Environmental effects on a host-pathogen system: frogs and *Batrachochytrium dendrobatidis* in wet and dry habitats

Thesis submitted by
Robert Puschendorf Hons, MSc, UCR
September 2009

For the degree of Doctor of Philosophy
School of Marine and Tropical Biology
James Cook University

ELECTRONIC COPY

I, the undersigned, and author of this work, declare that the electronic copy of this thesis provided to the James Cook University Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

_____ _____

Signature Date

STATEMENT OF ACCESS

I, the undersigned, author of this work, understand that James Cook University will make this thesis available for use within the University Library and, via the Australian Digital Theses network, for use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and;

I do wish to place a 12-month embargo on my thesis starting at final acceptance, after which there will be no further restriction on access to this work.

_____ _____

Signature Date

STATEMENT OF THE CONTRIBUTION OF OTHERS

This thesis was supervised by Ross Alford, Jeremy VanDerWal and Lee Skerratt. As such they had significant input on the design, execution and analysis of this project, as well as reviewing the individual chapters of this thesis. Ana Carnaval, Conrad Hoskin, Jamie Voyles, Scott Cashins, Sara Bell, Caroline Palmer, Federico Bolaños, Gerardo Chaves, Kris Murray, Stephen Garland, Jodi Rowley and Keith McDonald provided editorial assistance on individual chapters. PCR diagnostics for *Batrachochytrium dendrobatidis* were performed by Ruth Campbell and Stephen Garland. Conrad Hoskin confirmed the identity of *Litoria lorica* through morphological and molecular techniques. Sam Young performed the necropsies of dead and diseased frogs found in the field.

This work was funded by an International Postgraduate Research Scholarship from the Australian Department of Education, Science and Training, by the School of Marine and Tropical Biology at James Cook University, the Graduate Research School at James Cook University and by a research contract from the Australian Department of the Environment and Water Resources. Also, funding the Skyrail Foundation and a Research and Exploration grant from the National Geographic Society (#8608-09) made this work possible. For all these institutions I am incredibly grateful for without their support this work would not have been possible.

DECLARATION OF ETHICS AND PERMITS

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *National Statement on Ethics Conduct in Research Involving Human* (1999*)*, the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics*. *Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (A 1283). This research was conducted under permits WISP05056508, WITK05056608 and ATH08/027 granted by the Queensland Parks and Wildlife Services.

_____ _____

Signature Date

ACKNOWLEDGMENTS

I spent my MSc working on the effects of chytridiomycosis on Costa Rican amphibians under the excellent guidance of Bill Eberhard, one of my former supervisors. It was on Bill's advice that I decided to come to Australia and work with Ross. It was good advice, thank you Bill!

I would like to thank each of my supervisors, Ross Alford, Jeremy VanDerWal and Lee Skerratt. Your contributions, each from different fields, significantly enriched my PhD and my overall research experience throughout the last 3 years.

Ana Carnaval, Sara Bell, Scott Cashins, Jamie Voyles, Steve Williams, Caroline Palmer, Conrad Hoskin, Keith McDonald, Kris Murray, Lin Schwarzkopf, Stephen Garland and Alan Pounds were all kind enough to spend their valuable time discussing my ideas and proof-reading papers and chapters. Thank you all for your time and contagious enthusiasm.

With such a significant field component, this work would not have been possible without the help of many volunteers and friends. Noriko Iwai, Rebecca Sears, Ana Carnaval, Alan Pounds, Brian Colin Parton, Andrés Merino-Viteri, Kate Winters, Christie Youngs, David Scott, Ainslie Langdon, Adrian Arias, Katrin Schmidt, HyoJin Yang, Stephen M. Zozoya, Arnaud Gourret, Katrin Schmidt, Rhiannon Dalrymple, Josephine Laura Green, Vanessa Valdez, Michael Siegfried, Sara Gutowski, Russel Standford, Noriko Iwai, Steve Prutman, Nadeesha Perera, Leighlan Phillips, Alexander Young, Danielle Saye, Adrián Arias, Adriana Chacón, Caroline Palmer, Megan Higgie and Steve Richards. Katrin Schmidt tested different frog marking techniques on several of my field trips. Thank you for the good company Kat! Also, thank you to Lorena Falconi for helping me to produce publishable maps towards the end of my PhD.

Everybody involved in the Amphibian Disease Ecology Group (ADEG) were incredibly helpful throughout this project. Stephen Garland, Andrea Phillot, Sam

Young, Jodi Rowley, Rebecca Webb, Lee Berger, Rick Speare, thank you for all your help and support.
Keith McDonald, Tina Langford, David Sherwell and Michelle Nissen from the Environmental Protection Agency were incredibly helpful and supportive of my work. Robert and Jennifer Waterhouse of the Mount Carbine Caravan Park always made me feel at home during my field trips in Mount Carbine, thank you for all your kindness.

A lot of things happened in these three years, both good and bad. I was very fortunate to have been surrounded by such amazing friends and family, always supportive and present when it mattered. To Jamie Voyles, Scott Cashins, Ana Carnaval, Sara Bell, Jeremy VanDerWal, Andrés Merino-Viteri, Adrián Arias, Alan Pounds, Conrad Hoskin, Yvette Williams, Christopher Peter, Michael Siegfried, Brian Kubicki and Caroline Palmer, thank you. Caroline, thank you for putting up with the madness of the last few months. To my family, Mom, Dad, Caroline, Axel, Melissa, Sebastian and Luciana, thank you for all your support and love.

ABSTRACT

The global increase in emerging infectious diseases poses a substantial threat to wildlife, especially for small, fragmented and populations exposed to novel pathogens. The emergence of the chytrid fungus *Batrachochytrium dendrobatidis* is one of the clearest cases of this to date. *Batrachochytrium dendrobatidis* colonises keratinised cells of the amphibian epidermis and causes the disease chytridiomycosis when pathogen populations on hosts reach a threshold density. Host mortality usually occurs shortly after clinical disease symptoms become evident. Chytridiomycosis is fatal to a wide range of amphibian species, while other, less vulnerable species can serve as asymptomatic carriers. This has allowed chytridiomycosis to cause widespread local and global declines as well as extinctions in a wide range of amphibians. Many of the affected species occur in relatively pristine, protected areas; declines of these species have been termed “enigmatic” declines. Previous research has demonstrated that the physical environment plays a major role in determining whether infected hosts recover, coexist with sublethal infections or develop chytridiomycosis and die. The optimal environment for *B. dendrobatidis* is cool and moist, as is typical of tropical high elevation rainforests, where amphibian diversity is usually high. In tropical Central America and Australia, lowland populations of species that are widely distributed along elevational gradients often persist, while upland populations decline or suffer local extinction. It appears that the warmer, sometimes drier or more seasonal environments of lowlands are less favourable for the pathogen, permitting otherwise susceptible species to coexist with *B. dendrobatidis*. These areas are therefore climatic refuges from disease-driven amphibian declines and extinctions. The aim of this thesis was to gain an increased understanding of the operation of climatic refuges. I addressed this aim using three objectives: a) to determine how the climate within refuges affects the biology of *B. dendrobatidis*, b) to compare the epidemiology of the interaction between *B. dendrobatidis* and its amphibian hosts between refugial and non-refugial sites, and c) to elucidate the underlying mechanisms that enable amphibians in climatic refuges to coexist with this potentially lethal pathogen.

To attain the first objective, I used species distribution models for *B. dendrobatidis* to predict areas that are unsuitable for the pathogen and should therefore have low probabilities of suffering from disease-driven amphibian declines. I initially examined data from Costa Rica, collected during my Masters' research. In Costa Rica, areas of low probability of pathogen occurrence are mostly dry forests, which coincidentally support a large population of *Craugastor ranoides*, a critically endangered species that has disappeared from most of its range (in rainforest) in conjunction with outbreaks of chytridiomycosis. As predicted data on prevalence between dry and wet areas suggest, *B. dendrobatidis* is much more common in wet areas. This supports the proposed hypothesis that areas with environments that are hostile to *B. dendrobatidis* can serve as refuges for frogs that have broader environmental niches. I collected preliminary survey data that indicated that dense populations of the frog *Litoria nannotis*, which had declined across its distribution range above 400 m elevation in rainforest of the Australian Wet Tropics, occurred in dry forest areas adjacent to the western boundary of the rainforest. Species distribution models for *B. dendrobatidis* constructed using data on its known Australian distribution, predicted that these areas were not suitable for the pathogen. However, surveys of the prevalence of *B. dendrobatidis* in frogs in these dry forest areas, which were the first to be conducted showed that *B. dendrobatidis* is present at very high prevalences, higher than in adjacent wet forest areas. This contrasts strongly with low elevation refuges in the Australian Wet Tropics and with the dry-forest refuges in Costa Rica, where prevalences are lower than at high elevation sites. These results suggest that the mechanisms by which high elevation dry forests adjacent to the Australian Wet Tropics serve as climatic refuges differ from those in other areas. Rather than excluding *B. dendrobatidis* or limiting its prevalence, the high-elevation dry forests promote coexistence between frogs and the pathogen.

To better understand how the host-pathogen interaction differs between areas where frogs were extirpated and now occur in low abundance (wet sites) and adjacent refugial areas where dense frog populations persist (dry sites), I carried out a comparative epidemiological study. Despite the wet and dry study sites being less

than 1 km apart, the pathogen-susceptible *L. nannotis* were up to five times more abundant in the dry site than in the wet site. The intensities of infections in animals positive for *B. dendrobatidis* did not differ significantly between the sites, however chytridiomycosis-induced mortality was only detected in wet environments. Surveys carried out over a 15-month period showed that prevalence at the dry forest site remained consistently high, significantly higher than at the wet forest site. Also, dry forest frog populations remained much denser than wet forest populations. Given the very high prevalences of *B. dendrobatidis* infection occurring at the dry forest site, this strongly indicates that infected frogs at this location can tolerate infections for extended periods. The suggestion that the environment at the dry forest site leads to increased tolerance is supported by my discovery of a population of *Litoria lorica* a short distance downstream. This species was considered a rainforest endemic; it disappeared from all known localities in the early 1990s, had not been seen in 18 years, and was generally thought to be extinct. This newly-discovered population is dense and tolerates high prevalences of *B. dendrobatidis* infection, similar to those of dry forest *L. nannotis*. This discovery also demonstrates that it is essential to look for poorly-known species outside the "limits" of their distributions; in this case, it is clear that *L. lorica* is not, as had been thought a rainforest endemic, but is endemic to high elevation torrents and waterfalls, rather than the surrounding terrestrial habitat.

To further understand the mechanisms leading to the differences in epidemiological patterns between wet and dry habitats, I examined frog behaviour such as microhabitat use. In contrast to the wet forest, where *L. nannotis* use moist rock crevices as diurnal retreat sites, most tracked *L. nannotis* in the dry forest spent the day submerged under the water, in fast flowing sections of the stream. This could reduce rates of re-infection rates of individuals by flushing zoospores away from the frog as they are released, and thus reduce the rate of growth of pathogen populations on the hosts. The open canopy at dry forest sites leads to substantial heating of the large boulders that form the substrate usually occupied by frogs during nocturnal activity. When dry forest frogs emerge from their diurnal aquatic retreat sites at dusk, the rocks that they perch on, having been heated in the sun, are dry and substantially warmer than the air temperature. This has the potential to control pathogen loads on their ventral surfaces, the body region most susceptible to infection. The nocturnal perches of wet forest frogs receive little direct sunlight, and are typically moist and

cool when frogs emerge, which are conditions conducive to the build up of pathogen populations on the ventral surface.

At present, captive breeding is presumed to be the only viable conservation strategy to prevent chytridiomycosis from causing the extinction of many susceptible amphibian species. Climatic refuges that exclude the pathogen or enhance the ability of frogs to coexist with it, may allow natural amphibian populations to persist and eventually to evolve increased resistance and potentially recolonise non-refugial areas from which they have been extirpated. Any threat, such as chytridiomycosis, which is most severe in the core habitat of many species, can make populations living on the margins of species geographic or ecological ranges, vital to species' persistence.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE: INTRODUCTION

Diseases are an important evolutionary force being a natural component of every ecosystem, and they have profoundly influenced recorded human history. In cases such as the outbreaks of bubonic plague that killed a third of the European population in the 1300's (Kelly 2006) to the introduction of smallpox and influenza into the new world by the conquistadores, wiping out entire cultures (Diamond 1997), diseases have been a significant factor in shaping the world we live in. In ecological studies, the importance of diseases was largely overlooked until recently. Ecologists and conservationists have only recognized the importance of wildlife diseases as a major evolutionary force in the last two decades (Dobson and May 1986; May 1988; McCallum and Dobson 1995; Scott 1988).

In the field of ecology, host-pathogen systems are traditionally thought to be in equilibrium. Disease-related population crashes and single species extinctions have only been recorded in a handful of cases, such as island birds (van Riper III et al. 1986; Warner 1968), wild dogs (Ginsberg et al. 1995), the black-footed ferret (Thorne and Williams 1988) and snails (Daszak and Cunningham 1999). Most of these examples have other confounding factors, such as small population size (Shaffer 1981), isolation (Ouborg 1993) and inbreeding (Saccheri et al. 1998), which make them more susceptible to stochastic events that can lead to extinction (Lande 1993; Thomas 1994). However, the natural world has undergone, and continues to undergo, dramatic anthropogenic changes, which many authors have suggested are leading to a mass extinction event (Şengör et al. 2008). One aspect of these changes is shifts in natural systems, altering inter-organism dynamics including many host-pathogen interactions and leading to the establishment of new diseases or increased pathogenicity and virulence of established diseases (Anon 2000; Daszak et al. 1999; Daszak et al. 2000b; Harvell et al. 1999; Harvell 2004; Morse 1995; Reed et al. 2003). These emerging infectious diseases have recently been recognized as a threat, not only to humans, but also to global biodiversity (Cunningham et al. 2003; Daszak et al. 2000; Epstein et al. 2003; Harvell et al. 1999), with the most dramatic example being the worldwide amphibian population declines and species

extinctions (Berger et al. 1998; Berger et al. 1999a; Bosch and Martinez-Solano 2006; Briggs et al. 2005; Lips et al. 2006; Rachowicz et al. 2006).

In this introduction I firstly review the evidence for disease as a cause of extinction. Secondly I examine the role of the environment in host-pathogen interactions and how environmental conditions may benefit either the host or the pathogen. Finally I review the factors that make the causal agent of the disease chytridiomcyosis, the fungus *Batrachochytrium dendrobatidis*, such an effective frog killer, with a focus on environmental factors that affect the relationship between *B. dendrobatidis* and its amphibian hosts (Berger et al. 1998; Berger et al. 1999a; Bosch and Martinez-Solano 2006; Briggs et al. 2005; Lips et al. 2006; Rachowicz et al. 2006; Rosenblum et al. 2009; Voyles et al. 2007), culminating the debate of the role of climate change in these declines (Alford et al. 2007; Di Rosa et al. 2007; Laurance 2008; Lips et al. 2008; Pounds et al. 2006; Pounds and Puschendorf 2004; Skerratt et al. 2007).

Extinction risk due to disease

The ability of infectious diseases to extirpate local populations, modify community dynamics and decrease host ranges is well documented (Burdon 1991; Daszak and Cunningham 1999; de Castro and Bolker 2005; Dobson 1988; Harvell et al. 1999; McCallum and Dobson 1995; Walsh et al. 2003), but reports on their involvement in species extinctions have been relatively scarce. This is highlighted in a review by Smith et al. (2006) of the IUCN's Red List species database and the literature available, which found that diseases were involved in less than 4 % of total species extinctions recorded. It is likely that this is an underestimate, due in part to the difficulty of studying wildlife diseases. For example, carcasses decompose quickly, so disease outbreaks in wildlife can easily remain undetected (e.g. Balcomb 1986; Tobin and Dolbeer 1990; Ward et al. 2006; Wobeser and Wobeser 1992). Problems detecting diseases (Scott 1988) and a lack of established monitoring projects and funding (Aguirre et al. 2002) contribute to low detection rates. Monitoring programs are essential to the field of wildlife disease ecology; surprisingly even lacking in zoonotic diseases that threaten human health (e.g. Smith et al. 2009; Wang and Palese 2009). Studying and monitoring wildlife disease is

increasingly important, given the increased number of infectious diseases emerging in recent times (Aguirre et al. 2002).

Pathogen pollution, the introduction of new diseases into naive host populations, is one of the main drivers of disease-mediated population declines, and one of the principal causes of the recent emergence of many diseases (Cunningham et al. 2003; Daszak et al. 2000b; Epstein et al. 2003; McCallum et al. 2003; Wilcox and Gubler 2005). There are several well-documented cases of species population collapses and extirpations due to introduced pathogens (e.g. Ginsberg et al. 1995; Plowright 1962; Plowright 1982; Thorne and Williams 1988). However even the introduction of a virulent pathogen might need several other factors for it to cause extinction.

Many diseases caused by microparasites (usually bacteria, viruses and protozoa), have a single host species and depend on direct transmission to infect new hosts. Mathematical models developed for these relatively simple systems suggest that highly virulent strains of the pathogen will tend to deplete host populations to the point at which transmission becomes unlikely, and the pathogen may go extinct; this suggests that less virulent strains should be favoured, particularly in the later stages of an outbreak (Anderson 1979; Anderson and May 1979; McCallum and Dobson 1995). A classic example for this is the introduction of the myxoma virus to control introduced rabbit populations in Australia (Fenner and Ratcliffe 1965). After introduction the virus killed off 99 % of the rabbit population, with a fraction of the host population persisting. Subsequent experiments with recovered viral samples from the wild demonstrated that the virus had lost some of its virulence, supporting the hypothesis that pathogen virulence and transmission are intrinsically linked (Anderson and May 1982; Fenner and Marshall 1957; Massad 1987; May and Anderson 1983).

However, some pathogens do completely extirpate host populations. One of the key factors enabling this is the presence of a pathogen reservoir within the system (Gog et al. 2002; Plowright 1982; Thorne and Williams 1988; van Riper III et al. 1986). One of the best examples of the direct role of pathogen reservoirs in the demise of a species is the case of the black-footed ferret (*Mustela nigripes*), which is threatened by two diseases

caused by microparasites: canine distemper and plague (Thorne and Williams 1988; Williams et al. 1994; Williams et al. 1988). During the 1970's *M. nigripes* was thought to be extinct due mostly to habitat loss until a small population was rediscovered in 1981 (Anderson et al. 1986). Disease was then identified as the main threat to this species, because of its lack of immunity and small population size. In order to prevent extinction of this remnant population from disease it was necessary to secure a colony in captivity, from which natural populations have been re-established (Thorne and Williams 1988). The success of this program is still hampered by frequent die-offs in reintroduced populations (Thorne and Williams 1988; Williams et al. 1994; Williams et al. 1988). *M. nigripes* has not developed immunity to either disease. There are many alternative hosts for canine distemper and plague within the system, including the ferret's main food source, prairie dogs (Thorne and Williams 1988; Williams et al. 1994; Williams et al. 1988). Since prairie dogs are essential for the maintenance of the black-footed ferret, it is unlikely that long-term viable populations can be re-established unless the species increases its tolerance or becomes immune to these diseases.

One of the best-documented cases of disease causing extinction of some of its host species is the introduction of avian malaria into Hawaii's native bird fauna (van Riper III et al. 1986; Warner 1968). Historically, migratory birds infected with avian malaria could have reached the Hawaiian Islands on occasion, but the natural establishment of the diseases was unlikely because of the lack of the disease vector, the mosquito *Culex quinquefasciatus* (Warner 1968). Introduction of the vector occurred in 1826, which was followed by the introduction of avian malaria with the introduction of several bird species but the exact introduction date of the malaria parasite remains unknown, as the etiology of the disease was not known at the time (Warner 1968). The problem of declining birds became apparent in the early 1900's during a period in which many non-native bird species established populations in Hawaii, after being introduced by humans (van Riper III et al. 1986; Warner 1968). Avian malaria swept across the system affecting many species, some of which were completely extirpated from their habitat (van Riper III et al. 1986; Warner 1968). Endemic Hawaiian birds are extremely susceptible to the strain of malaria introduced, as infections of the parasite, *Plasmodium relictum*, can cause mortality rates of 65-90 % after infection (Atkins et al. 2002; Atkinson et al. 2000;

Atkinson et al. 2001; Atkinson et al. 1996). Several environmental and biological conditions were necessary for this disease to become so lethal. The mosquito species that functions as the main parasite vector was easily established as there is plenty breeding habitat on the islands (van Riper III et al. 1986; Warner 1968). Hawaiian birds lacked immune defences to the introduced pathogen, and the large number of introduced bird species with higher resistance to the disease served as pathogen reservoir, contributing to the demise of the endemic species (Atkins et al. 2002; Atkinson et al. 2000; Atkinson et al. 2001; Atkinson et al. 1996). Noteworthy in this case is the magnitude of the disease's effects across taxa, something that rarely occurs in natural systems (Atkins et al. 2002; Atkinson et al. 2000; Atkinson et al. 2001; Atkinson et al. 1996; van Riper III et al. 1986; Warner 1968).

The black-footed ferret and the declining endemic bird populations in Hawaii exemplify how serious the threat of disease becomes when a reservoir hosts is present in the system. The most effective reservoir hosts are those in which the virulence of the pathogen is very low, so it can occur at high prevalence (Dobson and Foufopoulos 2001; Dobson and May 1986; McCallum 1994; McCallum and Dobson 1995; McCallum and Dobson 2002). Under these conditions, if the pathogen produces high mortality in the susceptible host species, and the reservoir host species is abundant in the system, the threat of extinction due to disease can be extremely high. The presence and quality of pathogen reservoirs strongly affects the probability of a pathogen driving susceptible hosts to extinction, however host susceptibility is also a major factor determining vulnerability. Susceptibility can be affected by environmental factors, which play a vital role in regulating many host-pathogen interactions (Bannister et al. 1996; Blanford et al. 2003; Colhoun 1973; Dowell 2001; Elliot et al. 2002; Roberts 2003; Rossignol et al. 2006; Snieszko 1974). In some instances the environment in which the host-pathogen interaction is initiated can determine whether host populations will persist or disappear (Berger et al. 1998; Berger et al. 2004; Hall et al. 2006; Harvell et al. 1999; Maniero and Carey 1997; McCallum et al. 2003; Roy et al. 2004; Simms and Triplett 1994; Woodhams and Alford 2005; Zell 2004).

The environment and its effect on disease seasonality and pathogenicity

The environment plays a key role in determining the balance between hosts and pathogens (e.g. Altizer et al. 2006; Berger et al. 1998; Berger et al. 2004; Dowell 2001; Grassly and Fraser 2006; Harvell et al. 2002; Hosseini et al. 2004; Kriger and Hero 2006b; Plytycz and Seljelid 1997; Woodhams and Alford 2005). One of the most studied effects of the environment on host-pathogen interactions is the seasonality of many diseases (Altizer et al. 2006; Dowell 2001; Grassly and Fraser 2006; Hosseini et al. 2004). In humans, seasonal outbreaks of influenza in temperate climates during winter or the increase of malaria outbreaks during the wet season in the tropics are well-documented examples (Dowell 2001). Seasonality can generate geographic differences in the timing and severity of diseases (Cook et al. 1990; Randolph et al. 2000). A significant increase in the severity of disease can occur under environmental conditions in which the host's defences are suppressed, allowing the pathogen to flourish (Harvell et al. 2002; Pascual et al. 2002; Patz et al. 2005; Patz and Olson 2006). Even seasonal changes in host behaviour such as aggregation can have profound consequences for disease dynamics and host susceptibility. For example, outbreaks of bird conjunctivitis caused by *Mycoplasma gallisepticum* were strongly influenced by seasonal changes in behaviour by its host, the house finch *Carpocadus mexicanus* (Randolph et al. 2000). Bird feeders are a key resource enabling their survival during winter months in northern latitudes, and cause birds to aggregate, which increases pathogen transmission rates (Randolph et al. 2000). In another example, harlequin frogs of the genus *Atelopus* suffered a significant increase in attacks by a parasitic fly when they chose microenvironments close to spray zones around waterfalls (Pounds and Crump 1987). A diversity of environments might also be beneficial for the host. For example, in Hawaii, avian malaria is less pathogenic in xeric than in mesic habitats (van Riper III et al. 1986). The wet forest consistently supports higher disease prevalence, which is related to an increase in the availability of breeding sites for the disease’s vector (van Riper III et al. 1986). As a consequence, xeric habitats also supported a higher number of susceptible native birds than wet forest habitats, which was the inverse of what occurred before the invasion of the *Plasmodium* parasite into Hawai'i (van Riper III et al. 1986).

Changes in host behaviour that increase body temperature in response to infection are well documented in ectothermic organisms; this process is called behavioural fever (Blanford and Thomas 1999a, b, 2000; Blanford et al. 1998; Blanford et al. 2003; Thomas and Blanford 2003). In grasshoppers and locusts, individuals modify their behaviour when infected, increasing their preferred body temperature (Elliot et al. 2002). The increase in temperature negatively affects parasite growth, and also augments immune responses to infection (Ouedraogo et al. 2003). Even if hosts do not alter thermoregulatory behaviour in response to infection, many amphibians engage in behaviours such as basking, which lead to elevated body temperatures. Differences among species and among individuals within species in their proclivity to, or opportunity for, basking could strongly affect susceptibility to disease, even in species that do not exhibit behavioural fever (Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b; Woodhams et al. 2003).

A brief history of amphibian declines

Mass amphibian declines and extinctions have unfolded during the last three decades around the globe, leading to the current recognition of amphibians as the most threatened vertebrate class (Stuart et al. 2004). One feature of this dramatic species loss is that many species have suffered declines and extinctions in protected and pristine areas around the world (Blaustein and Wake 1990; Collins and Crump 2009; Stuart et al. 2004; Wake 1991). Initially it was debated whether these losses were part of natural population fluctuations, but it rapidly became clear that these “enigmatic declines” were in fact population crashes and potential species extinctions (Alford et al. 2001; Alford and Richards 1999; Blaustein et al. 1994; Heyer et al. 1988; Pechmann et al. 1991; Pechmann and Wilbur 1994; Pounds et al. 1997). The magnitude, timing and geography of the problem pointed to a common and recent worldwide phenomenon. Although many hypotheses were suggested, few are still considered relevant today by amphibian biologists studying this problem (Alford and Richards 1999; Blaustein et al. 1994; Collins and Crump 2009; Collins and Storfer 2003; Davidson et al. 2002; Skerratt et al. 2007; Vredenburg 2004). Most of the research today is focused on understanding the biology of one pathogen and its interaction with its amphibian host.

Enigmatic amphibian declines and chytridiomycosis

The most accepted hypothesis by amphibian biologists and wildlife managers working on the problem of the "enigmatic" worldwide amphibian declines today, is that the introduction of the chytrid fungus *Batrachochytrium dendrobatidis* (Longcore et al. 1999) into naive amphibian populations has caused the worldwide phenomenon of "enigmatic" amphibian declines (Berger et al. 1998; Collins and Crump 2009; Lips et al. 2006; Lips et al. 2008; Rachowicz et al. 2006; Skerratt et al. 2007; Stuart et al. 2004). *Batrachochytrium dendrobatidis* is the causal agent of the disease known as chytridiomycosis (Berger et al. 1998) and is the only member of the primitive fungi family Chytridiomycota known to be pathogenic to vertebrates (James et al. 2006; Longcore et al. 1999). *Batrachochytrium dendrobatidis* grows on the keratinized skin of amphibians (frog skin, tadpole mouthparts) but it is not restricted to keratin as a substrate (Altig 2007; Berger et al. 1998; Berger et al. 2005c; Fellers et al. 2001; Olsen et al. 2004; Piotrowski et al. 2004; Rachowicz and Vredenburg 2004; Rosenblum et al. 2008; Smith and Weldon 2007; Symonds et al. 2008). Although *B. dendrobatidis* DNA has been found in the environment (Lips et al. 2006; Walker et al. 2007), so far in nature it has only been shown to reproduce on the amphibian host (e.g. Rowley et al. 2006; Rowley et al. 2007a; Rowley et al. 2007b).

After the discovery of *B. dendrobatidis*, Koch's postulates (Koch 1893) were fulfilled demonstrating that *B. dendrobatidis* can kill amphibian hosts and is the causal agent of chytridiomycosis (Longcore et al. 1999; Nichols et al. 2001). This induced symptomatic disease chytridiomycosis develops in the adult stages, as a generalized skin infection (Berger et al. 1998; Berger et al. 2005c). Disease intensity can be explained by the increased population growth of the pathogen on the amphibian host (Carey et al. 2006). This infection disrupts normal epidermal functioning, producing an osmotic imbalance through loss of electrolytes, followed by death when the infection reaches an upper threshold at which basic physiological functions do not occur anymore (Voyles et al. 2007; Voyles 2009). It has also been proposed that *B. dendrobatidis* produces a toxin (Berger et al. 1998; Blaustein et al. 2005; Rosenblum et al. 2009), although no conclusive evidence exists at present to support this hypothesis.

Laurence et al. (1996) were the first to propose a link between the dramatic amphibian declines later referred to by Stuart et al. (2004) as “enigmatic” and an introduced disease. They hypothesized that the amphibian declines along the east coast of Australia (Ingram and McDonald 1993; McDonald and Alford 1999; Richards et al. 1993; Tyler 1991) could be explained by the spread of an introduced pathogen, which swept through this area from south to north, into naive amphibian populations. A similar hypothesis was later proposed for the Central American and South American declines (Lips et al. 2006; Lips et al. 2008; Lips et al. 2004). Although the idea that disease could be responsible for these declines was challenged early on (Alford and Richards 1997) and there are still many untested assumptions underlying the spreading-wave hypothesis, the discovery of *B. dendrobatidis* and its implication in worldwide amphibian declines has been well demonstrated.

The most solid evidence of *B. dendrobatidis* being an introduced, spreading pathogen comes from molecular work on the microparasite. This shows low genetic variability from samples collected across the world that is consistent with the pattern expected for an organism that has recently greatly expanded its geographic range (James et al. 2009; Morehouse et al. 2003; Morgan et al. 2007). Current molecular evidence suggests a North American origin for *B. dendrobatidis*, but further work with a wider variety of samples is necessary to conclusively determine this (James et al. 2009). Since its discovery, *B. dendrobatidis* has been identified in museum specimens collected immediately prior to species disappearances around the globe (Berger et al. 1998; Berger et al. 2004; Berger et al. 1999a; Bosch and Martinez-Solano 2006; Briggs et al. 2005; Kusrini et al. 2008; Lips et al. 2006; Merino-Viteri et al. 2006; Muths et al. 2002; Ouellet et al. 2005; Puschendorf 2003; Weldon and Du Preez 2004). However, because the high rate of false negatives in histology samples, and the lack of systematic spatiotemporal sampling in museum collection, it is hard to reach definitive conclusions on the origins of the pathogen (Berger et al. 1998; Berger et al. 1999a; Bosch and Martinez-Solano 2006; Briggs et al. 2005; Merino-Viteri et al. 2006; Muths et al. 2002; Ouellet et al. 2005; Puschendorf and Bolaños 2006; Puschendorf et al. 2006a; Weldon and Du Preez 2004).

Consistent with previously described pathogen-induced population crashes (Thorne and Williams 1988; van Riper III et al. 1986; Warner 1968; Williams et al. 1994; Williams et al. 1988), one of the key factors facilitating the lethality of chytridiomycosis is the presence of several reservoir hosts in most amphibian systems. One type of reservoir for *B. dendrobatidis* are species with limited susceptibility to the pathogen that persist despite being infected (e.g., Lips et al. 2006; McDonald and Alford 1999; Puschendorf et al. 2006a). Additionally, tadpoles of most amphibian species can serve as reservoirs, because *B. dendrobatidis* does not appear to commonly cause mortality in this life-history stage (but see Blaustein et al. 2005). The seasonally high abundance of tadpoles in many amphibian systems may therefore lead to high transmission rates (Briggs et al. 2005; Rachowicz and Briggs 2007; Rachowicz et al. 2006; Woodhams and Alford 2005).

Batrachochytrium dendrobatidis has been detected in hundreds of amphibian species (Olson and Ronnenberg 2008), from some that are now considered to be extinct (e.g., La Marca et al. 2005), to others that do not suffer from significant die offs in nature and clearly act as pathogen reservoir (Daszak et al. 2004; Garner et al. 2006; Mazzoni 2003; Peterson et al. 2007). The question of why some species succumb to this pathogen while others persist has been the focus of much recent research (Retallick et al. 2004; Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b; Woodhams et al. 2003; Woodhams et al. 2007a; Woodhams et al. 2007b; Woodhams et al. 2005; Woodhams et al. 2006). In addition to varying among species within sites, susceptibility to chytridiomycosis-related mortality varies among sites within species, usually in a manner clearly correlated with environmental gradients such as elevation (McDonald and Alford 1999; Richards et al. 1993; Young et al. 2001). This suggests that susceptibility is governed in at least some species by environmental conditions.

Environmental effect on *B. dendrobatidis* and its hosts

In tropical regions, the amphibian groups most threatened by chytridiomycosis are species closely associated with riparian habitats, especially in higher elevation rainforests (Lips et al. 2003b; McDonald and Alford 1999; Williams and Hero 1998). This is consistent with the biology of *B. dendrobatidis*, which includes an aquatic infective

stage, the zoospore, and therefore is likely to infect amphibians through contact with water containing zoospores (Berger et al. 2005a; Berger et al. 1998; Berger et al. 2004; Berger et al. 1999a; Rachowicz and Briggs 2007; Rachowicz and Vredenburg 2004). Additionally, *B. dendrobatidis* thrives in moist and wet environments, as it does not tolerate desiccation (Johnson and Speare 2003), making rainforests one of the most suitable habitats for this pathogen to exist (Ron 2005).

In order to elucidate the potential environmental conditions that favour the pathogen, laboratory studies working on *B. dendrobatidis* in pure culture have shown that the upper thermal threshold ranges from 28 °C where *B. dendrobatidis* stops growing, to 30 °C where the pathogen dies (Piotrowski et al. 2004). Optimum growth occurs between 17 and 25 °C, although population growth continues at lower temperatures (Piotrowski et al. 2004). Woodhams et al. (2008) demonstrated how *B. dendrobatidis* adjusts its life-history traits to compensate for slower development at lower temperatures via extended zoospore viability and higher zoospore production. As might be expected from the thermal biology of *B. dendrobatidis*, disease prevalence often increases, and disease-related mortality is more common, over winter. Thermal effects may also explain why rainforest amphibian populations at high elevations are the most affected (Berger et al. 2004; Bradley et al. 2002; Woodhams and Alford 2005). However, the documentation of species that have declined and subsequently recovered (e.g., Richards and Alford 2005), in environments where other species declined and disappeared suggests, some degree of resistance might have evolved in those populations or the virulence of the pathogen might have decreased after the initial outbreaks or a combination of both. These differences in susceptibility might be linked to the specific behaviour and microenvironment use among species, even when these occur in sympatry.

Under experimental conditions frogs can rid themselves of infection if they are able to attain body temperatures above 30 °C (Woodhams et al. 2003), consistent with the upper thermal tolerance limit of the pathogen (Piotrowski et al. 2004). The ability to thermally control *B. dendrobatidis* infections is therefore likely to depend on species-specific behaviour, such as basking and microhabitat selection; behavioural differences may therefore produce interspecific differences in susceptibility. The effect of temperature on

sympatric species of riparian frogs has been well studied in the Wet Tropics of Australia with the differential microenvironments utilized by each species directly influencing their susceptibility to disease (Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b). Consistent with Woodhams et al. (2003), in nature, species that were able to elevate their body temperature above 30 °C suffered little or no population declines, whereas the species that do not escape the environmental envelope of pathogen growth and reproduction have been the hardest hit (McDonald and Alford 1999; Richards and Alford 2005; Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b).

Amphibians' immune systems are notably sensitive to temperature and, since they are ectotherms, the functioning of their immune systems is affected by the environment they experience (Carey et al. 1996; Cooper et al. 1992; Maniero and Carey 1997; Matutte et al. 2000; Raffel et al. 2006). This has been well demonstrated in hibernating species of frogs that, during winter, have diminished immune capacity (Cooper et al. 1992). Colder temperatures make amphibians more susceptible to disease because their immune system becomes compromised (Maniero and Carey 1997). In the case of chytridiomycosis, this is a potentially lethal combination of host and pathogen responses to environmental conditions. Lower temperatures, such as the ones experienced at high elevation rainforest habitats and during winter months, have a synergetic effect, augmenting pathogen growth in addition to inhibiting host immune responses. In an experiment by Andre et al. (2008) 50 % of *B. dendrobatidis* infected individuals of *Rana muscosa* kept at 22 °C survived, whereas all infected frogs kept at 17 °C died from chytridiomycosis. This contrasts with the results of Woodhams et al. (2008), which suggest that frogs should die more quickly at 22 °C than at 17 °C, since the population growth of *B. dendrobatidis* is faster at the higher temperature. This suggests that reductions in immune function at the lower temperature may more than compensate for the reduced rate of pathogen population growth, therefore increasing the mortality rate. The effects of host immunity to chytridiomycosis are now being researched in greater detail. In whole-genome microarrays Rosenblum et al. (2009) monitored the transcriptional responses of several tissue types from *Xenopus tropicalis*, a susceptible frog species, to *B. dendrobatidis*. They present evidence suggesting that the immune system is not responding to infection, but they acknowledge that the experiment was conducted at low temperatures, where an

immune response of the host is unlikely. They propose three non-mutually exclusive hypotheses for the lack of immune response: a) *B. dendrobatidis* is able to evade/ suppress the immune system, b) susceptible species have a reduced immune response to this pathogen and c) there is a reduced immune response under certain environmental conditions. It would be extremely useful to repeat this experiment using different temperature treatments and including a non-susceptible model species such as *Rana catesbiana*. Understanding the immune response to *B. dendrobatidis* is a pivotal step for the future if for example, vaccines are to be developed.

Chytridiomycosis: past, present and future research directions

Most of the research on how the environment affects interactions between amphibians and *B. dendrobatidis* has focused on factors affecting the emergence of the pathogen and initial responses of host species to its emergence. The state of this research as of 2005 was summarised by Rachowicz et al. (2005), who defined a dichotomy between the novel and endemic pathogen hypotheses. They argued that if the pathogen was widely endemic, it is likely that environmental factors trigger disease outbreaks, while if the pathogen is spreading from a single origin, disease outbreaks may occur when it encounters naïve populations, regardless of local environmental conditions. The spreading hypothesis is also based on the assumption that *B. dendrobatidis* causes immediate outbreaks as it invades into the naive populations (Lips et al. 2008; Skerratt et al. 2007). The discussion became even more polarized when Pounds et al. (2006) proposed the chytrid-thermal optimum hypothesis, which suggests that an increase in cloud cover in mountainous regions due to global warming has increased the likelihood of disease outbreaks and therefore heavily involved in the declines. The hypothesis advanced by Pounds et al. (2006) has been heavily contested, especially as an origin of the emergence of this pathogen (Lips et al. 2008; Rohr et al. 2008; Skerratt et al. 2007), and modifications to the initial proposed hypothesis have been suggested (Alford et al. 2007; Di Rosa et al. 2007; Laurance 2008). A key aspect in the discussion is the timing of the declines, it is widely assumed that the arrival of *B. dendrobatidis* causes immediate outbreaks and population declines (e.g., Lips et al. 2006), but at least one case in Italy suggests that this assumption might have exceptions (Di Rosa et al. 2007). When endemic, outbreaks of *B.*

dendrobatidis are closely linked to environmental conditions, and even then, the effects of the pathogen will depend on the climate of the area, the best example being lowland areas where frogs persist despite being infected, in contrast to higher elevations in the same mountain where the same species goes extinct (Berger et al. 1998; McDonald and Alford 1999; Puschendorf et al. 2006a; Woodhams and Alford 2005). Based on this evidence, I argue that environmental conditions are extremely important in the host-pathogen interaction despite the spreading nature of this pathogen.

Recent genetic work (James et al. 2009; Morgan et al. 2007) indicates that the pathogen has recently spread from a single origin (Collins and Crump 2009; Lips et al. 2008; Rohr et al. 2008; Skerratt et al. 2007). Most current efforts at managing amphibian populations threatened by chytridiomycosis are focused on captive breeding (Mendelson et al. 2006), based on the assumption that there is nothing to be done with naive populations under the threat of this disease in the wild. This near-exclusive focus on captive management ignores an important feature of this host-pathogen system the existence of climatic refuges.

Climatic refuges are areas in which susceptible host species persist in association with pathogens, because environmental conditions are not conducive to disease development. These refuges therefore represent areas of high importance for species' conservation since they could function as source populations. For example, after the introduction of avian malaria into Hawaii, birds persisted in higher abundance in xeric habitats than mesic ones, because breeding habitat for the mosquito vector is limited in xeric habitats (van Riper III et al. 1986). Since then, many species of endemic birds have recovered, despite being heavily infected at present, suggesting that they have evolved tolerance to the disease while persisting in refuges (Woodworth et al. 2005). A similar phenomenon may be occurring in some amphibian populations. Many species with wide elevational ranges have declined to the point of local extinction at upland sites but persisted in coexistence with *B. dendrobatidis* at lowland sites (McDonald and Alford 1999; Puschendorf et al. 2006a). Although mortality still occurs at lower elevations, particularly when environmental conditions are most conducive for disease development (Murray et al. 2009), lowland populations of susceptible species of frogs on the east coast of Australia

have been persisting with infection for at least 15-20 years since the initial outbreaks of chytridiomycosis (Murray et al. 2009). Even when these refuges are not perfect because some pathogen-induced mortality still occurs, these lowland areas may be allowing resistance for disease to evolve. Populations of some susceptible frog species have recovered (Richards and Alford 2005) or have begun to recolonise (Collins and Crump 2009) sites from which they were extirpated during initial outbreaks of chytridiomycosis. Lowland environmental refuges can only benefit species with wide elevational ranges. A second type of climatic refuge is possible; as with the Hawaiian avifauna, the existence of xeric habitats at higher elevations might provide a refuge from the worst effects of chytridiomycosis. At the outset of my study, it was known that there were populations of the susceptible species *Litoria nannotis* in high elevation dry sclerophyll forests adjacent to the rainforest habitats from which it had disappeared, suggesting that this might be a possible climatic refuge (Williams 2006; McDonald and Alford, unpublished).

Aims of this study

Most of the research on chytridiomycosis and declining tropical frogs has occurred in rainforest habitats (e.g., Berger et al. 1998; Lips 1999; Lips et al. 2006; Lips et al. 2005a; Lips et al. 2005b; Lips et al. 2004; Lips et al. 2003b; McDonald and Alford 1999; McDonald et al. 2005; Puschendorf et al. 2006a; Puschendorf et al. 2006b; Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b; Rowley et al. 2007b; Woodhams 2003; Woodhams and Alford 2005; Woodhams et al. 2008a; Woodhams et al. 2007b; Woodhams et al. 2005; Woodhams et al. 2006). Previous work from Costa Rica, and the existence of high elevation populations of *Litoria nannotis* in dry habitats in the Australian tropics, suggested that dry forests might serve as a refuge from amphibian declines (Puschendorf et al. 2005); details of the host-pathogen interaction have not been studied outside the rainforest. The Australian Wet Tropics region is an ideal model system in which to examine the importance of climatic refuges because there are dramatic changes in habitat type over a short distances along streams. On the western edge of the mountains, the habitat changes quickly from rainforest and wet sclerophyll to dry sclerophyll defined by the type of soil, topography, precipitation and fire regimes (Ash 1988). The overall aim of this study was to compare the epidemiology of

chytridiomycosis in susceptible frog species between wet and adjacent dry forest environments and to determine whether the dry forest functions as a climatic refuge from disease driven amphibian declines. To address this general aim I designed this project to addressed the following specific aims:

1) To examine the predictions of bioclimatic models for the distribution of *B. dendrobatidis* in wet and dry forest habitats, and to compare the abundance of susceptible frog species in adjacent wet and dry high elevation forests.
2) To compare the epidemiology of the disease at high elevations between wet and adjacent dry environments in susceptible stream-dwelling species.
3) To compare behaviour and microenvironments used by *Litoria nannotis* between wet and dry forest populations and use to this information to understand the underlying mechanisms that lead to a decrease in susceptibility in dry environments.

The main challenge of developing this thesis was the lack of information available on host-pathogen interactions of *B. dendrobatidis* and tropical amphibians outside of rainforest habitats. This thesis is written a series of stand-alone chapters. Chapter two uses a species distribution model to examine the distribution of *B. dendrobatidis* across the Costa Rican landscape. It tests whether it is likely that *B. dendrobatidis* can exist in the area where populations of the extremely chytridiomycosis-susceptible frog *Craugastor ranoides* persist (Puschendorf et al. 2005; Sasa and Solórzano 1995), the dry forest of the Santa Elena Peninsula, Guanacaste province, Costa Rica. Chapter three uses a similar approach, creating a species distribution model for *B. dendrobatidis* in the Australian Wet Tropics region, based on the information available at the time. This prediction of the pathogen's distribution in the region is used as a null model, and is tested by measuring the presence of the pathogen in unsuitable habitat. This chapter also assesses pathogen prevalence and abundance of the host species in dry forest for the first time. The fourth chapter compares the epidemiology of the disease and the abundances of frogs between adjacent dry and wet environments. Chapter five is based on the rediscovery of *Litoria lorica*, an "endemic rainforest species" that was thought to be extinct. This chapter describes a population that was found a few kilometers downstream from the main study site used in chapter three, and its interaction with *B. dendrobatidis*

and the conservation implications of this discovery are also examined. Chapter six compares the biology of a susceptible host, *L. nannotis*, between adjacent dry and wet environments and proposes a mechanism for the different susceptibilities to disease discovered between both environments. Chapter eight summarizes these findings and puts them into context with the current knowledge of disease-mediated amphibian declines and the conservation implications derived from these findings.

