculata* retreat sites were the driest, with models desiccating rapidly, *L. lesueuri* retreat sites were of intermediate humidity, and *L. nannotis* retreat sites were continually saturated or near saturated. The highly desiccating nature of *L. genimaculata* microenvironments is therefore likely to slow or halt the development of chytridiomycosis, while *L. nannotis* microenvironments are likely to provide optimal hydric conditions for the development of chytridiomycosis. As a result, the species-specific microenvironmental conditions experienced by amphibians are likely to affect their susceptibility to chytridiomycosis-related declines. Differences in the microenvironments available to frogs may also explain temporal and spatial variation in infection prevalence and host mortality. In addition, relatively subtle changes in the availability of microenvironments due to climate change or habitat alteration may be capable of precipitating disease-related amphibian population declines and extinctions.

Synthesis and conclusion

This thesis provides the first empirical confirmation of the fact that behaviour and availability of microenvironmental conditions are likely to affect the susceptibility in nature of amphibians to chytridiomycosis-related declines. The behaviour of the most susceptible species I examined in terms of chytridiomycosis related declines was most favourable to the transmission, growth and survival of *B. dendrobatidis,* while the least susceptible species behaved in ways least conducive to *B. dendrobatidis* transmission, growth and survival (Table 8.1). Interspecific variation in behaviour may therefore play a role in determining why chytridiomycosis drives populations of some species to extinction and not others. Species-specific differences in behaviour may also explain why infected individuals of some species experience rapid mortality in the laboratory, yet are able to carry infections for extended periods in the field.

Conservation implications

The results of this thesis have several important implications for the management and conservation of my study species. While the species differ in their susceptibility to decline from *B. dendrobatidis* epidemics*,* this particular threat appears to have subsided at my study sites, with *B. dendrobatidis* becoming endemic in the region (McDonald et al. 2005). In contrast, other factors such as habitat modification and climate change are likely to increasingly threaten these species, particularly *L. nannotis*, which has already experienced significant population declines and range reductions. The ability of each species to persist under such threats is likely to be at least partially related to

behaviour. The high dispersal ability and wide habitat tolerance of *L. lesueuri* are likely to allow this species to persist in modified landscapes, and to be relatively tolerant of habitat modification, habitat fragmentation and climate change. In contrast, the sedentary nature and narrow habitat tolerance of *L. nannotis* should make the species highly vulnerable to disturbance, likely to become isolated in forest fragments and unable to persist in the face of climate change. While *L. genimaculata* was capable of moving large distances within and between streams, the species showed habitat specificity similar to that of to *L. nannotis*, never moving outside of intact rainforest. Therefore, maintenance of large terrestrial vegetation buffers along streams and connectivity between forest fragments is likely to be extremely important for the long-term conservation of both *L. nannotis* and *L. genimaculata*.

This research also has practical implications for the interpretation of population surveys of *L. lesueuri*, *L. genimaculata* and *L. nannotis*, the majority of which are carried out by using visual encounter surveys along a stream transect. As the observability of these species declines with distance from the stream and time spent in sheltered locations, population estimates will vary. At any time, a proportion of the population will be undetectable, and this will vary depending on species, sex and season. If, as my data suggest may be the case for *L. lesueuri* and *L. genimaculata,* some species are nomadic (Chapter 6), the already difficult problem of conducting censuses in fixed plots becomes more difficult, since even with mark-recapture data it will be difficult to distinguish between emigration and mortality, and population size estimates will be inflated by the immigration of unmarked individuals between surveys.

The results of my study also have implications for the management and conservation of other amphibian species. First, it suggests that information on amphibian behaviour and microenvironment use may be useful in predicting susceptibility to decline due to chytridiomycosis in species or populations that are thought to be naïve to *B. dendrobatidis.* By carrying out short-term studies of the behavioural ecology of the amphibians at a site, it may be possible to gain sufficient information to predict which species are most susceptible to declines caused by chytridiomycosis, thereby allowing the concentration of management efforts towards these species. An understanding of the movement patterns and habitat specificity of species may also allow the prediction of species most likely to decline from other threatening processes and aid in the design of reserves, or creation of habitat corridors or buffer zones along streams.