CHAPTER TWO: DISTRIBUTION MODELS FOR THE AMPHIBIAN CHYTRID *BATRACHYCHYTRIUM DENDROBATIDIS* IN COSTA RICA: PROPOSING CLIMATIC REFUGES AS A CONSERVATION TOOL

Abstract

Amphibian populations have declined and disappeared in protected and apparently undisturbed areas around the world. In Costa Rica, most of these declines have occurred above 500 m of altitude and have been linked to outbreaks of *Batrachochytrium dendrobatidis*. I used available information about the distribution of this pathogen to model its ecological niche within Costa Rica. Maxent, a machine learning technique, was used to create 100 predictions, of which the most accurate ten (based on areas under their ROC curves) were chosen to create a composite distribution model. I used this approach because it increased confidence in the generated predictions, differentiating between areas of high predictability and low variability (higher confidence) to those with high predictability but high variability (lower confidence) among models. Distribution patterns were not uniform throughout Costa Rica's mountains, where most amphibian declines have occurred. The pathogen was predicted to more likely occur on the Caribbean slopes compared to the Pacific slopes. This was particularly true for lowlands; the Caribbean had much higher predictions than the Pacific. While high temperature constrains the distribution of this pathogen, areas that also had low precipitation during the driest period of the year had low probabilities of *B. dendrobatidis* occurrence. *Craugastor ranoides*, which is a member of a taxonomic grouping of frogs sensitive to chytridiomycosis outbreaks, persists in the hot and seasonally dry Santa Elena Peninsula but disappeared in the nearby colder and more humid Guanacaste Volcanic Chain. This model predicts Santa Elena and the Central Valley to have low probability of *B. dendrobatidis* occurrence, suggesting that these could be refuges for amphibians from chytridiomycosis outbreaks. This could mean that the amphibian chytrid is either absent from the area, or present at such low prevalence as to render an outbreak of chytridiomycosis unlikely. This information suggests that climatic refuges, where environmental conditions prevent disease outbreaks, could be an important component in amphibian conservation.

Introduction

Amphibians are now considered the most threatened group of vertebrates, with a significant proportion of species declining in seemingly undisturbed habitats (Stuart et al. 2004; Wake 1991). Costa Rica has several examples of such enigmatic declines, including the well-documented amphibian population collapse observed in Monteverde (Pounds and Crump 1994; Pounds et al. 1997), where 40 % of the frog species disappeared in a short period of time. Unexplained extirpations of several amphibian species have also occurred at Las Tablas, located southeast of Monteverde (Lips 1998). Amphibian populations distributed throughout most of the country's montane areas (> 500 m above sea level, or asl) have followed a similar pattern, with 13 species suffering significant population declines and ten species not being reported for more than a decade (Bolaños 2002).

Many enigmatic amphibian declines worldwide have been associated with outbreaks of the chytrid fungus *Batrachochytrium dendrobatidis* (Berger et al. 1998; Bosch et al. 2001; Collins and Storfer 2003; La Marca et al. 2005; Lips et al. 2003a; Merino-Viteri et al. 2006). In amphibians, *B. dendrobatidis* causes the disease known as chytridiomycosis (Berger et al. 1998; Longcore et al. 1999). Field evidence from El Copé, Panama, showed that an outbreak of chytridiomycosis in a community of frogs that previously appeared to be pathogen-free led to a dramatic increase in amphibian mortality and the collapse of many local populations (Lips et al. 2006). Temperature (Berger 2001; Berger et al. 2004; Piotrowski et al. 2004; Woodhams 2003; Woodhams et al. 2008b) and moisture (Johnson and Speare 2003) have been suggested as two important environmental factors influencing growth and survival of *B. dendrobatidis* under laboratory conditions. Seasonal variation of both temperature and humidity has been likewise suggested to control the prevalence of the pathogen and the timing of chytridiomycosis outbreaks in the wild (Berger et al. 2004; Bradley et al. 2002; Kriger and Hero 2006b; McDonald et al. 2005; Retallick et al. 2004; Woodhams and Alford 2005).

Knowledge of the ecological and geographic limits of *B. dendrobatidis* is important for amphibian conservation. In the absence of the knowledge necessary to mechanistically model disease dynamics or the distribution of *B. dendrobatidis*, correlative-modelling

approaches can be applied. These approaches assume that correlative associations between where a species occurs and the environmental conditions of those occurrences provide useful information regarding the ecological requirements of the modelled species. This permits predictions of potential amphibian refugial areas, where environmental variables could limit pathogen impact on amphibian species.

A global predictive model of the potential distribution of *B. dendrobatidis* already exists (Ron 2005). Ron's (2005) model was of critical importance at the time of its publication, motivating inventories in previously unexplored regions (e.g., Carnaval et al. 2006; Puschendorf et al. 2006a). However, that model was developed under a relatively coarse resolution (2.5 minutes latitude and longitude) and over a larger geographic extent, not permitting the finer scale study aimed by the present chapter. As evidence of the role of the amphibian chytrid fungus in amphibian declines accumulates, it has become evident that there is a need for models with finer resolution, particularly in topographically complex countries such as Costa Rica. These models will also profit from the inclusion of additional information on the occurrence of *B. dendrobatidis* generated after Ron's (2005) study.

I used Maxent (Phillips et al. 2006) to create climate-based models of the potential distribution of *B. dendrobatidis* in Costa Rica, based on a set of eight environmental layers that reflect factors known or suspected to constitute environmental constraints for this fungus. Despite being a relatively novel approach in species modelling, Maxent has been shown to outperform other methods traditionally used in this type of study (Elith et al. 2006) and is robust even when models are created with small sample sizes (Hernandez et al. 2006; Phillips et al. 2006; Wisz et al. 2008). Given that these predictions were generated from a relatively small dataset, I incorporated uncertainty in my model predictions by generating a summary map with average likelihoods of pathogen occurrence across the ten most accurate models, as well as a map depicting the variability among model predictions. This approach allowed me to distinguish between areas where the pathogen was predicted to be found with great confidence (i.e., areas of high predictability and low variability among the best runs) and those areas showing high average probability, but also high variability among models. I quantified the influence of each environmental

layer on the models created (Phillips et al. 2006), to test whether the environmental factors previously proposed to have greater effects on the biology of the pathogen had greater influence in my models. Finally, I examined the implications of the predictions of my model and commented on their potential ramifications for amphibian conservation.

Methods

I used information about the occurrence of *B. dendrobatidis* in Costa Rica based on literature records (Lips et al. 2003a; Pounds et al. 2006; Puschendorf 2003; Puschendorf and Bolaños 2006; Puschendorf et al. 2006a) and new histological data. Thirty-five amphibian species and 647 individuals were screened for *B. dendrobatidis* (Appendix 1, 2, 3). The resulting dataset included 156 localities, 21 of which corresponded to sites of *B. dendrobatidis* occurrence. The sampled sites represented all of Costa Rica's ecosystems, with the exception of the higher elevations of the Talamanca mountain range (Figure 2.1).

Based on current knowledge of potential limiting factors for *B. dendrobatidis*, I used the following eight climatic layers to predict its distribution in the country: mean annual temperature, coefficient of variation of monthly temperature, maximum temperature of the warmest month, minimum temperature of the coldest month, mean annual precipitation, coefficient of variation of monthly precipitation, precipitation of the wettest month, and precipitation of the driest month. I obtained these data as environmental layers at 30 s (~1 km) resolution, which were downloaded from the WorldClim (version 1.4) dataset (Hijmans et al. 2005). I cropped the layers to include only Costa Rica and a small area of Nicaragua and Panama (N 7.750-11.500, W 82.150-86.250).

I created distribution models using Maxent, a species distribution modelling program that uses a maximum entropy algorithm to make predictions from presence-only data (Phillips et al. 2006); beta version 3.0.4). The use of presence-only data is crucial in this amphibian chytrid model as to avoid possible complications due to false negatives, which are commonly observed in histological assays due to the low sensitivity of the test (Hyatt et al. 2007; Puschendorf and Bolaños 2006) and which could also occur when surveys are

not systematic (Berger et al. 2004; McDonald et al. 2005; Skerratt et al. 2008; Woodhams and Alford 2005).

Because testing the accuracy of a proposed distribution model of *B. dendrobatidis* is key for conservation purposes, and given that my training data set was small, I designed my analysis to examine the sensitivity of my conclusions to the choice of positive sites used in model training. To that end I generated 100 models in total. For each model, I split the original occurrence data set into two sets: a training set with 75 % (16 out of 21 localities) of the positive sites, which were randomly selected, and a testing set with the remaining 25 % (5 localities). From these 100 models, I chose the ten most robust models as defined by the area under the receiver operating characteristic curve (AUC of the testing data). See Elith et al. (2006) and Hanley and McNeil (1982) for further analysis and discussion.

Based on the ten most robust models, I estimated the mean and the standard deviation of Maxent's raw probability of *B. dendrobatidis* occurrence at each grid cell. These values serve as a proxy for the mean likelihood that an area contains *B. dendrobatidis*-infected frogs and describe the variability amongst models generated from different sub-samples of the total dataset, respectively.

To gain insight into the factors determining the distribution patterns of *B. dendrobatidis* in Costa Rica, I examined the contribution of each environmental variable to the models generated by Maxent. This value is provided by Maxent, which quantifies and sums the contribution of every environmental layer in each iteration of its training runs. I examined the mean and standard deviation of the contribution of each environmental layer to the ten best models. *Craugastor fitzingeri*, the most densely sampled species in Costa Rica to date, is a common species that occurs in lowland to premontane areas of the country (0-1,520 m asl) (Savage 2002). Three hundred and fifty individuals of this species were sampled to compare the proportion of infected individuals relative to the total number of surveyed samples between the Pacific and Atlantic versants.

Results

The accuracy of the modelled distributions of *B. dendrobatidis* was better than random (AUC based on the testing data of 100 runs 0.7994 ±0.0742, AUC based on the testing data of ten most accurate 0.9260 ±0.0166). Precipitation of the driest period and annual mean temperature had the greatest contribution in defining areas of *B. dendrobatidis* occurrence in Costa Rica (Table 2.1). While much of the mountainous and foothill regions of the country were often predicted as suitable for *B. dendrobatidis*, the likelihood of occurrence in these areas varied greatly among regions and models (Figure 2.2). In the volcano peaks of the northwestern region (Guanacaste Volcanic Chain), suitable habitat for *B. dendrobatidis* appears to increase with altitude equally on both slopes. This pattern changes further south. Sites in the Central Caribbean Slopes (east of Chompipe) were consistently modelled as having high suitability for *B. dendrobatidis* occurrence (i.e., showing large likelihood of occurrence and low variability across models), even those located near sea level. In contrast, on the slope facing towards the Pacific region, a significant decrease in suitability was predicted with increasing altitude, and predicted probabilities of occurrence were much lower than in any site located on the Caribbean side (Figure 2.2). In general, the Pacific slopes in Costa Rica were predicted to be much less suitable for *B. dendrobatidis*. Also the likelihood of occurrence in the Caribbean lowlands seems to be much higher than in the Pacific lowlands. The largest continuous area predicted to be unsuitable for *B. dendrobatidis* in Costa Rica was the Pacific Northwest. Proposed refugial areas in Costa Rica are located at the extreme dry and hot Santa Elena Peninsula (Figure 2.3).

Table 2.1 Analysis of variable contribution in the ten best models run

Environmental layer	Average Gain (STDEV)
Precipitation of the Driest Period	49.89 (±6.90)
Annual Mean Temperature	40.55 (±7.07)
Minimum Temperature of the Coldest Period	3.54 (±1.72)
Annual Precipitation	2.84 (±2.58)
Precipitation of the Wettest Period	1.82 (±1.50)
Precipitation seasonality	1.17 (±2.55)
Temperature seasonality	0.15 (±0.22)
Maximum Temperature of the Coldest Period	0.03 (±0.09)

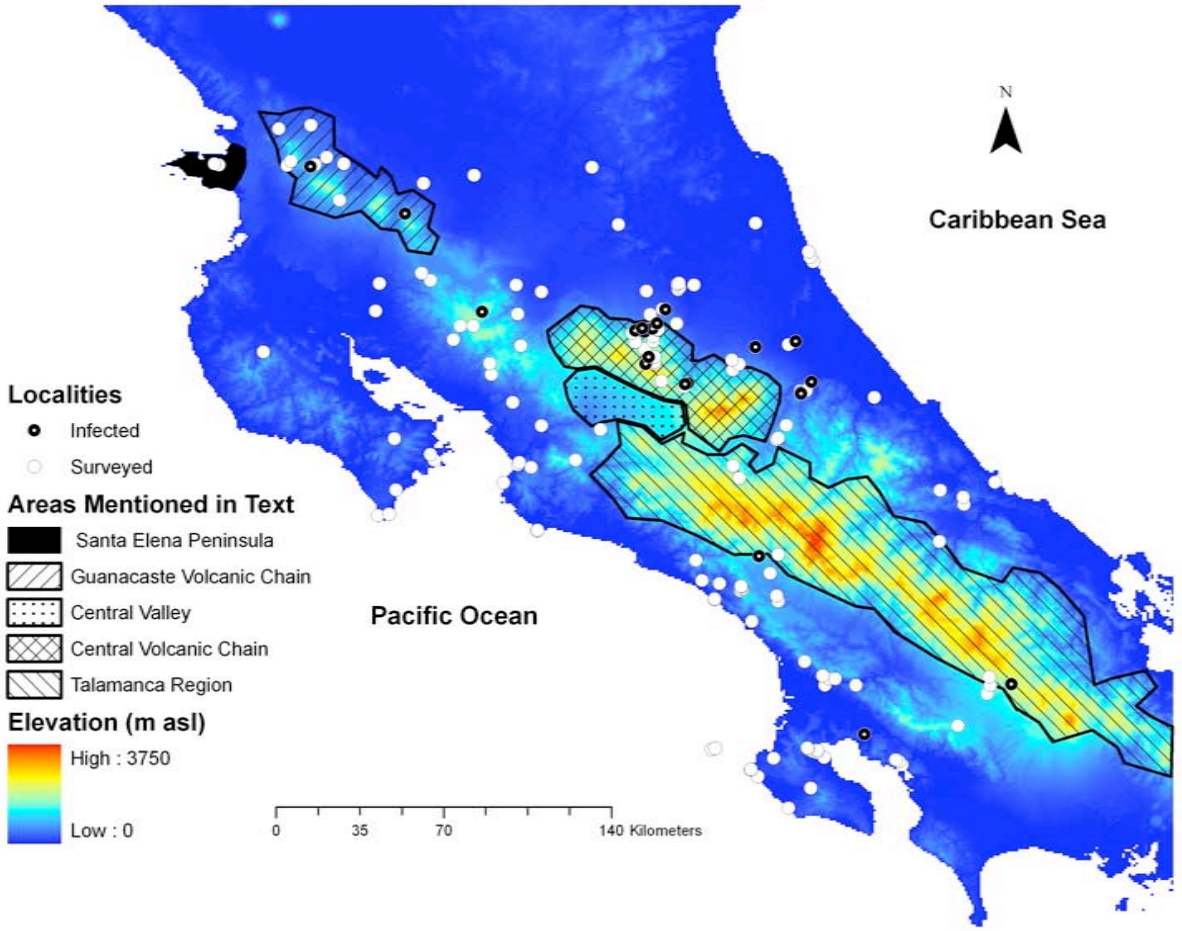


Figure 2.1 Map of localities where *Batrachochytrium dendrobatidis* was surveyed in Costa Rica. Dark circles depict areas in which the fungus was found to occur. Geographic regions of interest (and discussed in the text) are also shown.

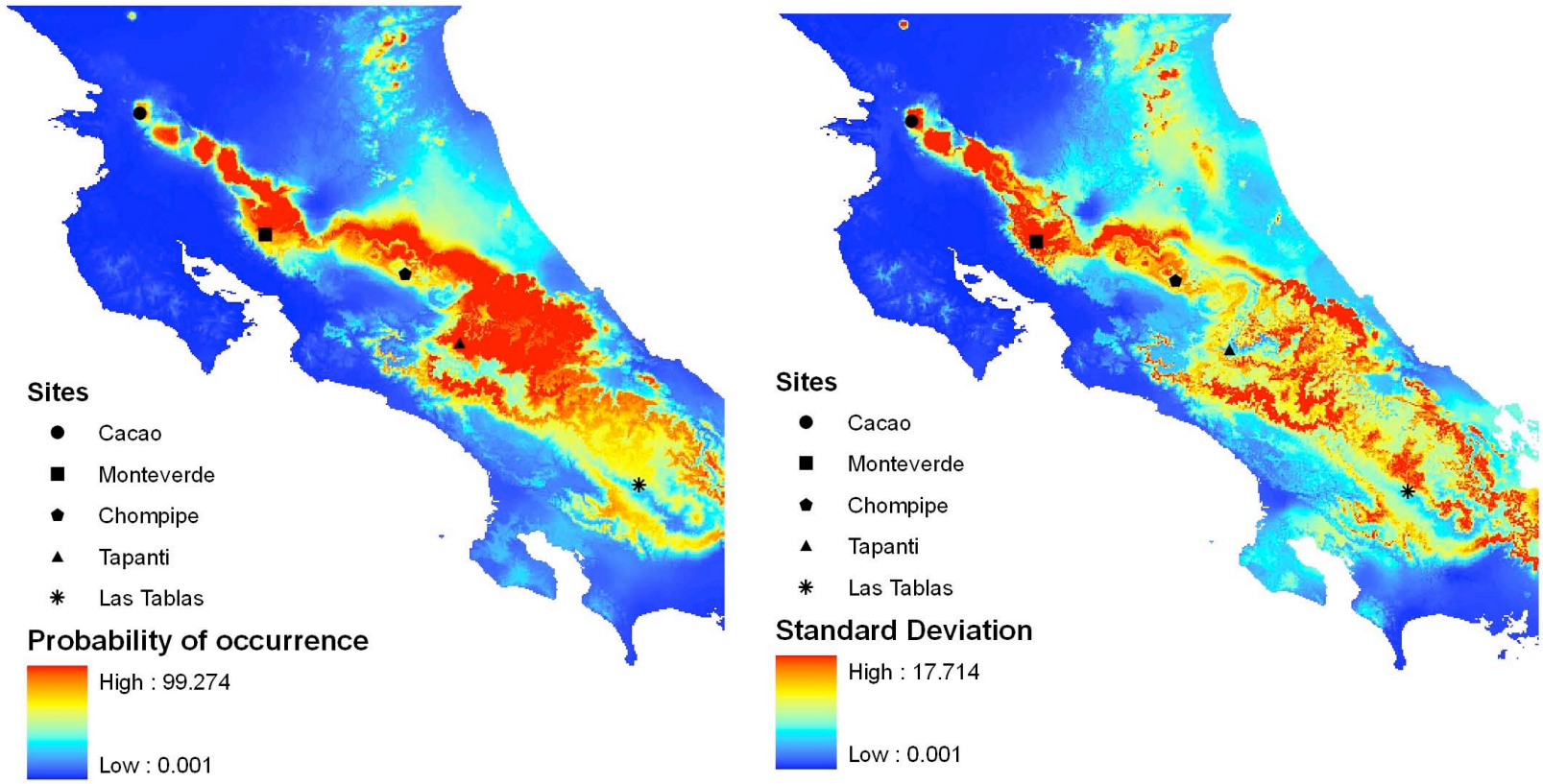


Figure 2.2 Average probabilities of occurrence (left) and standard deviation (right) based on ten best models of *Batrachochytrium dendrobatidis* infection occurrence in Costa Rica. Known decline sites are also depicted in the map as solid black symbols.

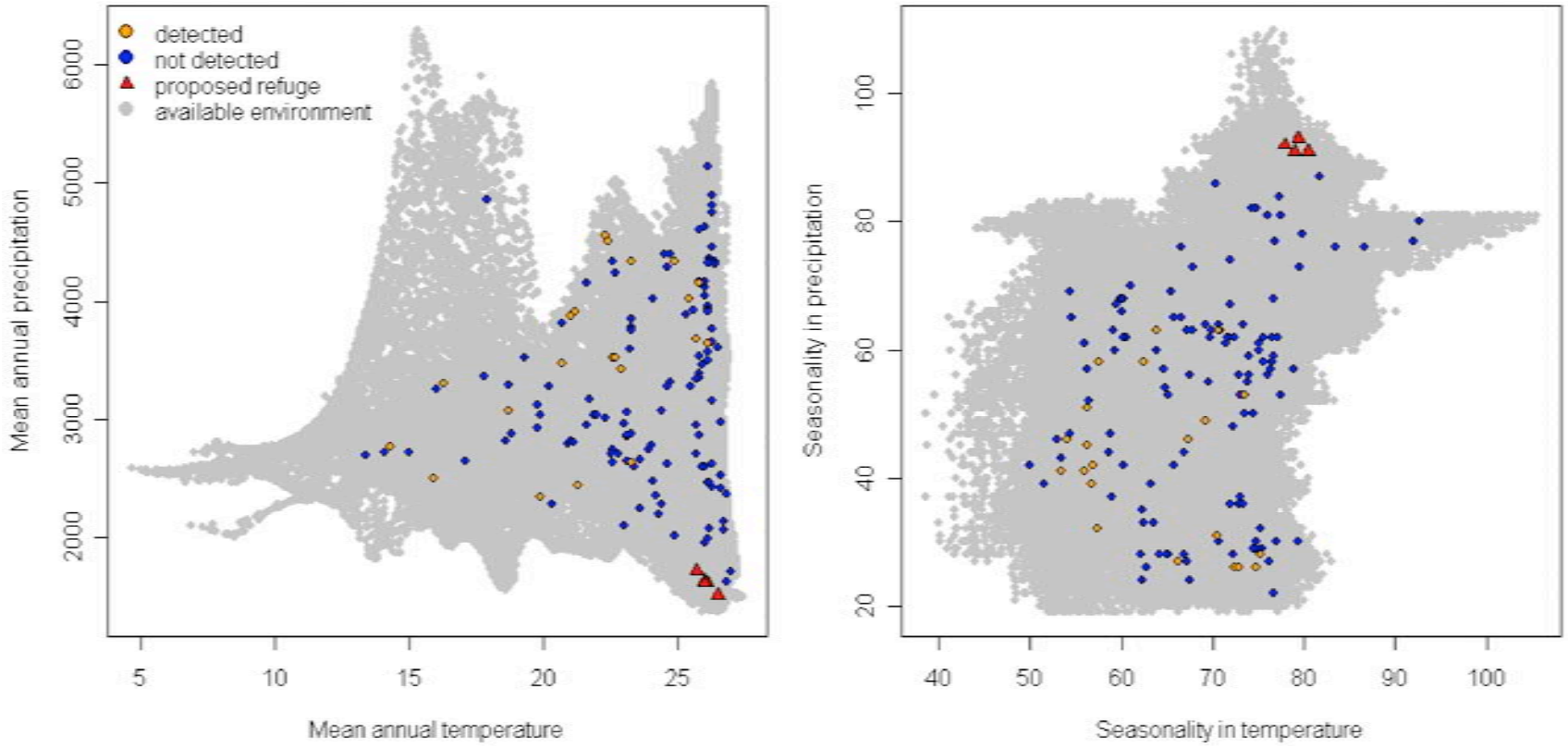


Figure 2.3 Plots of the environmental space of study area (grey circles), surveyed sites (orange circles depict sites where *Batrachochytrium dendrobatidis* infection was detected, blue circles represent sites where the pathogen was not detected) and the area of one proposed refuge, known where the susceptible *Craugastor ranoides* sites is known to occur in the Santa Elena Peninsula (red triangles)

I surveyed three hundred and fifty individuals of *Craugastor fitzinger* for the disease (22 infected/176 surveyed for Caribbean versant; 1/174 for the Pacific versant). Infection prevalence is significantly higher on the Caribbean slopes (χ^2=14.152, DF= 1, P= 0.0002; lower confidence limit for Caribbean slopes 0.092, upper confidence limit 0.208; lower confidence limit for Pacific slopes > 0.001, upper confidence limit 0.032).

Discussion

Predicting the occurrence of a pathogen capable of causing mass mortality in amphibian communities (Berger et al., 1998; Lips et al., 2006) can be challenging in an area as biologically and topographically diverse as Costa Rica. This is particularly true for models of *B. dendrobatidis*, about which only basic information is available. A precautionary approach therefore seems warranted when generating distribution models of *B. dendrobatidis*, and interpreting their results. In order to create robust predictions about this pathogen's distribution in Costa Rica and account for model uncertainty, I decided neither to use all known chytrid occurrence points as training data, nor to use the single most accurate Maxent run (i.e. that with the highest AUC value). Instead, I produced 100 models based on random subsets of the data and generated a map based on a subset of the ten models with the highest AUC values (Hanley & McNeil, 1982). This enabled me to recognize not only regions predicted to have high or low suitability for the pathogen, but also to estimate the level of uncertainty in the models. Some areas were predicted to have high suitability for *B. dendrobatidis* with low variance across the 10 best models. Other areas were predicted to have high suitability by some of the ten best models. The higher variance among models for these areas reduces confidence that those areas are truly suitable for *B. dendrobatidis*. Most importantly, some areas were consistently predicted to have low suitability for the pathogen. These areas may serve as environmental refuges for amphibians.

In Costa Rica, most areas above 500 m asl were predicted to be suitable for *B. dendrobatidis*: this is congruent with local patterns of amphibian declines (Bolaños 2002; Lips 1998; Pounds et al. 1997) and with decline records throughout Central America (Young et al. 2001). However, not all regions suitable for *B. dendrobatidis* were located at high elevations. For instance, the fungus is known to coexist with apparently stable frog populations in the lowlands of Costa Rica (Puschendorf et al. 2006a). In these areas my models predicted the

pathogen to occur with high probability and, in some areas, low variability across models. These data counter the common misconception that *B. dendrobatidis* is unable to occupy and move through lowland areas of Central and South America (e.g., Lips et al. 2008) and call for a re-examination of the routes of spread and spatiotemporal patterns of *B. dendrobatidis* dispersal recently proposed for the region (Lips et al. 2008).

Relatively warm sites located in the Central and Northern Pacific Coast of Costa Rica were consistently predicted to have low suitability for *B. dendrobatidis* infection. This agrees with observations from North America and Australia, where infection prevalence is lower at low elevations and during the (warmer) summer and conversely, most mortality events due to chytridiomycosis occur during the winter, when temperatures are lower (Bradley et al., 2002; Berger et al., 2004; Retallick et al., 2004; McDonald et al., 2005; Woodhams & Alford, 2005). Because local environmental conditions in Costa Rica suggest that several areas of the Pacific Coast are unfavourable to the pathogen, I propose their recognition as potential refuges for chytrid-susceptible amphibian species.

Of particular interest is the hot and dry Santa Elena Peninsula, in the Guanacaste Conservation Area (Figures 2.1, 2.2). Several streams in this region constitute the last known populations of *Craugastor ranoides* (Puschendorf et al. 2005; Sasa and Solórzano 1995; Zumbado-Ulate et al. 2007), a species once commonly distributed throughout the country and which has declined at all elevations throughout the remainder of its range in Costa Rica. By examining the temperature and precipitation of each sampled and infected locality found by the present study, it is clear that the climatic profile of the areas where *C. ranoides* occurs in Santa Elena does not overlap with that of any infected site (Figure 2.3). Moreover, Santa Elena is located at the edge of the environmental space included in my models. Similarly to *C. ranoides*, several populations of *Agalychnis annae* declined in the Central Volcanic Chain, but persist in the warmer and drier Central Valley, mostly in coffee plantations (F. Bolaños and G. Chaves unpublished data). This region is also predicted by my models to be less suitable for *B. dendrobatidis* (Figure 2.2) and could likewise be functioning as a refuge for local frogs.

An examination of the contributions of each environmental variable towards model prediction suggests that both temperature and precipitation influence the distribution of *B. dendrobatidis* (Table 2.1). This is consistent with the biology of the organism, which seems

to be adapted to an aquatic environment and not tolerant to desiccation (Johnson et al., 2003). The joint roles of temperature and humidity are also highly consistent with the patterns of predicted *B. dendrobatidis* occurrence across Costa Rica's Caribbean and Pacific versants. The origin of this pattern might lie in the different humidity regimes experienced on these coasts. The Pacific versant has lower precipitation during the driest period as compared to the Caribbean slopes. Cold fronts arrive during the northern hemisphere's winter months, producing increased rainfall on the Caribbean foothills and mountains. However, this phenomenon has a reverse effect on the Pacific area, producing a dry period extending from November to April (Herrera 1985). The difference between these areas is even stronger in the lowlands, which is also reflected in the predicted distribution maps. In the Australian Wet Tropics, McDonald et al. (2005) reported a decline in pathogen prevalence from the years 2000 to 2002, noting a potential relationship with the decreased precipitation observed during those years. This supports my suggestion that the disease dynamics and distribution of the amphibian chytrid are affected not by temperature alone, but by an interaction between temperature and rainfall or humidity.

Because histology can have low sensitivity when compared to other techniques (Hyatt et al. 2007; Puschendorf and Bolaños 2006), one could suggest that the apparent low predictability of *B. dendrobatidis* occurrence in the Pacific versant may be a result of incomplete and insufficient sampling. However, a simple comparison of the numbers of infected individuals between the Caribbean and the Pacific side of Costa Rica based on the most intensely sampled species occurring from low to mid elevations (*Craugastor fitzingeri*) shows a significant difference in prevalence (χ^2=14.152, DF= 1, P= 0.0002, see Appendix 2).

Amphibian declines linked to outbreaks of *B. dendrobatidis* have received a great amount of attention, with suggestions for captive breeding as a pivotal conservation strategy to preserve threatened species (Mendelson et al. 2006). In most cases, captive breeding is only a short-term solution; additional conservation measures need to be examined. Further studies are needed to validate the hypothesis that the persistence of *C. ranoides* in the Santa Elena Peninsula and of *A. annae* in the Central Valley are explained by local climatic conditions that render these regions unsuitable for *B. dendrobatidis* or prevent high prevalence and virulence of the fungus. However, the data indicate that the consideration of potential climatic refugial areas may have an important role in amphibian conservation. To that end,

distribution models can be invaluable tools to identify regions whose climatic profiles facilitate or hinder chytrid outbreaks. Management activities that aim to provide emergency responses to chytridiomycosis outbreaks in favourable areas and protect natural habitats in regions that are similarly unfavourable to the amphibian chytrid could be important in Middle America and elsewhere.

This discussion also raises the issue of the biological impacts of climate change, which can be not only detrimental to local frog populations, but also alter the location of potential refuges as proposed. Unfortunately, refuge stability is difficult to predict. Enquist (2002) modelled different future climate change scenarios for Costa Rica and concluded that dry forest areas (such as those in Santa Elena) should be more sensitive to temperature and precipitation changes than most other biomes. For example, a drier climate could change the hydrology of the area, hence threatening the survival of stream-dwelling frogs such as *C. ranoides*. Conversely, a moister and cloudier environment could tilt the balance towards the pathogen, through mechanisms similar to those suggested for other mountainous areas in the region (Pounds et al. 2007; Pounds and Puschendorf 2004). While a novel idea and worthy of attention, climatic refuge protection for frog species will only be effective if we take climate and land use change into consideration when designing amphibian conservation strategies research projects worldwide.

This chapter has been published with modifications as:

Puschendorf, R., Carnaval, A.C.O.Q., VanDerWal, J., Zumbado-Ulate, H., Chaves, G., Bolaños, F., Alford, R.A. 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. Diversity & Distributions 15, 401-408.

CHAPTER THREE: CLIMATIC REFUGIA CAN BE HABITATS THAT PROMOTE COEXISTANCE OF *BATRACHOCHYTRIUM DENDROBATIDIS* AND AMPHIBIANS

Abstract

The surrounding environment usually has a strong influence on host-pathogen interactions; this is true for the interaction between amphibian hosts and the pathogenic chytrid fungus *Batrachochytrium dendrobatidis*. Global amphibian declines, extirpations and extinctions due to *B. dendrobatidis* are well documented; in most cases these occur in environments that provide optimal or near-optimal conditions for pathogen growth. Less is known of the dynamics of the host-pathogen system in environments that provide sub-optimal conditions for *B. dendrobatidis*. *Batrachochytrium dendrobatidis* thrives in relatively cool, moist environments (such as montane tropical rainforests). Warmer, drier environments may serve as refugia from disease-mediated extinctions. Known occurrences of the fungus were used with an ecological niche modelling technique to model the pathogen's potential distribution in the Australian Wet Tropics (AWT) and surrounding areas. This model indicated that dry forests on the western boundary of the wet tropics were unsuitable for *B. dendrobatidis*. Data on frog populations and the prevalence and intensity of *B. dendrobatidis* infections were collected at two pairs of sites spanning the ecotone between wet and dry forests to test the model's predictions. Frogs and tadpoles were found in greater abundances in the drier 'highly unsuitable' sites indicating that they do serve as refugia. However, *B. dendrobatidis* occurred at all sites, with higher prevalences in the dry forest areas that the initial models predicted were highly unsuitable for it. Rather than providing refugia that exclude *B. dendrobatidis*, the high elevation dry forest environment in the AWT alters the host-pathogen interaction, allowing frogs to coexist with the pathogen. Updating the distribution model with the new localities showed high overlap between the two models with the predicted core distribution of *B. dendrobatidis* still focused on wet forests, but substantial extensions predicted into the dry forests peripheral to the rainforst. High elevation dry forest habitats deserve increased attention for amphibians threatened by extinction due to chytridiomycosis. Detailed understanding of the host-pathogen interaction in these systems may provide new strategies for managing this disease in the wild.

Introduction

Refugia across space and time are considered vital for the long-term persistence of many species (e.g., Fedorov and Stenseth 2002; Harrison et al. 2008; Lessa et al. 2003; Schneider and Moritz 1999). This is increasingly important in light of the variety of detrimental environmental issues facing species today. One such issue is the apparent increase in wildlife diseases (Daszak et al. 2000b). Disease poses a significant risk for many species, in many cases threatening them with extinction (Burdon 1991; Daszak and Cunningham 1999; de Castro and Bolker 2005; Dobson 1988; Harvell et al. 1999; McCallum and Dobson 1995; Walsh et al. 2003) and as such, the importance of disease management is increasing in conservation (e.g., Atkinson et al. 1996; Barlow 1996; Chomel et al. 2007; Crawshaw 1992; Cunningham 1996; Daszak et al. 2000b, 2001; Deem et al. 2001; Smith and Dobson 1992; Viggers et al. 1993). One conservation strategy is the identification, protection and use of refugia from pathogens, which can permit threatened species to persist in the absence of pathogens (Puschendorf et al. 2009). Refugia may also occur where the environment favours host-pathogen coexistence; in such refugia tolerance or resistance to the disease may evolve (e.g., van Riper III et al. 1986; Woodworth et al. 2005).

Amphibians in many regions of the globe are succumbing to outbreaks of the fungus *Batrachochytrium dendrobatidis*, which causes the disease chytridiomycosis (Berger et al. 1998; Longcore et al. 1999; Skerratt et al. 2007; Stuart et al. 2004). In tropical regions, most of these declines have occurred in high elevation rainforest habitats, which tend to be areas of high amphibian diversity and endemism (Berger et al. 1998; Berger et al. 2004; La Marca et al. 2005; Lips et al. 2006; McDonald and Alford 1999; Puschendorf et al. 2006a; Richards et al. 1993; Skerratt et al. 2007; Woodhams and Alford 2005; Young et al. 2001). These montane regions provide ideal conditions for disease outbreaks because *B. dendrobatidis* thrives under cool and moist conditions. The pathogen can survive temperatures down to near-zero °C, and populations of the pathogen can increase rapidly at temperatures between approximately 12 and 25 °C. Population growth is inhibited by temperatures above 28 °C and by desiccation (Johnson and Speare 2003; Piotrowski et al. 2004). The only current conservation strategy for amphibians threatened by extinction caused by chytridiomycosis is captive breeding (Mendelson et al. 2006), although other strategies such as eradication of the pathogen and stopping pathogen spread have been suggested (OIE 2007; Speare 2006).

Recently, Puschendorf et al. (2009) proposed an alternative conservation stategy for amphibians: protection of refugia which, while suitable for the amphibians, are unsuitable for the fungus. In these refugia significant disease outbreaks are unlikely, protecting amphibians from population declines and extinctions. The authors proposed that reserves should be established in regions in which this situation appears to exist. Although Puschendorf et al. (2009) were the first to propose that such areas be set aside as refuges, many other studies have already identified similar refugial areas in other regions. For example, tropical lowlands have been identified as regions in which populations of many species of amphibians have persisted while populations of the same species have declined or been extirpated at higher elevations (Berger et al. 1998; Berger et al. 2004; McDonald and Alford 1999; Puschendorf et al. 2006a). It has been proposed that amphibian persistence at low elevations is likely to be due to warmer temperatures and more seasonal precipitation being sub-optimal for significant pathogen growth, and therefore disease development (Carey et al. 2006).

Here, I sought to identify refugial areas in the Australian Wet Tropics (AWT) region. Many frog populations in the AWT underwent dramatic declines in the late 1980s and early 1990s due to outbreaks of *B. dendrobatidis*, primarily in high elevation rainforest habitats; seven endemic species of frogs suffered local extinctions, and four were thought to be globally extinct (Berger et al. 1998; McDonald and Alford 1999; Richards et al. 1993). Lowland (< ca. 400 m) populations of the remaining three species persisted, apparently unaffected by declines (Berger et al. 1998; McDonald and Alford 1999; Richards et al. 1993). These areas serve as environmental refuges despite the presence of *B. dendrobatidis* (McDonald and Alford 1999; Woodhams and Alford 2005); it appears that the warmer temperatures at lower elevations tip the balance between host and pathogen in favour of the host, so that prevalence of infection is generally low and host mortality is rare (Woodhams and Alford 2005).

Populations of at least one species also survived along the western margin of the AWT, along an upland ecotonal transition between wet, closed rainforest and dry, open forest (Williams 2006). I hypothesized that the western ecotonal region might provide an environmental refuge via mechanisms similar to those operating in dry habitat in Costa Rica (Puschendorf et al. 2009; chapter 2), providing environmental conditions that simply make the area unsuitable for the pathogen.

The hypothesis that the western-edge refugia in the Australian Wet Tropics provide a relatively *B. dendrobatidis* -free habitat was initially examined by modelling the distribution of *B. dendrobatidis* using known occurrences of the pathogen. I then tested the model by sampling for chytrid on four species of *B. dendrobatidis*-susceptible frogs (Berger et al. 1998; Berger et al. 1999a): *Litoria nannotis, Litoria genimaculata, Litoria jungguy* and *Mixophyes carbinensis* in areas predicted as highly suitable and of very low suitability (effectively unsuitable) for *B. dendrobatidis* along this western upland ecotonal transition. The original model was then updated to include the 'new' information provided by this data, which showed that the western-edge refugia are suitable for both *B. dendrobatidis* and frogs. I then examined other physiological, behavioral or environmental factors that could influence the host-pathogen interaction, permitting the coexistance of the host and pathogen in a high elevation ecosystem in which frog populations persist in great abundance.

Methods

The Australian Wet Tropics Region is one of the best-studied tropical systems in the world (Williams 2006). There is a high endemism of vertebrate species in the region; most of these are restricted to high elevation areas (Williams 2006). Biogeographically this region is an island of rainforests on the northeast coast of Australia that is surrounded by ocean on the east and drier, hotter environments on all other sides (Figure 1).

To test the concept that the western edge provided a series of refugia inhospitable to *B. dendrobatidis*, the distribution of the fungus was modelled using known occurrences and a presence-only distribution method, Maxent (ver. 3.3.0; Phillips et al. 2006). Maxent is a robust species distribution modelling technique that is commonly used and can out-perform more traditional modelling techniques (Elith et al. 2006). Included in the model were fifty-one unique occurrences from pathogen surveys of wild amphibian population and from opportunistic collection of diseased and dying frogs with severe infections of *B. dendrobatidis* (database maintained by a group of amphibian and wildlife disease researchers in north Queensland; Murray et al. unpublished; Figure 3.1).

The model used 35 climatic layers and a vegetation layer. Although many of these climatic layers are highly correlated, Maxent is robust enough to deal with correlated variables, however, interpretation of variables important in defining the model becomes more difficult

(Phillips et al. 2006). The climatic layers included quarterly and annual summaries of temperature, precipitation and solar radiation; these were created using Anuclim version 5.1 (McMahon et al. 1995) and an 80-m^2 digital elevation model for the Wet Tropics Region (resample from GEODATA 9-Second DEM, ver. 2; Geoscience Australia, http://www.ga.gov.au/). The vegetation data consisted of floristically classified broad vegetation groups (BVG at a 1:2 million resolution; Accad et al. 2006).

Sampling for *B. dendrobatidis* at four sites set tested the resulting model up in pairs of predicted suitable and unsuitable (very-low predicted suitability) habitats on the western edge of the AWT (Figure 3.1). The four sites represented two in rainforest / wet sclerophyll forest sites and two in open dry forest, hereafter described as Blencoe Falls (dry), Kirrama Uplands (wet) and Mt. Spurgeon National Park (wet and dry); these were sampled in September of 2006. Adults of *Litoria nannotis, Litoria genimaculata, Litoria jungguy* and tadpoles of *Mixophyes carbinensis* were sampled along a 400 m transect at each of these four sites. All frogs observed were captured (if possible) and swabbed for detection of *B. dendrobatidis* using quantitative PCR (qPCR) (Hyatt et al. 2007). I captured frogs by inverting an unused 15 x 15 cm plastic bag over my hand, grasping the frog, and drawing the bag inside out; this allowed me to capture the animals without touching them directly. After capture I quickly released frogs into larger 21 x 30 cm press-seal bags. Individual frogs were identified to species; each was handled with a new pair of low powder vinyl gloves (Livingstone) as it was swabbed for diagnostic qPCR. Swabbing involved firmly stroking a cotton swab on the frog's ventral skin three times on each of the following areas: (a) the back of each hindfoot; (b) the pelvic patch on both legs; (c) the ventral surface of the abdomen on each side and in the middle (9 strokes for the abdomen); (d) each forefoot, for a total of 27 strokes. Tadpoles of *Mixophyes carbinensis* were sampled by dip netting. Individuals were swabbed by gently stroking the tadpole's mouthparts for 20 seconds while it was held inverted in the palm of a gloved hand, with its tail gently secured between the forefinger and thumb. After swabbing, the frogs and tadpoles were immediately released at the point of capture. No sick or diseased frogs were found during these sampling trips.

Swabs were analysed for the presence of *B. dendrobatidis* using a real-time quantitative Taqman PCR assay at James Cook University, Townsville, Australia (Boyle et al. 2004). Because this was a preliminary screening to determine whether *B. dendrobatidis* occurred in these "unsuitable" habitats, and PCR is expensive, samples were run batched as suggested by

(Hyatt et al. 2007); frog samples were run in batches of four and tadpole samples in batches of three. Batched samples only contained individuals of a single species. Each pooled sample was run in triplicate. Samples were considered positive if two replicate wells contained *B. dendrobatidis* DNA. Prevalence of infection and confidence intervals were calculated from batched samples with variable pool sizes if only a proportion of the batches were infected using the method of Williams and Moffit (2001). If none were infected, only the upper confidence interval could be calculated using this method. If at least one individual in all batches was infected, I estimated a conservative lower 95 % binomial confidence limit for prevalence by iteratively solving for the minimum prevalence that would produce the given number of batches of size N, each containing at least one infected individual, in at least 2.5% of sets of batches sampled. The methodology I used to estimate prevalence and confidence limits for variable pool sizes assumed 100 % test sensitivity and specificity (see Williams and Moffitt 2001 for further details). Measures of intensity of infection derived from the qPCR were ignored for further analysis, since the samples were batch processed and interpreting data on intensity of infection is impossible unless samples are re-run separately.

The test data set indicated that the original model produced very low probabilities of occurrence for some sites at which *B. dendrobatidis* was common. The distribution model was thus updated (rerun) including the test data points. The two distribution models were compared using the I-statistic (Warren et al. 2008) using ENM Tools (http://enmtools.com/). The I-statistic estimates the amount of overlap between two distributions for which values range from 0 (no overlap) to 1 (perfect overlap) of the distributions. Significant deviance from random overlap of the distributions using the I-statistic was determined by bootstrapping (100x) the random resampling of distribution predictions to give a null distribution. Further identification of areas that differed significantly were identified by comparing the difference between predictions of any single cell with the mean and variance of the differences across all cells; in other words, I calculated the significance of the difference between the distributions for each cell relative to the mean and variance of differences across all cells (as per Januchowski et al. in press).