This study also highlights the need to have basic ecological and behavioural data on amphibians. In order to form effective conservation strategies aimed at reducing biodiversity loss, it is necessary to understand the causes of extinction and why some species are more vulnerable to extinction than others (Kotiaho et al. 2005). However, to do this, basic, species-specific ecological and behavioural information is required; information that is currently unavailable for the vast majority of species (Greene 1994). Even superficially similar species occurring in sympatry may differ greatly

in terms of movement patterns and habitat use and experience vastly different microenvironments, as this study reveals. Thus, broad classifications of habitat use and macroenvironmental conditions may fail to capture important differences between species that can only be gained via detailed ecological studies.

Future directions

Due to the relatively recent discovery of *B. dendrobatidis*, many aspects of its basic biology and ecology are unknown, particularly in the wild. For example, the distribution and abundance of *B. dendrobatidis* in potential environmental reservoirs is almost completely unknown. Because the presence of environmental reservoirs may be one factor causing the extremely high transmission rates seen in chytridiomycosis outbreaks in some systems (Berger et al. 1998; Lips et al. 2006), research investigating the distribution and abundance of *B. dendrobatidis* in the environment is urgently required. In addition, while *B. dendrobatidis* DNA has been detected on environmental substrate samples during epidemics (Lips et al. 2006), it is not known whether these substrates contain viable zoospores. Experimental infection trials using individuals of susceptible species and infected substrate samples from the field may determine whether environmental substrates contain viable zoospores and thus act as reservoirs for the pathogen. In addition, by comparing the ability of different substrate types to harbour *B. dendrobatidis,* it may be possible to more accurately predict the infection risk of a species based on species-specific microhabitat selection.

Interactions between *B. dendrobatidis,* its amphibian hosts, and environmental conditions are also relatively poorly understood. Although laboratory studies have been vital in determining the potential importance of host behaviour in host-pathogen interactions, conditions maintained in the laboratory are generally highly unrealistic. Laboratory experiments examining the growth of *B. dendrobatidis in vitro* or the development and outcome of *B. dendrobatidis* infection *in vivo* have typically maintained environmental conditions constant and often at levels optimal to either the pathogen or host. There is an urgent need for studies examining the growth and development of different strains of *B. dendrobatidis* under realistic, fluctuating environmental conditions simulating the microenvironmental patterns encountered by frogs in the wild, in both *in vitro* and *in vivo* experiments. There is also a need for more quantitative data on the effects of multiple environmental factors (ie. the combined effects of temperature and humidity) on the outcome of infections. The microenvironmental conditions described in this thesis for three species of frog during two seasons may provide the basis of a number of future studies addressing this gap in knowledge both *in vitro* and *in vivo*.

As microenvironment varies spatially, it is likely that infection prevalence and susceptibility to decline in amphibians will also vary spatially with factors such as elevation and habitat type.

Elevation above sea level is generally positively correlated with infection prevalence and host mortality in the field (McDonald et al. 2005; Woodhams & Alford 2005), and declines and local extinctions in amphibians attributable to chytridiomycosis have occurred more often at higher elevations (Lips et al. 2004; Lips et al. 2003b; McDonald & Alford 1999; Pounds et al. 2006). Habitat type has also been suggested as explaining variation in infection prevalence and host mortality in the field (Briggs et al. 2005; Retallick et al. 2004), and chytridiomycosis-related declines in a number of species including *L. nannotis* appear to have been less severe at non-rainforest sites throughout their range (Ingram & McDonald 1993; Williams 2006). Therefore, sites with environmental conditions unfavourable to the growth and survival of *B. dendrobatidis* (ie. low elevation or non-rainforest) may act as refuges for species susceptible to chytridiomycosis. However, due to behaviour, it is also possible that amphibians select microenvironments in a manner that allows them to maintain similar microenvironment conditions regardless of elevation or habitat type (ie. by selecting relatively cooler or more thermally buffered microenvironments at warmer sites). Further research examining amphibian behaviour and microenvironmental selection at higher elevation and in non-rainforest habitats will lead to a greater understanding of the interaction between amphibians and *B. dendrobatidis*, and in the ability of amphibian species to buffer themselves against environmental change. In addition, investigating whether aspects of movement and habitat use in amphibians vary with respect to elevation or habitat type may elucidate the particular aspects of behaviour that are most important in influencing the susceptibility of a species to experience chytridiomycosis-related declines.