Results

The accuracy of the initial distribution model for *B. dendrobatidis* was much better than random (AUC 0.978). It predicted that *B. dendrobatidis* should occur in most rainforest areas

in the Wet Tropics Region, both at higher elevations and in many lowland areas between Cairns and Townsville (Figure 3.2). It indicated that distributional limits to the north, south and west for this pathogen occur at the abrupt boundary between rainforest and drier environments (Figure 3.2); this is consistent with the hypothesis that drier environments should restrict pathogen growth

The test data showed that the distributional limits predicted by the initial model were incorrect. Dry forest sites predicted to be unsuitable (very low predicted suitability) for *B. dendrobatidis* contained frogs with relatively high prevalences of infection of the fungus. At Blencoe Falls, the prevalence of *B. dendrobatidis* infection in *L. nannotis* was 36.25 % (N = 17, 95 % CI = 12.30-69.59 %). Only three frogs were sampled at Kirrama Uplands, none of which was infected (N = 3, 95 % CI = 0.00-70.75 %). In the dry sclerophyll at Spurgeon Creek all pooled samples of *L. nannotis* came back positive. Prevalence for *L.a genimaculata* at this site was 20.11% (N = 27, 95 % CI = 6.58-42.06 %) and for *L. jungguy* 16.27 % (N = 23, 95 % CI = 4.26-37.74 %). Tadpoles of *M. carbinensis* had a prevalence of 11.21 % (N= 20, 95 % CI = 1.95-30.99 %). At the wet site the three sampled *L. nannotis* individuals were not infected. *L. genimaculata*'s prevalence was 16.45 % (N = 12, 95 % CI = 2.90-43.71 %) and all of the 18 batch processed samples of *M. carbinensis* tadpoles were infected, yielding a 95 % CI of 43.00-100 %. Species shared between site pairs were more abundant in the dry sclerophyll transects than in the wet sclerophyll/ rainforest sites (Table 3.1). The starkest contrast was for *L. nannotis* at Mt. Spurgeon, which was 16 times more abundant in the dry sclerophyll than the wet. Additionally, in the dry sclerophyll, *L. nannotis* was generally found in clusters around waterfalls whereas in the wet sclerophyll area only solitary individuals were located in the same microhabitat.

When the model was rerun including these new dry forest localities, the predicted distribution of the *B. dendrobatidis* expanded into the drier environments along the western boundary of the AWT (Figure 2). The I-statistic shows significant overlap between the two models (I=0.807, p<0.0001). Only small areas changed substantially (Figures 3.2 &3.3). Changes were most obvious in the marginal, ecotonal environments.

Table 3.1 Structure of pooled samples analysed with qPCR diagnostics for *B. dendrobatidis* at the three sampled sites.

Site	Species	Pool size	Number of pools tested	Number of pools positive
Blencoe (dry)	*Litoria nannotis*	4	3	2
	Frogs	3	1	1
		2	1	1
	Potentially infected			13
	Total sampled		17	
Kirrama (wet)	*Litoria nannotis*	3	1	0
	Frogs			
	Potentially infected			0
	Total sampled		3	
Spurgeon (dry)	*Litoria nannotis*	4	12	12
	Frogs			
	Potentially infected			48
	Total sampled		48	
	Litoria genimaculata	4	6	4
	Frogs	3	1	0
	Potentially infected			16
	Total sampled		27	
	Litoria jungguy	4	3	2
	Frogs	3	3	1
		2	1	0
	Potentially infected			11
	Total sampled		23	
	Mixophyes carbinensis	3	6	2
	Tadpoles	2	1	0
	Potentially infected			6
	Total sampled		20	
Spurgeon (wet)	*Litoria nannotis*	4	3	2
	Frogs	3	1	1
		2	1	1
	Potentially infected			11
	Total sampled		15	
	Litoria genimaculata	4	3	1
	Frogs	3	1	1
	Potentially infected			4
	Total sampled		12	
	Mixophyes carbinensis	3	11	11
	Tadpoles			
	Potentially infected			33
	Total sampled		33	

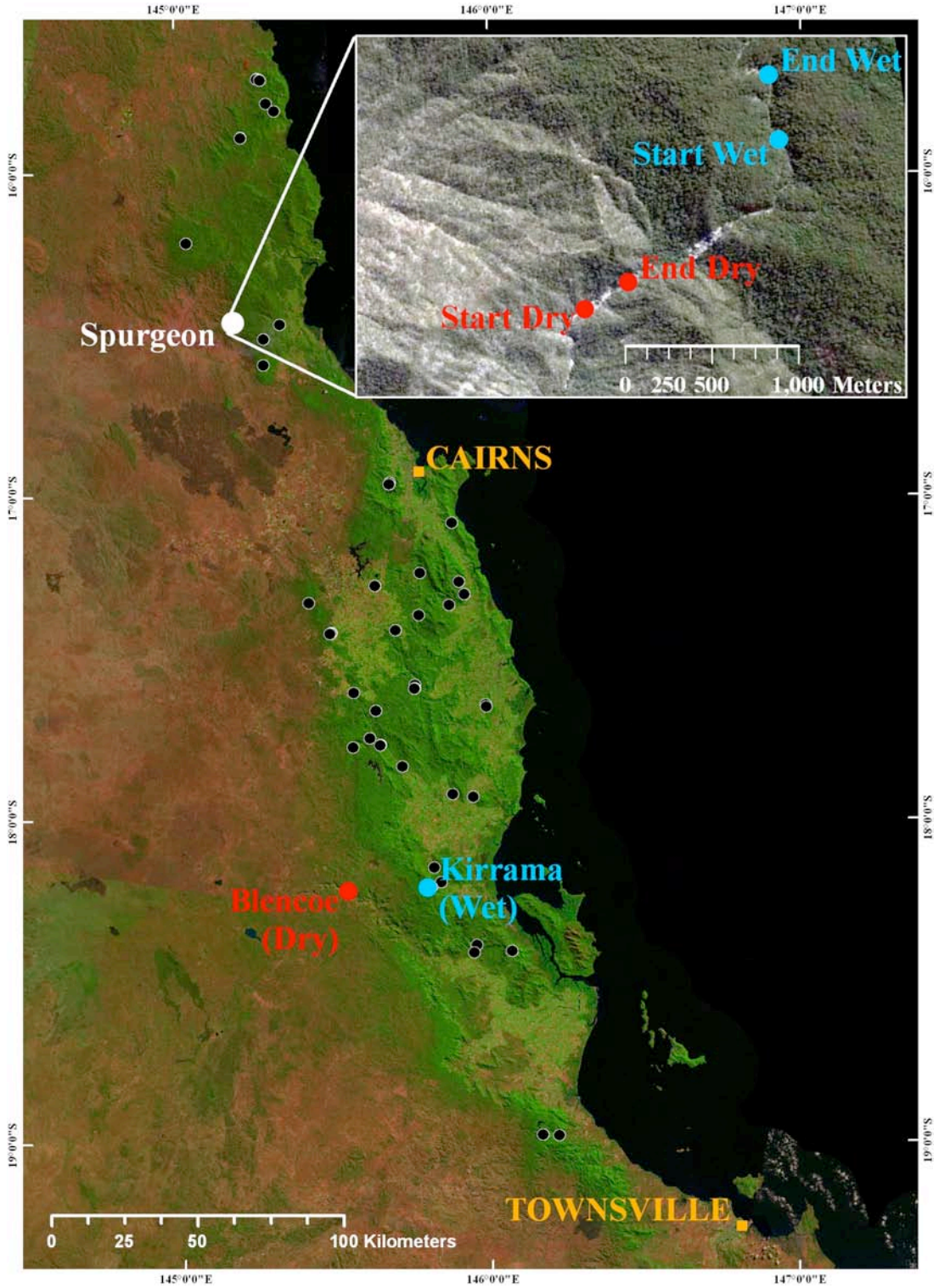


Figure 3.1 The Australian Wet Tropics, depicting known localities of *B. dendrobatidis* occurrence (dark circles), and the pairs of study sites at Blencoe Falls, Kirrama Uplands and Mt. Spurgeon National Park.

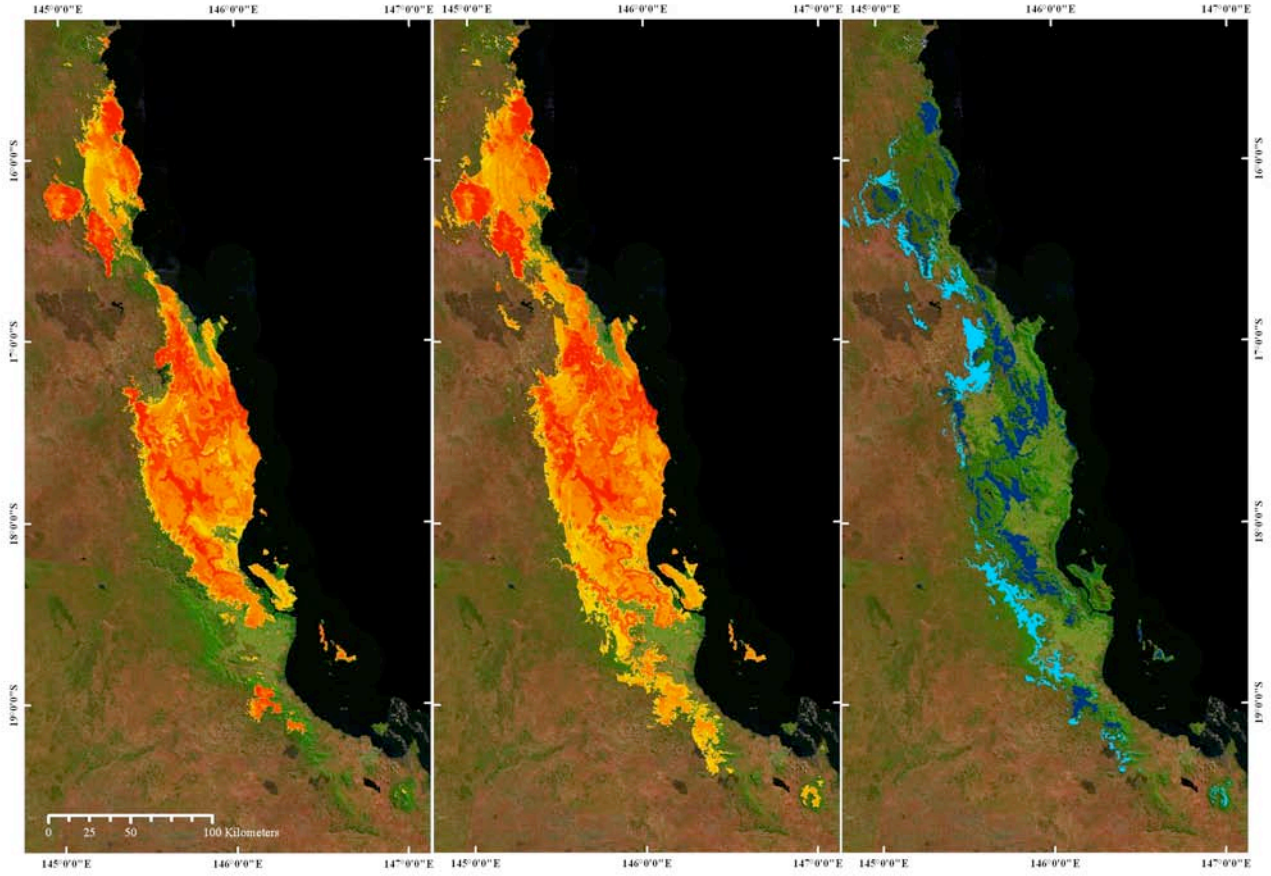


Figure 3.2 Original distribution model for *B. dendrobatidis* (left), and new distribution model for *B. dendrobatidis* (middle) generated with the new sampled localities. Suitable areas are depicted on a scale of red (most suitable) to yellow (less suitable). Areas of significant difference between the models are shown in the right panel with significant increases in model predictions shown in light blue and significant decreases in dark blue. For visualization purposes, unsuitable areas were defined by a conservative threshold, which ensured inclusion of all known occurrence records in the prediction.

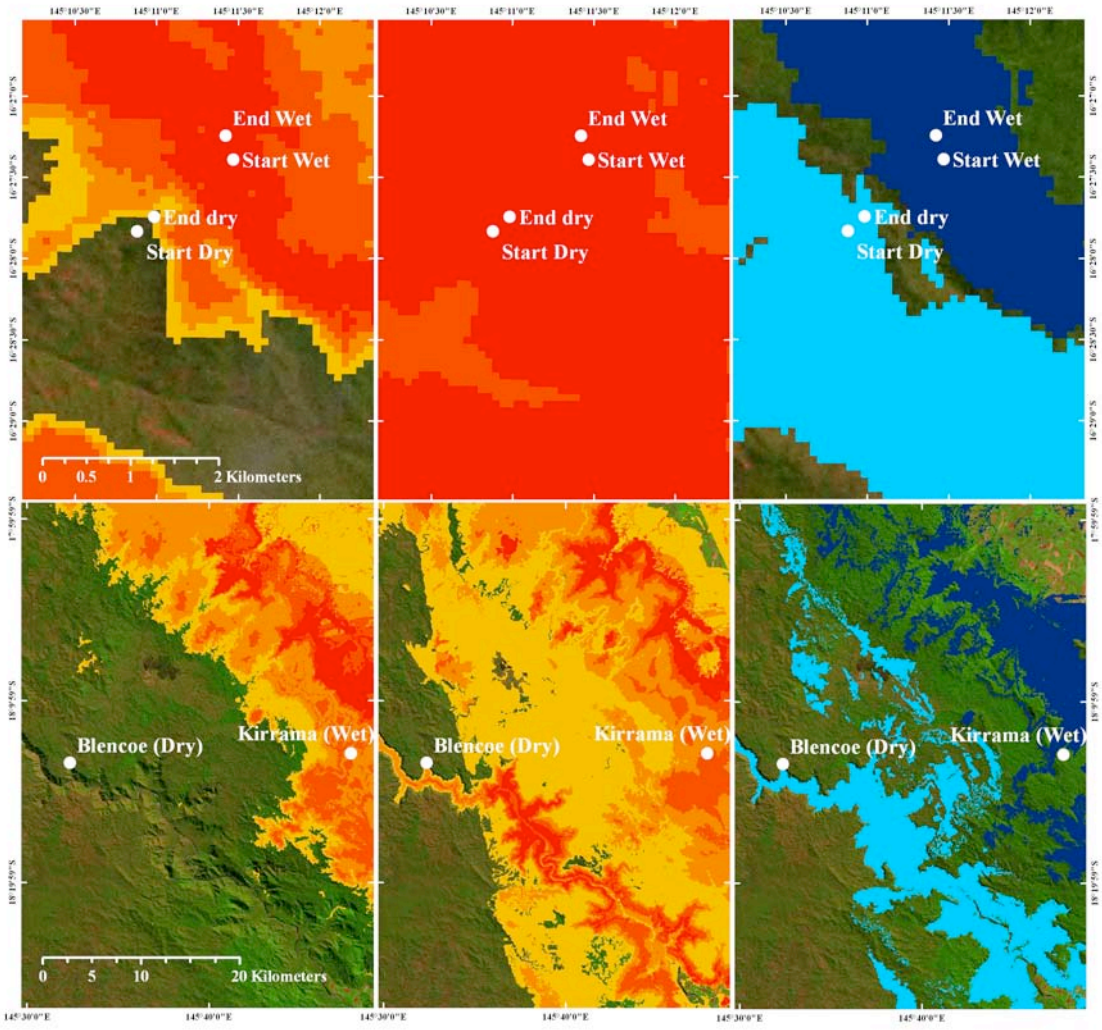


Figure 3.3 Sampled localities with the original (left) and new (middle) distribution model for *B. dendrobatidis* for the study areas (Mt. Spurgeon transects top, Blencoe Falls-Kirrama Uplands transects bottom). Suitable areas are depicted on a scale of red (most suitable) to yellow (less suitable). Areas of significant difference between the models are shown in the right panels with significant increases in model predictions shown in light blue and significant decreases in dark blue. For visualization purposes, unsuitable areas were defined by a conservative threshold, which ensured inclusion of all known occurrence records in the prediction.

Discussion

Most research on amphibian declines linked to outbreaks of *B. dendrobatidis* has focused on rainforest habitats (e.g., Berger et al. 1998; Berger et al. 1999a; Lips et al. 2006; Lips et al. 2003a; Lips et al. 2004; Puschendorf et al. 2006a; Puschendorf et al. 2006b; Woodhams and Alford 2005). In part, this is because mass declines of amphibian populations have been particularly severe in high elevation rainforest habitats, which are often areas of high amphibian diversity and endemism (Lips et al. 2006; Lips et al. 2005b; McDonald and Alford 1999; Young et al. 2001). This focus has, however, caused a significant bias in efforts directed at sampling for the presence of *B. dendrobatidis.* This bias accounts for the under-prediction of the pathogen's distribution in the initial model. Although this study did not conduct systematic sampling along the entire western edge of the wet tropics region into savannah areas that surround it, it clearly demonstrates that both *B. dendrobatidis* and some of the "rainforest" frogs can live outside of the rainforest, and both can occur at relatively high densities in such habitats.

Frogs, particularly the most susceptible of the sampled species, *L. nannotis* (Berger et al. 1998; Berger et al. 1999a; McDonald and Alford 1999), were more abundant in the dry habitats, despite relatively high prevalences of infection by *B. dendrobatidis*. This suggests that frogs in these areas have higher resistance to the development of disease, or increased tolerance to the pathogen, compared to frogs in the wet environments. Another non exclusive alternative is that the fungus is less virulent in those environments. Understanding the epidemiology of chytridiomycosis in dry environments and comparing it to the epidemiology of frogs and the pathogen in adjacent rainforest habitat is a necessary first step in understanding why susceptible species survive in greater abundance in dry environments.

Tadpoles of the frog genus *Mixophyes* have been suggested as an ideal target organism for *B. dendrobatidis* surveillance across Australia's east coast (Symonds et al. 2007). These tadpoles spend multiple years in the stream, and do not develop the disease when infected, allowing them to persist with high infection rates and release infective zoospores over long periods of times, potentially transmitting the infection to other amphibians that frequent the stream (Symonds et al. 2007). They are very

likely to be a significant source of zoospores in the system, allowing populations of frogs to decline and be continuously threatened by infection since these tadpoles are a pathogen-tolerant reservoir.

Host mortality rates can vary among strains of *B. dendrobatidis* (Berger et al. 2005b; James et al. 2009; Retallick and Miera 2007). However, this is unlikely to explain differences in disease prevalence and mortality rates between wet and dry sites. Strain differences are unlikely to occur between sites such as those at Mt. Spurgeon, because the wet site is immediately upstream of the dry site, and both occur along the same stream within a relatively short distance (Figure 3.1). For the same reason, it seems unlikely that genetic differentiation among frogs explains differences between sites at this spatial scale. It is more likely that differences in the host-pathogen relationship between these sites are caused by differences in host behaviour or microenvironment use.

The behaviour of *L. nannotis* has been well studied in rainforest areas, where it is mostly restricted to waterfalls during the adult stages (Hodgkison and Hero 2001, 2003; Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b; Rowley et al. 2007b). This microenvironment preference of *L. nannotis* has been used to explain why its susceptibility to *B. dendrobatidis* is so high, since it spends most of its life in cool and moist conditions, ideal for disease outbreaks (Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b; Rowley et al. 2007b). However, the behaviour of this species has not been studied in peripheral dry forest populations. In these areas, where the canopy is more open, this species might modify its behaviour by basking, and therefore control pathogen loads, keeping them under the threshold that causes mortality (Carey et al. 2006; Woodhams et al. 2003).

Knowing the distribution of any organism, especially that of a virulent pathogen, is a fundamental step in understanding its biology. In the case of a pathogen, it is only with this information that it is possible to predict its impact across different landscapes. Sampling for *B. dendrobatidis* in the AWT has been clearly biased towards rainforest habitats. Although this project's objective was not to do systematic sampling outside of the rainforest, it strongly suggests that *B. dendrobatidis* is extremely common in areas peripheral to the rainforest. Previous substantial sampling

effort has not detected infection in some localities in savannah frogs in western Queensland and Cape York (L. Skerratt and K. McDonald pers. comm.). Future research using a systematic protocol will demonstrate the exact boundaries of this fungus in the region, a pivotal first step for future disease management strategies (Skerratt et al. 2008). For example, it remains unclear whether *B. dendrobatidis* could colonize rainforest patches in the Cape York region, an area of high frog endemism separated by lowland dry forest from the AWT (Cogger 2000).

The differences between the first and second pathogen distribution model are a good example of the danger of creating species distribution models without having sampled the full environmental space available. The models failed to predict the full potential distribution of the species. This is crucial for managing the impact of disease. Previous sampling was driven by the past focus on rainforest environments, because the impact of *B. dendrobatidis* has been particularly strong in these habitats. Although surveys have failed to detect *B. dendrobatidis* in hot and dry areas of Australia such as the Northern Territory and inland Queensland (Skerratt et al. 2008), dry areas adjacent to the rainforest seem extremely suitable for *B. dendrobatidis.* A survey protocol for detecting *B. dendrobatidis* has been developed (Skerratt et al. 2008), and its implementation in the region would be extremely useful to determine the finer scale distribution of *B. dendrobatidis* west from these dry forest were *B. dendrobatidis* has been located with this study.

The importance of peripheral populations in conservation biology is the subject of ongoing debate (Lesica and Allendorf 1995; Nielsen et al. 2001; Peterson 2001). Species of frogs susceptible to declines and extinction persist in abundance in peripheral dry forest areas in Costa Rica (Puschendorf et al. 2009) and Australia (Williams 2006), suggesting that these environments protect them from mass mortality due to chytridiomycosis. Climatic refuges exist in areas where the environment makes pathogen establishment and therefore disease development unlikely (Puschendorf et al. 2009). This chapter demonstrates that they also exist in areas where frogs and pathogen coexist, even when the pathogen is present at high prevalences. These newly described Australian climatic refuges from extinction are especially interesting, since they occur at high elevations, in close proximity to the rainforest habitats where many species have gone missing since the initial outbreaks

of chytridiomycosis. Studying these dry forest systems will not only increase my knowledge of these frogs' biology, but also of their interaction with *B. dendrobatidis*, in environments other than rainforests. Understanding the environment-host-pathogen interaction in these dry forest systems seems crucial for amphibian conservation and for the development of *in situ* management tools for declining amphibian populations.

CHAPTER FOUR: COMPARATIVE EPIDEMIOLOGY OF *BATRACHOCHYTRIUM DENDROBATIDIS* INFECTION IN WET AND DRY FOREST POPULATIONS OF STREAM FROGS

Abstract

Most studies of the recent amphibian declines in seemingly pristine and protected areas that have been linked to outbreaks of the fungus *Batrachochytrium dendrobatidis* have been carried out in rainforest sites. This pathogen is strongly affected by environmental conditions; in the laboratory, it grows most rapidly in cool, moist conditions. Many studies have noted that in the Australian Wet Tropics, there is an elevational gradient in virulence of the pathogen; several susceptible species declined to local extinction during initial outbreaks at high elevations while low-elevation populations were not obviously affected. Studies of host-pathogen dynamics in rainforest along this gradient have confirmed that prevalences are higher and morbidity and mortality are more common at high elevations. Several species of Wet Tropics frogs have ranges that extend into the environmentally distinct dry sclerophyll forests that occur at high elevations on the western boundary of the Wet Tropics region. Initial data showed that in that habitat, species that are susceptible to chytridiomycosis can be very abundant, despite high prevalences of the pathogen. The aim of this project was to compare the epidemiology of *B. dendrobatidis* between frogs inhabiting high-elevation dry forest systems and the same species inhabiting adjacent wet systems, where chytridiomycosis has its strongest effects. Two transects were set up in each habitat, located less than one kilometer apart along the same creek. Individuals of *Litoria nannotis* and *Litoria genimaculata* were surveyed, and population disease status was determined between March 2007 and July 2008. On each transect, continuous hourly air temperature and relative humidity were recorded. Logistic regression and best-subset regression analyses were used to evaluate the effects of multiple variables on prevalence and intensity of infection. Abundance of *L. nannotis* was five times higher in the dry than in the wet transect, whereas densities of *L. genimaculata* were similar between the two habitats. Frogs with obvious signs of chytridiomycosis and disease-related mortality were only found in the wet forest site. These consisted only of juvenile *L. nannotis* and male *L. genimaculata.* Prevalence

and intensity of infection were significantly negatively related to minimum daily temperature; higher temperatures decreased both measures. Habitat type was the second strongest predictor of prevalence. Frogs in the dry habitat had a much higher pathogen prevalence than those in the wet habitat. Intensity was not significantly affected by habitat type. Prevalence was also significantly affected by species; *L. nannotis* was 5.45 times more likely to be infected than *L. genimaculata.* Intensity of infection was not significantly affected by species. Age and gender group significantly affected both prevalence and intensity of infection. Juveniles of both species had significantly higher intensities of infection than males and females. The selective pressure exerted by chytridiomycosis appears to be higher in wet than dry environments. The higher prevalence of *B. dendrobatidis* in the dry sclerophyll, in conjunction with the lack of observed mortality in this habitat, suggests that this environment increases the tolerance of frogs to pathogen infections.

Introduction

Amphibian population declines and species extinctions due to *Batrachochytrium dendrobatidis*, the organism that causes outbreaks of chytridiomycosis in tropical regions, have primarily been studied in rainforest areas (Berger et al. 1998; Lips et al. 2006; Murray et al. 2009; Skerratt et al. 2007; Woodhams and Alford 2005). Temperature and humidity can both affect the outcome of disease; in culture, the pathogen thrives at temperatures between 17 and 25 °C, but dies after prolonged exposures over 30 °C (Piotrowski et al. 2004). Moisture is another important factor, since this pathogen does not tolerate desiccation (Johnson and Speare 2003). These environmental parameters affect pathogen growth and development, and explain why this disease has its strongest effects on amphibian populations in winter and in high elevation rainforest habitats, where cool and moist conditions allow it to thrive (Berger et al. 2004; Hines et al. 1999; Lips et al. 2006; Woodhams 2003).

In the Australian Wet Tropics, *B. dendrobatidis* has caused declines of *L. genimaculata, L. nannotis* and *Litoria rheocola* and the potential extinctions of *Taudactylus acutirrostris, Taudactylus rheofilus, Litoria lorica* and *Litoria nyakalensis* (McDonald and Alford 1999). The first three were originally found across all available elevations (0 – 1200 m above sea level (m asl) in rainforest habitats, and

completely disappeared from elevations above 400 m asl during epizootics of *B. dendrobatidis* between the late 1980s and early 1990s (Hines et al. 1999). The four missing species were considered to be high elevation rainforest endemics and have not been seen for more than 15 years (Hines et al. 1999). *Litoria genimaculata* has recovered to pre decline abundances (Richards and Alford 2005). Although now found again at some rainforest sites above 400 m asl, *L. nannotis* and *L. rheocola* remain relatively rare at these higher elevations (K. McDonald and R. Alford pers. comm.). Seasonal increases in mortality caused by enzootic chytridiomycosis still occur in wild amphibian populations even years after the first epidemic outbreaks (Murray et al. 2009), mostly during winter months when environmental conditions promote disease development (Berger et al. 2004; Murray et al. 2009; Woodhams and Alford 2005). Susceptible amphibians living in environments unfavourable for disease development have a higher chance of survival than those in disease-favourable environments. The persistence of all known lowland rainforest populations of *L. nannotis, L. rheocola* and *Nyctimisted dayi,* despite being infected with chytridiomycosis, is a good example (Hines et al. 1999). Climatic refugia thus seem to be important for the conservation of amphibian species threatened by chytridiomycosis, since they allow susceptible species to coexist with the pathogen (Puschendorf et al. 2009). Lowland refuges are irrelevant for species endemic only to high elevations. However, climatic regimes that allow coexistence of vulnerable species with *B. dendrobatidis* can occur at higher elevations.

On the western slopes of several mountains located between Townsville and Cooktown, Queensland, there is an abrupt, mostly fire-mediated boundary between rainforest and dry sclerophyll habitats (Ash 1988). In the high elevation dry forest habitats, *L. nannotis* can be extremely abundant (Williams 2006), in stark contrast to the neighbouring rainforest where they were previously common and now are at best considered rare (Hines et al. 1999; Richards et al. 1993; Williams 2006; chapter 3). The goal of this project was to compare baseline epidemiological data between wet and adjacent dry habitats in a montane area and determine a) whether frogs are more abundant in dry habitats than in wet ones, b) whether there is a clear indication *B. dendrobatidis* is shaping differences in abundance and c) whether differences in epidemiology of *B. dendrobatidis* exist between wet and dry habitats.

Methods

Field sites and environmental data

Sampling occurred at Mt. Spurgeon National Park. I marked two 400 meter transects in the areas where preliminary surveys had been conducted (chapter 3). The first transect was set up in dry sclerophyll forest (beginning of transect: 16° 27.823'S 145°, 10.865'E, 795 m asl); this transect extended to the edge of the gallery forest immediately before Spurgeon Falls (end of transect: 16° 27.709'S, 145° 11.032'E, 861 m asl). The second permanent transect was set up in wet sclerophyll/ rainforest habitat (beginning of transect; 16° 27.368'S, 145° 11.457'E, 1067 m asl; end of transect 16° 27.193'S, 145° 11.488'E, 1113 m asl). The dry and wet forest transects were separated by 900 m along the creek and 206 m in elevation, but the environments along them were dramatically different. The site layout was designed so that I could sample the sites on subsequent nights. The sampling interval was initially planned to be bi-weekly during winter and every month in summer, but because of access issues this was not always accomplished (Table 4.1).

Four Hygrochron™ data loggers (model DS1923, Dallas Semiconductor) were set up along each transect. The loggers were placed in tea strainers and hung on vegetation 1 m away from the stream and approximately 1 m above the level of the stream, in areas where direct sunshine would never hit them, but they would be exposed to ambient conditions. The loggers were set up to record air temperature (AT) and relative humidity (RH) at 1-hour intervals.

Table 4.1 Sampling dates at Mt. Spurgeon National Park by habitat, highlighted by individual trips. Left column is Spurgeon Wet (SPW) and right column Spurgeon Dry (SPD).

SPW	SPD
7-Apr-07	7-Apr-07
	16-Jun-07
26-Jul-07	27-Jul-07
26-Aug-07	27-Aug-07
8-Oct-07	9-Oct-07
18-Jan-08	19-Jan-08
27-Mar-08	28-Mar-08
8-May-08	9-May-08
21-May-08	22-May-08
15-Jun-08	16-Jun-08
6-Jul-08	7-Jul-08

Study species

I chose to study the green-eyed treefrog (*Litoria genimaculata*) and the waterfall frog (*Litoria nannotis*). These species were chosen because there is good baseline information on their biology and decline history, mostly based on studies in rainforest habitats. (e.g., Alford et al. 2007; Alford and Richards 1999; Cunningham 2002; Haas and Richards 1998; Hines et al. 1999; Hodgkison and Hero 2003; Hoskin 2007; Hoskin et al. 2005; Hoskin and McCallum 2007; King et al. 1990; Liem 1974; Richards 2002; Richards and Alford 2005; Rowley and Alford 2007b; Rowley et al. 2007b; Williams and Hero 2001). Because disease diagnostics are expensive, systematic disease sampling was initially restricted to *L. nannotis*. This formerly occurred across all elevations in the Wet Tropics and was completely extirpated from elevations above 400 m asl in rainforest, but persisted at lowland rainforest sites. This indicates that it is extremely susceptible to chytridiomcyosis but can be protected by environmental conditions (Hines et al. 1999; McDonald et al. 2005). Individual *L. genimaculata* were sampled from March to August 2008 (all observed individuals were processed for disease surveys), but sporadically sampled during the rest of the study. *L. genimaculata* is also known to be susceptible to chytridiomycosis, but did not decline to local extinction even at high elevations (Richards et al. 1993) and high elevation populations have recovered since the initial chytridiomycosis epizootics (Richards and Alford 2005).

Frog sampling

Sampling occurred after 7:00 pm in winter (May-September) and 7:30 in summer (October-April), to allow frogs to emerge from their diurnal retreat sites and be available for capture. When sampling, I walked along the transect, spotlighting for frogs. I captured frogs by inverting an unused 15 x 15 cm plastic bag over my hand and grasping the animal while drawing the bag inside out, thus capturing the frog in the bag without touching it. Whenever I had five or more individuals captured, I processed them. I used a new pair of low powder vinyl gloves (Livingstone) to handle each individual as it was swabbed for diagnostic qPCR (Hyatt et al. 2007), measured and weighed. After swabbing, I recorded each individual's sex, age class status (juvenile or adult: male, female), snout-vent length and mass.

Clinical signs of chytridiomycosis reported in the literature and observed in the field include lethargy, skin sloughing and loss of muscle strength (the main sign is the inability to retract legs; Berger et al. 1999b). If a frog had any of these signs, I tested its righting reflex by manually positioning it on its dorsal side and checking if it was able to reposition itself back to normal. All dead frogs were preserved in 10 % formalin and sent to Dr. Sam Young in Cairns who performed necropsies to determine cause of death. I compared numbers of diseased and dying animals to the numbers of aclinical individuals caught on the same transect (including animals sampled in the studies reported in chapter 3). I calculated prevalence and 95 % confidence intervals for diseased and dead frogs across habitats. I used the program Winpepi (Abramson 2004) to calculate Fisher's odds-ratio confidence intervals (Fisher 1962).

Pathogen sampling and diagnostics

Frogs were sampled for *B. dendrobatidis* by firmly stroking a cotton swab on their ventral skin three times on each of the following regions: (a) the ventral surface of each hindfoot; (b) the pelvic patch area in the anterior ventral region of each leg; (c) the ventral surface of the abdomen; and (d) the ventral surface of each forefoot, for a total of 27 strokes. After measurement and swabbing, the frog was immediately released at the point of capture. Swabs were analysed for the presence of *B. dendrobatidis* using a real-time quantitative Taqman PCR assay (Boyle et al. 2004) at James Cook University, Townsville, Australia. Each sample was run in triplicate. Samples were considered positive if two replicate wells were found to have *B. dendrobatidis* DNA. Negative samples were rerun with an internal positive control to confirm that inhibition did not cause the negative results (Hyatt et al. 2007).

Statistical analysis for prevalence and intensity of infection

To calculate the relative abundance of *L. nannotis* at each site, I used all captures regardless of class (Table 4.2), since all classes (males, females and juveniles) spend the majority of their time near waterfalls (Hodgkison and Hero 2001; Rowley and Alford 2007b). *Litoria genimaculata* was only sampled systematically between March and August 2008 (6 trips). Relative abundance for this species was calculated for that time period and for males only, since females and juveniles were only occasionally

found along the stream (Richards and Alford 2005). I report prevalence of infection as the percentage of infected individuals of each species with 95 % confidence intervals (CI). I used a binary logistic regression to evaluate the effects of several variables on infection status (i.e., absence or presence of infection in a given individual). Three categorical variables were included: species, class (male, female, juvenile) and site. Continuous covariates were mean, minimum (min) and maximum (max) air temperature (AT) and relative humidity (RH) averaged over the 3, 7, 14, 21 and 28 day periods previous to the capture of each frog, giving a total of 30 continuous variables. The goal was to predict which factors, alone or in combination, have a significant effect on infection status. I constructed models based on the recommendations of Hosmer and Lemeshow (2000) and conducted the analysis using SPSS (version 16, SPSS Inc.). I first examined the relationship between infection status and the 33 variables independently. I excluded variables for which p was > 0.25, eliminating eight of the continuous variables through this process (Table 4.2). The remaining 22 were examined for co-linearity. All continues variables were highly correlated, therefore I chose 28-day min AT and 28-day mean RH, because they had the highest Wald statistics scores and significance for AT and RH when run in univariate form (Table 4.2).

I fitted a combined model that included all five selected variables. Only variables with a significance of $P<0.05$ were selected. Of the five variables, species, site, class and 28-day minimum AT were significant; 28-day mean RH was eliminated. Then I fitted the individual combinations of the three categorical variables testing for significant interactions. Finally I ran the four single variables and the significant interaction variables individually. Only one of the interactions remained significant when included in the same model with the single variables and thus was included in the final model.

Table 4.2 Variables tested by univariate logistic regression for infection status. Variables have been ranked according to their significance within variable types (continuous and categorical). Variables selected for further analysis have been highlighted in gray. Environmental variables depict mean temperature (AT) and relative humidity (RH).

Variable	Beta	S.E.	Wald statistic	df	Sig.
Species	-1.29797	0.146907	78.06325558	1	>0.0001
Site	1.262752	0.148814	72.00271717	1	>0.0001
Class			13.42458174	2	0.0012
28 mean RH	-0.09547	0.010674	79.98972299	1	>0.0001
14 mean RH	-0.09009	0.01028	76.79940867	1	>0.0001
21 mean RH	-0.08702	0.01017	73.22016582	1	>0.0001
28 min RH	-0.05085	0.006022	71.28729129	1	>0.0001
14 min RH	-0.04887	0.006018	65.93566241	1	>0.0001
21 max RH	-0.20094	0.024942	64.90605171	1	>0.0001
28 max RH	-0.20549	0.025817	63.35191843	1	>0.0001
14 max RH	-0.18134	0.022923	62.57832454	1	>0.0001
7 max RH	-0.17843	0.022574	62.47461095	1	>0.0001
7 mean RH	-0.07991	0.010183	61.57932746	1	>0.0001
21 min RH	-0.04396	0.005689	59.71128501	1	>0.0001
7 min RH	-0.03864	0.005949	42.19211007	1	>0.0001
3 max RH	-0.09617	0.015833	36.89156677	1	>0.0001
3 mean RH	-0.05286	0.008804	36.05328446	1	>0.0001
3 min RH	-0.02013	0.005205	14.95378008	1	0.0001
28 min AT	-0.08515	0.029649	8.248761672	1	0.0041
21 min AT	-0.07491	0.030107	6.190095668	1	0.0128
14 max AT	0.043186	0.020188	4.576272403	1	0.0324
14 min AT	-0.05789	0.029371	3.88482843	1	0.0487
28 max AT	0.036982	0.020953	3.115162227	1	0.0776
21 max AT	0.031194	0.020443	2.328323703	1	0.1270
7 max AT	0.026272	0.019017	1.90858598	1	0.1671

Measures of infection intensity include only infected individuals and were log transformed prior to analysis (Zar 1999). I used a univariate analysis of variance to test the effects of species, class, season and site on infection intensity. Season was divided into summer (October-April) and winter (May-August). I calculated the mean intensity of infection per trip and used univariate regression analysis (Minitab; version 16, Minitab Inc.) to test the effects of the environmental variables used for the analysis of disease prevalence that best predicted intensity of infection.

Results

Environmental differences between wet and dry forest transects

Although temperatures were significantly higher in the dry site (median = 19.675 n = 10103) than in the wet site (median = 16.675, n = 10103; Wilcoxon Signed Rank Test, z= -85.649, p < 0.0001), ambient temperatures remained mostly in the range of optimum growth temperatures for the pathogen in the laboratory (Figure 4.1), although a substantial proportion (12.3 % dry forest, 1.6% wet forest) of recorded temperatures in the dry forest were outside the optimal range for the pathogen (≥ 25°C). In the case of humidity, differences were much greater (dry forest median = 85.15 %, n = 10103; wet forest median = 97.95, n = 10103; Wilcoxon Signed Rank Test, z = -81.559, p = < 0.0001) with the wet site having recorded relative humidities above 90 % up to 5 times more often than the dry site (Figure 4.2).

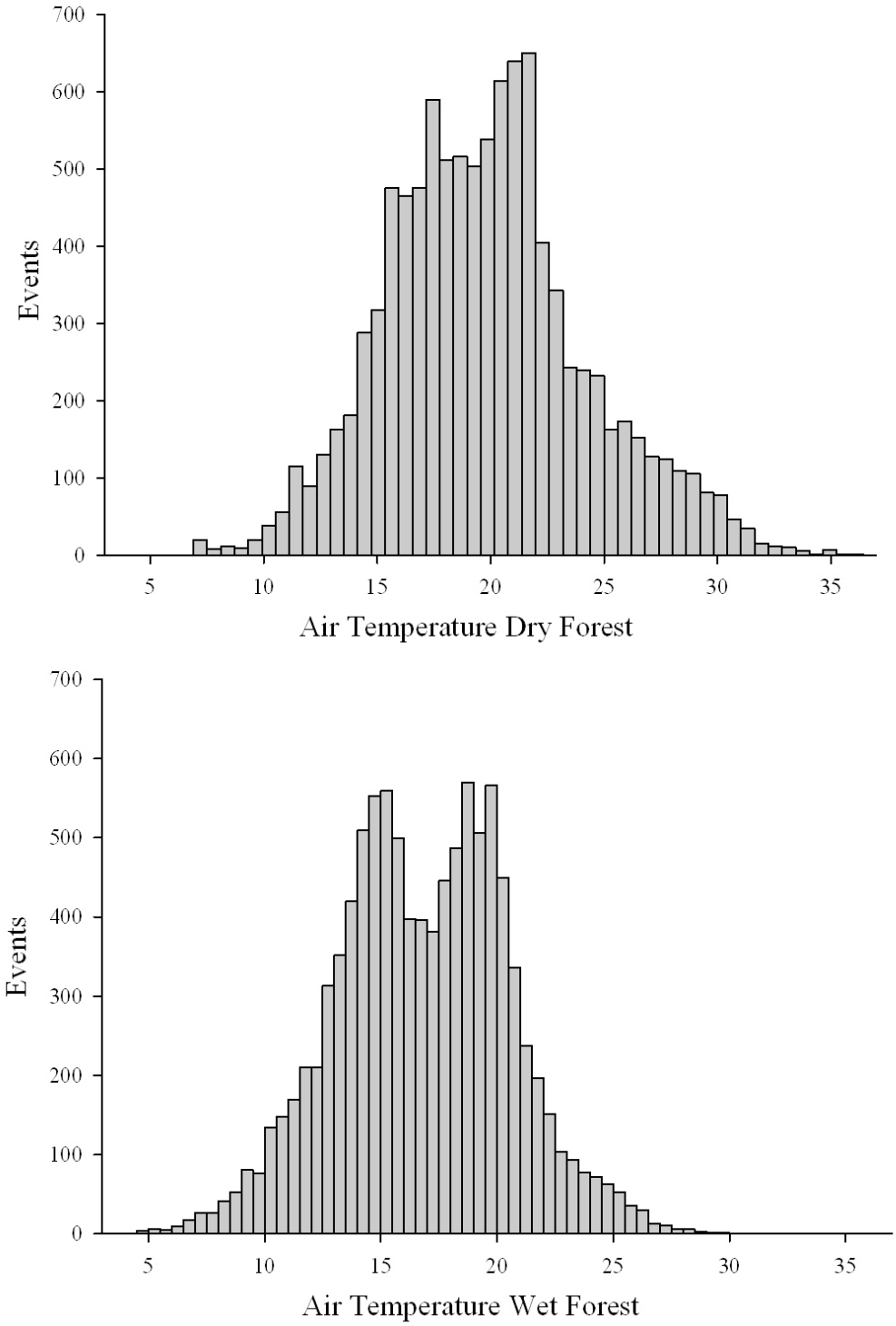


Figure 4.1 Distributions of air temperature of dry (top) and wet (bottom) transects during the study period.

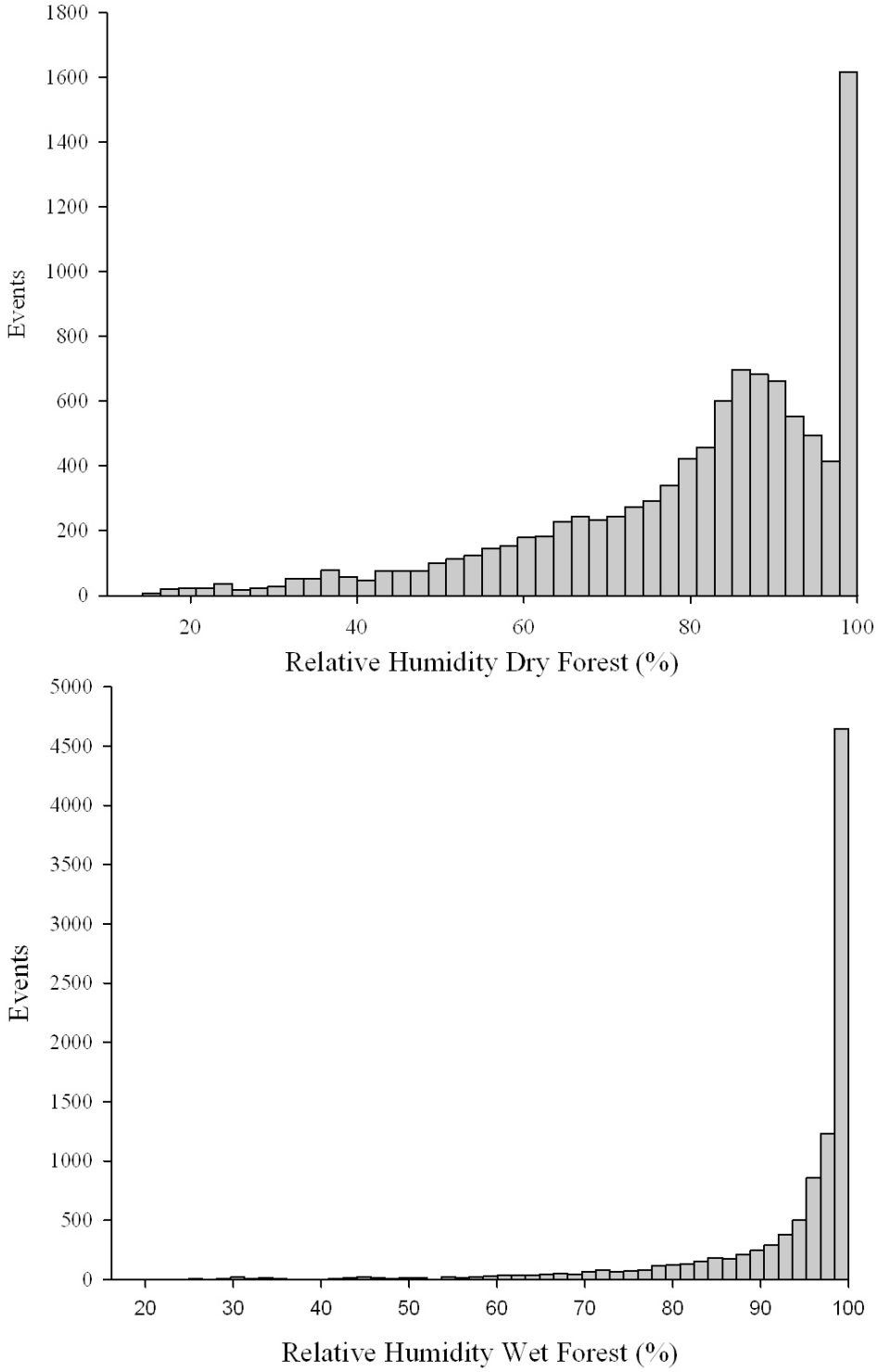


Figure 4.2 Distributions of relative humidity for the dry (top) and wet (bottom) transects during the study period.

Frog abundance between forest types

Litoria nannotis was consistently more abundant in the dry forest than in the wet forest (Kruskall-Wallis test: Chi square = 39.49, df = 1, $p < 0.001$; Figure 4.3). The abundance of *L. genimaculata*, although generally similar between both transects (t = 0.329, df = 10, p = 0.729; Figure 4.4), fluctuated between sampling events, especially in the wet forest site.

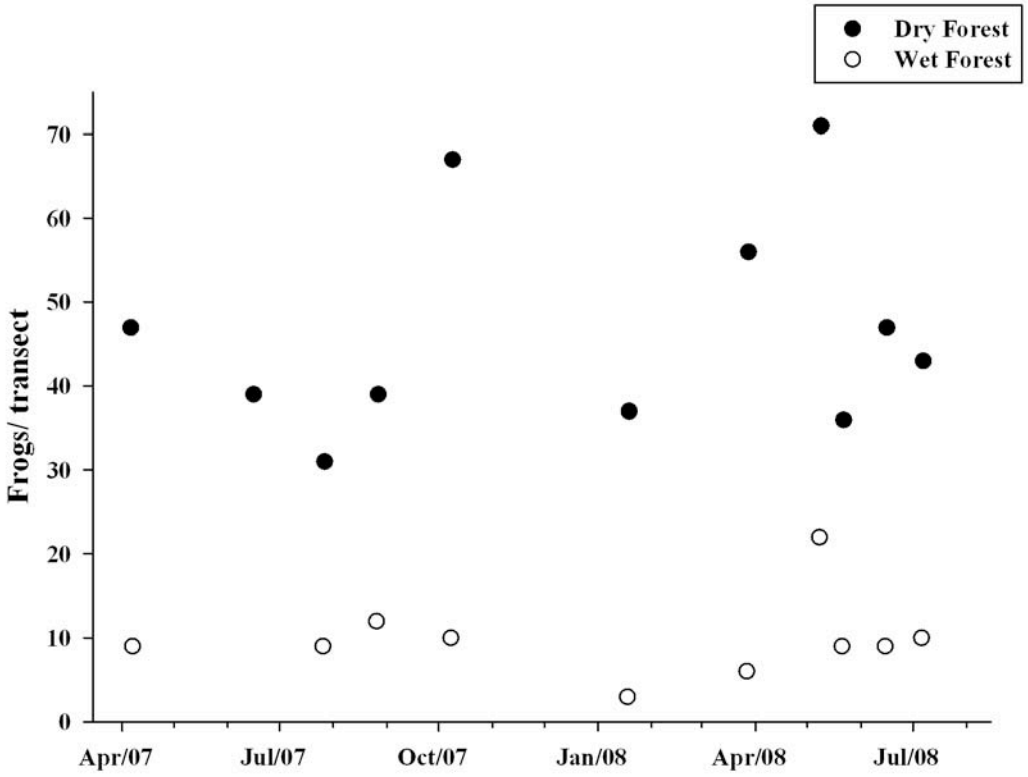


Figure 4.3 Abundance (count data) of *Litoria nannotis* from April 2007-July 2008.

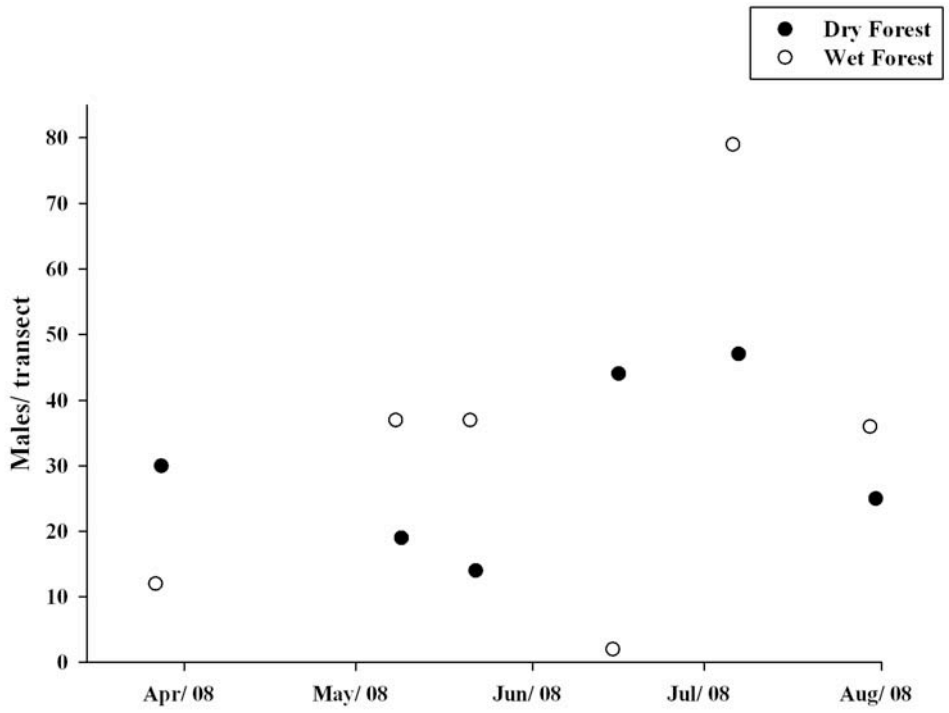


Figure 4.4 Abundance (count data) of *Litoria genimaculata* between March-August, 2008.

Morbidity and mortality patterns

Diseased frogs with signs of chytridiomycosis were found only in the wet forest and only during winter sampling (Table 4.3). All diseased frogs with clear signs of chytridiomycosis died during handling. At that time, *L. nannotis* were approximately five times more abundant in the dry forest than in the wet forest (Figure 4.3) and none of them had any of the clinical signs of disease. A Fisher's odds ratio 95 % confidence interval indicates that the likelihood of finding a diseased or dying *L. nannotis* in the dry forest is at least 9.2 times lower than in the wet forest. For *L. genimaculata*, the upper 95 % binomial confidence interval for the occurrence of clinical disease in the dry forest is very low, but within the confidence interval of observed mortality in the wet forest transect (Table 4.3). All diseased *L. nannotis* were juveniles, whereas all diseased *L. genimaculata* were males.

Table 4.3 Numbers of diseased (clinical signs of chytridiomycosis) individuals and total numbers of animals sampled without any signs of disease by species and site. Prevalence and 95 % binomial confidence intervals are included in the table; lower confidence interval (LCL) and upper confidence interval (UCL).

	Litoria genimaculata		*Litoria nannotis*	
	Dry Forest	Wet Forest	Dry Forest	Wet Forest
Diseased	0	5	0	8
Total	234	241	580	106
Prevalence	0.00	2.07	0.00	7.55
LCL %	0.00	0.67	0.00	3.31
UCL %	1.56	4.78	0.01	14.33

Half of the diseased animals were encountered on the wet forest transect during a single night (July 26th, 2007). Although diseased animals were also found in the winter of 2008, only six more animals were encountered, despite a 5 fold increase sampling effort compared to 2007. In diseased animals, intensity of infection in *L. genimaculata* was significantly higher than in *L. nannotis* ($t = 2.514$, $df = 10$, $p = 0.031$; table 4.4).

Table 4.4 Diseased frogs collected in the wet forest transect during the winters of 2007 and 2008 with species, class, intensity of infection and collecting date.

Species	Class	Zoospore equivalents	Date
Litoria genimaculata	Male	70705	26/07/07
Litoria nannotis	Juvenile	3845	26/07/07
Litoria genimaculata	Male	13012	26/07/07
Litoria genimaculata	Male	7569	26/07/07
Litoria nannotis	Juvenile	15130	26/07/07
Litoria nannotis	Juvenile	2631	26/07/07
Litoria nannotis	Juvenile	21620	6/05/08
Litoria nannotis	Juvenile	1440	6/05/08
Litoria nannotis	Juvenile	638	6/05/08
Litoria genimaculata	Male	77908	6/05/08
Litoria nannotis	Juvenile	1917	13/06/08
Litoria nannotis	Juvenile	12520	13/06/08

Prevalence of infection

The final logistic regression model in the assessment of the possible impacts of 33 variables on infection status contained four independent variables: species, class, site and 28-day min AT and the interaction of two variables: species x class (Table 4.5). The full model containing all predictors was statistically significant (chi square = 252.28, df = 7, $p < 0.0001$) and showed no lack of fit (Hosmer and Lemeshow goodness of fit-test; chi square = 5.314, df = 8, $p = 0.724$), correctly classifying 76 % of cases. The strongest predictor for infection status was the mean of the minimum temperature recorded for the 28 days previous to sampling. For each decrease of 1 °C the odds of being infected increase by a factor of 1.51 all else being equal (Table 4.5). The second variable of importance was habitat type (wet or dry forest); the probability of a frog being infected in the dry forest was 5.55 times higher than in the wet forest (see prevalence data in Table 4.6). Third in importance was the species factor, with the odds of *L. nannotis* 5.45 higher to be infected than *L. genimaculata.* Class was also important; the odds of juveniles were 2.54 higher than adults to be infected and in the case of *L. nannotis* juveniles the odds were 6.05 higher than any other combination of species and class, all else being equal (Tables 4.5, 4.6; Figures 4.5, 4.6).

Table 4.5 Final logistic regression model for individual infection status incorporating all variables found significant in previous analysis and significant interactions.

Variable	B	SE	Wald	d.f.	P	Odds Ratio	95.0% C.I. for Odds Ratio	
							Lower	Upper
28 min AT	-0.41	0.04	87.03	1	> 0.0001	0.66	0.61	0.72
Wet Forest	-1.72	0.21	68.42	1	> 0.0001	0.18	0.12	0.27
Litoria nannotis	1.70	0.35	23.22	1	> 0.0001	5.45	2.73	10.86
Class			22.20	2	> 0.0001			
Females	-0.70	0.48	2.12	1	0.1456	0.50	0.19	1.27
Juveniles	0.95	0.32	8.61	1	0.0034	2.57	1.37	4.84
Species x Class			20.78	2	> 0.0001			
Litoria nannotis x Juveniles	1.80	0.56	10.19	1	0.001	6.05	2.00	18.28
Litoria nannotis x Females	-0.56	0.42	1.75	1	0.186	0.57	0.25	1.31
Constant	5.92	0.73	65.49	1	> 0.0001	371.14		

Table 4.6 Prevalence of infection (% infected) and 95% binomial confidence intervals for the significant categorical variables included in the final logistic regression model for prevalence.

Variables	Infected	Total	Prevalence	LCL	UCL
Dry Forest	436	584	74.7	70.9	78.1
Wet Forest	140	308	45.5	39.8	51.2
Litoria nannotis (Ln)	395	513	77.0	73.1	80.6
Litoria genimaculata (Lg)	181	379	47.8	42.6	52.9
Juvenile	165	221	74.7	68.4	80.3
Female	154	259	59.5	53.2	65.5
Male	257	412	62.4	57.5	67.1
Ln x juvenile	155	177	87.6	81.8	92.0
Ln x female	135	197	68.5	61.5	74.9
Ln x male	105	139	75.5	67.5	82.4
Lg x juvenile	10	44	22.7	11.5	37.8
Lg x female	19	62	30.6	19.6	43.7
Lg x male	152	273	55.7	49.6	61.7

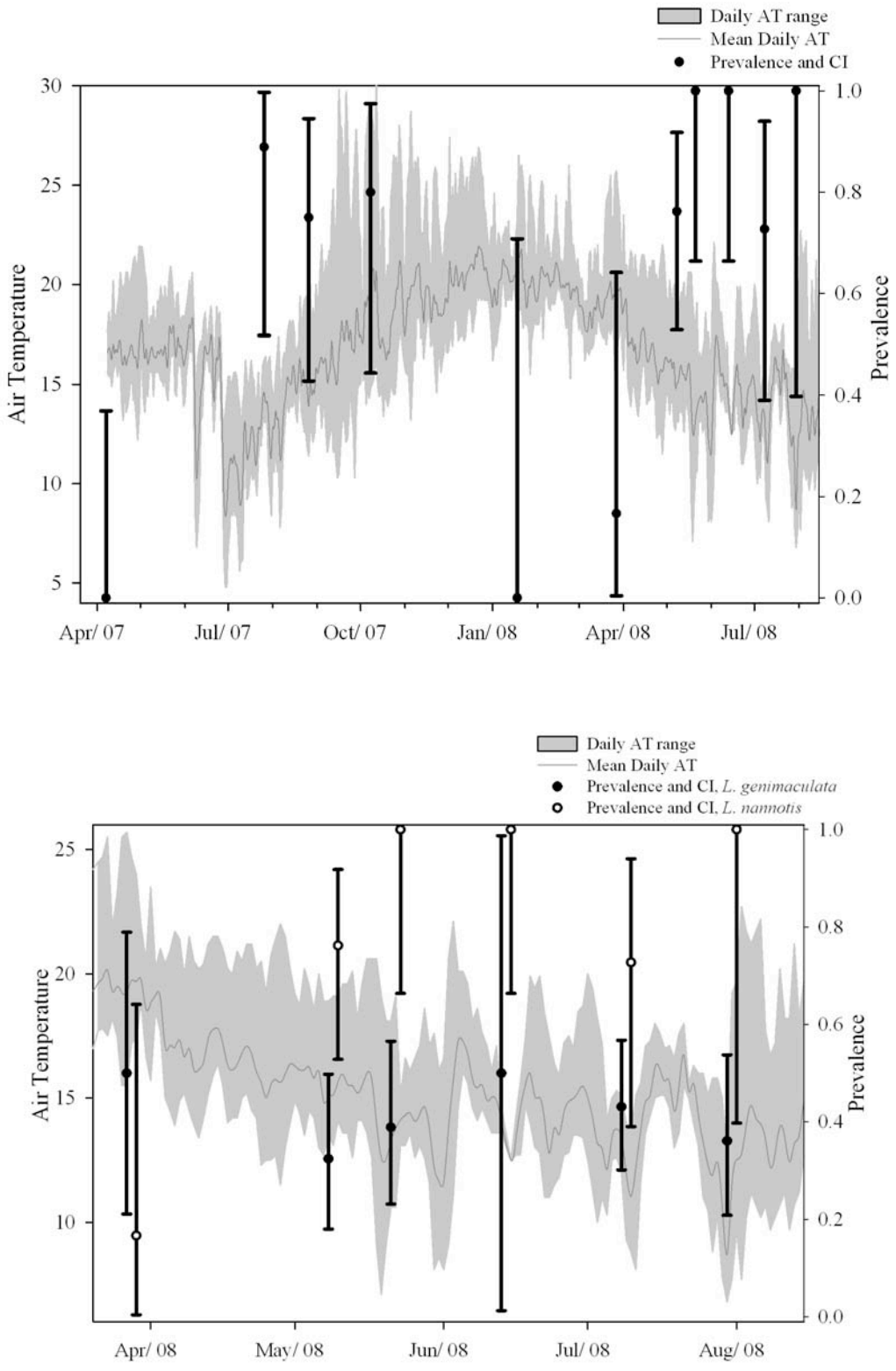


Figure 4.5 Prevalence of infection (+/- 95 % C.I.) in *Litoria nannotis* and air temperature (AT) at the wet forest site, 2007 – 2008 (top). Prevalence of infection in *Litoria genimaculata* and *Litoria nannotis* and air temperature at the wet forest site in 2008 (bottom).

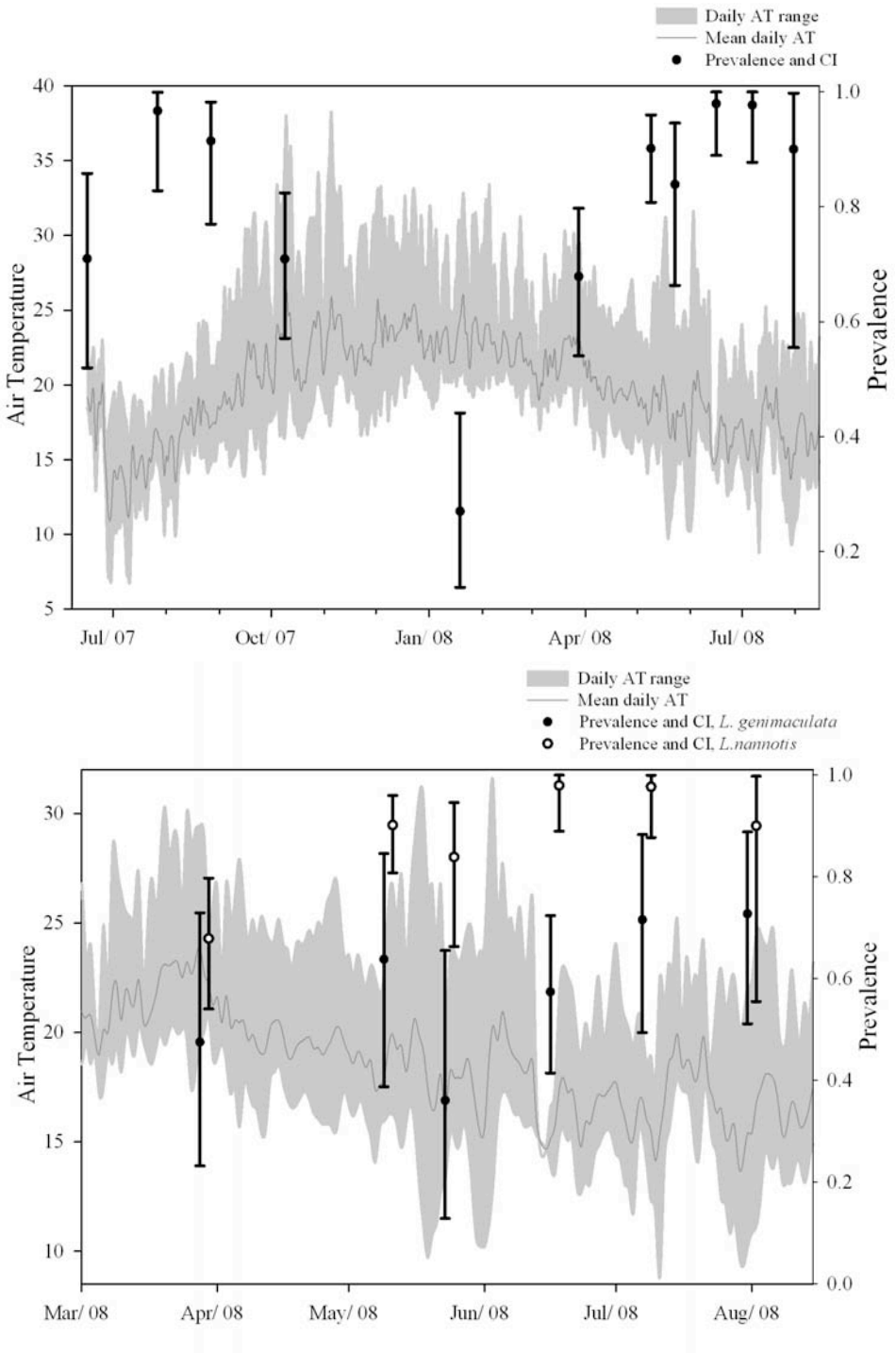


Figure 4.6 Prevalence of infection (+/- 95 % C.I.) in *Litoria nannotis* and air temperature in the dry forest site, 2007-2008 (top). Prevalence of infection in *Litoria genimaculata* and *L. nannotis* and air temperature in the dry forest site in 2008 (bottom).

Intensity of infection

There were significant effects of class, season and the interaction of species and sex on intensity of infection (Table 4.7). Post-hoc comparisons using the Tukey test indicated that juveniles had significantly more intense infections than males or females (Figure 4.7). Frogs sampled in winter also had significantly greater intensities of infection (Figure 4.8). The univariate regressions indicated that the effect of temperature on intensity is stronger than the effect of humidity. Minimum temperature over 21 days was the most important variable (Figure 4.9); higher ambient air temperatures produced lower intensities of infection ($R^2 = 0.411$, n = 21, p = 0.002). The best humidity variable was the maximum humidity of the last 3 days, but it was not significant ($R^2 = 0.122$, n = 21, p = 0.081). The highest intensities occurred in July 2007 at both sites. In that month, sampling occurred after a cold front passed through the area and minimum daily temperatures were extremely low for several weeks (Figure 4.10). Temperatures this low and intensities this high were not recorded in 2008.

Table 4.7. Univariate analysis of variance examining the effects of species, class, season and their interactions on log-transformed intensity of infection.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	116.18	9	12.91	13.77	> 0.0001
Intercept	324.85	1	324.85	346.52	> 0.0001
Species	0.05	1	0.05	0.05	0.8192
Class	27.33	2	13.66	14.58	> 0.0001
Site	2.84	1	2.84	3.03	0.0820
Season	21.77	1	21.77	23.22	> 0.0001
Species x Class	8.93	2	4.46	4.76	0.0089
Species x Site	3.23	1	3.23	3.45	0.0638
Species x Season	0.18	1	0.18	0.19	0.6612
Error	558.73	596	0.94		
Total	3021.68	606			
Corrected Total	674.92	605			

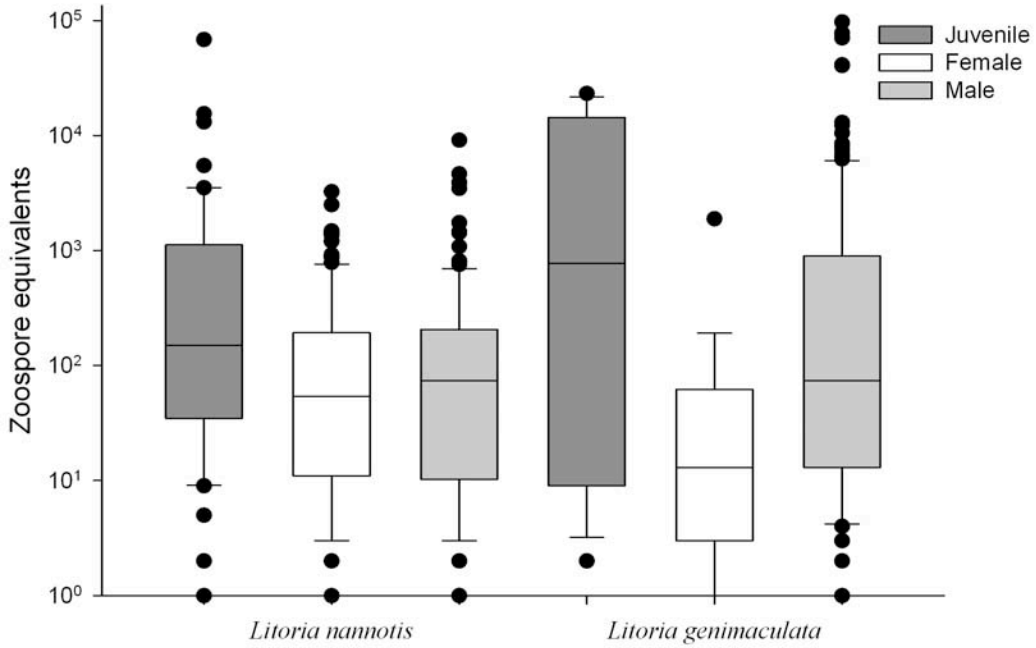


Figure 4.7 Tukey (1977) boxplot of intensity of infection by species and class

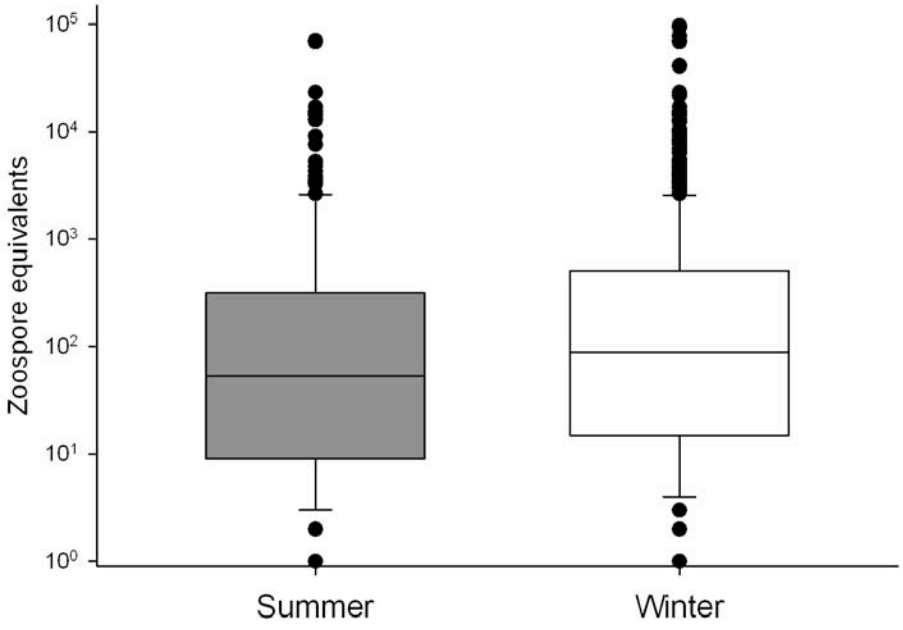


Figure 4.8 Tukey (1977) boxplots of intensity of infection by season

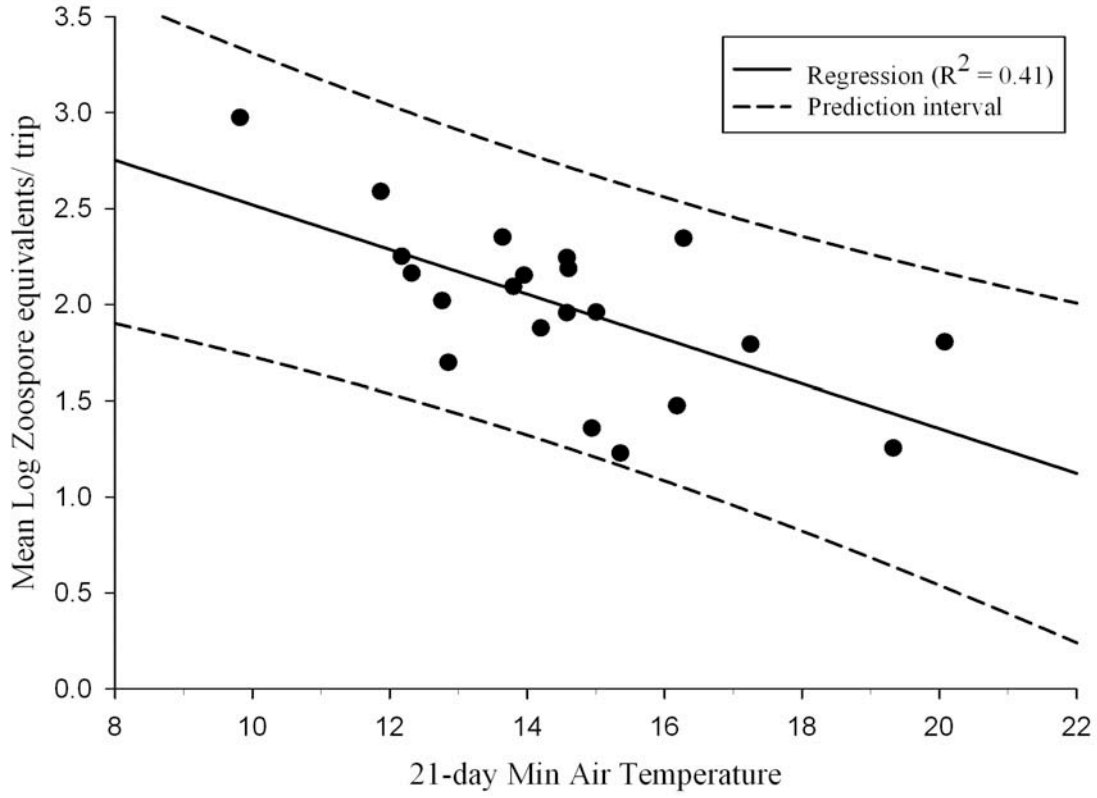


Figure 4.9 Linear regression of the mean of the $\log_{10}$ (number of zoospore equivalents) for each trip against 21-day mean of minimum temperature.

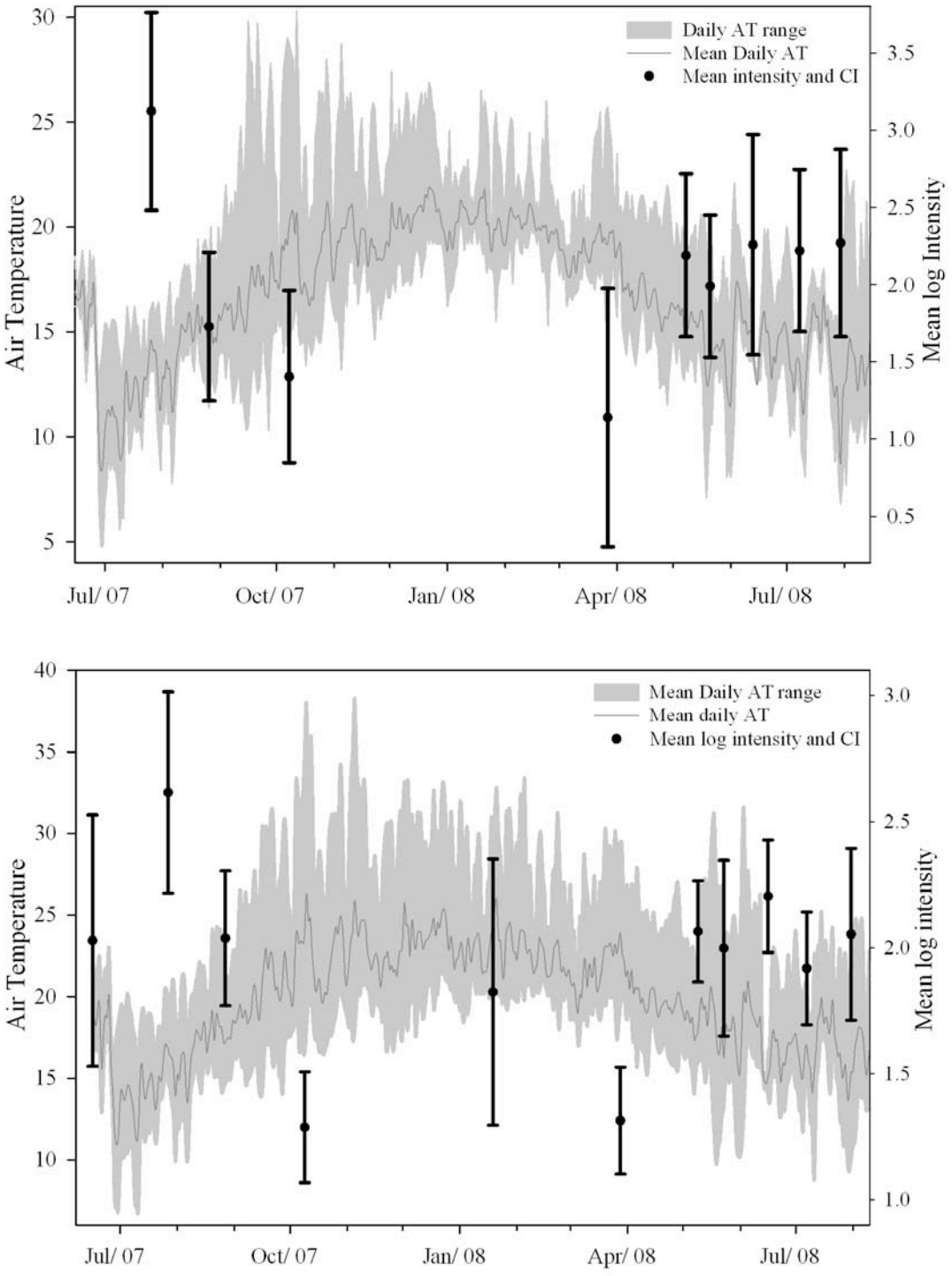


Figure 4.10 Mean intensity of infection in *Litoria nannotis* for each trip (+/- 95% C.I.) and air temperature (AT) in the wet forest (top) and the dry forest (bottom), 2007-2008.

Discussion

The Australian Wet Tropics amphibian fauna is one of the best studied in tropical regions; its evolutionary history, biology and recent decline history are all well documented (e.g., Berger et al. 1998; Berger et al. 2004; Hines et al. 1999; Hoskin et al. 2005; Richards et al. 1993; Schneider and Moritz 1999; Williams 2006; Williams and Hero 2001; Williams and Pearson 1997; Woodhams and Alford 2005). The signature of the amphibian declines that occurred between the mid 1980s and 1990s is still evident in the stream breeding amphibian populations in this area today, at least in one of the two study species. *Litoria nannotis* was extirpated from rainforests at elevations above 400 m asl and *L. genimaculata* declined in the same areas, but has since recovered (Hines et al. 1999; Richards and Alford 2005; Richards et al. 1993). This fits well with the abundance patterns found in this study and the suggestion that the dry forest acts as a refuge from decline (Puschendorf et al. 2009), with *L. genimaculata* having similar abundance in dry and wet habitats, while *L. nannotis* was rare in the wet forest as compared to the dry forest or to lower elevation rainforest sites where they persisted during the declines (Hines et al. 1999; Woodhams 2003). If chytridiomycosis is responsible for shaping these differences in abundance, the epidemiology of the disease should differ between habitats giving dry forest frog populations an advantage over wet forest populations.

I observed diseased linked mortality only in wet environments and never in dry environments. This does not preclude the possibility that chytridiomycosis is having an effect in dry forest populations. Observed mortality in wildlife will always underestimate natural mortality in a population, since diseased animals are often difficult to detect and carcasses decompose quickly. In experiments using birds, carcasses disappeared completely in 5-12 days (Balcomb 1986; Tobin and Dolbeer 1990; Ward et al. 2006; Wobeser and Wobeser 1992). Anurans, being smaller, probably disappear even more rapidly. However, observed mortality should be strongly correlated with actual mortality rates, so it is likely that the periods of observed mortality corresponded to maximum mortality rates in the field. The case is especially strong for *L. nannotis* since the likelihood of mortality in this species is at least 9 times greater in the wet than in dry environments, strongly suggesting that chytridiomycosis-induced mortality rates are very different between both sites.

Intensities of infection in diseased individuals (clear signs of chytridiomycosis) differed significantly between species. Intensities of infection in *L. genimaculata* were much higher than in *L. nannotis*, probably because the former is less susceptible than the later (Hines et al. 1999), however this is confounded with the ages of diseased individuals encountered. All diseased *L. nannotis* were juveniles, in which susceptibility could be increased by an underdeveloped immune system, less capable of deterring infections by *B. dendrobatidis* (Rollins-Smith 1998). All morbid *L. genimaculata* were males, but they were also the only class consistently sampled in this species, since juveniles and females were rarely encountered, a common pattern for this species (Richards and Alford 2005). Mortality in juvenile *L. genimaculata* could be higher than that observed in males, since their intensities of infection were higher than those of adults.

Results from the logistic regression analysis on the effects of the environment on prevalence of infection suggest that temperature has the greatest effects on host-pathogen dynamics. Prevalence fluctuated through the seasons in concert with temperature, but *B. dendrobatidis* was always detectable in the population, unlike other studies in the region where *B. dendrobatidis* became "undetectable" (Kriger and Hero 2006b) during summer. Low temperatures, usually within the optimum range for *B. dendrobatidis* and the close association of these species to streams can provide an environment that is near optimal for the pathogen. Mortality was only evident at the wet site during winter when prevalence was highest, clearly linking environmental conditions to disease outbreaks as has been reported in other cases (Berger et al. 2004; Bradley et al. 2002; Woodhams and Alford 2005). Although temperature varied significantly across sites, temperatures in the dry forest site seemed to be closer to the thermal optimum for the pathogen than the ones in the wet forest where the median was slightly below this range (Piotrowski et al. 2004; Woodhams et al. 2008a).

Habitat type was the second variable of importance; prevalence was significantly higher in the dry than in the wet forest. Clearly the pathogen thrives in the dry forest during winter, since close to 100 % of the sampled individuals were infected. This is probably because *L. nannotis*, the species with higher prevalence, never experiences the highest temperatures. All current evidence suggests that *B. dendrobatidis* is specific to amphibians (Berger et al. 2005a; Berger et al. 2005c; Rowley et al. 2006;

Rowley et al. 2007a; Rowley et al. 2007b) making it likely that frog-frog and frog-water contact are the main transmission modes. The higher density of *L. nannotis* in the dry forest should therefore produce higher transmission rates, explaining the very high prevalence at this site.

The comparisons of prevalence and frog numbers between the dry and wet forest sites reveal an apparent inconsistency; in the dry forest site, where frog abundance is greatest, a higher proportion of animals are infected with this virulent pathogen. Mathematical models of the dynamics of microparasitic disease suggest that pathogens are unlikely to occur at high prevalences if they have significant negative effects on populations of their hosts (Anderson and May 1979; McCallum and Dobson 1995; McCallum and Dobson 2002). The answer thus may lie in higher pathogen tolerance by the host, lower virulence of the pathogen in these environments, or both. Although *B. dendrobatidis* can continue to produce mortality in individual anurans years after emerging (Murray et al. 2009), the difference in pathogen prevalence and host abundance I found between habitats indicates strongly that the frog-pathogen interaction in the dry forest allows frogs to persist better, despite the disease being abundant in the system.

The odds of *L. nannotis* to be infected were 5.45 higher than *L. genimaculata*. This difference in infection likelihood may be driven at least in part by interspecific behavioural differences. *Litoria nannotis* generally live clustered around waterfalls, in contact with stream water from which they could become infected by aquatic zoospores (Rowley and Alford 2007a). In contrast, *L. genimaculata* spend little time in water and often elevate their body temperatures above 30 °C; these temperatures can kill off the pathogen (Rowley 2006).

Likelihood of being infected also differed significantly between age classes. The odds of infection are 2.57 higher in juveniles (6.05 times higher when only *L. nannotis* is considered), suggesting that they are more susceptible to infection. Amphibian larvae and adults of less susceptible species, are the only known carriers of *B. dendrobatidis* and obvious disease signs and death do not occur during the larval stage (Berger et al. 1998; Berger et al. 1999a), but sublethal effects of the pathogen on amphibian larvae might have a significant effect during development. Tadpoles infected by *B.*

dendrobatidis take longer to reach metamorphosis and when they do, they are smaller in size than uninfected ones (Parris and Cornelius 2004). Infected tadpoles can also be more susceptible to predation (Parris 2006). The sublethal effects may be carried on from the larval stages through metamorphosis, could be enhanced by a weaker immune system, which is reorganized during metamorphosis and is less developed in younger individuals (Pasquier et al. 1989; Rollins-Smith 1998). At this stage the anuran skin is also reorganized, with increased keratinisation and becomes more susceptible to infection by *B. dendrobatidis* (Berger et al. 1998; Berger et al. 1999a; Berger et al. 2005c; Davidson et al. 2003). Their less-developed immune system may decrease the chances of surviving infection of metamorphs and juveniles. Juveniles are also more prone to desiccation than adults and in *L. nannotis*, juveniles seem to be entirely restricted to moist environments near waterfalls (Puschendorf unpublished), where rates of transmission from water are likely to be higher. Adults can forage in forest habitats, occasionally escaping microenvironments in which amphibians are more prone to disease development (Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b).

In addition to prevalence, intensity of infection was also inversely related to ambient temperature. For example, the sampling event in July of 2007 in which the highest mean intensity of infection was recorded occurred during a period of prolonged cold, reaching minimum temperatures of 5 °C. These temperature regimes never occurred during the winter of 2008, when intensities never reached similar levels. Intensities of infection were also greatest on juveniles. The pattern was especially consistent in *L. nannotis*, for which the sample size of juveniles was relatively high. It was common to find infected animals with thousands of zoospore equivalents, whereas in adults this was rare, with intensities usually in the hundreds. This pattern further reinforces the suggestion that disease may exert the strongest selective pressure on juveniles, affecting recruitment into the population and therefore may slow population recovery.

Differences in virulence and infection outcomes among *B. dendrobatidis* strains are well documented (Berger et al. 2005b; Retallick and Miera 2007), but it is unlikely that strains differed between the study sites. The distance between transects was short and they are highly connected by sharing the same stream, with the wet forest site immediately above the dry forest site. At typical flow rates (ca. 10-50 cm/sec),

zoospores would be transported from the wet to the dry forest site within minutes. Infection is known to occur through contact with zoospores in water (Rachowicz and Vredenburg 2004). Because strain differences are highly unlikely, it appears that frogs in the dry habitat have greater tolerance to chytridiomycosis infections. This is likely to be due to environmental conditions that inhibit pathogen growth or augment immune responses. Even for a species such as *L. nannotis* that is unlikely to bask (Rowley 2006), small increases in temperature could result in an increase in survival.

Most of the research looking at the temperature effects on host survival has focused on the upper threshold of pathogen tolerance of 30 °C at which *B. dendrobatidis* dies. Nevertheless, a recent publication demonstrates that even small temperature differences can significantly affect disease outcomes, even when temperature ranges are within the optimum for pathogen growth (Andre et al. 2008). The amphibian immune system is very sensitive to temperature changes and even a slight increase in temperature could make a significant difference in disease outcome, if chytridiomycosis is affected by the immune system (Cooper et al. 1992; Maniero and Carey 1997).

The environmental conditions in which amphibian populations exist may determine whether they persist successfully when faced with the threat of chytridiomycosis. In the present study, it is clear that dry forest populations are coexisting more successfully with the pathogen. As suggested by Puschendorf et al (2009), dry forest populations should be included in any amphibian conservation and management plans to further guarantee the survival of these species in the wild. Further research is necessary to determine the mechanisms by which dry forest populations gain increased tolerance to *B. dendrobatidis*.

CHAPTER FIVE: REDISCOVERY OF AN "EXTINCT" AUSTRALIAN STREAM FROG (*LITORIA LORICA*) IN DRY FOREST HABITAT

Abstract

Chytridriomycosis, caused by the fungus *Batrachochytrium dendrobatidis*, is one of the main drivers in the decline and extinction of amphibians in pristine and undisturbed tropical areas. It was believed that the armoured mist frog (*Litoria lorica*) succumbed to chytridiomycosis as, despite considerable search effort, it was last observed in 1991 coinciding with chytridiomycosis-induced declines of six other species in the region. However, a previously unknown population of *L. lorica* was discovered in June 2008, with high prevalence of *B. dendrobatidis* infections in terrestrial stages of *L. lorica* (80.4 %), sympatric *L. nannotis* (80.8 %) and in tadpoles of *L. nannotis* and *L. rheocola* (both 100 %). Despite this, *L. lorica* and *L. nannotis* were relatively abundant and remained so through to the most recent surveys (late March 2009) in which prevalence and intensities of infections in frogs remained similar to those in winter months. This population of *L. lorica* is apparently coexisting with *B. dendrobatidis*, which is thought to have caused the extinction of other populations of the species. Environmental characteristics at the site of rediscovery differ from the rainforest sites where extirpation occurred, being drier, hotter and more seasonal. These environmental conditions may have enabled the population of *L. lorica* to persist despite being considered "marginal". In the face of emerging diseases and environmental changes, it is important to fully understand the ecological range of species under threat and to ensure that conservation and survey efforts are not solely focused on what are thought to be "core" environments.