The results of this thesis may also be used to address the general question of how climate change is likely to affect species' use of the environment and interactions with other organisms. The vast majority of studies predicting the effects of climate change on a species' future distribution and interactions have considered only mean ambient environmental conditions. However, as this thesis reveals, microenvironmental conditions experienced by organisms may differ greatly from ambient, and from other species in the same habitat. Therefore, this thesis may serve as the basis for future studies that will explore how macroenvironmental shifts in climate and weather are filtered by the microenvironmental relationships of species to produce changes in species interactions. In addition, a better understanding of the full host-environment-pathogen system is needed to evaluate the hypothesis that *B. dendrobatidis* outbreaks may be precipitated as global climate change causes environmental conditions to cross particular thresholds (Pounds et al. 2006). Such studies will enhance our ability to predict the consequences of these changes on amphibians around the world.

Table 8.1. Summary of the likely influences of behaviour on vulnerability to chytridiomycosis-related declines.

	Litoria lesueuri No population declines	***Litoria genimaculata*** Population declines, recovery	***Litoria nannotis*** Persistent population extinctions
Opportunities for transmission of *B. dendrobatidis*			
Frog-to-frog	Low	Medium	High
Contact with water	Low	Low	High
Movement and habitat use			
Mobility	High	Medium	Low
Habitat specificity	Low	Medium	High
Stream association	Low	Medium	High
Microenvironmental conditions for *B. dendrobatidis* growth and survival			
Thermal conditions	Poor	Sub-optimal	Optimal
Hydric conditions	Sub-optimal	Poor	Optimal
Theoretical susceptibility to chytridiomycosis-related declines based on behaviour	**LOW**	**MEDIUM**	**HIGH**

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APPENDIX 1: NON-CONTACT INFRARED THERMOMETERS CAN ACCURATELY MEASURE AMPHIBIAN BODY TEMPERATURES

*Rowley, J. J. L. and Alford, R. A. (In press) Non-contact infrared thermometers can accurately measure amphibian body temperatures. *Herpetological Review*.

Introduction

Body temperature affects almost all biochemical and physiological processes in ectothermic organisms (Hutchinson and Dupré 1992), and thus affects movement, habitat selection and thermoregulatory behaviour (Bartelt and Peterson 2005). A variety of devices have been used to determine the body temperatures of amphibians in the field. The most typical is a thermocouple probe connected to a quick-reading thermometer, which has been used to measure skin (Brattstrom 1963; Heath 1975; Lillywhite 1970; Navas 1996; Tracy 1976), oral (Brattstrom 1963; Lillywhite et al. 1973; Lillywhite et al. 1998), or cloacal (Brattstrom 1963; Cabanac and Cabanac 2004; John-Alder et al. 1988; Passmore and Malherbe 1985; Thorson 1955; Vences et al. 2002) temperature. These methods require manual handling of each individual, which may artificially elevate its body temperature (due to conduction; Navas and Araujo 2000), or may alter the behaviour of individuals in studies of a longer duration (ie. by capture stress).

To date, non-invasive methods of measuring amphibian body temperature that do not require the manual handling of individuals have included the attachment of thermally sensitive radio-transmitters (Bradford 1984; Heath 1975; Seebacher and Alford 2002) and the use of physical models (Bartelt and Peterson 2005; Hasegawa et al. 2005; Navas and Araujo 2000; O'Connor and Tracy 1987; Seebacher and Alford 2002). More recently, an additional method of measuring amphibian body temperature has become available, via the use of hand-held, non-contact, infrared thermometers (e.g., Young et al. 2005). These thermometers have been widely adopted in the medical sciences, and have been shown to accurately measure body temperature in humans (Koçak et al. 1999; Rotello et al. 1996; Terndrup et al. 1997). Despite their promise as a rapid, non-invasive method of measuring amphibian body temperature, their accuracy for measuring amphibian body temperature has not been quantified. I designed this study to determine whether a non-contact infrared thermometer can be used to accurately measure the body temperatures of amphibians.

Methods

This experiment was designed to allow the measurement of body temperatures of frogs experiencing a wide range of thermal environments in a laboratory setting. I set up three opaque plastic containers (60 x 40 x 40 cm), each with a small water bowl in the center and a metal fly-screen lid. The containers were housed in a constant temperature room, which maintained ambient temperature

between 19.5 and 21.5º C. Relative humidity fluctuated between 64 and 96% (mean 74%). A 150-watt heat lamp was provided at one end of the container during the day (0930-2130 h) in order to provide a heat gradient within the normal temperature range of the species.

Twelve adult common green or White's tree frogs (*Litoria caerulea*) were captured near Townsville, Queensland, Australia. They ranged from 74.1-91.8 mm SVL and 26-65 g body mass. Prior to experiments, they were maintained in the constant temperature room in which the experiments were carried out, but in smaller containers with no access to heat lamps. Frogs were fed crickets *ad libitum*. Each frog was used in a single run of the experiment. I ran four temporal replicates of the experiment, creating a total of twelve sets of measurements of frog and model temperatures for comparison. Each replicate ran for three days.