Introduction

Currently, amphibians are the most threatened group of vertebrates (Stuart et al. 2004). In addition to the effects of many anthropogenic factors on amphibian populations, species disappearances in pristine and protected areas are of particular concern (Stuart et al. 2004). The primary cause of these declines and extinctions has been attributed to chytridiomycosis (e.g., Berger et al. 1998; Bosch et al. 2006; Lips et al. 2006; Rachowicz et al. 2006; Schloegel et al. 2006; Skerratt et al. 2007). In

Australia, *Batrachochytrium dendrobatidis* (Longcore et al. 1999), the pathogen that causes chytridiomycosis, has been found on sick and dying frogs (Berger et al. 1998; Berger et al. 2004; Berger et al. 1999a) and is thought to have caused the decline and extirpation of several frog species (Berger et al. 1998; Berger et al. 1999a; Hines et al. 1999; Schloegel et al. 2006; Skerratt et al. 2007).

The Wet Tropics region of northern Queensland, Australia, comprises approximately 1 million hectares of rainforest along with sclerophyll communities dominated by *Melaleuca* and *Eucalyptus* (Williams 2006) and supports 27 endemic frog species (Hoskin and Hero 2008). At present, four Wet Tropics stream frogs are classified as critically endangered, three as endangered and one as near threatened (IUCN, 2008). Chytridiomycosis is considered the primary cause of the declines of these species, as several well-documented populations vanished following outbreaks of *B. dendrobatidis* (Hines et al. 1999; Laurance et al. 1996; Schloegel et al. 2006). The four species classified as critically endangered were presumed extinct by local researchers because no individuals had been observed for at least 18 years, despite intensive searches, although they had previously been locally abundant (Hines et al. 1999; McDonald unpublished).

One of these missing species, the armoured mist frog (*Litoria lorica*), is considered an endemic to the Thornton Uplands and Carbine Tableland (Cogger 2000; Cunningham 2002; Davies and McDonald 1979; Hoskin and Hero 2008). It was last seen in 1991 and was thus thought to be extinct (Covacevich and McDonald 1993; Cunningham 2002). Its disappearance coincided with declines and disappearances suffered by sympatric species such as *Litoria nannotis, Litoria rheocola, Nyctimystes dayi* and *Taudactylus acutirostris*. Necropsies on dead and dying individuals of these species collected in the area at the time showed that they died of chytridiomycosis (Berger et al. 1998; Berger et al. 1999a). It is highly likely that an epidemic of chytridiomycosis was responsible for the disappearance of those species, including *L. lorica*.

In June 2008, 18 years after it had last been observed, I discovered a population of frogs I believed to be *L. lorica* outside its formerly known distribution. *Litoria lorica* was thought to be a rainforest specialist (Williams and Hero 1998, 2001); however, the population discovered was outside the rainforest in an adjacent dry sclerophyll

habitat. The work presented here had three goals: (1) to confirm the species' identity using morphological and genetic analyses; (2) to determine the prevalence and intensity of infections by *B. dendrobatidis* in the newly discovered population and in sympatric populations of other species; (3) to characterize the environment occupied by the newly discovered population and determine whether environmental effects might be responsible for this population surviving the epidemic outbreaks of chytridiomycosis that caused other populations to become locally extinct in the early 1990s. I then discuss the broader implications of my results for efforts towards the conservation of other species of amphibians and for conservation of relatively poorly known species of other taxa.

Methods

Identification of *Litoria lorica*

Litoria lorica was identified using morphological and genetic data. The morphology of potential *L. lorica* individuals was compared against the species description (Davies and McDonald 1979) and specific key diagnostic traits outlined in later publications (Cunningham 2002; Hoskin and Hero 2008). *L. lorica* is one of four species comprising the Australian 'torrent frogs'. It is easily distinguished from two of these species, *L. rheocola* and *L. nyakalensis*, by a number of traits (Cunningham 2002; Hoskin and Hero 2008) but could be confused with *L. nannotis*, a sympatric species that is generally larger in size but otherwise of very similar morphology and ecology. Individuals of each species were examined in the field and the snout-vent length (SVL) and weight of each individual were measured.

Toe-pad tissue was taken from three *L. nannotis* and three suspected *L. lorica* individuals for genetic analysis. These samples were sequenced for a segment of the mitochondrial cytochrome oxidase I (*CO1*) gene. Extraction and sequencing followed standard techniques and the primers used were *Cox and Coy* (Hoskin et al. 2005; Schneider et al. 1998). These sequences were added to extensive sequence data for *L. nannotis* and *L. rheocola* from the phylogeographic studies of (Schneider et al. 1998). A neighbour-joining tree was constructed from 510 base pairs (bp) of *CO1*, with *Litoria genimaculata* and *Nyctimystes dayi* used as outgroups. Bootstrap analysis was

performed to test the support for relationships. *Litoria lorica* had not previously been sequenced because all samples collected pre-decline were formalin-fixed.

Litoria lorica and *Litoria nannotis* abundance surveys

Historical records indicate that *Litoria lorica* occurred in rainforests between 640-1000 m asl in the Thornton Peak Uplands and Carbine Tableland (Cogger 2000; Covacevich and McDonald 1993; Cunningham 2002; Davies and McDonald 1979; Hoskin and Hero 2008) but provide no information on the species' abundance. I conducted abundance surveys along a 400 m transect, which consisted of three discrete waterfall and cascade sections (primary habitat for both species) separated by deeper pools in the upstream section of the site. The riparian habitat along the transect was dry sclerophyll woodland on granite hills. Abundance surveys were conducted three times in July 2008, once in September 2008 and once in March 2009 and involved counting and identifying all *L. nannotis* and *L. lorica* individuals located along the transect at night. Because the species proved to be morphologically distinct (see results), individuals could be identified without handling. The area of the three occupied sections was approximated, so that abundance per unit area could be estimated.

Detection of *Batrachochytrium dendrobatidis* in frogs and tadpoles

Frogs and tadpoles were sampled for infection status at the peak of winter (July 2008) when the air temperature is coolest and prevalence and intensity of infection were expected to be greatest. A second set of samples was collected at the end of summer (March 2009) when prevalence and intensity were expected to be lowest (Berger et al. 1998; Bradley et al. 2002; Kriger and Hero 2006b; McDonald et al. 2005; Retallick et al. 2004; Woodhams 2003).

I captured frogs by inverting an unused 15 x 15 cm plastic bag over my hand and grasping the animal while drawing the bag inside out, thus capturing the frog in the bag without touching it. Captured frogs were then quickly released into larger 21 x 30 cm press seal bags.

I attempted to catch all visible animals at each localised waterfall or cascade area. I used a new pair of low powder vinyl gloves (Livingstone) to handle each individual as

it was swabbed for (diagnostic qPCR; (Hyatt et al. 2007), measured and weighed. The exact capture locality of each frog was recorded using a Garmin 60CSX GPS. After swabbing I recorded each individual's sex, age class status, (juvenile or adult), snout-vent length and mass. Frogs were sampled for *B. dendrobatidis* by firmly stroking a cotton swab on their ventral skin three times in each of the following parts: (a) the back of each hindfoot; (b) the pelvic patch on both legs; (c) the ventral surface of the abdomen on each side and in the middle (9 strokes for the abdomen); and (d) each forefoot, for a total of 27 strokes. After measurement and swabbing, the frog was immediately released at the point of capture.

Tadpoles were captured by dip netting and quickly transferred them into individual press seal bags, avoiding any direct handling. I used a new pair of well-rinsed vinyl gloves for each individual and observed tadpoles for any ill effects after handling as suggested by Cashins et al. (2008). Tadpoles were poured from the bags into the palm of a gloved hand and secured on the ventral surface between the forefinger and thumb. They were then sampled by gently stroking a swab over the mouthparts; 8 times horizontally across the upper and lower tooth rows and jaw sheath and 8 times vertically across all rows for a total of 24 strokes. Following sampling, tadpoles were returned to the individual bags and held for at least 15 minutes to confirm their condition before release. Tadpoles were identified according to (Richards 1992). Tadpoles of *L. nannotis* and another torrent frog, *L. rheocola*, were captured and swabbed but no *L. lorica* tadpoles were found.

Swabs were analysed for the presence of *B. dendrobatidis* using a real-time quantitative Taqman PCR assay (Boyle et al. 2004) at James Cook University, Townsville, Australia. Each sample was run in triplicate. Samples were considered positive if three replicate wells were found to have *B. dendrobatidis* DNA. Negative samples were rerun with an internal positive control to confirm inhibition did not cause the negative results (Hyatt et al. 2007). I chose this stringent criterion to minimise the false positive rate, therefore my prevalence estimates are conservative, especially under the current circumstances. I report prevalence of infection as the percentage of infected individuals of each species with 95 % confidence intervals (CI). Measures of infection intensity include only infected individuals and were log

transformed prior to analysis (Zar 1999), but are reported as zoospore equivalents to improve clarity.

Environments of the original and newly discovered *Litoria lorica* localities

Beyond site descriptions of the environments of the extirpated and newly discovered populations of *L. lorica* and *L. nannotis*, I further examined differences with respect to long-term climate means. The climate values used were mean annual temperature and precipitation and the annual seasonality, which were derived using Anuclim 5.1 software (McMahon et al. 1995) and an 80 m^2 resolution DEM (resampled from GEODATA 9-Second DEM, ver. 2; Geoscience Australia, http://www.ga.gov.au/). The locations of populations of *L. lorica* and *L. nannotis*, which were obtained from published data including Davies and McDonald (1979), Cunningham (2002) and Williams (2006) and institutional database of the Centre for Tropical Biodiversity and Climate Change (James Cook University, Townsville, Australia).

Results

Identification of *Litoria lorica* and habitat

The newly discovered population fits all the morphological traits for *L. lorica*, including those that distinguish this species from the most morphologically similar species, *L. nannotis* (Cunningham 2002; Davies and McDonald 1979; Hoskin and Hero 2008).

Adult *L. lorica* (SVL 30-40 mm, mean = 35.2 mm, SD = 2.7, N = 45) are significantly smaller than adult *L. nannotis* (SVL 53-62 mm, mean = 56.4 mm, SD = 3.3, N = 30). . Sub-adult *L. nannotis* overlap in size with adult *L. lorica*, but *L. lorica* are readily diagnosed by the presence of nuptial pads and accessory spines on the chest and chin (obvious in males and present, but less prominent in females), a truncate snout shape, ventral colouration (white *versus* cream with grey or brown areas in *L. nannotis*) and dorsal pattern (more distinctly blotched *versus* more mottled in *L. nannotis*). Dorsally, individuals of *L. lorica* are generally light brown to grey, with darker, distinct blotches or mottling. *L. nannotis* tend to be yellowish green with darker mottling patterns than *L. lorica*.

Genetic analysis supported morphological identification of the population as *L. lorica* with samples representing a highly distinct genetic lineage that clearly falls within the Australian 'torrent frog' species group. All three *L. lorica* individuals sequenced shared the same *CO1* haplotype and these samples formed a well-supported sister lineage to *L. nannotis* (Hoskin, unpublished data). Mean sequence divergence (510 bp *CO1*, Kimura two-parameter) between *L. lorica* and *L. nannotis* was 19.8 % and between *L. lorica* and *L. rheocola* was 24.7 %. Additionally, three *L. nannotis* individuals were sequenced from the *L. lorica* site. These *L. nannotis* fell neatly amongst *L. nannotis* sequences from other sites on the Carbine Tableland (Hoskin, unpublished data).

The new population of *L. lorica* was located along two kilometres of rocky river with perennial flow around cascades and large waterfalls. This study, however, was restricted to the upper 400 m of its distribution. The surrounding vegetation in the area is dry sclerophyll woodland, with the nearest well-developed rainforest 6 km upstream. All previously known localities for *L. lorica* were in rainforest. This site is less than 3 km from the dry sclerophyll transect and 4 km from the wet sclerophyll sites downstream from Mt. Spurgeon (see previous chapters). Biplots of environmental variables indicate this site is characterized by having much higher precipitation seasonality and being much drier than the historical sites at which this species was previously found in the rainforest (Figure 5.1).

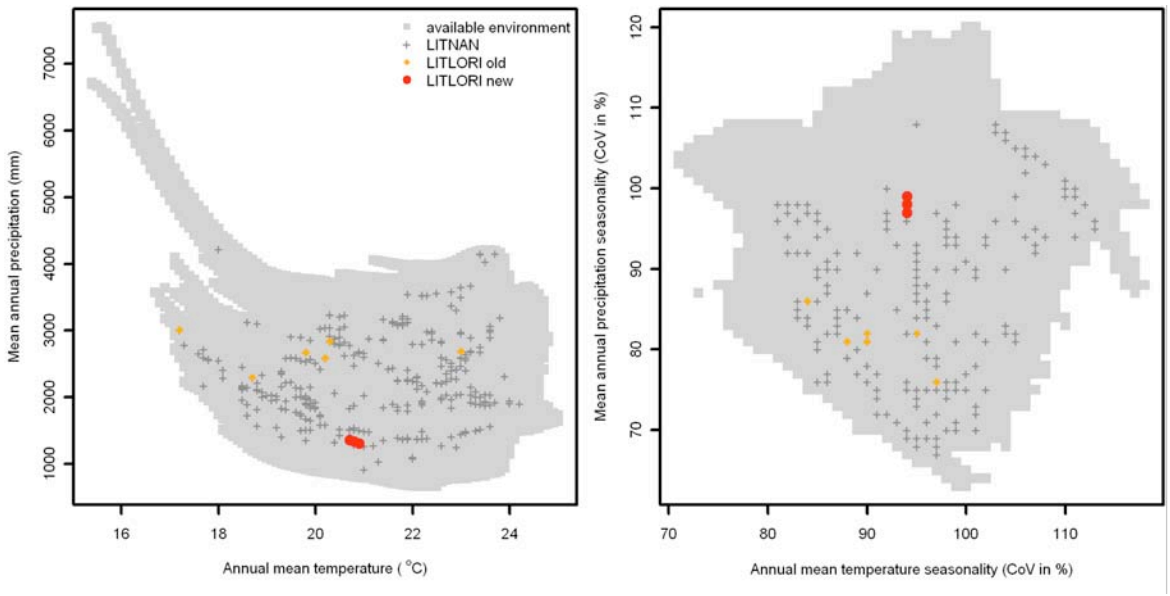


Figure 5.1 Plots of the environmental space of the Australian Wet Tropics Bioregion (grey); sites where *Litoria nannotis* is found (dark-grey crosses); historical sites for *Litoria lorica* (orange); and points from the site where *L. lorica* was rediscovered in this study (red circles).

Frog abundance estimates

On the 400 m transect, both *L. nannotis* and *L. lorica* were found only in three discrete waterfall/cascade sections, totalling approximately 150 m^2. Frogs were highly clustered, with *L. lorica* and *L. nannotis* co-occurring on rocks next to waterfalls and cascades. Within the small areas in which they were found, both species occurred at high densities, with *L. nannotis* (mean = 0.251 individuals/ m^2, SD = 0.059) at higher densities than *L. lorica* (mean = 0.159 individuals / m^2, SD= 0.064). The maximum number of frogs observed on a single sampling occasion was 35 *L. lorica* and 50 *L. nannotis*, observed in March, 2009. In total, I sighted *L. nannotis* individuals 188 times and *L. lorica* individuals 119 times during abundance surveys. None of the frogs sighted showed clinical signs of chytridiomycosis such as lethargy, skin sloughing or loss of righting reflex (Berger et al. 1999a).

Prevalence and intensity of infection of *Batrachochytrium dendrobatidis* in frogs and tadpoles

Analysis of skin swabs from winter 2008 revealed that 80.9 % (N = 89, 95% CI = 71.2-88.5%) of the samples from terrestrial (adult and juvenile) *L. nannotis* were positive for *B. dendrobatidis* while prevalence on terrestrial *L. lorica* was 80.4 % (N = 46, 95 % CI = 66.1-90.6 %). Prevalence in terrestrial *L. nannotis* (69.0 %, N = 29, 95 % CI = 49.2-84.7 %) and *L. lorica* (80.5 %, N = 41, 95% CI = 65.1-91.1; for details see Table 5.1) was similar in summer 2009. There was no significant effect of species, class (male, female, juvenile), season or the interaction of species and class or species and season with regards to infection status (Table 5.2). All of the 57 tadpoles swabbed (Fig. 5.2, N = 24 *L. nannotis* and N = 33 *L. rheocola*) were infected with *B. dendrobatidis* (Table 5.1). No *L. lorica* tadpoles were captured or sampled. Infection prevalence was significantly higher in *L. nannotis* tadpoles than in terrestrial frogs (Chi square = 12.44, $P < 0.001$, df = 1). One infected metamorph of *L. lorica* was sampled during the summer sampling (Table 5.1).

Intensity of infection did not differ significantly between species, class, season and the factorial interactions among them (Table 5.3). Infected tadpoles of *L. nannotis* had significantly higher intensities of infection than infected terrestrial *L. nannotis* ($F_{(1,110)} = 79.995$, $p < 0.001$; Figure 5.2; Table 5.1). As in the abundance surveys, none of the sampled individuals showed any of the clinical signs of chytridiomycosis (Berger et al. 1999a).

Table 5.1 Summarized frog and pathogen data for winter 2008 and summer 2009 sampling.

		Winter			Summer	
		Litoria lorica	*Litoria nannotis*	*Litoria rheocola*	*Litoria lorica*	*Litoria nannotis*
Female	Percentage infected	77.8	82.9		66.7	63.6
	Lower 95% CI	57.7	67.9		38.4	30.8
	Upper 95% CI	91.4	92.8		88.2	89.1
	Average Intensity	207	125		89	156
	STDEV Intensity	345	186		162	177
	Min Intensity	8	2		3	6
	Max Intensity	1616	657		566	552
	Subtotal sampled	27	41		15	11
Male	Percentage infected	86.7	77.8		86.4	62.5
	Lower 95% CI	59.5	40.0		65.1	24.5
	Upper 95% CI	98.3	97.2		97.1	91.5
	Average Intensity	198	395		1015	220
	STDEV Intensity	331	740		1236	177
	Min Intensity	5	5		8	4
	Max Intensity	1288	2191		3662	421
	Subtotal sampled	15	9		22	8
Juvenile	Percentage infected	75.0	79.5		100.0	80.0
	Lower 95% CI	19.4	63.5		29.2	44.4
	Upper 95% CI	99.4	90.7		-	97.5
	Average Intensity	47	913		403	637
	STDEV Intensity	18	2066		267	777
	Min Intensity	27	5		158	8
	Max Intensity	71	10094		774	2228
	Subtotal sampled	4	39		3	10
Metamorph	Percentage infected				100.0	
	Lower 95% CI				2.5	
	Upper 95% CI				-	
	Intenity of infection				24	
	Subtotal sampled				1	
Tadpoles	Percentage infected		100.0	100.0		
	Lower 95% CI		85.8	89.4		
	Upper 95% CI		-	-		
	Average Intensity		3709	2291		
	STDEV Intensity		3564	2899		
	Min Intensity		515	273		
	Max Intensity		13188	15178		
	Subtotal sampled		24	33		
	Total sampled	46	113	33	41	29

Table 5.2 Generalized linear model with a binary logistic link function to test the effect of species, class, season and their interaction on prevalence of infection.

	Wald Chi-Square	df	Sig.
(Intercept)	29.742	1	.000
Species	.970	1	.325
Class	.705	2	.703
Season	1.144	1	.285
Species * Class	1.779	2	.411
Species * Season	.250	1	.617

Table 5.3 Univariate analysis of variance to test the effect of species, class, season and their interaction on intensity of infection.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	13.898a	11	1.263	2.176	0.019
Intercept	339.957	1	339.957	585.412	0.000
Species	0.068	1	0.068	0.117	0.733
Class	2.996	2	1.498	2.580	0.079
Season	1.259	1	1.259	2.168	0.143
Species * Class	1.031	2	0.516	0.888	0.414
Species * Season	0.228	1	0.228	0.393	0.532
Class * Season	1.681	2	0.841	1.448	0.238
Species * Class * Season	2.855	2	1.428	2.458	0.089
Error	86.526	149	0.581		
Total	708.268	161			
Corrected Total	100.424	160			

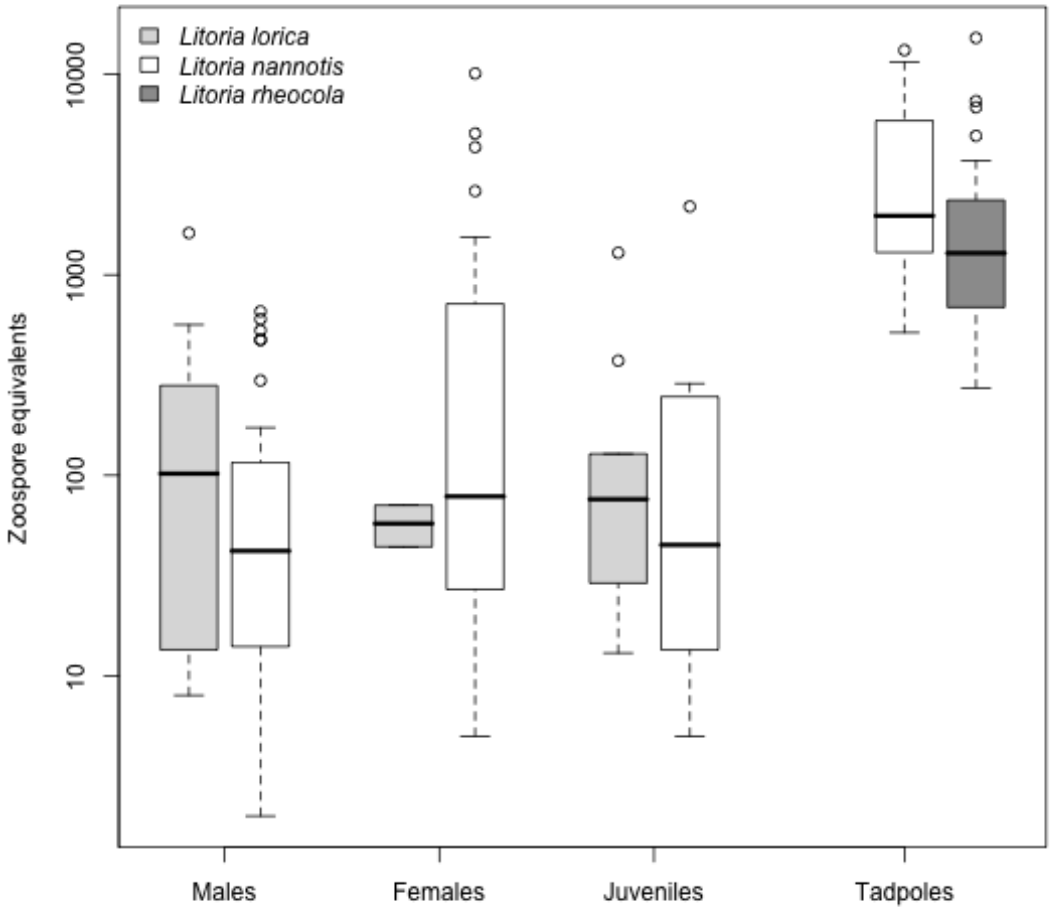


Figure 5.2 Intensity of infection in winter 2008 of *B. dendrobatidis* positive terrestrial individuals of *Litoria lorica*, *Litoria nannotis* and *Litoria rheocola* and tadpoles of *L. nannotis* and *L. rheocola.*

Discussion

Little was known about the distribution, ecology, or behaviour of *L. lorica* before it disappeared from rainforest sites. After it disappeared *L. lorica* was listed as critically endangered (Covacevich and McDonald 1993; Cunningham 2002; Hines et al. 1999), however it was generally thought to be extinct because despite repeated surveys of previously known populations, the species had not been observed for 18 years (Williams 2006, McDonald, unpublished; see previous chapters). Morphological and genetic analyses clearly show that the population I discovered is *L. lorica.* The most

parsimonious explanation for the current presence of *L. lorica* at this site is that historical surveys had not documented the full extent of its geographic and environmental range. *L. lorica* was probably present at the site throughout the period in which rainforest populations declined. The rediscovery of this species supports the precautionary approach taken in listing missing amphibian species as critically endangered when there is uncertainty about the species' distribution and ecology.

The prevalences reported in this study (and previous chapters) are higher than any that have been measured and reported in the literature using molecular techniques to diagnose infection where *B. dendrobatidis* is enzootic (e.g., Brem and Lips 2008; Kriger and Hero 2006a; Kriger and Hero 2006b), especially considering a stringent criterion was used when determining infection to minimize false positives. This study shows that the average prevalence across *L. nannotis* and *L. lorica* (77.1 %, N=121, 95 % CI = 70.9- 82.6 %) was extremely high, suggesting that these populations have persisted in association with this highly virulent pathogen.

It is not surprising that prevalence and intensity of infection did not differ significantly between these species, sex and age class, since both species and all classes of individuals appear to be ecologically and behaviourally similar, coexisting in the same areas; splash zones of cascades and waterfalls. The close association between individuals in a common microhabitat should mean that transmission rates are similar across species and classes (Rowley and Alford 2007a; Rowley and Alford 2007b). Until now, no high elevation populations of *L. nannotis* had been intensively sampled for *B. dendrobatidis* in the Wet Tropics region, since most populations of this species at high elevation rainforest presently occur at low densities (Hines et al. 1999; Richards et al. 1993). Previous information regarding the pathogen's seasonality was generated in lowland areas where frogs are still abundant and summer temperatures are more to likely reach levels lethal to *B. dendrobatidis*, leading to decreases in prevalence during the summer months (Berger et al. 2004; Kriger and Hero 2006b; Woodhams and Alford 2005). It is though very different from what was found at Mt. Spurgeon (see previous chapters) in which seasonality in prevalence was evident.

Tadpoles and terrestrial frogs do not share identical microhabitats and differed significantly in both prevalence and intensity of infection. All sampled tadpoles were infected and they had significantly higher intensities of infection than terrestrial frogs. These comparisons are confounded by the fact that the body regions sampled differ between tadpoles and frogs. However, they indicate that aquatic tadpoles are an important part of the host-pathogen system. Frogs of both species frequently contact water (Rowley and Alford 2007a; R. Puschendorf, unpublished) and transmission could occur in either direction via water-borne zoospores.

A few of the Australian frog species that suffered large declines during the original outbreaks of chytridiomycosis now coexist with the pathogen where it has become endemic (Australian Government Department of the Environment and Heritage 2006). While this may indicate that a change in the host-pathogen relationship favouring host survival has occurred (for example if host immunity has improved or pathogen virulence has decreased), several species persist in restricted ranges and/or at lower abundances. In these cases, ongoing disease-associated mortality may inhibit full population recovery long after epidemics have subsided (e.g., Murray et al. 2009) and species survival may be locally facilitated by host range overlap with environmental refugia that limit the pathogen's growth (Puschendorf et al. 2009) or virulence (Woodhams et al. 2008a) or augment the host species' immune response (Andre et al. 2008; Richmond et al. 2009). In the present case, the high prevalences and infection intensities I measured suggest that environmental conditions for *B. dendrobatidis* are appropriate. However, although both species are clearly vulnerable to chytridiomycosis-related population declines, this study populations do not appear to have crashed or to be declining and no sick or dying frogs have been found. Diseased *L. nannotis* are, however, currently found during winter months in the adjacent rainforest (less than 4 kilometers from this site, see previous chapters). The results of this study suggest that although the pathogen persists throughout the year in this study populations, aspects of the environment, the organisms, or both are preventing infection intensities from increasing to the level that causes disease (Carey et al. 2006).

The newly discovered *L. lorica* site has higher surface temperatures, lower annual precipitation and higher seasonality than the original rainforest occurrence localities

(Figure 5.1). It has been experimentally demonstrated that exposure to high ambient temperatures can cure frogs of infection by *B. dendrobatidis* (Retallick and Miera 2007; Woodhams et al. 2003). In rainforest areas the sympatric *L. nannotis* seems to choose moist and buffered microhabitats ideal for chytridiomycosis, which has been suggested as one reason why this species suffered major declines in the rainforest during the initial chytridiomycosis outbreaks (Rowley and Alford 2007a; Rowley and Alford 2007b). Even in July, diurnal substrate temperatures at my site are substantially higher than in adjacent closed-canopy rainforest; this may be critical in preventing pathogen populations from increasing on hosts to the point at which they cause disease.

Maintaining elevated body temperatures and having the option to choose dry areas, even for short periods of time, might both slow pathogen population growth since the pathogen does not tolerate warmer temperatures and grows best in moist conditions (Johnson and Speare 2003; Piotrowski et al. 2004). In temperate areas, frogs' immune systems are suppressed over winter and recover when temperatures are elevated (Cooper et al. 1992; Maniero and Carey 1997). This does not indicate that infected frogs never develop symptomatic (and possibly lethal) chytridiomycosis. However, the fact that abundance surveys in March 2009 indicated that populations were at sizes similar to those in June 2008, despite very high prevalences of *B. dendrobatidis* infection, indicates that many infected frogs do not develop fatal chytridiomycosis, even over extended periods. These data therefore indicate that at this field site, populations of *L. lorica* and *L. nannotis* are presently coexisting with *B. dendrobatidis*. This coexistence may be tenuous; the mechanisms responsible for it and the extent to which the pathogen may be affecting survival and recruitment are unknown. A mark recapture study, coupled with disease sampling similar to (Murray et al. 2009), would shed light on the impacts of *B. dendrobatidis* on this population.

Litoria lorica remains critically endangered, since the rediscovered population is relatively common locally but is restricted to a very small area within a single catchment. In regards to management, it must be first determine whether this is the only population. Considerable survey effort has been conducted since the original disappearance of the species in 1991 but all of this has been at rainforest sites (McDonald, unpublished). Since the species' rediscovery, surveys of stream systems

in similar dry sclerophyll habitats elsewhere in the same catchment and in a neighbouring catchment have failed to find additional populations (Puschendorf and Hoskin, unpublished data). More surveys of dry forest stream habitats downstream from rainforest are required in the Carbine Tableland, Thornton Uplands and Windsor Tableland regions before it can be concluded that the rediscovered population is the only remaining population of *L. lorica*.

Assuming for now that this is the only population and that this population has persisted with *B. dendrobatidis* for some time, I outline the following threats and management suggestions. At present the site is open to public use and subject to a variety of impacts. Entrance to the site should be closed to the public and protected to minimised human impacts. The *L. lorica* population should be closely monitored using low-impact techniques, so that any changes in status are detected before they become irreversible. Because the population is clearly persisting despite high prevalences of infection by *B. dendrobatidis* and because of its very unique biology, I do not believe that individuals should be immediately removed to captive species-assurance populations. In conjunction with monitoring, additional data should be collected on the basic biology of the species, which is largely unknown at present; this will facilitate more detailed conservation planning, which might eventually include translocations and the development of captive maintenance techniques. Because captive techniques for torrent frogs do not exist, their reproductive biology is poorly known and could be difficult to replicate in captivity. Development of captive maintenance techniques should therefore commence immediately using the closely related and much more common *L. nannotis* as a surrogate. This approach would make any emergency response more likely to be successful, if monitoring determines that declines are in process (Mendelson et al. 2006).

These results highlight the importance of environmental refugia from disease to amphibian conservation. In both tropical Australia and Costa Rica, species that were thought to be extinct survive in dry forest areas adjacent to rainforest sites. At least in the case of the Australian refuge, where frogs seem to tolerate chytrid infections better than in the rainforest, environmental refugia may allow frogs to evolve other mechanisms of resistance since the lesser effects of the disease in these environments may allow time for selection to act. Protecting refuge areas adjoining rainforest is

important for the survival of *L. lorica* and is likely to be important for many other frog species around the world.

These results also highlight the fact that known localities may fail to encompass either or both of the full geographical and environmental ranges of species. Survey efforts focused exclusively on habitats with environmental characteristics matching existing records may therefore fail to detect populations that can be important for species' conservation. Survey efforts should therefore focus on documenting the full extent of the geographical and ecological ranges of species by including areas at or outside the margins of their known ecological ranges. In particular, dry forest habitats bordering rainforest should be seen as targets for survey effort for apparently extinct and declined rainforest amphibian populations globally.

In summary, the rediscovery of *L. lorica* highlights the importance of accurately determining the distribution of threatened or presumed extinct species. It illustrates the need to take a precautionary approach when listing the status of species when there is uncertainty regarding their distribution and ecology. It also highlights the need to look for and conserve populations across the range of environmental conditions that may be occupied by species, because this will reduce the chance of a single threatening process causing species' global extinctions. The rediscovery of *L. lorica* gives hope that small populations of other species that appear to have been driven extinct by chytridiomycosis may persist in environmental refugia outside their former known distributions.

CHAPTER SIX: BEHAVIOUR AND MICROENVIRONMENTS EXPERIENCED BY *LITORIA NANNOTIS* IN WET AND DRY ENVIRONMENTS: WHY ARE RAINFOREST FROGS MORE SUSCEPTIBLE TO CHYTRIDIOMYCOSIS?

Abstract

Dense populations of the chytrid fungus *Batrachochytrium dendrobatidis* on hosts cause the disease known as chytridiomycosis, which can be lethal to many amphibian species. This pathogen grows best between 17 and 25 ℃ and under moist conditions. In the tropics it has its most significant detrimental effects on amphibian populations in high elevation rainforest habitats. Even within these habitats, amphibians of different species experience a wide range of different microenvironments. It has been suggested that this may explain some or most interspecific variation in vulnerability to *B. dendrobatidis*; amphibians that experience conditions outside the optimum range for *B. dendrobatidis* may be less vulnerable. A comparison of the epidemiology of *B. dendrobatidis* between high-elevation wet and dry forest environments, using *L. nannotis* as a model host species, suggested that the dry forest is a refuge from significant disease-linked population collapses. In this chapter, I compare differences in microenvironment use and behaviour in this species between these habitats to develop hypotheses for the mechanisms that produce the observed differences in host-pathogen dynamics. The research was carried out within a three km section of connected flowing streams in a single catchment. I monitored *L. nannotis* in one wet forest and two dry forest populations, for ten days in July 2008. To quantify differences in microenvironmental use I constructed frog models made out of agar with an embedded temperature logger, placed in frogs' diurnal retreat sites. Although median and minimum retreat site temperatures were similar across sites, retreat sites in dry forest habitats had significantly higher maximum temperatures than those in the wet forest habitat. Behaviour of dry forest *L. nannotis* also differed significantly from those in the rainforest. They spent a substantial amount of time submerged under the water and when they emerged at dusk they perched on rocks that have been warmed by long-term exposure to direct sunlight. The likelihood of zoospore reinfection should be reduced when frogs are submerged in fast flowing water and nocturnal perching on still-warm rocks could both increase immuno-competence

and directly reduce pathogen loads. The observed behavioural differences may therefore explain why *L. nannotis* is more tolerant to *B. dendrobatidis* infections in the dry forest. It is likely that in other species there are similar differences in behaviour and microenvironment use between populations in core and peripheral habitats. This study demonstrates the importance of understanding the biology of organisms throughout their ranges, rather than only in "core" habitats.

Introduction

Most enigmatic amphibian declines have now been linked to outbreaks of the disease chytridiomycosis (Berger et al. 1998; Collins and Crump 2009; Lips et al. 2006; Rachowicz et al. 2006; Skerratt et al. 2007). The causal agent, *Batrachochytrium dendrobatidis* (Longcore et al. 1999) infects the keratinized epidermis of amphibians and can be lethal to postmetamorphic frogs by causing a generalized skin infection (Berger et al. 1998; Berger et al. 2005c). This infection disrupts normal epidermal functioning, producing an osmotic imbalance through loss of electrolytes (Voyles et al. 2007). When the infection reaches a threshold level, this process leads to death (Carey et al. 2006; Voyles et al. 2007).

In the laboratory this pathogen's optimum temperature is between 17 and 25 ℃, but population growth continues at lower temperatures (Piotrowski et al. 2004). Its' upper threshold starts at 25 ℃, where growth slows. At 28 ℃ it stops growing and it dies if continuously exposed to temperatures above 30 ℃. (Piotrowski et al. 2004). *B. dendrobatidis* is restricted to moist and wet environments, as its aquatic reproductive zoospores cannot tolerate desiccation (Johnson and Speare 2003). These physiological thresholds for pathogen growth and reproduction explain why environmental conditions play a significant role in regulating disease dynamics. In the tropics, high elevation rainforest amphibian species are most strongly affected by this pathogen and mortality caused by chytridiomycosis increases during the winter months (Berger et al. 1998; Berger et al. 2004; Berger et al. 1999a; Kriger and Hero 2006b; McDonald and Alford 1999; Woodhams 2003; Woodhams and Alford 2005).

Temperature affects not only the pathogen, but also the host's immunity. The amphibian immune system is notably sensitive to temperature changes. In temperate areas during winter months, the immune system of hibernating amphibians shuts down, but quickly bounces back during warmer temperatures in spring (Cooper et al. 1992). Amphibians are more susceptible to pathogen infections at lower temperature because of decreased immune function (Maniero and Carey 1997). In the case of chytridiomycosis, lower temperatures, such as the ones experienced in high elevation rainforest habitats in the tropics, probably inhibit immune responses and may thus contribute to the development of epidemics. The effect of temperature on chytrid infections in riparian frogs has been well studied in the wet tropics region and it is known that the microenvironments used by each species can influence their susceptibility to disease (McDonald and Alford 1999; Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b).

In the wet tropics region of Queensland, species that are able to elevate their body temperature above 30 ℃ suffered little or no population declines, while species that do not escape the environmental envelope of pathogen growth and reproduction have been the hardest hit, mostly persisting in the lowlands (McDonald and Alford 1999; Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b). One of these susceptible species is the waterfall frog *Litoria nannotis* (Andersson 1916), a species belonging to the group of torrent frogs, endemic to the wet tropics region (Cunningham 2002; Liem 1974).

Litoria nannotis spends most of its lifecycle on waterfalls or fast flowing sections of creeks and rivers, where individuals congregate on rocks around the splash zones, occasionally venturing out into the adjacent forest at night (Hodgkison and Hero 2001; Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b). Most of what is known about the biology of this species, including the recent history of population collapses associated with outbreaks of chytridiomycosis, comes from studies in rainforest habitats (Berger et al. 1998; McDonald and Alford 1999; Richards et al. 1993; Rowley and Alford 2007a; Woodhams and Alford 2005). In rainforests, *L. nannotis* does not bask; it spends the day in wet and moist retreat sites associated with waterfalls, only emerging from these microhabitats at night when

the higher diurnal temperatures have receded (Hodgkison and Hero 2001; Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b). The status, behaviour and ecology of this species where it occurs in dry forests to the west of the wet tropics rainforest were largely unstudied, although it appears that populations in these areas survived the initial outbreaks of chytridiomycosis that eliminated high elevation rainforest populations (Williams 2006).

It is very likely that within catchments, local populations of both hosts and the pathogen are not genetically differentiated, since there are minimal barriers to the movement of frogs, frog larvae and chytrid zoospores are carried by water (Johnson and Speare 2003; Richards and Alford 1993). It therefore appears likely that when local frog populations show different degrees of vulnerability to chytridiomycosis within a catchment (Chapters 4 and 5), the differences are caused by environmental factors. The first aim of this chapter was to compare the microenvironments *L. nannotis* experience in two habitat types, their "typical" rainforest habitat and dry forest. Because this species is nocturnal, the highest temperatures it experiences should be in diurnal retreat sites. If retreat site temperatures in dry habitats are significantly warmer than retreat sites in wet habitats, this could explain some or even all of the differences in disease mortality between these two habitats. The second aim was to study the behaviour of *L. nannotis* in dry forest habitats and compare it to what is known of its behaviour in the rainforest. If dry forest frogs bask, even for short periods of time, this might explain their lower vulnerability to mortality caused by chytridiomycosis.

Methods

Study sites

This study was conducted at three sites, two in dry sclerophyll and one in wet sclerophyll/ rainforest habitat. All sites were less than 3 km from each other, within or just outside the boundary of Mt. Spurgeon National Park, Queensland, Australia. The wet sclerophyll/ rainforest site was along Spurgeon Creek at the top of Spurgeon Falls (Spurgeon Wet; start of transect: 16° 27.368'S, 145° 11.457'E, 1067 meters above sea level (m asl); end of transect 16° 27.193'S, 145° 11.488'E, 1113 m asl). The first dry

sclerophyll site (Spurgeon Dry) was less than 900 m downstream on Spurgeon Creek (beginning of transect: 16° 27.823'S 145°, 10.865'E, 795 m asl; end of transect: 16° 27.709'S, 145° 11.032'E, 861 m asl). Populations of frogs and pathogen dynamics were monitored at these sites between April 2007 and July 2008 (chapter 4). Spurgeon Creek is a tributary of the McLeod River. The second dry sclerophyll transect was set up along a section of riffles and waterfalls on the McLeod River in dry sclerophyll habitat (McLeod Dry), where *Litoria lorica* was recently rediscovered (chapter 6; beginning of transect: 16° 27.841'S, 145° 9.003'E, 660 m asl; end of transect: 16° 28.002'S, 145° 9.164'E, 671 m asl). Populations of frogs and pathogen dynamics were monitored at this site between July 2008 and March 2009 (chapter 5).

The habitat in which frogs spend most of their time (waterfalls and riffles) is very similar across the three sites, but the environment immediately adjacent to the river is starkly different between wet and dry environments. Dense vegetation and almost complete canopy cover characterize the wet habitat, with little or no sunshine reaching the substrate where *L. nannotis* usually occur. The dry forest sites have little (Spurgeon Dry) or no (McLeod Dry) canopy cover shading the riffles and waterfalls that frogs inhabit. The McLeod site differs from the Spurgeon sites in that the streambed consists of solid granite with very few cracks and crevices available for frogs to use as retreat sites, yet *L. nannotis* and *L. lorica* are very abundant there (chapter 5). All sites are located above 400 m asl, at elevations where the environment in rainforests is ideal for *B. dendrobatidis* to thrive (Berger et al. 1998; Berger et al. 1999a; Longcore et al. 1999; Piotrowski et al. 2004; Woodhams and Alford 2005).

Environmental monitoring and identification of retreat sites

Four Hygrochron^{TM} data loggers (model DS1923, Dallas Semiconductor) were set up along each transect. The loggers were placed in tea strainers and hung 1 m away from the stream at about 1 m of elevation in shaded areas. The loggers were set up to record hourly readings of air temperature (AT) and relative humidity (RH). Recording times were synchronized across all data loggers. Rainfall data were obtained as interpolated data from the National Climate Centre, Australian Bureau of Meteorology (Jones et al. 2007).

During previous monitoring of frog populations and pathogen dynamics at the field sites (chapters 4 and 5), commonly used diurnal retreat sites were identified. While monitoring populations, I searched for the crack or crevice nearest to the nocturnal location of each individual frog. The following day, using a head torch, I inspected these potential retreat sites, in most cases finding several *L. nannotis* in them as described in the literature (Hodgkison and Hero 2001; Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b; Rowley et al. 2007b).

Physical frog models and rock temperatures

To define the upper and lower boundaries of possible body temperatures for *L. nannotis* occupying their diurnal microenvironment, physical models representing frogs in their water conserving posture were constructed out of agar with an embedded temperature logger (Thermochron iButton, Dallas Semicondictor, Dallas, Texas USA) (Rowley and Alford 2009). As recommended by Rowley and Alford (2009), pairs of models were set up in each retreat site, an impermeable one coated in clear PLASTI DIP® (PLASTI DIP International Inc., Blaine, Minnesota USA) and a uncoated, permeable one. In Spurgeon Wet and Spurgeon Dry, most permeable models suffered significant damage, most likely by White-tailed Rats (*Uromys caudimaculatus*), therefore only data collected from the impermeable models were used. Because all retreat sites in which these models were deployed were completely shaded from the sun, in moist cracks and crevices with very low wind velocities, it is unlikely that there were substantial temperature differences between permeable and impermeable models. The data from impermeable models should therefore be a reasonable representation of body temperatures available to frogs. Data loggers were programmed to record temperatures every hour and were synchronized with the data loggers recording ambient air temperatures outside the stream. In total the synchronized microenvironment and ambient temperature loggers recorded hourly information from 24/7/08 through 17/8/08.

Rock temperatures at sites where frogs were found perching at night were recorded every half hour during one 34 - hour period by directly adhering temperature loggers (Thermochron iButtons™) to the rocks, using silver-coloured duct tape, which completely covered the loggers. The relatively high reflectance of the tape, coupled

with the relatively high thermal conductance of granite, should mean that the temperatures recorded reflected the temperature of the rock substrate, not the temperature due to direct sunlight.

Tracking

Previous projects have studied the behaviour of *L. nannotis* in rainforest habitats (Rowley 2006; Rowley and Alford 2007a; Rowley and Alford 2007b; Rowley et al. 2007b). My tracking efforts were therefore focused on populations living in dry forest habitat. Tracking occurred during a 10-day period at the end of July 2008 at McLeod Dry. Twelve adult *L. nannotis* (all weighing more than 12 g) were tracked via radio telemetry. The radio transmitters used (BD-2NT; Holohil Systems Ltd., Ontario, Canada) were attached to a harness made of silicone tubing, designed to minimise restrictions on movement. The transmitter and harness weight was 0.67 g. Frogs did not carry harnesses and associated equipment that weighed more than 5 % of their total body weight. This percentage is half of the maximum that has been recommended in the literature (Richards et al. 1994). Tracking devices were fitted in the field and frogs were released at point of capture after less than ten minutes of handling.