Body temperatures of frogs were recorded five times per day (0900, 1100, 1300, 1500, and 1700 h), producing fifteen measurements for each of the twelve frogs. The first time (0900) was chosen because at that time the frogs had received no source of heat for almost 12 h, and their body temperature should have been similar to a nocturnal reading in the field. Each run of the experiment was set up at least 60 minutes before the first temperature reading was taken, allowing frogs to reach a thermal steady state.

At each reading, frog body temperature was recorded using three techniques. Firstly, temperature was recorded by holding a Raytek ST80 Pro-Plus Non-contact thermometer (RAYST80; "IR thermometer") approximately 5 cm away from the frog and aiming at the lower dorsal area near the thigh. The model of IR thermometer used in this study had a distance to spot ratio of 50:1, and the area measured is delineated by a circle of laser diodes. Emissivity was set on the IR thermometer at 0.95, as it is generally accepted that amphibians have a long-wave emissivity of approximately 0.95-0.97 (Carroll et al. 2005; Tracy 1976), regardless of their colour (Nussear et al. 2000; Tracy 1979). In initial trials, I determined that measured frog body temperatures did not vary by more than 0.1ºC when the emissivity setting of the IR thermometer was varied between 0.95-1.0.

After taking a reading using the IR thermometer, I then measured skin and cloacal temperatures (in that order) using a small, chromel-alumel "K" type thermocouple (diameter approx. 1 mm) with the tip coated in plastic, attached to a digital thermometer type 90000. To measure skin temperature, the thermocouple was held firmly against the skin on the lower dorsal area near the thigh while the frog remained in its original position in the container. During cloacal temperature measurement, each frog was held by a single leg, whilst still in the container, and the thermocouple was inserted 10-20mm inside the cloaca and the reading was taken when the indicated temperature stablized.

Frogs were usually in the water conserving posture immediately prior to temperature measurement, except for several instances at 0900 h. It is probable that there are small differences

among individual frogs, related to body size or individual behaviour, that affect how temperatures measured using the three techniques are correlated, so that the measurements for each individual frog are not entirely statistically independent. I therefore did not carry out any hypothesis tests, but concentrated on modelling the relationships between measurements taken using the three techniques, and determining how well my models fit the data. Because all three variables are measured with error, I constructed models of their relationships using major axis regression (Sokal and Rohlf 1995). I determined how well my regression models fit the data by calculating coefficients of determination, using standard correlation analysis.

Room temperatures were recorded every 30 minutes using thermal data loggers (Thermochron iButtons by Dallas Semicondictor, Dallas, Texas USA). Data loggers and thermocouples were calibrated against a high precision mercury thermometer in a magnetically stirred water bath.

Results

Body temperatures measured using the IR thermometer and both skin and cloacal temperatures measured using the thermocouple probe were highly correlated. The major-axis regression lines relating each type of temperature measurement to the others were all very similar to the line of equality (Figure A1). The major-axis regression lines never predict a mean difference greater than approximately 0.5^{o}C between any two temperatures in the range 18 to 34 ^{o}C for any pair of measurements.

Discussion

The surface of basking animals may reach slightly higher temperatures faster and decrease more rapidly after basking ceases than the body core (Remmert 1985). However, I found that skin temperatures measured either by contact thermocouple or IR thermometer were almost always within 0.5^{o}C of cloacal temperatures (Figure A1); this appears to be relatively common in small ectotherms such as frogs (Wygoda 1984), and other small ectotherms such as lizards (<10-20g; Jones and Avery 1989). It is likely that the small number of points which depart to a larger-than-usual extent from the lines of equality and regression lines in Figure A1 were measured on animals that had recently changed from basking to non-basking or the reverse.

We found that cloacal temperature was slightly better predicted by surface temperature as measured by the IR thermometer than it was by skin temperature measured using a thermocouple. This indicates that surface temperatures measured using the IR thermometer should provide accurate indicators of internal body temperatures in most amphibians.