Frogs fitted with radio transmitters were tracked using a HABIT Research HR2500 Osprey VHF Receiver (HABIT Research, Victoria, B.C., Canada), with a three-element folding Yagi antenna (A.F. Antronics, White Heath, Illinois, USA). During surveys, the location of each frog was determined once during the day (0900-1800 h) and once at night (1900-0400 h). Variables recorded for each tracked individual when it was located were distance moved from last location, whether the animal was exposed or not and the microenvironment where it was located. I excluded the data from the first day after transmitter attachment, since there can be short-term effects of tag attachment on behaviour (Langkilde and Alford 2002), which disappear after 24 hours (Rowley and Alford 2007c).

Analysis

To avoid pseudoreplication when analysing data on thermal models, I used separate retreat sites as replicates and compared summary statistics calculated for each retreat site. I compared median, maximum and minimum temperatures between localities and also compared diurnal and nocturnal temperatures, since frogs primarily use these retreat sites during the day.

As for the models, summary statistics from individual frogs were used to avoid pseudoreplication. I used the median, rather than the mean, when looking at distances moved between each sampling period since infrequent large movements bias the average (Rowley and Alford 2007b). I compared movement patterns between days in which rainfall occurred and days in which rain was absent because previous studies suggested that behaviour was likely to differ between wet and dry periods. Only eight frogs were used for this comparison, since the rest had been tagged the night before it started raining and the data for the first night following tag attachment were not used, as described above. I also compared diurnal and nocturnal microenvironments for all 12 frogs tracked during the ten-day period. I used nonparametric statistics for all the analyses, using SPSS (version 16, SPSS Inc.).

Results

Eighteen separate diurnal retreat sites were located for *L. nannotis*, five at Spurgeon Wet, eight at Spurgeon Dry and five at McLeod Dry. The temperature ranges of all retreat sites were buffered when compared to ambient temperatures. The extreme case was McLeod Dry, where ambient air temperatures ranged from 5 ℃ to 29 ℃, but retreat site temperatures always remained within 9.5 ℃ to 21 ℃ (Figure 6.1). Retreat site temperatures differed significantly among the transects, (Kruskal-Wallis test; chi square = 12.358, d.f. = 2, p= 0.002; Figure 6.2), the median retreat site temperature at McLeod Dry was 3 ℃ higher than that at Spurgeon Wet. A paired comparison indicated that median diurnal and nocturnal temperatures did not differ within retreat sites (Wilcoxon Signed Rank Test; z = -1.294, N = 18, p = 0.196). Paired comparisons of minimum and maximum temperatures indicated that these differed between day and night (Wilcoxon Signed Rank Test; z = -3.727, N = 18, p < 0.001), Kruskal-Wallis tests indicated that minimum temperatures do not differ significantly across

sites (Kruskal-Wallis test; chi square = 2.780, d.f. = 2, p= 0.249), but maximum temperatures are significantly different across sites (Kruskal-Wallis test; chi square = 13.629, d.f. = 2, p= 0.001). Maximum temperatures were higher at Spurgeon Dry and McLeod Dry than at Spurgeon Wet, usually in the afternoon (Figure 6.3).

Tracked individuals used three different microenvironments. They perched on wet rocks or dry rocks and they spent extended periods submerged under water. The pattern of microenvironment use differed between day and night (Friedman Test on change in percent use of each substrate between day and night by each individual; chi square = 18.766, d.f.= 2, $p < 0.0001$; Table 6.1). Frogs were never found exposed to direct sunshine or even in the open during the day. Most of the tracked individuals spend the day submerged under water and emerged at night to sit on rocks next to or within riffles and waterfalls. Rock temperatures on which frogs perched at dusk were much warmer than the temperatures they would experience at the same time in their retreat sites, reaching 31 ℃ shortly before frogs emerged and 25-26 ℃ at the times when frogs are perching on them (Figure 6.4). Given that these data were collected over a short period, it is likely that frogs encounter both higher and lower rock temperatures; even short periods with higher temperatures may strongly affect the course of *B. dendrobatidis* infections (Woodhams et al. 2003). Rain only occurred during the 2nd and 3rd days of tracking, with a total of 31.7 mm of rainfall during that period. Frogs moved significantly longer distances during that period than over the later dry days (Wilcoxon Signed Rank Test comparing mean distance moved during and after rain for each frog; z = -2.521, N = 8, p = 0.012; Table 6.2). All movement was restricted to the creek; frogs never ventured into the adjacent vegetation.

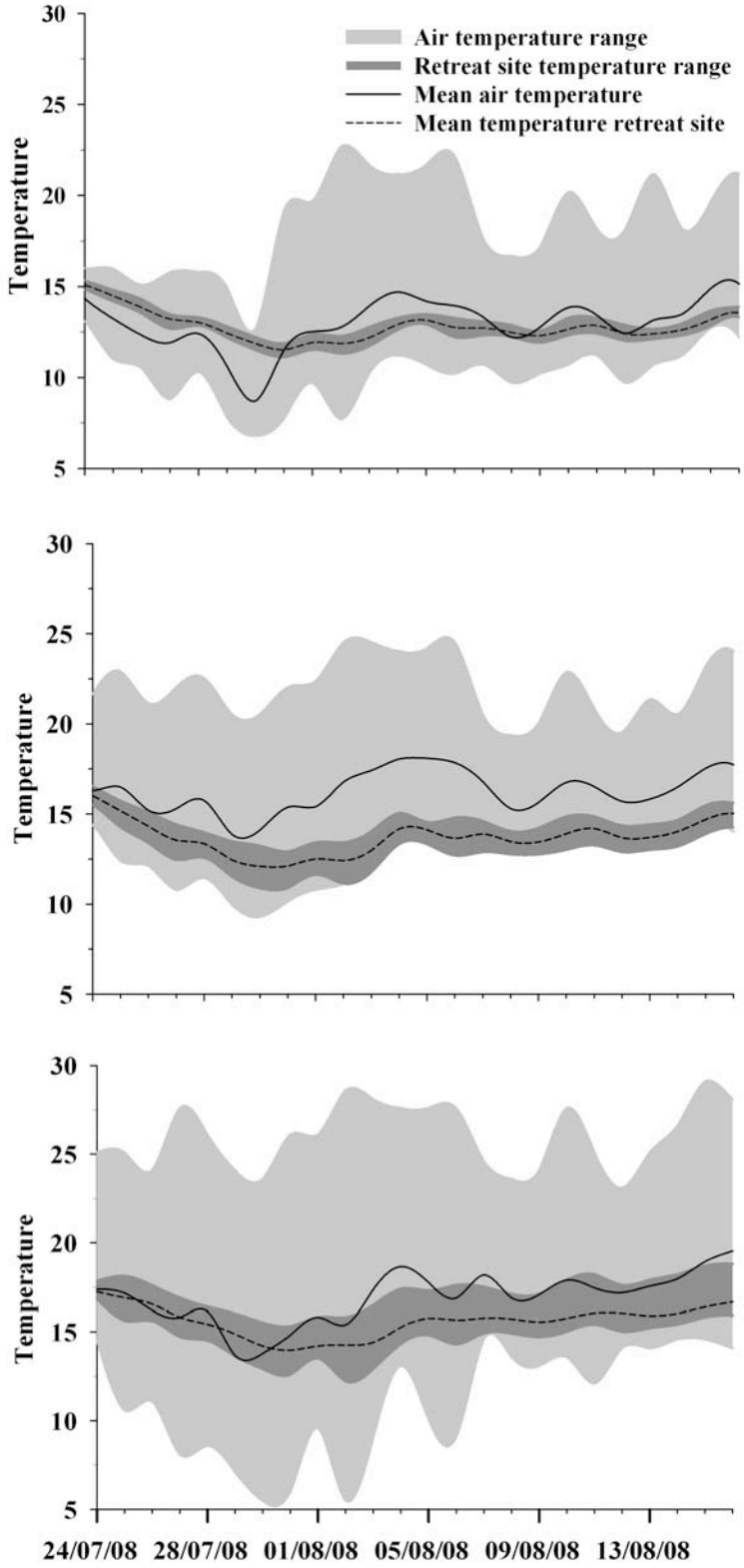


Figure 6.1. Means (lines) and maxima and minima (shading) of ambient and retreat site temperatures at the Spurgeon Wet (top), Spurgeon Dry (mid) and McLeod Dry (bottom) transects from 24/ 07/08 through 16/ 08/08. Maximum and minimum temperatures have not been averaged.

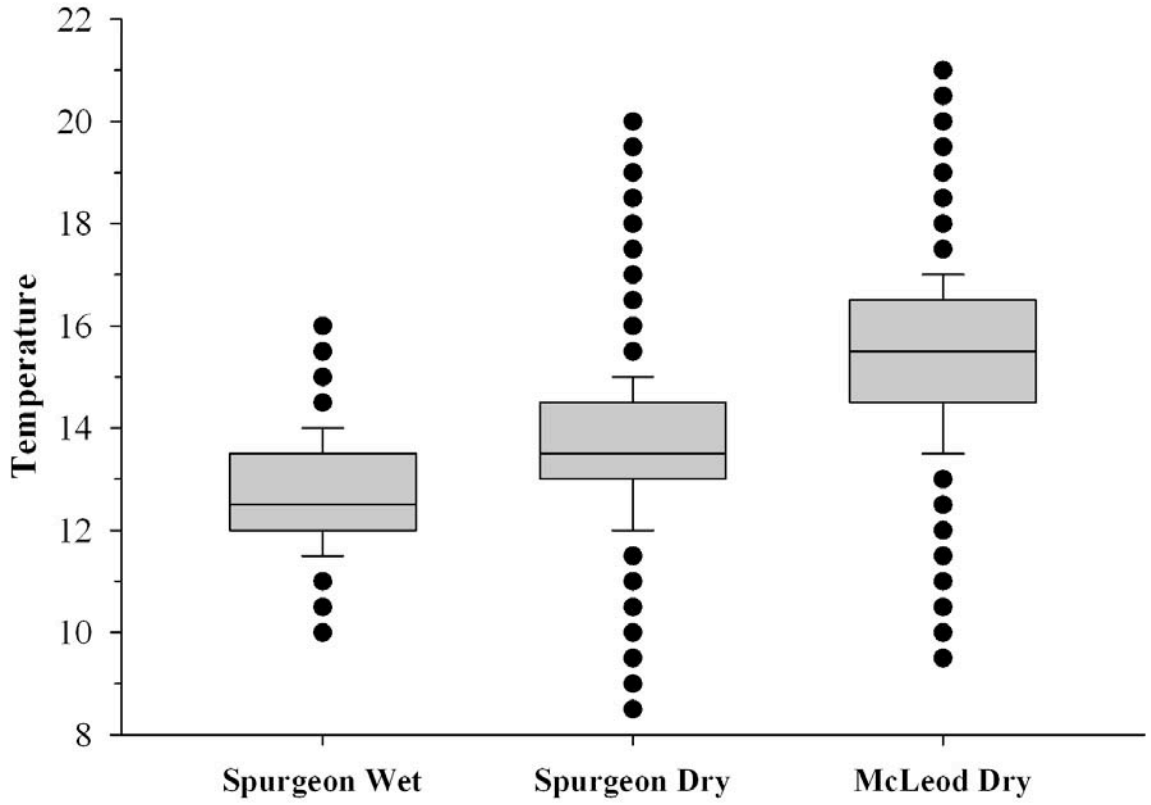


Figure 6.2. Tukey (1977) boxplot of diurnal and nocturnal retreat site temperatures at Spurgeon Wet, Spurgeon Dry and McLeod Dry using individual data points from all models.

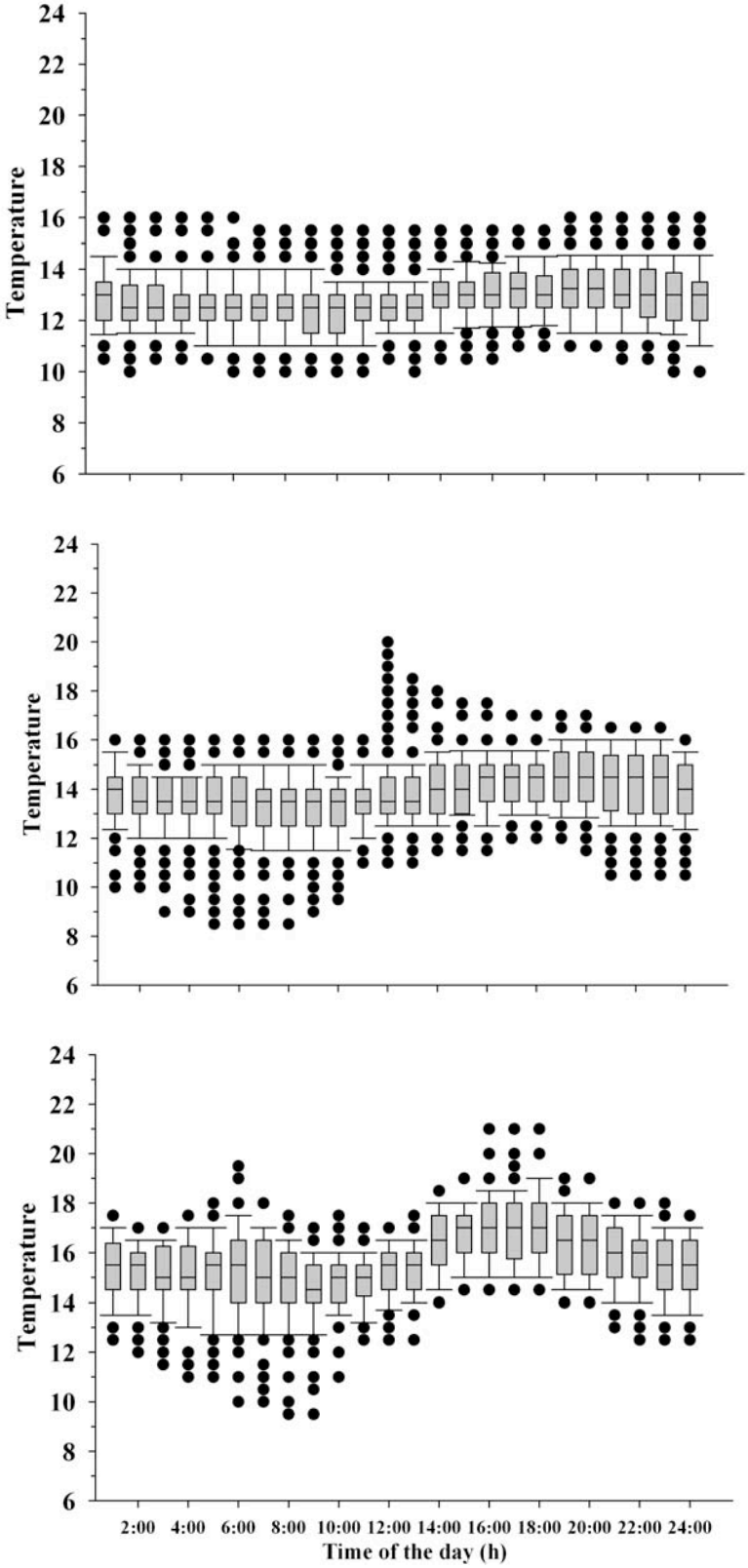


Figure 6.3. Tukey (1977) boxplots of temperatures measured using impermeable models in *L. nannotis* retreat sites. Boxplots represent hourly temperatures of all models for each of the three localities, Spurgeon Wet (top), Spurgeon Dry (mid), McLeod Dry (bottom).

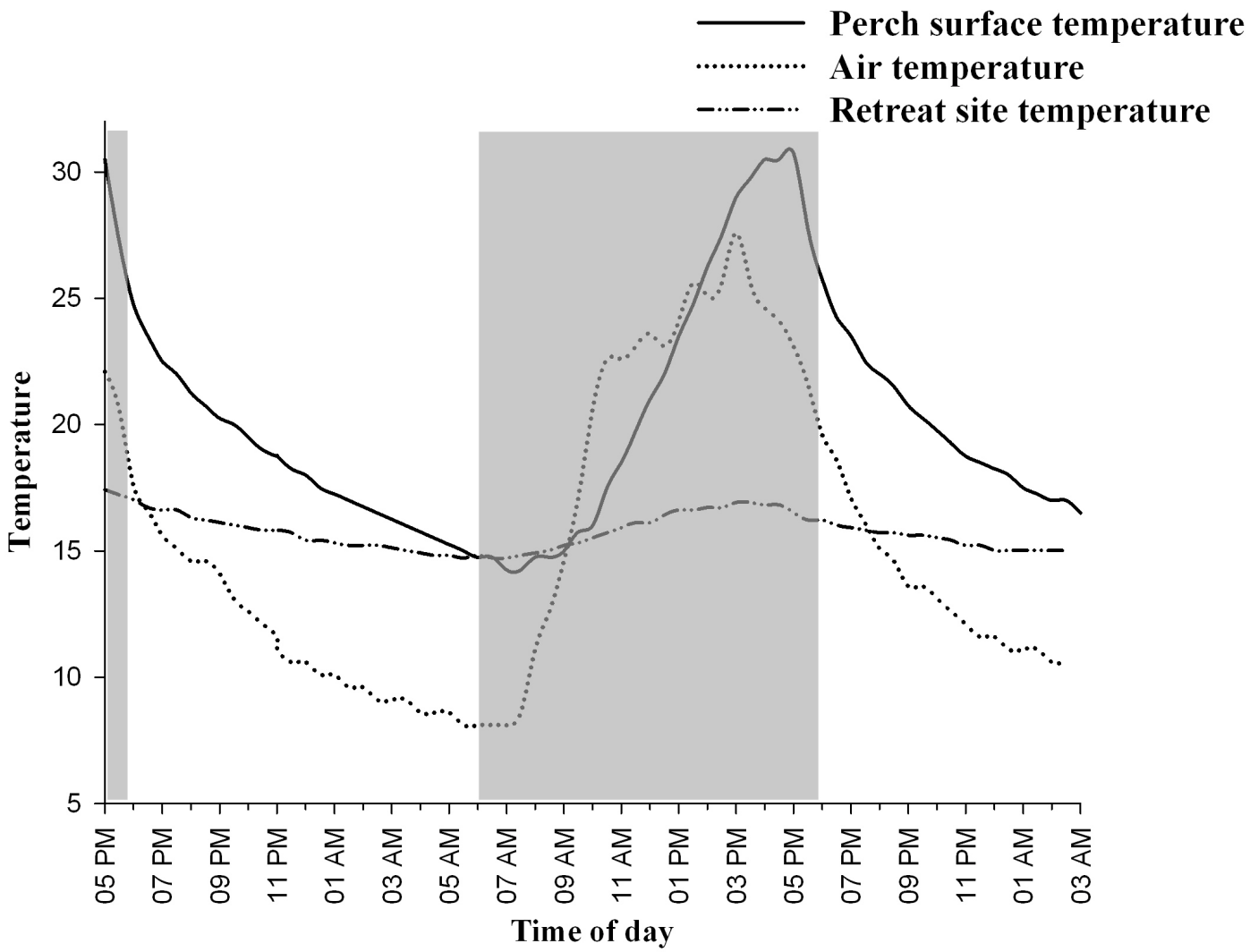


Figure 6.4. Perch surface, air and retreat site temperatures during a period of 34 hours at McLeod Dry. Shaded areas represent the times of the day when frogs are sheltered in their retreat sites, after which they come out and perch on the rocks where perch surface temperatures were measured, which are next to or within waterfalls.

Table 6.1. Mean and range of percent of observations on each substrate type, using individual frogs as replicates.

Time	Substrate	Mean	Minimum	Maximum
Day	Dry rock	0	0	0
Day	Under water	97	80	100
Day	Wet Rock	3	0	20
Night	Dry Rock	51	25	80
Night	Under water	5	0	20
Night	Wet Rock	45	20	75

Table 6.2. Movement patterns of *Litoria nannotis* at McLeod Dry. Values represent means (and ranges) of data obtained using individual frogs as replicates.

Parameters for each individual	Median	Minimum	Maximum
Median distance moved between surveys (m)	1.0	0.5	3.5
Maximum distance moved between surveys (m)	6.0	2.0	65.0
Median distance moved rainy days (m)	7.5	4.0	35.0
Maximum distance moved rainy days (m)	8.0	4.0	65.0
Median distance moved between surveys dry days (m)	1.0	0.3	2.3
Maximum distance moved between surveys dry days (m)	3.8	2.5	5.0

Discussion

Fatal chytridiomycosis seems to be directly linked to the size of the pathogen population on the host (Carey et al. 2006), therefore the effects of *B. dendrobatidis* should be the strongest where the pathogen can grow and reproduce the fastest. The temperatures experienced by *L. nannotis* in their retreat sites across the three localities are mostly within or just below the optimum thermal range for pathogen growth. Even at the dry forest site, where frogs were more abundant and there was no observed mortality (chapters 5-6), air temperature never reached the threshold at which *B. dendrobatidis* stops developing (Piotrowski et al. 2004). In contrast to the strong effects of temperatures above the thermal optimum, temperatures below 17 ℃ can still lead to rapid pathogen growth. Woodhams et al. (2008) showed that *B. dendrobatidis* responds to colder temperature by increasing fecundity. These adaptations should allow rapid population growth at a wide range of temperatures, including those experienced by frogs in all of the sampled retreat sites.

Retreat sites were warmer at Spurgeon Dry and McLeod Dry than Spurgeon Wet, where the impacts of disease seem to be more attenuated (chapters 5-6), but the range of temperatures experienced at these dry sites should actually increase the rates of pathogen population growth, since they approach 23 ℃, the optimum temperature for pathogen population growth (Woodhams et al. 2008). This suggests that if site differences are related to temperature, they are not caused by long term effects of temperature on pathogen population growth, but may be due to either or both of short-term effects of occasional temperature extremes, or longer-term effects of temperature on host defences.

Andre et al. (2008) showed experimentally that if infected individuals of *Rana muscosa* were kept at 22 ℃, 50 % of them survived, whereas all infected frogs kept at 17 ℃ died from chytridiomycosis. The results of Woodhams et al. (2008) suggest that frogs at 22 ℃ should have died faster than those at 17 ℃, since the population growth of *B. dendrobatidis* is more rapid at the former temperature. The results of Andre et al. thus suggest that the hosts' immune response was playing a significant role at 22 ℃. The warmer temperatures experienced by *L. nannotis* in their retreat sites at McLeod Dry might similarly augment immune responses, but it seems

unlikely that this alone could explain higher pathogen tolerance at this dry forest locality. Other factors need to be considered to explain the differences in the epidemiology of *B. dendrobatidis* between environments.

The behaviour of *L. nannotis* in the dry forest differs in several aspects from what has been described in rainforest habitats. In the rainforest, *L. nannotis'* retreat sites are cracks and crevices above the waterline next to waterfalls, in which frogs usually congregate during the day (Hodgkison and Hero 2001; Rowley 2006; Rowley and Alford 2007b). Although *L. nannotis* continues using these types of retreat sites when they are available in the dry forest, they also spend a significant amount of time submerged under the water during the day. Preliminary observations suggest they do not need to surface to breathe, as one frog located under the water was observed continuously for six hours, before it emerged at nightfall (Puschendorf unpublished). Torrent frogs seem to be extremely aquatic in the dry forest, which probably increases transmission rates of this waterborne disease (Rowley and Alford 2007a). This is clearly the case as most of the sampled torrent frogs at Spurgeon Dry and Mcleod Dry were infected (chapters 5-6), but it could also explain why they seem to be more tolerant to *B. dendrobatidis.* In an experiment in which frogs were infected and kept in mist, continuous simulated rainfall and dry air, the individuals kept in continuous rainfall developed disease at rates lower than those in mist, but higher than those in dry air (Woodhams 2003). In the fast flowing aquatic environments occupied by dry forest frogs during the day, a relatively high proportion of zoospores may be flushed away, reducing reinfection rates and keeping the parasite load below lethal levels. This behaviour also explains how *L. nannotis* and *L. lorica* can occur at McLeod Dry in high abundance, although few retreat sites of the type usually used in rainforest habitats are available. It is likely that a substantial proportion of the population spends the day submerged.

Submergence in flowing water may partially explain lower rates of pathogen population growth, however, there may also be an advantage to living in an environment where the lack of canopy cover allows more sunlight to reach the substrate on which frogs perch. Although this species does not bask during the day, it may effectively do so at night, by exposing the body regions most susceptible to becoming infected to conductive heating. Infections of *B. dendrobatidis* start on the

ventral side of the frog (Berger et al. 2005; North and Alford 2008; Puschendorf and Bolaños 2006), because this is the first area that comes in contact with the infectious stage of the pathogen, the zoospores. *Litoria nannotis* usually emerge around dusk from their diurnal retreat sites to sit on rocks. In the Dry Forest site, these rocks have been exposed to direct sunshine for most of the day and when frogs perch on them, rock temperatures during the short period in which they were monitored still averaged 26 to 28 ℃. Seasonal and shorter-term variation in weather and insolation undoubtedly extend this range to both higher and lower temperatures. Tracked frogs also spent approximately half of the time sitting on dry rocks, which for a fungus that does not tolerate desiccation should have a significant detrimental effect. In combination, perching on warm, dry rocks could substantially increase pathogen tolerance, by lowering pathogen loads. Previous work on frog immunity also suggests that the host should greatly benefit from nocturnal "basking", as even small elevations in body temperature can greatly increase the activity of the immune system against pathogens (Carey et al. 1996; Cooper et al. 1992; Maniero and Carey 1997; Matutte et al. 2000; Raffel et al. 2006).

My results suggest several non-exclusive mechanisms by which frogs may increase their tolerance to chytridiomycosis in dry forest habitats; any or all of these could explain the persistence in these habitats of a species (*L. lorica*) that is extremely vulnerable to chytridiomycosis (chapter 5). The balance of factors that favours host survival in these dry forest systems may be tenuous. Ongoing seasonal mortality in adjacent rainforest habitat suggests that innate resistance in these populations is still low (chapter 5). Substantial disease-induced mortality may occur even in the dry forest localities, during seasonal enzootics which have not been detected (Murray et al. 2009). Still, the rediscovery of *L. lorica* and the high abundance of *L. nannotis* at these marginal dry forest localities raise hope that other species such as *Litoria nyakalensis*, thought to be extinct could be located in similar habitat sometime in the future.

There are many conservation implications for these dry forest refugia. High elevation rainforest populations of torrent frogs were completely extirpated by the initial chytridiomycosis outbreaks (Berger et al. 1998; McDonald and Alford 1999; Richards et al. 1993), but *L. nannotis* and *L. rheocola* presently exist at the Spurgeon Wet

locality at low densities. This is the highest locality in their known present (post-decline) range (McDonald and Alford unpublished), suggesting recolonization or *in situ* recovery. If these are self-perpetuating populations, it is possible that the individuals in them have evolved increased resistance to chytridiomycosis, although this resistance is not completely efficient, as I observed mortality due to chytridiomycosis at this site (chapter 4). It is also possible that these are "sink" populations, maintained by migration from the dense populations occurring in the dry forest. Understanding the source and nature of these apparently recolonised or recovering populations is necessary to determine their implications for host-pathogen biology and management.

Comparing frog behaviour between dry and wet environments also illustrates how important it is to study the biology of species across their entire ranges. In the rainforest *L. nannotis* commonly moves into the adjacent vegetation to forage, especially during rainy periods (Hodgkison and Hero 2001, 2003; Rowley 2006; Rowley and Alford 2007b). In the dry forest, although tracked individuals moved greater distances during wet periods they never left the creek, only moving between nearby waterfalls, suggesting that their main source of food must come from the riparian environment. At another site, gravid females in the dry forest were commonly observed hunting for *Macrobrachium australiense*, a freshwater prawn (Puschendorf unpublished), a food source not documented for this species in rainforest habitats (Hodgkison and Hero 2003). As researchers we need to break away from the circularity of studying organisms only in their core habitats; marginal populations can yield important information on what sets species' range boundaries and how organisms may adapt to current and future threats such as disease and climate change.

CHAPTER SEVEN: GENERAL DISCUSSION AND FUTURE DIRECTIONS

Disease can be a powerful evolutionary force, depressing host populations and changing the course of evolution of entire biological systems (Lips et al. 2006; O'Brien and Evermann 1988). Such massive biological changes are in train now: amphibians in many parts of the world are disappearing as a consequence of the emergence of chytridiomycosis, caused by the aquatic fungus *Batrachochytrium dendrobatidis* (Berger et al. 1998; Daszak et al. 1999; Longcore et al. 1999). Because of its enormous impact, chytridiomycosis has been identified in Australia as a key threatening process for amphibians under the federal Environmental Protection and Biodiversity Conservation Act (DEH 2006) and listed as a notifiable disease by the World Organisation of Animal Health (OIE 2007). Numerous amphibian species' disappearances and potential extinctions can be primarily attributed to chytridiomycosis. However, during my Ph.D. research I discovered surviving, indeed thriving, populations of species thought to have become locally and even globally extinct as a result of the disease. Understanding how these populations have persisted was the focus of this research.

Proposing climatic refugia from disease-driven amphibian extinctions

Climatic refugia from chytridiomycosis-driven amphibian extinctions had previously been documented, for example the persistence of vulnerable species frogs in lowlands in the Australian Wet Tropics (AWT). Chapter 2 formally introduces this concept using the example of a climatic refuge in Costa Rica (Puschendorf et al. 2009). I hypothesize that *Craugastor ranoides*, an species extremely susceptible to chytridiomycosis and only persisting in the dry forest of Costa Rica, is able to do so because climatic conditions in that habitat make pathogen establishment or persistence on hosts less likely. As a result, even when *B. dendrobatidis* is present its prevalence on hosts are very low. This hypothesis is supported by bioclimatic modelling. I argue that conserving similar habitats around the world, in which frogs thrive, but pathogen establishment and growth is inhibited, will allow *in situ* conservation for susceptible species threatened by this disease.

Similar climatic refugia might also occur at high elevation sites, where most of the declines and extinctions caused by chytridiomycosis have occurred. In the AWT my surveys showed that *Litoria nannotis* is present in large numbers at high elevation dry forest sites, whereas it declined to local disappearance in adjacent rainforest and most high-elevation rainforest populations are either still absent or only present in low numbers. The relatively high numbers of *L. nannotis* at dry forest sites occur despite high prevalences of infection with *B. dendrobatidis*. Therefore I propose a new type of refuge, one in which frogs persist in relatively high numbers, despite high prevalences of infection. This suggests that these populations have either acquired a degree of resistance to the development of disease or have higher tolerance than neighbouring rainforest frog populations to infection by *B. dendrobatidis*. These findings argue against the commonly held perception that vulnerable species in high elevation populations are always threatened and once again highlights the importance of conserving the full range of diversity of habitats across which species exist.

Epidemiology of *B. dendrobatidis* in high elevation dry forests of the AWT

The significantly higher abundance and lower observed mortality of *L. nannotis* in the dry forest contrasts strongly with the situation in adjacent wet forest habitats, where populations were sparse and diseased and where dead frogs were found during winter months. This suggests that dry forest sites constitute high elevation climatic refugia, where vulnerable species can persist even though they are only a short distance downstream from threatened populations in the wet forest.

Unlike low-elevation climatic refugia, where *B. dendrobatidis* prevalences are typically lower (Woodhams and Alford 2005), prevalence in the high-elevation refugia is among the highest recorded in nature, with 80 % or more of individuals infected. Intensities of infection are similar to those in wet forest habitats. The persistence of these populations, despite very high prevalences and high intensities of *B. dendrobatidis* infection, indicates that frogs must have higher resistance to the development of disease as a consequence of infection in these habitats. The rediscovery of an "extinct" species, *Litoria lorica,* in a dry forest refuge highlights the importance of these dry forest habitats for *in situ* conservation of amphibians threatened by disease. It also highlights a circularity in our reasoning that needs to

change; previous efforts to locate populations of frogs that have declined due to disease outbreaks have focused on known "core" habitats, but for many species it appears that core habitats favour development of disease. It is important to survey habitats spatially and bioclimatically outside of, but adjacent to, species' core habitats, as these may be serving as climatic refugia. For example, torrent frogs such as *L. nannotis* and *L. lorica* are not rainforest specialists; therefore it is worth looking for them outside this habitat. Another species in the same group as *L. nannotis* and *L. lorica*, *Litoria nyakalensis*, which is also thought to be extinct (McDonald and Alford 1999), could persist in similar habitats at another location or locations in dry forest adjacent to the AWT.

Mechanisms for the increased ability of dry forest frogs to coexist with *B. dendrobatidis*

The greater ability of frogs in dry forest refugia to coexist with *B. dendrobatidis* may be explained by differences in behaviour and microhabitat availability from wet forest populations. In dry forest, *L. nannotis* spends the day submerged under riffles in the stream. This may occur because the deep rock cracks and crevices they use in the wet forest are less available in the dry forest. It is likely that this reduces rates of reinfection of *B. dendrobatidis* by washing zoospores away before they recolonise the host. Slowing reinfection rates would slow the growth of *B. dendrobatidis* populations on hosts, decreasing rates of disease development and mortality (Woodhams 2003). It is also likely that "basking" by conductance from sun-warmed rock substrates shortly after frogs emerge in the early evening, decreases pathogen loads and increases immune activity. This cannot occur in the rainforest, because the substrate along the creek is mostly protected from direct sunlight by the closed forest canopy. Further studies could test these potential mechanisms and propose new management strategies based on them.

Future directions

Many amphibian species exist as metapopulations (Alford and Richards 1999). That is, they form a mosaic of partially isolated breeding subpopulations, each with a local extinction probability and connected to other subpopulations by dispersing individuals

(Hanski and Simberloff 1997; Levins 1969, 1970). Assuming that *Litoria nannotis* fits this pattern, the few frogs presently appearing at rainforest decline sites might be immigrants from dry forest refuges, so that apparently recolonised rainforest populations are entirely "sink" populations. Alternatively, they may be descendents of either immigrants from refuges or remnants of the pre-infection populations, which have survived strong natural selection and acquired increased immunity to chytridiomycosis. Distinguishing between these possibilities should be a major focus of future research, since it is presently unknown whether frogs are capable of evolving increased resistance to the disease. If recovered or recolonised populations have evolved increased resistance, studying them may reveal new mechanisms for reducing the impact of the disease and suggest strategies for increasing the resistance of captive-bred frogs prior to reintroduction.

At present, captive breeding features strongly as the main conservation strategy to mitigate chytridiomycosis-driven amphibian declines (Mendelson et al. 2006). In the near future, environmental refuges from disease outbreaks in the wild may be extremely important. They potentially not only serve as refuges for existing populations, giving them a greater opportunity to evolve increased resistance to the disease and hence be able to permanently recolonise rainforest sites, but may also be a good starting point for reintroductions of frog species that have been bred in captivity. Identifying, understanding and conserving these refugial areas could significantly improve the probabilities of many threatened amphibian species to survive in the wild.

REFERENCES

Abramson, J., 2004. WINPEPI (PEPI-for-Windows): computer programs for epidemiologists. Epidemiologic Perspectives & Innovations 1, 6.

Accad, A., Neldner, V.J., Wilson, B.A., Niehus, R.E., 2006. Remnant vegetation in Queensland. Analysis of remnant vegetation 1997-1999-2000-2001-2003, including regional ecosystem information. Brisbane, Queensland Herbarium, Environmental Protection Agency.

Aguirre, A.A., Ostfeld, R.S., Tabor, G.M., House, C., Pearl, M.C., 2002. Conservation Medicine: ecological health in practice. Oxford University Press, New York.

Alford, R.A., Bradfield, K.S., Richards, S.J., 2007. Ecology: Global warming and amphibian losses. Nature 447, E3-E4.

Alford, R.A., Dixon, P.M., Pechmann, J.H.K., 2001. Ecology: Global amphibian population declines. Nature 412, 499-500.

Alford, R.A., Richards, S.J., 1997. Lack of evidence for epidemic disease as an agent in the catastrophic decline of australian rain forest frogs. Conservation Biology 11, 1026-1029.

Alford, R.A., Richards, S.J., 1999. Global amphibian declines: A problem in applied ecology. Annual Review Of Ecology and Systematics 30, 133-165.

Altig, R., 2007. Comments on the descriptions and evaluations of tadpole mouthpart anomalies. Herpetological Conservation and Biology 2, 1-4.

Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M., Rohani, P., 2006. Seasonality and the dynamics of infectious diseases. Ecology Letters 9, 467-484.

Anderson, E., Forrest, S.C., Clark, T.W., Richardson, L., 1986. Paleobiology, biogeography, and systematics of the black-footed ferret *Mustela nigripes* (Audubon and Bachman), 1851. Great Basin Naturalist Memoirs 8, 11-62.

Anderson, R.M., 1979. Parasite pathogenicity and the depression of host population equilibria. Nature 279, 150-152.

Anderson, R.M., May, R.M., 1979. Population biology of infectious diseases: Part I. Nature 280, 361-367.

Anderson, R.M., May, R.M., 1982. Coevolution of hosts and parasites. Parasitology 85, 411-426.

Andersson, L.G., 1916. Results of Dr. Mjoeberg's Swedish Scientific Expeditions to Australia, 1910-1913. IX, Batrachians from Queensland. K. svenska Vetensk. Akad. Handl. 52, 1-20.

Andre, S.E., Parker, J.M., Briggs, C.J., 2008. Effect of temperature on host response to *Batrachochytrium dendrobatidis* infection in the Mountain Yellow-legged Frog (*Rana muscosa*). Journal Of Wildlife Diseases 44, 716-720.

Anon, 2000. Report: Emerging infectious diseases of wild animals are threat to biodiversity, human health. World Disease Weekly.

Ash, J., 1988. The location and stability of rainforest boundaries in North-Eastern Queensland, Australia. Journal Of Biogeography 15, 619-630.

Atkins, N., Jones, S.M., Edwards, A., 2002. Fecal testosterone concentrations may not be useful for monitoring reproductive status in male blue-tongued lizards (*Tiliqua nigrolutea* : Scincidae). Journal Of Herpetology 36, 106-109.

Atkinson, C., Dusek, R., Woods, K., Iko, W., 2000. Pathogenicity of avian malaria in experimentally-infected Hawaii Amakihi. Journal of Wildlife Diseases 36, 197-204.

Atkinson, C.T., Lease, J.K., Drake, B.M., Shema, N.P., 2001. Pathogenicity, serological responses, and diagnosis of experimental and natural malarial infections in native Hawaiian Thrushes. The Condor 103, 209-218.

Atkinson, C.T., Woods, K.L., Dusek, R.J., Sileo, L.S., Iko, W.M., 1996. Wildlife disease and conservation in Hawaii: pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected Iiwi (*Vestiaria coccinea*). Parasitology 111, S59-69.

Balcomb, R., 1986. Songbird carcassess disappear rapidly from agricultural fields. Auk 103, 817-820.

Bannister, B.A., Begg, N.T., Gillespie, S.H., 1996. Infectious Disease. Blackwell Science, Oxford, U.K.

Barlow, N.D., 1996. The ecology of wildlife disease control: Simple models revisited. The Journal of Applied Ecology 33, 303-314.

Berger, L., 2001. Diseases in Australian Frogs. James Cook University, Townsville.

Berger, L., Hyatt, A.D., Speare, R., Longcore, J.E., 2005a. Life cycle stages of the amphibian chytrid *Batrachochytrium dendrobatidis*. Diseases Aquatic Organisms 68, 51-63.

Berger, L., Marantelli, G., Skerratt, L.F., Speare, R., 2005b. Virulence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* varies with the strain. Diseases Of Aquatic Organisms 68, 47-50.

Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G., Parkes, H., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proceedings of the National Academy of Science USA 95, 9031-9036.

Berger, L., Speare, R., Hines, H.B., Marantelli, G., Hyatt, A.D., McDonald, K.R., Skerratt, L.F., Olsen, V., Clarke, J.M., Gillespie, G., Mahony, M., Sheppard, N., Williams, C., Tyler, M.J., 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Australian Veterinary Journal 82, 434-439.

Berger, L., Speare, R., Hyatt, A., 1999a. Chytrid fungi and amphibian declines: Overview, implications and future directions. In: Declines and disappearances of Australian Frogs. ed. A. Campbell, pp. 23-33. Environment Australia, Canberra.

Berger, L., Speare, R., Kent, A., 1999b. Diagnosis of chytridiomycosis of amphibians by histological examination. Zoos Print Journal 15, 184-190.

Berger, L., Speare, R., Skerratt, L.F., 2005c. Distribution of *Batrachochytrium dendrobatidis* and pathology in the skin of green tree frogs *Litoria caerulea* with severe chytridiomycosis. Diseases of Aquatic Organisms 68, 65-70.

Blanford, S., Thomas, M.B., 1999a. Host thermal biology: the key to understanding host-pathogen interactions and microbial pest control? Agricultural and Forest Entomology 1, 195-202.

Blanford, S., Thomas, M.B., 1999b. Role of thermal biology in disease dynamics. Aspects of Applied Biology 53, 73-82.

Blanford, S., Thomas, M.B., 2000. Thermal behavior of two acridid species: Effects of habitat and season on body temperature and the potential impact on biocontrol with pathogens. Environmental Entomology 29, 1060-1069.

Blanford, S., Thomas, M.B., Langewald, J., 1998. Behavioural fever in a population of the Sengalese grasshopper, *Oedaleus senegalensis*, and its implications for biological control using pathogens. Ecological Entomology 23, 9-14.

Blanford, S., Thomas, M.B., Pugh, C., Pell, J.K., 2003. Temperature checks the Red Queen? Resistance and virulence in a fluctuating environment. Ecology Letters 6, 2-5.

Blaustein, A.R., Romansic, J.M., Scheessele, E.A., Han, B.A., Pessier, A.P., Longcore, J.E., 2005. Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. Conservation Biology 19, 1460-1468.

Blaustein, A.R., Wake, D.B., 1990. Declining amphibian populations: A global phenomenon. Trends In Ecology & Evolution 5, 203-204.

Blaustein, A.R., Wake, D.B., Sousa, W.P., 1994. Amphibian Declines: Judging stability, persistence, and susceptibility of populations to local and global extinctions. Conservation Biology 8, 60-71.

Bolaños, F., 2002. Anfibios en Retirada. Ambientico 107, 12-13.

Bosch, J., Carrascal, L.M., Durán, L., Walker, S., Fisher, M.C., 2007. Climate change and outbreaks of amphibian chytridiomycosis in a montane area of central Spain; is there a link? Proceedings of the Royal Society of London - Series B: Biological Sciences 274, 253-260.

Bosch, J., Martinez-Solano, I., 2006. Chytrid fungus infection related to unusual mortalities of *Salamandra salamandra* and *Bufo bufo* in the Peñalara Natural Park, Spain. Oryx 40, 84-89.

Bosch, J., Martinez-Solano, I., Garcia-Paris, M., 2001. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. Biological Conservation 97, 331-337.

Boyle, D.G., Boyle, D.B., Olsen, V., Morgan, J.A.T., Hyatt, A.D., 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Diseases of Aquatic Organisms 60, 141-148.

Bradley, G.A., Rosen, P.C., Sredl, M.J., Jones, T.R., Longcore, J.E., 2002. Chytridiomycosis in native Arizona frogs. Journal Of Wildlife Diseases 38, 206-212.

Brem, F.M.R., Lips, K.R., 2008. *Batrachochytrium dendrobatidis* infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic. Diseases of Aquatic Organisms 81, 189-202.

Briggs, C.J., Vredenburg, V.T., Knapp, R.A., Rachowicz, L.J., 2005. Investigating the population-level effects of chytridiomycosis: an emerging infectious disease of amphibians. Ecology 86, 3149-3159.

Burdon, J.J., 1991. Fungal pathogens as selective forces in plant populations and communities. Australian Journal of Ecology 16, 423-432.

Carey, C., Bruzgul, J.E., Livo, L.J., Walling, M.L., Kuehl, K.A., Dixon, B.F., Pessier, A.P., Alford, R.A., Rogers, K.B., 2006. Experimental exposures of Boreal Toads (*Bufo boreas*) to a pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*). EcoHealth 3, 5-21.

Carey, C., Maniero, G.D., Stinn, J.F., 1996. Effect of cold on immune function and susceptibility to bacterial infection in toads (*Bufo marinus)*, In Adaptations to the Cold: Tenth International Hibernation Symposium. eds G.F. Hulbert, S.C. Nicol. University of New England Press, Armidale.

Carnaval, A.C.O.d.Q., Puschendorf, R., Peixoto, O.L., Verdade, V.K., Rodrigues, M.T., 2006. Amphibian chytrid fungus broadly distributed in the Brazilian Atlantic Rain Forest. EcoHealth 3, 41-48.

Cashins, S., Alford, R.A., Skerratt, L.F., 2008. Lethal effect of latex, nitrile, and vinyl gloves on tadpoles. Herpetological Review 39, 298-300.

Chomel, B.B., Belotto, A., Meslin, F.-X., 2007. Wildlife, exotic pets, and emerging zoonoses. Emerging Infectious Diseases 13, Available from http://www.cdc.gov/ncidod/EID/13/11/16.htm.

Cogger, H.G., 2000. Reptiles and Amphibians of Australia, 6th ed. Reed New Holland, Sydney, Australia.

Colhoun, J., 1973. Effects of environmental factors on plant disease. Annual Review of Phytopathology 11, 343-363.

Collins, J.P., Crump, M.L., 2009. Extinction in our times: global amphibian declines. Oxford University Press, New York.

Collins, J.P., Storfer, A., 2003. Global amphibian declines: sorting the hypotheses. Diversity and Distributions 9, 89-98.

Cook, S., Glass, R., LeBaron, C., Ho, M.-S., 1990. Global seasonality of rotavirus infections. Bulletin of the World Health Organization 68.

Cooper, E.L., Wright, R.K., Klempau, A.E., Smith, C.T., 1992. Hibernation alters the frog's immune system. Cryobiology 29, 616-631.

Covacevich, J.A., McDonald, K.R., 1993. Distribution and conservation of frogs and reptiles of Queensland rainforests. Memoirs of the Queensland Museum 34, 189-199.

Crawshaw, G.J., 1992. The role of disease in amphibian decline. In: Declines in Canadian amphibian populations: designing a national monitoring strategy. eds C.A. Bishop, K.E. Pettit, pp. 60-62. Canadian Wildlife Service, Ottawa, Canada.

Cunningham, A.A., 1996. Disease Risks of Wildlife Translocations. Conservation Biology 10, 349-353.

Cunningham, A.A., Daszak, P., Rodríguez, J.P., 2003. Pathogen pollution: defining a parasitological threat to biodiversity conservation. Journal of Parasitology 89 (Suppl.), S78-S83.

Cunningham, M., 2002. Identification and evolution of Australian torrent treefrogs (Anura: Hylidae: *Litoria nannotis* group). Memoirs of the Queensland Museum 48, 93-102.

Daszak, P., Berger, L., Cunningham, A.A., Hyatt, A.D., Green, D.E., Speare, R., 1999. Emerging infectious diseases and amphibian population declines. Emerging Infectious Diseases 5, 735-748.

Daszak, P., Cunningham, A.A., 1999. Extinction by infection. Trends In Ecology and Evolution 14, 279-279.

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2000. Wildlife ecology - Emerging infectious diseases of wildlife - Threats to biodiversity and human health. Science 287, 443-449.

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropical 78, 103-116.

Daszak, P., Strieby, A., Cunningham, A.A., Longcore, J.E., Brown, C.C., Porter, D., 2004. Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herpetological Journal 14, 201-207.

Davidson, C., Shaffer, H.B., Jennings, M.R., 2002. Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for california amphibian declines. Conservation Biology 16, 1588-1601.

Davidson, E.W., Parris, M., Collins, J.P., Longcore, J.E., Pessier, A.P., Brunner, J., 2003. Pathogenicity and transmission of chytridiomycosis in tiger salamanders (*Ambystoma tigrinum*). Copeia, 601-607.

Davies, M., McDonald, K.R., 1979. A new species of stream-dwelling hylid frog from Northern Queensland. Transactions of the Royal Society of South Australia 103, 169-176.

de Castro, F., Bolker, B., 2005. Mechanisms of disease-induced extinction. Ecology Letters 8, 117-126.

Deem, S.L., Karesh, W.B., Weisman, W., 2001. Putting theory into practice: Wildlife health in conservation. Conservation Biology 15, 1224-1233.

DEH, 2006. Threat Abatment plan: infection of amphibians with chytrid fungus resulting in chytridiomycosis. Commonwealth Department of Environment and Heritage.

Di Rosa, I., Simoncelli, F., Fagotti, A., Pascolini, R., 2007. Ecology: The proximate cause of frog declines? Nature 447, E4-E5.

Diamond, J., 1997. Guns, Germs and Steel. W.W. Norton.

Dobson, A.P., 1988. Restoring island ecosystems: the potential of parasites to control introduced mammals. Conservation Biology 2, 31-39.

Dobson, A.P., Foufopoulos, J., 2001. Emerging infectious pathogens of wildlife. Philosophical Transactions of the Royal Society, London: Biological Sciences 356, 1001-1012.

Dobson, A.P., May, R.M., 1986. Disease and conservation. In: Conservation biology: The science of scarcity and diversity. Ed. M. Soulé, pp. 345-365. Sinauer Assoc Inc., Sunderland (MA), USA.

Dowell, S.F., 2001. Seasonal variation in host susceptibility and cycles of certain infectious diseases. Emerging Infectious Diseases 7, 369-374.

Elith, J., H. Graham, C., P. Anderson, R., Dudik, M., Ferrier, S., Guisan, A., J. Hijmans, R., Huettmann, F., R. Leathwick, J., Lehmann, A., Li, J., G. Lohmann, L., A. Loiselle, B., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., McC. M. Overton, J., Townsend Peterson, A., J. Phillips, S., Richardson, K., Scachetti-Pereira, R., E. Schapire, R., Soberon, J., Williams, S., S. Wisz, M., E. Zimmermann, N., 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography 29, 129-151.

Elliot, S.L., Blanford, S., Thomas, M.B., 2002. Host, pathogen interactions in a varying environment: temperature, behavioural fever and fitness. Proceedings of the Royal Society of London Series B-Biological Sciences 269, 1599-1607.

Enquist, C.A.F., 2002. Predicted regional impacts of climate change on the geographical distribution and diversity of tropical forests in Costa Rica. Journal Of Biogeography 29, 519-534.

Epstein, P.R., Chivian, E., Frith, K., 2003. Emerging diseases threaten conservation. Environmental Health Perspectives 111, A506-508.

Fedorov, V.B., Stenseth, N.C., 2002. Multiple glacial refugia in the North American Arctic: inference from phylogeography of the collared lemming (*Dicrostonyx groenlandicus*). Proceedings of The Royal Society of London Series B-Biological Sciences 269, 2071-2077.

Fellers, G.M., Green, D.E., Longcore, J.E., 2001. Oral chytridiomycosis in the Mountain Yellow-Legged Frog (*Rana muscosa*). Copeia 2001, 945-953.

Fenner, F., Marshall, I.D., 1957. A comparison of the virulence for european rabbits (*Oryctolagus cuniculus*) of strains of myxoma virus recovered in the field in Australia, Europe and America. The Journal of Hygiene 55, 149-191.

Fenner, F., Ratcliffe, F.N., 1965. Myxomatosis. Cambridge University Press.

Fisher, R.A., 1962. Confidence limits for a cross-product ratio. Australian & New Zealand Journal of Statistics 4, 41-41.

Garner, T., Perkins, M., Govindarajulu, P., Seglie, D., Walker, S., Cunningham, A., Fisher, M., 2006. The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. Biological Letters 2, 455-459.

Ginsberg, J.R., Mace, G.M., Albon, S., 1995. Local extinction in a small and declining population: wild dogs in the Serengeti. Proceedings of The Royal Society of London Series B-Biological Sciences 262, 221-228.

Gog, J., Woodroffe, R., Swinton, J., 2002. Disease in Endangered Metapopulations: The Importance of Alternative Hosts. Proceedings of The Royal Society of London Series B-Biological Sciences 269, 671-676.

Grassly, N.C., Fraser, C., 2006. Seasonal infectious disease epidemiology. Proceedings of The Royal Society of London Series B-Biological Sciences 273, 2541-2550.

Haas, A., Richards, S.J., 1998. Correlations of cranial morphology, ecology, and evolution in Australian suctorial tadpoles of the genera *Litoria* and *Nyctimystes* (Amphibia: Anura: Hylidae: Pelodryadinae). Journal of Morphology 238, 109-141.

Hall, S.R., Tessier, A.J., Duffy, M.A., Huebner, M., Caceres, C.E., 2006. Warmer does not have to mean sicker: Temperature and predators can jointly drive timing of epidemics. Ecology 87, 1684-1695.

Hanley, J.A., McNeil, B.J., 1982. The meaning and use of the area under a receiver operating characteristic (ROC) Curve. Radiology 143, 29-36.

Hanski, I., Simberloff, D., 1997. The metapopulation approach, its history, conceptual domain, and application to conservation, In Metapopulation biology: ecology, genetics and evolution. eds I. Hanski, M. Gilpin, pp. 2-26. Academic press.

Harrison, S., Viers, J.H., Thorne, J.H., Grace, J.B., 2008. Favorable environments and the persistence of naturally rare species. Conservation Letters 1, 65-74.

Harvell, C.D., Kim, K., Burkholder, J.M., Colwell, R.R., Epstein, P.R., Grimes, D.J., Hoffman, E., Lipp, E.K., Osterhaus, A.D.M.E., Overstreet, R.M., Porter, J.W., Smith, G.W., Vasta, G.R., 1999. Emerging marine diseases: climate links and anthropogenic factors. Science 285, 1505-1510.

Harvell, C.D., Mitchell, C.E., Ward, J., Altizer, S., Dobson, A., Ostfeld, R.S., Samuel, M.D., 2002. Climate warming and disease risks for terrestrial and marine biota. Science 296, 2158-5162.

Harvell, D., 2004. Ecology and evolution of host-pathogen interactions in Nature. The American Naturalist 164, S1-S5.

Hernandez, P.A., Graham, C.H., Master, L.L., Albert, D.L., 2006. The effect of sample size and species characteristics on performance on different species distribution methods. Ecography 29, 773-785.

Herrera, W., 1985. Clima de Costa Rica. EUNED, San José, Costa Rica.

Heyer, W.R., Rand, A.S., da Cruz, C.A.G., Peixoto, O.L., 1988. Decimations, extinctions, and colonizations of frog populations in southeast Brazil and their evolutionary implications. Biotropica 20, 230-235.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25, 1965-1978.

Hines, H., Mahony, M., McDonald, K., 1999. An assessment of frog declines in wet subtropical Australia. In: Declines and disappearances of Australian Frogs. Ed. A. Campbell, pp. 44-63. Environment Australia, Canberra.

Hodgkison, S., Hero, J.-M., 2001. Daily behaviour and microhabitat use of the waterfall frog, *Litoria nannotis* in Tully Gorge, Eastern Australia. Journal Of Herpetology 35, 116-120.

Hodgkison, S., Hero, J.-M., 2003. Seasonal, sexual and ontogenic variations in the diet of the 'declining' frogs *Litoria nannotis, Litoria reheocola* and *Nyctimystes dayi*. Wildlife Research 30, 345-354.

Hoskin, C.J., 2007. Description, biology and conservation of a new species of Australian tree frog (Amphibia: Anura: Hylidae: *Litoria*) and an assessment of the remaining populations of *Litoria genimaculata* Horst, 1883: systematic and conservation implications of an unusual speciation event. Biological Journal of The Linnean Society 91, 549-563.

Hoskin, C.J., Hero, J.-M., 2008. Rainforest frogs of the wet tropics, north-east Australia. Griffith University, Gold Coast, Australia.

Hoskin, C.J., Higgie, M., McDonald, K.R., Moritz, C., 2005. Reinforcement drives rapid allopatric speciation. Nature 437, 1353-1356.

Hoskin, C.J., McCallum, H., 2007. Phylogeography of the parasitic fly *Batrachomyia* in the Wet Tropics of north-east Australia, and susceptibility of host frog lineages in a mosaic contact zone. Biological Journal Of The Linnean Society 92, 593-603.

Hosmer, D.W., Lemeshow, S., 2000. Applied logistic regression, 2nd edition. John Wiley & Sons, New York.

Hosseini, P.R., Dhondt, A.A., Dobson, A., 2004. Seasonality and wildlife disease: how seasonal birth, aggregation and variation in immunity affect the dynamics of *Mycoplasma gallisepticum* in house finches. Proceedings of the Royal Society of London. Series B: Biological Sciences 271, 2569-2577.

Hyatt, A.D., Boyle, D.G., Olsen, V., Boyle, D.B., Berger, L., Obendorf, D., Dalton, A., Kriger, K., Hero, M., Hines, H., Phillott, R., Campbell, R., Marantelli, G., Gleason, F., Colling, A., 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 73, 175-192.

Ingram, G.J., McDonald, K.R., 1993. An update on the decline of Queensland's frogs, In Herpetology in Australia: A Diverse Discipline. eds D. Lunney, D. Ayers, pp. 297-303. Transactions of the Royal Zoological Society of NSW, Surrey Beatty & Sons, NSW, Sydney, Australia.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H.T., Rauhut, A., Reeb, V., Arnold, A.E., Amtoft, A., Stajich, J.E., Hosaka, K., Sung, G.-H., Johnson, D.,

O/'Rourke, B., Crockett, M., Binder, M., Curtis, J.M., Slot, J.C., Wang, Z., Wilson, A.W., Schuszler, A., Longcore, J.E., O/'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P.M., Powell, M.J., Taylor, J.W., White, M.M., Griffith, G.W., Davies, D.R., Humber, R.A., Morton, J.B., Sugiyama, J., Rossman, A.Y., Rogers, J.D., Pfister, D.H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R.A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R.A., Serdani, M., Crous, P.W., Hughes, K.W., Matsuura, K., Langer, E., Langer, G., Untereiner, W.A., Lucking, R., Budel, B., Geiser, D.M., Aptroot, A., Diederich, P., Schmitt, I., Schultz, M., Yahr, R., Hibbett, D.S., Lutzoni, F., McLaughlin, D.J., Spatafora, J.W., Vilgalys, R., 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443, 818-822.

James, T.Y., Litvintseva, A.P., Vilgalys, R., Morgan, J.A.T., Taylor, J.W., Fisher, M.C., Berger, L., Weldon, C., du Preez, L., Longcore, J.E., 2009. Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. PLoS Pathog 5, e1000458.

Januchowski, S., VanDerWal, J., Edwards, A., Pressey, B. 2009. Characterizing errors in topographic models and associated costs. International Journal of Geographical Information Science. In Press.

Johnson, M.L., Speare, R., 2003. Survival of *Batrachochytrium dendrobatidis* in water: quarantine and disease control implications. Emerging Infectious Diseases 9, 922-925.

Jones, D.A., Wang, W., Fawcett, R., 2007. Climate data for the Australian Water Availability Project, In Australian Water Availability Project. National Climate Centre, Australian Bureau of Meteorology.

Kelly, J., 2006. The Great Mortality: An Intimate History of the Black Death, the Most Devastating Plague of all Times. Harper Perennial.

King, M., Contreras, N., Honeycutt, R.L., 1990. Variation within and between nucleolar organizer regions in Australian hylid frogs (Anura) shown by 18S + 28S in-situ hybridization. Genetica 80, 17-29.

Koch, R., 1893. Ueber den augenblicklichen Stand der bakteriologischen Choleradiagnose. Medical Microbiology and Immunology 14, 319-338.

Kriger, K., Hero, J.-M., 2006a. Survivorship in wild frogs infected with chytridiomycosis. EcoHealth 3, 1-7.

Kriger, K.M., Hero, J.-M., 2006b. Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. Journal of Zoology 271, 352-359.

Kusrini, M.D., Skerratt, L.F., Speare, R., 2008. Chytridiomycosis in frogs of Mount Gede Pangrango, Indonesia. Diseases of Aquatic Organisms 82, 406-413.

La Marca, E., Lips, K.R., Lotters, S., Puschendorf, R., Ibanez, R., Rueda-Almonacid, J.V., Schulte, R., Marty, C., Castro, F., Manzanilla-Puppo, J., Garcia-Perez, J.E.,

Bolaños, F., Chaves, G., Pounds, J.A., Toral, E., Young, B.E., 2005. Catastrophic population declines and extinctions in Neotropical Harlequin frogs (Bufonidae: *Atelopus*). Biotropica 37, 190-201.

Lande, R., 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. The American Naturalist 142, 911.

Langkilde, T., Alford, R.A., 2002. The tail wags the frog: Harmonic radar transponders affect movement Behavior in *Litoria lesueuri*. Journal Of Herpetology 36, 711-715.

Laurance, W.F., 2008. Global warming and amphibian extinctions in eastern Australia. Austral Ecology 33, 1-9.

Laurance, W.F., McDonald, K., Speare, R., 1996. Epidemic disease and the catastrophic decline of Australian rain forest frogs. Conservation Biology 10, 406-413.

Lesica, P., Allendorf, F.W., 1995. When are peripheral populations valuable for conservation? Conservation Biology 9, 753-760.

Lessa, E.P., Cook, J.A., Patton, J.L., 2003. Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. Proceedings of The National Academy of Sciences, USA100, 10331-10334.

Levins, R., 1969. Some demographic and genetic consequences of environmental heterogeneity for biological control. Bulletin of the Entomological Society of America 15, 237-240.

Levins, R., 1970. Extinction, In Some mathematical problems in biology. ed. M. Gerstenhaber, pp. 75-107. American Mathematical Society.

Liem, D.S., 1974. A review of the *Litoria nannotis* species group, and a description of a new species of *Litoria* from northern Queensland, Australia (Anura: Hylidae). Memoirs of the Queensland Museum 17, 151-168.

Lips, K.R., 1998. Decline of a tropical montane amphibian fauna. Conservation Biology 12, 106-117.

Lips, K.R., 1999. Mass mortality and population declines of anurans at an upland site in western Panama. Conservation Biology 13, 117-125.

Lips, K.R., Brem, F., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J., Carey, C., Livo, L., Pessier, A.P., Collins, J.P., 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proceedings of the National Academy of Sciences USA 103, 3165-3170.

Lips, K.R., Burrowes, P.A., Mendelson, J.R., Parra-Olea, G., 2005a. Amphibian Declines in Latin America: Widespread Population Declines, Extinctions, and Impacts. Biotropica 37, 163-165.

Lips, K.R., Burrowes, P.A., Mendelson, J.R., Parra-Olea, G., 2005b. Amphibian Population Declines in Latin America: A Synthesis. Biotropica 37, 222-226.

Lips, K.R., Diffendorfer, J., Mendelson, J.R., Sears, M.W., 2008. Riding the Wave: Reconciling the Roles of Disease and Climate Change in Amphibian Declines. PLOS Biology 6, e72.

Lips, K.R., Green, D.E., Papendick, R., 2003a. Chytridiomycosis in wild frogs from southern Costa Rica. Journal of Herpetology 37, 215-218.

Lips, K.R., Mendelson, J.R., Munoz-Alonso, A., Canseco-Marquez, L., Mulcahy, D.G., 2004. Amphibian population declines in montane southern Mexico: resurveys of historical localities. Biological Conservation 119, 555-564.

Lips, K.R., Reeve, J.D., Witters, L.R., 2003b. Ecological traits predicting amphibian population declines in Central America. Conservation Biology 17, 1078-1088.

Longcore, J.E., Pessier, A.P., Nichols, D.K., 1999. *Batrachochytrium dendrobatidis* gen et sp nov, a chytrid pathogenic to amphibians. Mycologia 91, 219-227.

Maniero, G.D., Carey, C., 1997. Changes in selected aspects of immune function in the leopard frog, *Rana pipiens*, associated with exposure to cold. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 167, 256-263.

Massad, E., 1987. Transmission rates and the evolution of pathogenicity. Evolution 41, 1127-1130.

Matutte, B., Storey, K.B., Knoop, F.C., Conlon, J.M., 2000. Induction of synthesis of an antimicrobial peptide in the skin of the freeze-tolerant frog, *Rana sylvatica*, in response to environmental stimuli. FEBS letters 483, 135-138.

May, R.M., 1988. Conservation and disease. Conservation Biology 2, 28-30.

May, R.M., Anderson, R.M., 1983. Epidemiology and genetics in the coevolution of parasites and hosts. Proceedings of the Royal Society of London. Series B. Biological Sciences 219, 281-313.

Mazzoni, R., 2003. Emerging pathogen of wild amphibians in frogs (*Rana catesbeiana*) farmed for international trade. Emerging Infectious Diseases 9, 995-998.

McCallum, H., 1994. Quantifying the impact of disease on threatened species. Pacific Conservation Biology 1, 107-117.

McCallum, H., Dobson, A., 1995. Detecting disease and parasite threats to endangered species and ecosystems. Trends in Ecology and Evolution 10, 190-194.

McCallum, H., Dobson, A., 2002. Disease, habitat fragmentation and conservation. Proceedings of the Royal Society of London. Series B: Biological Sciences 269, 2041-2049.

McCallum, H., Harvell, D., Dobson, A., 2003. Rates of spread of marine pathogens. Ecology Letters 6, 1062-1067.

McDonald, K., Alford, R.A., 1999. A review of declining frogs in northern Queensland, In Declines and disappearances of Australian Frogs. ed. A. Campbell, pp. 14-22. Environment Australia, Canberra.

McDonald, K.R., Méndez, D., Müller, R., Freeman, A.B., Speare, R., 2005. Decline in the prevalence of chytridiomycosis in frog populations in North Queensland, Australia. Pacific Conservation Biology 11, 114-120.

McMahon, J.P., Hutchinson, M.F., Nix, H.A., Ord, K.D., 1995. ANUCLIM users guide. Centre for Resource and Environmental Studies, Australian National University, Canberra.

Mendelson, J.R., III, Lips, K.R., Gagliardo, R.W., Rabb, G.B., Collins, J.P., Diffendorfer, J.E., Daszak, P., Ibanez D, R., Zippel, K.C., Lawson, D.P., Wright, K.M., Stuart, S.N., Gascon, C., da Silva, H.R., Burrowes, P.A., Joglar, R.L., La Marca, E., Lotters, S., du Preez, L.H., Weldon, C., Hyatt, A., Rodriguez-Mahecha, J.V., Hunt, S., Robertson, H., Lock, B., Raxworthy, C.J., Frost, D.R., Lacy, R.C., Alford, R.A., Campbell, J.A., Parra-Olea, G., Bolanos, F., Domingo, J.J.C., Halliday, T., Murphy, J.B., Wake, M.H., Coloma, L.A., Kuzmin, S.L., Price, M.S., Howell, K.M., Lau, M., Pethiyagoda, R., Boone, M., Lannoo, M.J., Blaustein, A.R., Dobson, A., Griffiths, R.A., Crump, M.L., Wake, D.B., Brodie Jr, E.D., 2006. BIODIVERSITY: Confronting amphibian declines and extinctions. Science 313, 48.

Merino-Viteri, A., Coloma, L.A., Almendariz, A., 2006. Los *Telmatobius* de los Andes de Ecuador y su disminución poblacional. Monografías de Herpetología 7, 9-37.

Morehouse, E.A., James, T.Y., Ganley, A.R., Vilgalys, R., Berger, L., Murphy, P.J., Longcore, J.E., 2003. Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. Molecular Ecology 12, 395-403.

Morgan, J.A.T., Vredenburg, V.T., Rachowicz, L.J., Knapp, R.A., Stice, M.J., Tunstall, T., Bingham, R.E., Parker, J.M., Longcore, J.E., Moritz, C., Briggs, C.J., Taylor, J.W., 2007. Population genetics of the frog-killing fungus *Batrachochytrium dendrobatidis*. Proceedings of the National Academy of Sciences 104, 13845-13850.

Morse, S.S., 1995. Factors in the emergence of infectious diseases. Emerging Infectious Diseases 1, 7-15.

Murray, K.A., Skerratt, L.F., Speare, R., McCallum, H., 2009. Impact and Dynamics of Disease in Species Threatened by the Amphibian Chytrid Fungus, *Batrachochytrium dendrobatidis*. Conservation Biology, published online.

Muths, E., P.S. Corn, A.P. Pessier, Green, D.E., 2002. Evidence for disease-related amphibian decline in Colorado. Biological Conservation 110, 357-365.

Nichols, D.K., Lamirande, E.W., Pessier, A.P., Longcore, J.E., 2001. Experimental transmission of cutaneous chytridiomycosis in dendrobatid frogs. J Wildl Dis 37, 1-11.

Nielsen, J.L., Scott, J.M., Aycrigg, J.L., 2001. Endangered species and peripheral populations: cause for conservation. (Letter to the Editor). Endangered Species Update 18, 194.

North, S., Alford, R., 2008. Infection intensity and sampling locality affect *Batrachochytrium dendrobatidis* distribution among body regions on green-eyed tree frogs *Litoria genimaculata*. Diseases of Aquatic Organisms 81, 177-188.

O'Brien, S.J., Evermann, J.F., 1988. Interactive influence of infectious disease and genetic diversity in natural populations. Trends In Ecology & Evolution 3, 254-259.

OIE, 2007. Report of the Meeting of the OIE Aquatic Health Standards Commission, Paris.

Olsen, V., Hyatt, A.D., Boyle, D.G., Mendez, D., 2004. Co-localisation of *Batrachochytrium dendrobatidis* and keratin for enhanced diagnosis of chytridiomycosis in frogs. Diseases of Aquatic Organisms 61, 85-88.

Olson, D., Ronnenberg, K., 2008. Global Bd mapping project. Partners in Amphibian and reptile conservation http://www.parcplace.org/bdmap2008update.html

Ouborg, J., 1993. Isolation, Population Size and Extinction: The Classical and Metapopulation Approaches Applied to Vascular Plants along the Dutch Rhine-System. Oikos 66.

Ouedraogo, R.M., Cusson, M., Goettel, M.S., Brodeur, J., 2003. Inhibition of fungal growth in thermoregulating locusts, *Locusta migratoria*, infected by the fungus *Metarhizium anisopliae* var acridum. Journal of Invertebrate Pathology 82, 103-109.

Ouellet, M., Mikaelian, I., Pauli, B.D., Rodrigue, J., Green, D.M., 2005. Historical evidence of widespread chytrid infection in North American amphibian populations. Conservation Biology 19, 1431-1440.

Parris, K.M., Cornelius, T.O., 2004. Fungal pathogen causes competetive and developmental stress in larval amphibian communities. Ecology 85, 3385-3395.

Parris, M.R., E; Storfer, A, 2006. Antipredator behavior of chytridiomycosis-infected northern leopard frog (*Rana pipiens*) tadpoles. Canadian Journal of Zoology 84, 58-65.

Pascual, M., Bouma, M.J., Dobson, A.P., 2002. Cholera and climate: revisiting the quantitative evidence. Microbes and Infection 4, 237-245.

Pasquier, L.D., Schwager, J., Flajnik, M.F., 1989. The immune system of *Xenopus*. Annual Review of Immunology 7, 251-275.

Patz, J.A., Campbell-Lendrum, D., Holloway, T., Foley, J.A., 2005. Impact of regional climate change on human health. Nature 438, 310-317.

Patz, J.A., Olson, S.H., 2006. Malaria risk and temperature: Influences from global climate change and local land use practices. Proceedings of the National Academy of Science USA 103, 5635-5636.

Pechmann, J.H.K., Scott, D.E., Semlitsch, R.D., Caldwell, J.P., Vitt, L.J., Gibbons, J.W., 1991. Declining amphibian populations the problem of separating human impacts from natural fluctuations. Science 253, 892-895.

Pechmann, J.H.K., Wilbur, H.M., 1994. Putting declining amphibian populations in perspective: natural fluctuations and human impacts. Herpetologica 50, 65-84.

Peterson, A.T., 2001. Endangered species and peripheral populations: Cause for reflection. Endangered Species Update 18, 30.

Peterson, J.D., Wood, M.B., Hopkins, W.A., Unrine, J.M., Mendonca, M.T., 2007. Prevalence of *Batrachochytrium dendrobatidis* in American Bullfrogs and southern Leopard frog larvae from wetlands on the Savannah River site, South Carolina. Journal of Wildlife Diseases 43, 450-460.

Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190, 231-259.

Piotrowski, J., Annis, S., Longcore, J.E., 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96, 9-15.

Plowright, W., 1962. Rinderpest virus. Annals of the New York Academy of Sciences 101, 548-563.

Plowright, W., 1982. The effects of rinderpest and rinderpest control on wildlife in Africa. Symposia of the Zoological Society of London 50, 1-28.

Plytycz, B., Seljelid, R., 1997. Rhythms of Immunity. Archivum Immunologiae et Therapiae Experimentalis 45, 157-162.

Pounds, A.J., Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.N., La Marca, E., Merino-Viteri, A., Puschendorf, R., Sanchez-Azofeifa, G.A., Still, C.J., Young, B.E., 2007. Global warming and amphibian losses; The proximate cause of frog declines? Nature 447, E5-E6.

Pounds, J.A., Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P., Foster, P.N., La Marca, E., Masters, K.L., Merino-Viteri, A., Puschendorf, R., Ron, S.R., Sanchez-Azofeifa, G.A., Still, C.J., Young, B.E., 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439, 161-167.

Pounds, J.A., Crump, M.L., 1987. Harlequin frogs along a tropical montane stream: aggregation and the risk of predation by frog-eating flies. Biotropica 19, 306-309.

Pounds, J.A., Crump, M.L., 1994. Amphibian declines and climate disturbance: The case of the Golden toad and the Harlequin Frog. Conservation Biology 8, 72-85.

Pounds, J.A., Fogden, M.P.L., Savage, J.M., Gorman, G.C., 1997. Tests of null models for amphibian declines on a tropical mountain. Conservation Biology 11, 1307-1322.

Pounds, J.A., Puschendorf, R., 2004. Ecology - Clouded futures. Nature 427, 107-109.

Puschendorf, R., 2003. *Atelopus varius* (Harlequin frog). Fungal infection. Herpetological Review 34, 355.

Puschendorf, R., Bolaños, F., 2006. Detection of *Batrachochytrium dendrobatidis* in *Eleutherodactylus fitzingeri*: effects of skin sample location and histologic stain. Journal of Wildlife Diseases 42, 301-306.

Puschendorf, R., Bolaños, F., Chaves, G., 2006a. The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica. Biological Conservation 132, 136-142.

Puschendorf, R., Carnaval, A.C.O.Q., VanDerWal, J., Zumbado-Ulate, H., Chavez, G., Bolaños, F., Alford, R.A., 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. Diversity & Distributions 15, 401-408.

Puschendorf, R., Castañeda, F., McCranie, J.R., 2006b. Chytridiomycosis in wild frogs from Pico Bonito National Park, Honduras EcoHealth 3, 178-181.

Puschendorf, R., Chaves, G., Crawford, A.J., Brooks, D.R., 2005. *Eleutherodactylus ranoides* (NCN). Dry Forest population, refuge from decline? Herpetological Review 36, 53.

Rachowicz, L.J., Briggs, C.J., 2007. Quantifying the disease transmission function: effects of density on *Batrachochytrium dendrobatidis* transmission in the mountain yellow-legged frog Rana muscosa. Journal Of Animal Ecology 76, 711-721.

Rachowicz, L.J., Hero, J.M., Alford, R.A., Taylor, J.W., Morgan, J.A.T., Vredenburg, V.T., Collins, J.P., Briggs, C.J., 2005. The novel and endemic pathogen hypotheses: Competing explanations for the origin of emerging infectious diseases of wildlife. Conservation Biology 19, 1441-1448.

Rachowicz, L.J., Knapp, R.A., Morgan, J.A.T., Stice, M.J., Vredenburg, V.T., Parker, J.M., Briggs, C.J., 2006. Emerging infectious disease as a proximate cause of amphibian mass mortality. Ecology 87, 1671-1683.

Rachowicz, L.J., Vredenburg, V.T., 2004. Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. Diseases Of Aquatic Organisms 61, 75-83.

Raffel, T.R., Rohr, J.R., Kiesecker, J.M., Hudson, P.J., 2006. Negative effects of changing temperature on amphibian immunity under field conditions. Functional Ecology 20, 819-828.

Randolph, S.E., Green, R.M., Peacey, M.F., Rogers, D.J., 2000. Seasonal synchrony: the key to tick-borne encephalits foci idendtified by satellite data. Parasitology 121, 15-23.

Reed, K.D., Meece, J.K., Henkel, J.S., Shukla, S.K., 2003. Birds, migration and emerging zoonoses: West Nile Virus, Lyme Disease, Influenza A and Enteropathogens. Clinical Medicine and Research 1, 5-12.

Retallick, R.W., Miera, V., 2007. Strain differences in the amphibian chytrid *Batrachochytrium dendrobatidis* and non-permanent, sub-lethal effects of infection. Diseases of Aquatic Organisms 75, 201-207.

Retallick, R.W.R., McCallum, H., Speare, R., 2004. Endemic infection of the amphibian chytrid fungus in a frog community post-decline. PLOS Biology 2, 1965-1971.

Richards, S.J., 1992. The tadpole of the Australian frog *Litoria nyakalensis* (Anura, Hylidae), and a key to the torrent tadpoles of northern Queensland. Alytes 10, 99-103.

Richards, S.J., 2002. Influence of flow regime on habitat selection by tadpoles in an Australian rainforest stream. Journal of Zoology, London 257, 273-279.

Richards, S.J., Alford, R.A., 1993. The tadpoles of two Queensland frogs (Anura: Hylidae, Myobatrachidae). Memoirs of the Queensland Museum 33, 337-340.

Richards, S.J., Alford, R.A., 2005. Structure and dynamics of a rainforest frog (*Litoria genimaculata*) population in northern Queensland. Australian Journal Of Zoology 53, 229-236.

Richards, S.J., McDonald, K.R., Alford, R.A., 1993. Declines in populations of Australia's endemic tropical rainforest frogs. Pacific Conservation Biology 1, 66-77.

Richards, S.J., Sinsch, U., Alford, R.A., 1994. Radio Tracking, In Measuring and monitoring biological diversity: Standard Methods for Amphibians. eds W.R. Heyer, M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, M.S. Foster, pp. 155-157. Smithsonian Institution Press, Washington.

Richmond, J.Q., Savage, A.E., Zamudio, K.R., Rosenblum, E.B., 2009. Toward Immunogenetic Studies of Amphibian Chytridiomycosis: Linking Innate and Acquired Immunity. Bioscience 59.

Roberts, .A., 2003. Pathogen, host and environmental factors contributing to the pathogenesis of listeriosis. CMLS-Cellular-and-Molecular-Life-Sciences 60, 904.

Rohr, J.R., Raffel, T.R., Romansic, J.M., McCallum, H., Hudson, P.J., 2008. Evaluating the links between climate, disease spread, and amphibian declines. Proceedings of the National Academy of Sciences 105, 17436-17441.

Rollins-Smith, L.A., 1998. Metamorphosis and the amphibian immune system. Immunological Reviews 166, 221-230.

Ron, S., 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. Biotropica 37, 209-221.

Rosenblum, E.B., Poorten, T.J., Settles, M., Murdoch, G.K., Robert, J., Maddox, N., Eisen, M.B., 2009. Genome-wide transcriptional response *Xenopus tropicalis* to infection with the deadly chytrid fungus. PLoS One 4, e6494.

Rosenblum, E.B., Stajich, J.E., Maddox, N., Eisen, M.B., 2008. Global gene expression profiles for life stages of the deadly amphibian pathogen *Batrachochytrium dendrobatidis*. Proceedings of the National Academy of Sciences USA105, 17034-17039.

Rossignol, P.A., Orme-Zavaleta, J., Rossignol, A.M., 2006. Global climate change and its impact on disease embedded in ecological communities. Environmental Geosciences 13, 55-63.

Rowley, J.J.L., 2006. Why does Chytridiomycosis drive some frog populations to extinction and not others? The effects of interspecific variation in host behaviour, In School of Marine and Tropical Biology. p. 120. James Cook University, Townsville.

Rowley, J.J.L., Alford, R.A., 2007a. Behaviour of Australian rainforest stream frogs may affect the transmission of chytridiomycosis. Diseases of Aquatic Organisms 77, 1-9.

Rowley, J.J.L., Alford, R.A., 2007b. Movement patterns and habitat use of rainforest stream frogs in northern Queensland, Australia: implications for extinction vulnerability. Wildlife Research 34, 371-378.

Rowley, J.J.L., Alford, R.A., 2007c. Techniques for tracking amphibians: the effects of tag attachment, and harmonic direction finding versus radio telemetry. Amphibia-Reptilia 28, 367-376.

Rowley, J.J.L., Alford, R.A., 2009. Models in field studies of temperature and moisture, In Amphibian Ecology and Conservation: A Handbook of Techniques. ed. C.K.J. Dodd. Oxford University Press.

Rowley, J.J.L., Alford, R.A., Skerratt, L.F., 2006. The amphibian chytrid *Batrachochytrium dendrobatidis* occurs on freshwater shrimp in rain forest streams in northern Queensland, Australia. EcoHealth 3, 49-52.

Rowley, J.J.L., Hemingway, V.A., Alford, R.A., Waycott, M., Skerratt, L.F., Campbell, R., Webb, R., 2007a. Experimental infection and repeat survey data indicate the amphibian chytrid *Batrachochytrium dendrobatidis* may not occur on freshwater crustaceans in Northern Queensland, Australia. EcoHealth 4, 31-36.

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

Roy, B.A., Gusewell, S., Harte, J., 2004. Response of plant pathogens and herbivores to a warming experiment. Ecology 85, 2570-2581.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W., Hanski, I., 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392, 491-494.

Sasa, M., Solórzano, A., 1995. The reptiles and amphibians of Santa Rosa National Park, Costa Rica, with comments about the herpetofauna of xerophytic areas. Herpetological Natural History 3.

Savage, J.M., 2002. The amphibians and reptiles of Costa Rica: a herpetofauna between two continents, between two seas. University of Chicago Press, Chicago.

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

Schneider, C., Moritz, C., 1999. Rainforest refugia and evolution in Australia's Wet Tropics. Proceedings of The Royal Society of London Series B-Biological Sciences 266, 191-196.

Schneider, C.J., Cunningham, M., Moritz, C., 1998. Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rainforests of Australia. Molecular Ecology 7, 487-498.

Scott, M.E., 1988. The impact of infection and disease on animal populations: Implications for conservation biology. Conservation Biology 2, 40-56.

Şengör, A.M.C., Atayman, S., Özeren, S., 2008. A scale of greatness and causal classification of mass extinctions: Implications for mechanisms. Proceedings of the National Academy of Sciences USA 105, 13736-13740.

Shaffer, M.L., 1981. Minimum population sizes for species conservation. Bioscience 31, 131-134.

Simms, E.L., Triplett, J., 1994. Costs and benefits of plant responses to disease: Resistance and tolerance. Evolution 48, 1973-1985.

Skerratt, L., Berger, L., Speare, R., Cashins, S., McDonald, K., Phillott, A., Hines, H., Kenyon, N., 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4, 125-134.

Skerratt, L.F., Berger, L., Hines, H., McDonald, K.R., Mendez, D., Speare, R., 2008. Survey protocol for detecting chytridiomycosis in all Australian frog populations. Diseases of Aquatic Organisms 80, 85-94.

Smith, G., Dobson, A.P., 1992. Sexually transmitted diseases in animals. Parasitology Today 8, 159-166.

Smith, G.J.D., Bahl, J., Vijaykrishna, D., Zhang, J., Poon, L.L.M., Chen, H., Webster, R.G., Peiris, J.S.M., Guan, Y., 2009. Dating the emergence of pandemic influenza viruses. Proceedings of the National Academy of Sciences USA 106, 11709-11712.

Smith, K.F., Sax, D.F., Lafferty, K.D., 2006. Evidence for the role of infectious disease in species extinction and endangerment. Conservation Biology 20, 1349-1357.

Smith, K.G., Weldon, C., 2007. A conceptual framework for detecting oral chytridiomycosis in tadpoles. Copeia 2007, 1024-1028.

Snieszko, S.F., 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. Journal of Fish Biology 6, 197-208.

Speare, R., 2006. Threat Abatement Plan: Infection of amphibians with chytrid fungus resulting in chytridiomycosis, p. 22. Department of the Environment and Heritage, Canberra.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., Waller, R.W., 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306, 1783-1786.

Symonds, E.P., Hines, H.B., Bird, P.S., Morton, J.M., Mills, P.C., 2007. Surveillance for *Batrachochytrium dendrobatidis* using *Mixophyes* (Anura: Myobatrachidae) Larvae. Journal Of Wildlife Diseases 43, 48-60.

Symonds, E.P., Trott, D.J., Bird, P.S., Mills, P., 2008. Growth characteristics and enzyme activity in *Batrachochytrium dendrobatidis* isolates. Mycopathologia 166, 143-147.

Thomas, C.D., 1994. Extinction, Colonization, and Metapopulations: Environmental Tracking by Rare Species. Conservation Biology 8, 373-378.

Thomas, M.B., Blanford, S., 2003. Thermal biology in insect-parasite interactions. Trends In Ecology & Evolution 18, 344-350.

Thorne, E.T., Williams, E.S., 1988. Disease and endangered species: The Black-Footed Ferret as a recent example. Conservation Biology 2, 66-74.

Tobin, M.E., Dolbeer, R.A., 1990. Disappearence and recoverability of songbird carcasses in fruit orchards. Journal of Field Ornithology 61, 237-242.

Tukey, J.W., 1977. Exploratory Data Analysis. Addison-Wesley, Reading, MA.

Tyler, M.J., 1991. Species at risk: our vanishing frogs. Habitat 19, 20-25.

IUCN, Conservation International, and NatureServe. 2008. An Analysis of Amphibians on the 2008 IUCN Red List <www.iucnredlist.org/amphibians>. Downloaded on 6 October 2008.

van Riper III, C., Riper, S.G.v., Goff, M.L., Laird, M., 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecological Monographs 56, 327-344.

Viggers, K.L., Lindenmayer, D.B., Spratt, D.M., 1993. The importance of disease in reintroduction programmes. Wildlife Research 20, 687-698.

Voyles, J., Berger, L., Young, S., Speare, R., Webb, R., Warner, J., Rudd, D., Campbell, R., Skerratt, L.F., 2007. Electrolyte depletion and osmotic inbalance in amphibians with chytridiomycosis. Diseases of Aquatic Organisms 77, 113-118.

Voyles, J.L., 2009. Virulence and pathogenesis of chytridiomycosis: A lethal disease of amphibians, In School of Public Health and Tropical Medicine. p. 151. James Cook University, Townsville.

Vredenburg, V.T., 2004. Reversing introduced species effects: Experimental removal of introduced fish leads to rapid recovery of a declining frog. Proceedings of The National Academy of Sciences USA 101, 7646-7650.

Wake, D.B., 1991. Declining amphibian populations. Science 253, 860-860.

Walker, S.F., Baldi Salas, M., Jenkins, D.M., Garner, T.W.J., Cunningham, A.A., Hyatt, A.D., Bosch, J., Fisher, M.C., 2007. Environmental detection of *Batrachochytrium dendrobatidis* in a temperate climate. Diseases Of Aquatic Organisms 77, 105-112.

Walsh, P.D., Abernethy, K.A., Bermejo, M., Beyers, R., De Wachter, P., Akou, M.E., Huijbregts, B., Mambounga, D.I., Toham, A.K., Kilbourn, A.M., Lahm, S.A., Latour, S., Maisels, F., Mbina, C., Mihindou, Y., Ndong Obiang, S., Effa, E.N., Starkey, M.P., Telfer, P., Thibault, M., Tutin, C.E.G., White, L.J.T., Wilkie, D.S., 2003. Catastrophic ape decline in western equatorial Africa. Nature 422, 611-614.

Wang, T.T., Palese, P., 2009. Unraveling the mystery of swine influenza virus. Cell 137, 983-985.

Ward, M.R., Stallknecht, D.E., Willis, J., Conroy, M.J., Davidson, W.R., 2006. Wild bird mortality and West Nile Virus surveillance: Biases associated with detection, reporting, and carcass persistance. Journal of Wildlife Diseases 42, 92-106.

Warner, R.E., 1968. The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. The Condor 70, 101-120.

Warren, D.L., Glor, R.E., Turelli, M., 2008. Environmental Niche equivalency versus conservatism: Quantitative Approaches to Niche Evolution. Evolution 62, 2868-2883.

Weldon, C., Du Preez, L., 2004. Decline of the Kihansi Spray Toad, *Nectophrynoides asperginis*, from the Udzungwa Mountains, Tanzania. Froglog 62, 2-3.

Wilcox, B.A., Gubler, J., 2005. Disease ecology and the global emergence of zoonotic pathogens. Environmental Health and Preventive Medicine 10, 263-272.

Williams, C.J., Moffitt, C.M., 2001. A critique of methods of sampling and reporting pathogens in populations of Fish. Journal of Aquatic Animal Health 13, 300-309.

Williams, E., Mills, K., Kwiatkowski, D., Thorne, E., Boerger-Fields, A., 1994. Plague in a black-footed ferret (*Mustela nigripes*). Journal of Wildlife Diseases 30, 581-585.

Williams, E., Thorne, E., Appel, M., Belitsky, D., 1988. Canine distemper in black-footed ferrets (*Mustela nigripes*) from Wyoming. Journal of Wildlife Diseases 24, 385-398.

Williams, S.E., 2006. Vertebrates of the Wet Tropics Rainforests of Australia: Species Distribution and Biodiversity. Cooperative Research Centre for Tropical Rainforest Ecology and Management, Cairns, Australia.

Williams, S.E., Hero, J.-M., 1998. Rainforest frogs of the Australian wet tropics: guild classification and the ecological similarity of declining species. Proceedings of the Royal Society of London - Series B: Biological Sciences 265, 597-602.