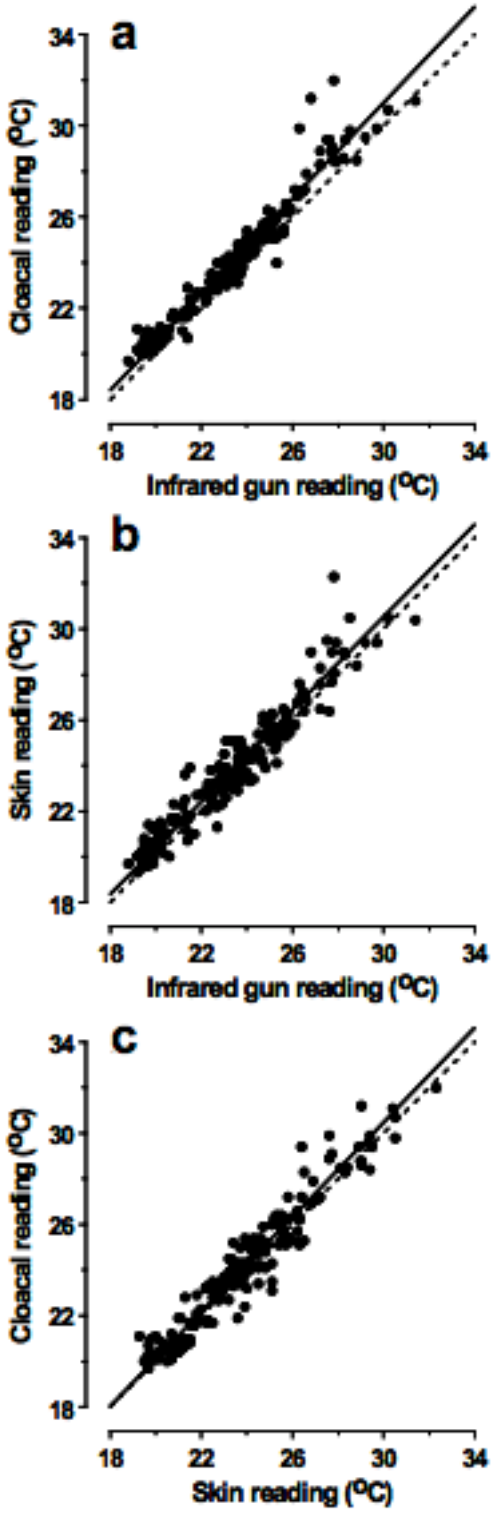

Figure A1. **a**. Measured cloacal temperature as a function of infrared gun reading. Dashed line is at y = x, solid line is the major axis regression $y = 1.052x - 0.535$ with $r^2 = 0.949$. **b**. Measured skin temperature as a function of infrared gun reading. Dashed line is at y = x, solid line is the major axis regression $y = 1.014x + 0.1049$, with $r^2 = 0.922$. **c**. Measured cloacal temperature as a function of skin temperature. Dashed line is at y = x, solid line is the major axis regression $y = 1.038x - 0.657$, with $r^2 = 0.932$.

Good quality IR thermometers have long-range optical resolution, allowing measurement of small targets at long distances. As the distance from the object increases, the spot size of the area measured by the unit becomes larger. Therefore, the smaller the target, the closer you must be to it in order to avoid measuring a combination of amphibian and background temperatures. Especially in the field, it is necessary to take the distance to spot ratio into account. When studying small frogs, it may only be possible to measure temperatures at short ranges (<0.5 m). As the laser is located above the sensor in many models, it is also important to take parallax effects into account when aiming the sensor, as at near distances the point of aim of the sensor will be displaced from the point of aim of the laser diode.

It is likely that IR thermometers will be useful in measuring other small animals, such as reptiles, in the field. As all plants and animals act almost as black-bodies in the middle infrared (Sustare 1979), having an emissivity nearing 1.0, no major changes in the technique will be necessary when used on different species. Indeed, I am presently using IR thermometers successfully for measuring the body temperatures of a number of amphibian species in the field (Rowley and Alford, unpubl. data).

We have shown that non-contact infrared thermometers can be used to accurately determine the body temperatures of amphibians. Benefits of this technique include relatively low cost (approximately US$340), small size and therefore high portability, and the ability to rapidly record the temperature of a large number of individuals. Perhaps the most important feature of the technique, however, is its ability to record the temperature of amphibians without handling them. This reduces disturbance, which can be important when the same individual is to be measured repeatedly, or when disturbance may cause an animal to abandon a retreat or basking site, exposing it to increased risks of predation or desiccation. In addition, such non-invasive methods of determining amphibian body temperature are likely to be increasingly important due to the need to minimise handling stress and the possibility of disease transmission, particularly when studying species of conservation concern.

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