Williams, S.E., Hero, J.-M., 2001. Multiple determinants of Australian tropical frog diversity. Biological Conservation 98, 1-10.

Williams, S.E., Pearson, R.G., 1997. Historical Rainforest Contractions, Localized Extinctions and Patterns of Vertebrate Endemism in the Rainforests of Australia's Wet Tropics. Proceedings of the Royal Society of London - Series B: Biological Sciences 264, 709-716.

Wisz, M.S., Hijmans, R.J., Li, J., Peterson, A.T., Graham, C.H., Guisan, A., 2008. Effects of sample size on the performance of species distribution models. Diversity And Distributions 14, 763-773.

Wobeser, G.A., Wobeser, A.G., 1992. Carcass disappearance and estimation of mortaility in simulated die-off of small birds. Journal Of Wildlife Diseases 28, 548-554.

Woodhams, D.C., 2003. The Ecology of Chytridiomycosis and the Emerging Infectious Disease of Australian Rainforest Frogs, In School of Tropical Biology. James Cook University, Townsville.

Woodhams, D.C., Alford, R.A., 2005. Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. Conservation Biology 19, 1449-1459.

Woodhams, D.C., Alford, R.A., Briggs, C.J., Johnson, M., Rollins-Smith, L.A., 2008a. Life history trade-offs influence disease in changing climates: strategies of an amphibian pathogen. Ecology 89, 1627-1639.

Woodhams, D.C., Alford, R.A., Marantelli, G., 2003. Emerging disease of amphibians cured by elevated body temperature. Diseases of Aquatic Organisms 55, 65-67.

Woodhams, D.C., Ardipradja, K., Alford, R.A., Marantelli, G., Reinert, L.K., Rollins-Smith, L.A., 2007a. Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. Animal Conservation 10, 409-417.

Woodhams, D.C., Kilburn, V.L., Reinert, L., Voyles, J., Medina, D., Ibàñez, R., Hyatt, A.D., Boyle, D.G., Pask, J., D., Green, D.M., Rollins-Smith, L.A., 2008b. Chytridiomycosis and amphibian population declines continue to spread eastward in Panama. EcoHealth 5, 268-274.

Woodhams, D.C., Rollins-Smith, L.A., Alford, R.A., Simon, M.A., Harris, R.N., 2007b. Innate immune defenses of amphibian skin: antimicrobial peptides and more. Animal Conservation 10, 425-428.

Woodhams, D.C., Rollins-Smith, L.A., Carey, C., Reinert, L., Tyler, M.J., Alford, R.A., 2005. Population trends associated with skin peptide defenses against chytridiomycosis in Australian frogs. Oecologia 146, 531-540.

Woodhams, D.C., Voyles, J., Lips, K.R., Carey, C., Rollins-Smith, L.A., 2006. Predicted disease susceptibility in a panamanian amphibian assembladge based on skin peptide defenses. Journal of Wildlife Diseases 42, 207-218.

Woodworth, B.L., Atkinson, C.T., LaPointe, D.A., Hart, P.J., Spiegel, C.S., Tweed, E.J., Henneman, C., LeBrun, J., Denette, T., DeMots, R., Kozar, K.L., Triglia, D., Lease, D., Gregor, A., Smith, T., Duffy, D., 2005. Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. Proceedings of The National Academy of Sciences USA 102, 1531-1536.

Young, B.E., Lips, K.R., Reaser, J.K., Ibanez, R., Salas, A.W., Cedeno, J.R., Coloma, L.A., Ron, S., La Marca, E., Meyer, J.R., Munoz, A., Bolaños, F., Chaves, G., Romo, D., 2001. Population declines and priorities for amphibian conservation in Latin America. Conservation Biology 15, 1213-1223.

Zar, J.H., 1999. Biostatistical Analysis, 4 edn. Prentice-Hall, Upper Saddle City, New Jersey.

Zell, R., 2004. Global climate change and the emergence/re-emergence of infectious diseases. International Journal of Medical Microbiology 293, 16-26.

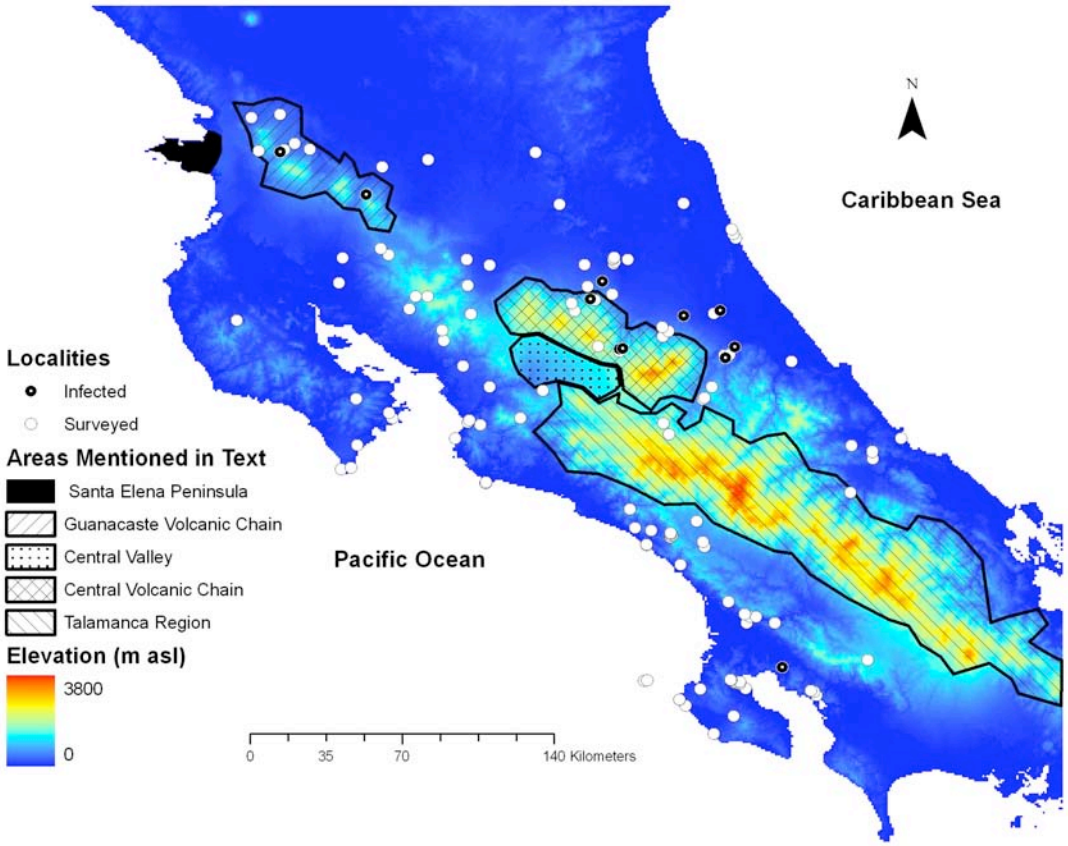
Zumbado-Ulate, H., Puschendorf, R., Chavarria, M.M., 2007. *Eleutherodactylus ranoides* (NCN). Distribution. Herpetological Review 38, 184-185.

Appendix 1. Species, percentage of individuals infected and total number of individuals surveyed for *Batrachochytrium dendrobatidis* in Costa Rica and used in chapter 2.

Species	% Infected	# Surveyed Individuals
Bufonidae		
Atelopus chiriquiensis	22.2	9
Atelopus senex	0.0	14
Atelopus varius	5.0	40
Incilius fastidiosus	0.0	2
Centrolenidae		
Centrolene prosoblepon	0.0	7
Hyalinobatrachium collymbiphyllum	0.0	2
Craugastoridae		
Craugastor andi	0.0	1
Craugastor bransfordii	17.6	17
Craugastor crassidigitus	6.3	16
Craugastor escoces	0.0	8
Craugastor fitzingeri	6.6	349
Craugastor megacephalus	0.0	5
Craugastor melanostictus	25.0	8
Craugastor podiciferus	5.3	19
Craugastor ranoides	0.0	9
Craugastor talamancae	40.0	5
Dendrobatidae		
Oophaga pumilio	16.7	6
Eleutherodactylidae		
Diasporus diastema	0.0	12
Diasporus hylaeformis	0.0	8
Hylidae		
Duellmanohyla rufioculis	0.0	2
Duellmanohyla uranochroa	16.7	6
Hylomantis lemur	0.0	1
Isthmohyla angustilineata	0.0	7
Isthmohyla calypsa	0.0	4
Isthmohyla pseudopuma	7.1	14
Isthmohyla rivularis	0.0	12
Smilisca phaeota	0.0	1
Smilisca sordida	0.0	7
Ranidae		
Lithobates vibicarius	52.4	21
Lithobates warszewitschii	0.0	4
Strabomantidae		
Pristimantis altae	0.0	2
Pristimantis caryophyllaceus	0.0	8
Pristimantis cerasinus	0.0	3
Pristimantis cruentus	0.0	15
Pristimantis ridens	0.0	3
Total	7.7	647

Appendix 2. Proportion of infected individuals of *Craugastor fitzingeri* between the Pacific and Atlantic versants used in chapter 2.

I compared the proportion of infected individuals relative to the total number of surveyed samples between the Pacific and Atlantic versants using *Craugastor fitzingeri,* the most densely sampled species in Costa Rica to date. *C. fitzingeri* is a common species that occurs in lowland to premontane areas of the country (0-1,520 m asl; Savage, 2002). Three hundred and fifty individuals were surveyed for the disease (22 infected/176 surveyed for Caribbean versant; 1/174 for the Pacific versant). Infection prevalence is significantly higher on the Caribbean slopes (χ^2=14.152, DF= 1, P= 0.0002; lower confidence limit for Caribean slopes 0.092, upper confidence limit 0.208; lower confidence limit for Pacific slopes > 0.001, upper confidence limit 0.032). The map below shows the distribution of infected and surveyed sites based on the *C. fitzingeri* data alone.



Appendix 3. Complete list of species and number of individuals utilized for the analysis in chapter 2.

Raw data used for the analysis. Latitude and longitude in decimal degrees. Elevation in m asl. Infected individuals depicted as one, non infected ones as zero.

Family	Species	Latitude	Longitude	Elevation	Infected
Bufonidae	*Atelopus chiriquiensis*	8.9456	-82.7574	1750	1
Bufonidae	*Atelopus chiriquiensis*	8.9456	-82.7574	1750	1
Bufonidae	*Atelopus chiriquiensis*	8.9456	-82.7574	1750	0
Bufonidae	*Atelopus chiriquiensis*	8.9456	-82.7574	1750	0
Bufonidae	*Atelopus chiriquiensis*	8.9456	-82.7574	1750	0
Bufonidae	*Atelopus chiriquiensis*	8.9456	-82.7574	1750	0
Bufonidae	*Atelopus chiriquiensis*	8.9456	-82.7574	1750	0
Bufonidae	*Atelopus chiriquiensis*	8.9456	-82.7574	1750	0
Bufonidae	*Atelopus chiriquiensis*	8.9456	-82.7574	1750	0
Bufonidae	*Atelopus senex*	10.0861	-84.0722	2100	0
Bufonidae	*Atelopus senex*	10.0861	-84.0722	2100	0
Bufonidae	*Atelopus senex*	10.0861	-84.0722	2100	0
Bufonidae	*Atelopus senex*	10.0861	-84.0722	2100	0
Bufonidae	*Atelopus senex*	10.0861	-84.0722	2100	0
Bufonidae	*Atelopus senex*	10.0861	-84.0722	2100	0
Bufonidae	*Atelopus senex*	10.0861	-84.0722	2100	0
Bufonidae	*Atelopus senex*	10.0861	-84.0722	2100	0
Bufonidae	*Atelopus senex*	10.0861	-84.0722	2100	0
Bufonidae	*Atelopus senex*	10.0861	-84.0722	2100	0
Bufonidae	*Atelopus senex*	10.0861	-84.0722	2100	0
Bufonidae	*Atelopus senex*	10.1778	-84.0972	2050	0
Bufonidae	*Atelopus senex*	10.1778	-84.0972	2050	0
Bufonidae	*Atelopus senex*	10.1778	-84.0972	2050	0
Bufonidae	*Atelopus varius*	10.2194	-84.6000	900	0
Bufonidae	*Atelopus varius*	10.2194	-84.6000	900	0
Bufonidae	*Atelopus varius*	10.2194	-84.6000	900	0
Bufonidae	*Atelopus varius*	10.2194	-84.6000	900	0
Bufonidae	*Atelopus varius*	10.2194	-84.6000	900	0
Bufonidae	*Atelopus varius*	10.2194	-84.6000	900	0
Bufonidae	*Atelopus varius*	10.2194	-84.6000	900	0
Bufonidae	*Atelopus varius*	10.2194	-84.6000	900	0
Bufonidae	*Atelopus varius*	10.2194	-84.6000	900	0
Bufonidae	*Atelopus varius*	10.2194	-84.6000	900	0
Bufonidae	*Atelopus varius*	10.7667	-85.2806	810	0
Bufonidae	*Atelopus varius*	10.7667	-85.2806	810	0
Bufonidae	*Atelopus varius*	10.9132	-85.4633	1000	0
Bufonidae	*Atelopus varius*	8.9417	-82.8361	1480	0
Bufonidae	*Atelopus varius*	8.9106	-82.8481	1300	0
Bufonidae	*Atelopus varius*	8.9417	-82.8361	1480	0
Bufonidae	*Atelopus varius*	8.9417	-82.8361	1480	0
Bufonidae	*Atelopus varius*	8.9417	-82.8361	1480	0
Bufonidae	*Atelopus varius*	8.9417	-82.8361	1480	0
Bufonidae	*Atelopus varius*	8.9735	-82.8403	1700	0
Bufonidae	*Atelopus varius*	8.9735	-82.8403	1700	0

Bufonidae	*Atelopus varius*	9.4333	-83.6333	1000	0
Bufonidae	*Atelopus varius*	9.4275	-83.7053	40	1
Bufonidae	*Atelopus varius*	8.9778	-83.4667	120	0
Bufonidae	*Atelopus varius*	8.9417	-82.8361	1480	0
Bufonidae	*Atelopus varius*	8.9417	-82.8361	1480	0
Bufonidae	*Atelopus varius*	8.9417	-82.8361	1480	0
Bufonidae	*Atelopus varius*	8.9417	-82.8361	1480	0
Bufonidae	*Atelopus varius*	10.2845	-84.1437	115	1
Bufonidae	*Atelopus varius*	10.2554	-84.1022	1300	0
Bufonidae	*Atelopus varius*	10.2554	-84.1022	1300	0
Bufonidae	*Atelopus varius*	10.2361	-84.1028	1500	0
Bufonidae	*Atelopus varius*	10.2361	-84.1028	1500	0
Bufonidae	*Atelopus varius*	10.2361	-84.1028	1500	0
Bufonidae	*Atelopus varius*	10.2361	-84.1028	1500	0
Bufonidae	*Atelopus varius*	10.2361	-84.1028	1500	0
Bufonidae	*Atelopus varius*	10.3556	-84.0556	280	0
Bufonidae	*Atelopus varius*	10.2554	-84.1022	1300	0
Bufonidae	*Atelopus varius*	10.2361	-84.1028	1500	0
Bufonidae	*Atelopus varius*	10.2554	-84.1022	1300	0
Bufonidae	*Incilius fastidiosus*	8.9456	-82.7574	1750	0
Bufonidae	*Incilius fastidiosus*	8.9456	-82.7574	1750	0
Centrolenidae	*Centrolene prosoblepon*	10.2785	-84.0853	960	0
Centrolenidae	*Centrolene prosoblepon*	10.2361	-84.1028	1500	0
Centrolenidae	*Centrolene prosoblepon*	10.2361	-84.1028	1500	0
Centrolenidae	*Centrolene prosoblepon*	10.2361	-84.1028	1500	0
Centrolenidae	*Centrolene prosoblepon*	10.2361	-84.1028	1500	0
Centrolenidae	*Centrolene prosoblepon*	10.2075	-84.1346	1700	0
Centrolenidae	*Centrolene prosoblepon*	10.2361	-84.1028	1500	0
Centrolenidae	*Hyalinobatrachium colymbiphyllum*	10.23610	-84.10280	1500	0
Centrolenidae	*Hyalinobatrachium colymbiphyllum*	10.23610	-84.10280	1500	0
Craugastoridae	*Craugastor andi*	10.2554	-84.1022	1300	0
Craugastoridae	*Craugastor bransfordii*	10.3556	-84.0556	280	0
Craugastoridae	*Craugastor bransfordii*	10.3350	-84.1158	700	0
Craugastoridae	*Craugastor bransfordii*	10.3350	-84.1158	700	0
Craugastoridae	*Craugastor bransfordii*	10.3350	-84.1158	700	0
Craugastoridae	*Craugastor bransfordii*	10.2833	-84.1056	960	0
Craugastoridae	*Craugastor bransfordii*	10.2357	-83.5673	80	1
Craugastoridae	*Craugastor bransfordii*	10.2357	-83.5673	80	0
Craugastoridae	*Craugastor bransfordii*	10.0431	-83.5486	700	1
Craugastoridae	*Craugastor bransfordii*	10.0431	-83.5486	700	0
Craugastoridae	*Craugastor bransfordii*	10.3556	-84.0556	280	0
Craugastoridae	*Craugastor bransfordii*	10.3556	-84.0556	280	0
Craugastoridae	*Craugastor bransfordii*	10.3556	-84.0556	280	0
Craugastoridae	*Craugastor bransfordii*	10.3556	-84.0556	280	1
Craugastoridae	*Craugastor bransfordii*	10.3566	-84.0674	280	0
Craugastoridae	*Craugastor bransfordii*	10.3566	-84.0674	280	0
Craugastoridae	*Craugastor bransfordii*	10.3350	-84.1158	700	0
Craugastoridae	*Craugastor bransfordii*	10.2554	-84.1022	1300	0
Craugastoridae	*Craugastor crassidigitus*	10.2787	-84.0850	960	0
Craugastoridae	*Craugastor crassidigitus*	10.2787	-84.0850	960	0
Craugastoridae	*Craugastor crassidigitus*	10.2785	-84.0853	960	0
Craugastoridae	*Craugastor crassidigitus*	10.0431	-83.5486	700	0

Craugastoridae	*Craugastor crassidigitus*	10.0431	-83.5486	700	0
Craugastoridae	*Craugastor crassidigitus*	10.0431	-83.5486	700	0
Craugastoridae	*Craugastor crassidigitus*	10.0431	-83.5486	700	0
Craugastoridae	*Craugastor crassidigitus*	10.0431	-83.5486	700	0
Craugastoridae	*Craugastor crassidigitus*	10.0431	-83.5486	700	1
Craugastoridae	*Craugastor crassidigitus*	10.2787	-84.0850	1000	0
Craugastoridae	*Craugastor crassidigitus*	10.2787	-84.0850	1000	0
Craugastoridae	*Craugastor crassidigitus*	10.2787	-84.0850	1000	0
Craugastoridae	*Craugastor crassidigitus*	10.2787	-84.0850	1000	0
Craugastoridae	*Craugastor crassidigitus*	10.2787	-84.0850	1000	0
Craugastoridae	*Craugastor crassidigitus*	10.2785	-84.0853	960	0
Craugastoridae	*Craugastor crassidigitus*	10.2785	-84.0853	960	0
Craugastoridae	*Craugastor escoces*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor escoces*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor escoces*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor escoces*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor escoces*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor escoces*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor escoces*	10.2554	-84.1022	1300	0
Craugastoridae	*Craugastor escoces*	10.1778	-84.0972	2050	0
Craugastoridae	*Craugastor fitzingeri*	10.4500	-84.0111	50	0
Craugastoridae	*Craugastor fitzingeri*	10.4500	-84.0111	50	0
Craugastoridae	*Craugastor fitzingeri*	10.4389	-84.0028	50	0
Craugastoridae	*Craugastor fitzingeri*	10.1658	-83.8076	440	0
Craugastoridae	*Craugastor fitzingeri*	10.0056	-84.6306	250	0
Craugastoridae	*Craugastor fitzingeri*	10.0056	-84.6306	250	0
Craugastoridae	*Craugastor fitzingeri*	10.4656	-84.9396	650	0
Craugastoridae	*Craugastor fitzingeri*	10.4656	-84.9396	650	0
Craugastoridae	*Craugastor fitzingeri*	10.4528	-85.1306	40	0
Craugastoridae	*Craugastor fitzingeri*	8.6917	-83.5056	25	0
Craugastoridae	*Craugastor fitzingeri*	8.6917	-83.5056	25	0
Craugastoridae	*Craugastor fitzingeri*	10.3500	-85.1444	45	0
Craugastoridae	*Craugastor fitzingeri*	8.9417	-83.4583	16	0
Craugastoridae	*Craugastor fitzingeri*	10.2667	-84.1833	830	0
Craugastoridae	*Craugastor fitzingeri*	10.2667	-84.1833	830	0
Craugastoridae	*Craugastor fitzingeri*	10.0644	-83.9851	1260	0
Craugastoridae	*Craugastor fitzingeri*	10.0644	-83.9851	1260	0
Craugastoridae	*Craugastor fitzingeri*	8.6944	-83.5083	40	0
Craugastoridae	*Craugastor fitzingeri*	8.6944	-83.5083	40	0
Craugastoridae	*Craugastor fitzingeri*	8.6944	-83.5083	40	0
Craugastoridae	*Craugastor fitzingeri*	9.8667	-83.6389	580	0
Craugastoridae	*Craugastor fitzingeri*	9.8722	-83.6333	600	0
Craugastoridae	*Craugastor fitzingeri*	8.6889	-83.4889	20	0
Craugastoridae	*Craugastor fitzingeri*	10.0644	-83.9851	1260	0
Craugastoridae	*Craugastor fitzingeri*	8.6972	-83.4861	2	0
Craugastoridae	*Craugastor fitzingeri*	9.7217	-83.7800	1555	0
Craugastoridae	*Craugastor fitzingeri*	9.7667	-83.8028	1200	0
Craugastoridae	*Craugastor fitzingeri*	8.7042	-83.8704	20	0
Craugastoridae	*Craugastor fitzingeri*	8.7042	-83.8704	20	0
Craugastoridae	*Craugastor fitzingeri*	8.7042	-83.8704	20	0
Craugastoridae	*Craugastor fitzingeri*	8.7042	-83.8704	20	0
Craugastoridae	*Craugastor fitzingeri*	8.7042	-83.8704	20	0
Craugastoridae	*Craugastor fitzingeri*	8.7042	-83.8704	20	0
Craugastoridae	*Craugastor fitzingeri*	8.7042	-83.8704	20	0

Craugastoridae	*Craugastor fitzingeri*	8.7042	-83.8704	20	0
Craugastoridae	*Craugastor fitzingeri*	8.7042	-83.8704	20	0
Craugastoridae	*Craugastor fitzingeri*	10.4918	-84.9722	485	0
Craugastoridae	*Craugastor fitzingeri*	10.4918	-84.9722	485	0
Craugastoridae	*Craugastor fitzingeri*	10.4918	-84.9722	485	0
Craugastoridae	*Craugastor fitzingeri*	10.4918	-84.9722	485	0
Craugastoridae	*Craugastor fitzingeri*	10.4918	-84.9722	485	0
Craugastoridae	*Craugastor fitzingeri*	10.4918	-84.9722	485	0
Craugastoridae	*Craugastor fitzingeri*	9.9178	-84.5222	180	0
Craugastoridae	*Craugastor fitzingeri*	9.9178	-84.5222	180	0
Craugastoridae	*Craugastor fitzingeri*	10.4556	-84.0056	50	0
Craugastoridae	*Craugastor fitzingeri*	10.4556	-84.0056	50	0
Craugastoridae	*Craugastor fitzingeri*	10.4918	-84.9722	485	0
Craugastoridae	*Craugastor fitzingeri*	10.4918	-84.9722	485	0
Craugastoridae	*Craugastor fitzingeri*	10.4918	-84.9722	485	0
Craugastoridae	*Craugastor fitzingeri*	9.2778	-83.6389	560	0
Craugastoridae	*Craugastor fitzingeri*	10.1528	-84.7167	850	0
Craugastoridae	*Craugastor fitzingeri*	9.2583	-83.6333	520	0
Craugastoridae	*Craugastor fitzingeri*	9.8111	-84.9389	12	0
Craugastoridae	*Craugastor fitzingeri*	9.8111	-84.9389	12	0
Craugastoridae	*Craugastor fitzingeri*	9.8111	-84.9389	12	0
Craugastoridae	*Craugastor fitzingeri*	10.1528	-84.7167	850	0
Craugastoridae	*Craugastor fitzingeri*	10.8972	-85.4778	750	0
Craugastoridae	*Craugastor fitzingeri*	10.4250	-84.1278	200	0
Craugastoridae	*Craugastor fitzingeri*	10.4250	-84.1278	200	0
Craugastoridae	*Craugastor fitzingeri*	10.4250	-84.1278	200	0
Craugastoridae	*Craugastor fitzingeri*	9.3139	-83.7722	880	0
Craugastoridae	*Craugastor fitzingeri*	11.0361	-85.5083	300	0
Craugastoridae	*Craugastor fitzingeri*	11.0361	-85.5083	300	0
Craugastoridae	*Craugastor fitzingeri*	11.0361	-85.5083	300	0
Craugastoridae	*Craugastor fitzingeri*	11.0361	-85.5083	300	0
Craugastoridae	*Craugastor fitzingeri*	11.0361	-85.5083	300	0
Craugastoridae	*Craugastor fitzingeri*	11.0361	-85.5083	300	0
Craugastoridae	*Craugastor fitzingeri*	8.5978	-83.7092	20	0
Craugastoridae	*Craugastor fitzingeri*	8.6250	-83.7357	80	0
Craugastoridae	*Craugastor fitzingeri*	8.6250	-83.7357	80	0
Craugastoridae	*Craugastor fitzingeri*	8.6239	-83.7365	20	0
Craugastoridae	*Craugastor fitzingeri*	8.6250	-83.7357	80	0
Craugastoridae	*Craugastor fitzingeri*	9.7056	-82.8194	25	0
Craugastoridae	*Craugastor fitzingeri*	9.3056	-83.7722	900	0
Craugastoridae	*Craugastor fitzingeri*	9.3056	-83.7722	900	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	10.2333	-84.1694	1200	0
Craugastoridae	*Craugastor fitzingeri*	8.9778	-83.4667	120	0
Craugastoridae	*Craugastor fitzingeri*	10.0644	-83.9851	1260	0
Craugastoridae	*Craugastor fitzingeri*	8.9667	-83.4417	30	0
Craugastoridae	*Craugastor fitzingeri*	8.9667	-83.4194	40	0
Craugastoridae	*Craugastor fitzingeri*	10.0250	-83.2722	19	0
Craugastoridae	*Craugastor fitzingeri*	9.7806	-84.6056	40	0
Craugastoridae	*Craugastor fitzingeri*	9.7806	-84.6056	40	0
Craugastoridae	*Craugastor fitzingeri*	9.7806	-84.6056	40	0
Craugastoridae	*Craugastor fitzingeri*	9.7806	-84.6056	40	0
Craugastoridae	*Craugastor fitzingeri*	9.7806	-84.6056	40	0
Craugastoridae	*Craugastor fitzingeri*	9.7806	-84.6056	40	0

Craugastoridae	*Craugastor fitzingeri*	9.7806	-84.6056	40	0
Craugastoridae	*Craugastor fitzingeri*	9.7806	-84.6056	40	0
Craugastoridae	*Craugastor fitzingeri*	9.7806	-84.6056	40	0
Craugastoridae	*Craugastor fitzingeri*	9.7806	-84.6056	40	0
Craugastoridae	*Craugastor fitzingeri*	8.9417	-83.3417	80	0
Craugastoridae	*Craugastor fitzingeri*	10.0774	-83.9723	1000	0
Craugastoridae	*Craugastor fitzingeri*	10.0774	-83.9723	1000	1
Craugastoridae	*Craugastor fitzingeri*	9.4833	-83.0278	700	0
Craugastoridae	*Craugastor fitzingeri*	9.4833	-83.0278	700	0
Craugastoridae	*Craugastor fitzingeri*	10.2944	-84.7778	1400	0
Craugastoridae	*Craugastor fitzingeri*	10.2944	-84.7778	1400	0
Craugastoridae	*Craugastor fitzingeri*	8.7889	-82.9583	1200	0
Craugastoridae	*Craugastor fitzingeri*	8.6694	-83.4611	4	0
Craugastoridae	*Craugastor fitzingeri*	10.0750	-83.9833	1400	0
Craugastoridae	*Craugastor fitzingeri*	10.0750	-83.9833	1400	1
Craugastoridae	*Craugastor fitzingeri*	10.0750	-83.9833	1400	0
Craugastoridae	*Craugastor fitzingeri*	10.0750	-83.9833	1400	0
Craugastoridae	*Craugastor fitzingeri*	10.0750	-83.9833	1400	1
Craugastoridae	*Craugastor fitzingeri*	10.0750	-83.9833	1400	0
Craugastoridae	*Craugastor fitzingeri*	9.9194	-83.6028	700	0
Craugastoridae	*Craugastor fitzingeri*	10.0861	-84.0722	2110	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	8.6422	-83.1694	5	0
Craugastoridae	*Craugastor fitzingeri*	8.6422	-83.1694	5	0
Craugastoridae	*Craugastor fitzingeri*	10.4472	-83.9500	40	0
Craugastoridae	*Craugastor fitzingeri*	10.4472	-83.9500	40	0
Craugastoridae	*Craugastor fitzingeri*	10.4472	-83.9500	40	0
Craugastoridae	*Craugastor fitzingeri*	9.5861	-85.0944	20	0
Craugastoridae	*Craugastor fitzingeri*	9.5861	-85.0944	20	0
Craugastoridae	*Craugastor fitzingeri*	9.5861	-85.0944	20	0
Craugastoridae	*Craugastor fitzingeri*	9.5861	-85.0944	20	0
Craugastoridae	*Craugastor fitzingeri*	9.5861	-85.0944	20	0
Craugastoridae	*Craugastor fitzingeri*	9.5861	-85.0944	20	0
Craugastoridae	*Craugastor fitzingeri*	9.5861	-85.0944	20	0
Craugastoridae	*Craugastor fitzingeri*	9.5861	-85.0944	20	0
Craugastoridae	*Craugastor fitzingeri*	10.5528	-83.5139	1	0
Craugastoridae	*Craugastor fitzingeri*	10.5361	-83.5028	1	0
Craugastoridae	*Craugastor fitzingeri*	10.5361	-83.5028	1	0
Craugastoridae	*Craugastor fitzingeri*	10.5361	-83.5028	1	0
Craugastoridae	*Craugastor fitzingeri*	10.5722	-83.5194	1	0
Craugastoridae	*Craugastor fitzingeri*	10.5722	-83.5194	1	0
Craugastoridae	*Craugastor fitzingeri*	10.5722	-83.5194	1	0
Craugastoridae	*Craugastor fitzingeri*	10.8611	-84.7750	35	0
Craugastoridae	*Craugastor fitzingeri*	10.9014	-85.3722	500	0
Craugastoridae	*Craugastor fitzingeri*	10.7171	-85.0338	700	0
Craugastoridae	*Craugastor fitzingeri*	10.7171	-85.0338	700	1
Craugastoridae	*Craugastor fitzingeri*	10.7171	-85.0338	700	0
Craugastoridae	*Craugastor fitzingeri*	10.8306	-84.9653	120	0

Craugastoridae	*Craugastor fitzingeri*	10.7171	-85.0338	700	0
Craugastoridae	*Craugastor fitzingeri*	10.9278	-85.3292	350	0
Craugastoridae	*Craugastor fitzingeri*	10.9278	-85.3292	350	0
Craugastoridae	*Craugastor fitzingeri*	11.0486	-85.3883	350	0
Craugastoridae	*Craugastor fitzingeri*	10.7171	-85.0338	700	0
Craugastoridae	*Craugastor fitzingeri*	10.9035	-85.2649	450	0
Craugastoridae	*Craugastor fitzingeri*	10.9035	-85.2649	450	0
Craugastoridae	*Craugastor fitzingeri*	10.9035	-85.2649	450	0
Craugastoridae	*Craugastor fitzingeri*	10.9035	-85.2649	450	0
Craugastoridae	*Craugastor fitzingeri*	10.9035	-85.2649	450	0
Craugastoridae	*Craugastor fitzingeri*	10.3028	-84.0139	440	0
Craugastoridae	*Craugastor fitzingeri*	10.0644	-83.9851	1260	0
Craugastoridae	*Craugastor fitzingeri*	9.7889	-84.9250	3	0
Craugastoridae	*Craugastor fitzingeri*	10.6803	-83.7184	100	0
Craugastoridae	*Craugastor fitzingeri*	10.6803	-83.7184	100	0
Craugastoridae	*Craugastor fitzingeri*	10.2230	-83.5946	100	0
Craugastoridae	*Craugastor fitzingeri*	9.5222	-84.5389	2	0
Craugastoridae	*Craugastor fitzingeri*	9.7889	-84.3944	900	0
Craugastoridae	*Craugastor fitzingeri*	9.5252	-84.5367	20	0
Craugastoridae	*Craugastor fitzingeri*	10.2194	-84.6000	900	0
Craugastoridae	*Craugastor fitzingeri*	8.4806	-83.5944	5	0
Craugastoridae	*Craugastor fitzingeri*	9.5252	-84.5367	20	0
Craugastoridae	*Craugastor fitzingeri*	8.5556	-83.5111	260	0
Craugastoridae	*Craugastor fitzingeri*	10.2194	-84.6000	900	0
Craugastoridae	*Craugastor fitzingeri*	10.2194	-84.6000	900	0
Craugastoridae	*Craugastor fitzingeri*	10.2194	-84.6000	900	0
Craugastoridae	*Craugastor fitzingeri*	10.2194	-84.6000	900	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	8.6972	-83.5139	40	0
Craugastoridae	*Craugastor fitzingeri*	8.6972	-83.5139	40	0
Craugastoridae	*Craugastor fitzingeri*	9.5861	-85.0944	20	0
Craugastoridae	*Craugastor fitzingeri*	8.6556	-83.1806	35	0
Craugastoridae	*Craugastor fitzingeri*	8.6556	-83.1806	35	0
Craugastoridae	*Craugastor fitzingeri*	8.6556	-83.1806	35	0
Craugastoridae	*Craugastor fitzingeri*	8.6556	-83.1806	35	0
Craugastoridae	*Craugastor fitzingeri*	8.6556	-83.1806	35	0
Craugastoridae	*Craugastor fitzingeri*	9.7889	-84.9250	3	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	8.7028	-83.5222	80	0
Craugastoridae	*Craugastor fitzingeri*	10.1955	-85.5668	600	0
Craugastoridae	*Craugastor fitzingeri*	9.5806	-85.1361	10	0
Craugastoridae	*Craugastor fitzingeri*	9.6750	-83.0250	100	0
Craugastoridae	*Craugastor fitzingeri*	8.7575	-83.3103	100	1
Craugastoridae	*Craugastor fitzingeri*	8.7575	-83.3103	100	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	10.4306	-84.0083	40	0
Craugastoridae	*Craugastor fitzingeri*	10.4222	-84.5222	80	0
Craugastoridae	*Craugastor fitzingeri*	10.4222	-84.5222	80	0
Craugastoridae	*Craugastor fitzingeri*	10.6803	-83.7184	100	0
Craugastoridae	*Craugastor fitzingeri*	10.6803	-83.7184	100	0
Craugastoridae	*Craugastor fitzingeri*	10.6803	-83.7184	100	0
Craugastoridae	*Craugastor fitzingeri*	10.6803	-83.7184	100	0
Craugastoridae	*Craugastor fitzingeri*	9.3639	-83.6639	670	0

Craugastoridae	*Craugastor fitzingeri*	10.6750	-84.2333	31	0
Craugastoridae	*Craugastor fitzingeri*	10.0836	-83.5078	62	1
Craugastoridae	*Craugastor fitzingeri*	10.0836	-83.5078	62	1
Craugastoridae	*Craugastor fitzingeri*	10.0836	-83.5078	62	1
Craugastoridae	*Craugastor fitzingeri*	10.0836	-83.5078	62	0
Craugastoridae	*Craugastor fitzingeri*	10.2194	-84.6000	900	0
Craugastoridae	*Craugastor fitzingeri*	10.8909	-84.3318	40	0
Craugastoridae	*Craugastor fitzingeri*	10.4472	-84.6167	140	0
Craugastoridae	*Craugastor fitzingeri*	10.2944	-84.8278	900	0
Craugastoridae	*Craugastor fitzingeri*	10.4472	-84.6167	140	0
Craugastoridae	*Craugastor fitzingeri*	10.4472	-84.6167	140	0
Craugastoridae	*Craugastor fitzingeri*	10.4472	-84.6167	140	0
Craugastoridae	*Craugastor fitzingeri*	8.6250	-83.7357	80	0
Craugastoridae	*Craugastor fitzingeri*	10.3389	-84.6111	625	0
Craugastoridae	*Craugastor fitzingeri*	9.6222	-82.9361	600	0
Craugastoridae	*Craugastor fitzingeri*	10.3556	-84.0556	280	1
Craugastoridae	*Craugastor fitzingeri*	10.3350	-84.1158	700	0
Craugastoridae	*Craugastor fitzingeri*	10.2833	-84.1056	960	1
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	1
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	9.6500	-82.9389	300	0
Craugastoridae	*Craugastor fitzingeri*	10.2417	-84.8528	170	0
Craugastoridae	*Craugastor fitzingeri*	10.2417	-84.8528	170	0
Craugastoridae	*Craugastor fitzingeri*	10.2417	-84.8528	170	0
Craugastoridae	*Craugastor fitzingeri*	9.8689	-85.0742	80	0
Craugastoridae	*Craugastor fitzingeri*	9.8689	-85.0742	80	0
Craugastoridae	*Craugastor fitzingeri*	9.8689	-85.0742	80	0
Craugastoridae	*Craugastor fitzingeri*	8.6611	-83.1917	17	0
Craugastoridae	*Craugastor fitzingeri*	10.1111	-84.7111	620	0
Craugastoridae	*Craugastor fitzingeri*	9.0306	-83.5336	560	0
Craugastoridae	*Craugastor fitzingeri*	9.0306	-83.5336	560	0
Craugastoridae	*Craugastor fitzingeri*	9.1822	-83.7323	220	0
Craugastoridae	*Craugastor fitzingeri*	9.2639	-83.8722	10	0
Craugastoridae	*Craugastor fitzingeri*	9.2667	-83.8722	100	0
Craugastoridae	*Craugastor fitzingeri*	9.2667	-83.8722	100	0
Craugastoridae	*Craugastor fitzingeri*	9.2667	-83.8722	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7611	-84.5611	500	0
Craugastoridae	*Craugastor fitzingeri*	9.7722	-84.6111	40	0
Craugastoridae	*Craugastor fitzingeri*	9.4125	-83.9419	428	0
Craugastoridae	*Craugastor fitzingeri*	9.4125	-83.9419	428	0
Craugastoridae	*Craugastor fitzingeri*	9.3028	-83.7667	1000	0
Craugastoridae	*Craugastor fitzingeri*	9.3028	-83.7667	1000	0
Craugastoridae	*Craugastor fitzingeri*	9.3361	-83.9194	350	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	9.2667	-83.8722	100	0
Craugastoridae	*Craugastor fitzingeri*	9.2667	-83.8722	100	0
Craugastoridae	*Craugastor fitzingeri*	9.3250	-83.8528	100	0
Craugastoridae	*Craugastor fitzingeri*	9.2972	-83.7739	700	0
Craugastoridae	*Craugastor fitzingeri*	10.1278	-83.8042	650	0
Craugastoridae	*Craugastor fitzingeri*	10.1500	-83.7806	650	0
Craugastoridae	*Craugastor fitzingeri*	10.2357	-83.5673	80	1
Craugastoridae	*Craugastor fitzingeri*	10.2357	-83.5673	80	1
Craugastoridae	*Craugastor fitzingeri*	10.2357	-83.5673	80	1

Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.3122	-83.7719	930	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.6767	-85.0693	100	0
Craugastoridae	*Craugastor fitzingeri*	8.6668	-83.6501	100	0
Craugastoridae	*Craugastor fitzingeri*	8.7006	-83.8837	120	0
Craugastoridae	*Craugastor fitzingeri*	8.7053	-83.5239	126	0
Craugastoridae	*Craugastor fitzingeri*	9.6767	-85.0693	100	0
Craugastoridae	*Craugastor fitzingeri*	9.6767	-85.0693	100	0
Craugastoridae	*Craugastor fitzingeri*	9.6767	-85.0693	100	0
Craugastoridae	*Craugastor fitzingeri*	9.6767	-85.0693	100	0
Craugastoridae	*Craugastor fitzingeri*	9.6767	-85.0693	100	0
Craugastoridae	*Craugastor fitzingeri*	9.6767	-85.0693	100	0
Craugastoridae	*Craugastor fitzingeri*	9.6767	-85.0693	100	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	1
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	1
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	1
Craugastoridae	*Craugastor fitzingeri*	10.8936	-85.3889	500	1
Craugastoridae	*Craugastor fitzingeri*	10.8936	-85.3889	500	1
Craugastoridae	*Craugastor fitzingeri*	10.8936	-85.3889	500	1
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	1
Craugastoridae	*Craugastor fitzingeri*	10.3556	-84.0556	280	1
Craugastoridae	*Craugastor fitzingeri*	10.3350	-84.1158	700	0
Craugastoridae	*Craugastor fitzingeri*	10.2785	-84.0853	960	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	9.7045	-84.6641	100	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0

Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.2140	-83.7184	200	0
Craugastoridae	*Craugastor fitzingeri*	10.8936	-85.3889	500	0
Craugastoridae	*Craugastor fitzingeri*	10.8936	-85.3889	500	0
Craugastoridae	*Craugastor fitzingeri*	10.8936	-85.3889	500	0
Craugastoridae	*Craugastor fitzingeri*	10.8936	-85.3889	500	0
Craugastoridae	*Craugastor fitzingeri*	10.8936	-85.3889	500	0
Craugastoridae	*Craugastor fitzingeri*	10.0511	-83.5286	513	0
Craugastoridae	*Craugastor fitzingeri*	10.0511	-83.5286	513	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	9.9043	-84.3012	550	0
Craugastoridae	*Craugastor fitzingeri*	10.0500	-83.5503	600	0
Craugastoridae	*Craugastor fitzingeri*	10.0500	-83.5503	600	0
Craugastoridae	*Craugastor fitzingeri*	10.0500	-83.5503	600	0
Craugastoridae	*Craugastor fitzingeri*	10.0500	-83.5503	600	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.0389	-83.5472	745	0
Craugastoridae	*Craugastor fitzingeri*	10.2194	-84.6000	900	0
Craugastoridae	*Craugastor fitzingeri*	10.89360	-85.38890	500	1
Craugastoridae	*Craugastor fitzingeri*	10.2194	-84.6000	900	0
Craugastoridae	*Craugastor megacephalus*	10.3566	-84.0674	280	0
Craugastoridae	*Craugastor megacephalus*	10.3350	-84.1158	700	0
Craugastoridae	*Craugastor megacephalus*	10.3350	-84.1158	700	0
Craugastoridae	*Craugastor megacephalus*	10.3566	-84.0674	280	0
Craugastoridae	*Craugastor megacephalus*	10.3350	-84.1158	700	0
Craugastoridae	*Craugastor melanostictus*	8.9456	-82.7574	1750	1
Craugastoridae	*Craugastor melanostictus*	8.9456	-82.7574	1750	1
Craugastoridae	*Craugastor melanostictus*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor melanostictus*	10.2075	-84.1346	1700	0

Craugastoridae	*Craugastor melanostictus*	8.9456	-82.7574	1750	0
Craugastoridae	*Craugastor melanostictus*	8.9456	-82.7574	1750	0
Craugastoridae	*Craugastor melanostictus*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor melanostictus*	10.2075	-84.1346	1700	0
Craugastoridae	*Craugastor podiciferus*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor podiciferus*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor podiciferus*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor podiciferus*	10.2075	-84.1346	1700	0
Craugastoridae	*Craugastor podiciferus*	10.2075	-84.1346	1700	0
Craugastoridae	*Craugastor podiciferus*	10.2075	-84.1346	1700	0
Craugastoridae	*Craugastor podiciferus*	10.2075	-84.1346	1700	0
Craugastoridae	*Craugastor podiciferus*	10.2075	-84.1346	1700	0
Craugastoridae	*Craugastor podiciferus*	10.1512	-84.1304	90	1
Craugastoridae	*Craugastor podiciferus*	10.2075	-84.1346	1700	0
Craugastoridae	*Craugastor podiciferus*	10.2075	-84.1346	1700	0
Craugastoridae	*Craugastor podiciferus*	10.2075	-84.1346	1700	0
Craugastoridae	*Craugastor podiciferus*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor podiciferus*	10.2361	-84.1028	1500	0
Craugastoridae	*Craugastor podiciferus*	10.2075	-84.1346	1700	0
Craugastoridae	*Craugastor podiciferus*	10.2554	-84.1022	1300	0
Craugastoridae	*Craugastor podiciferus*	10.2075	-84.1346	1700	0
Craugastoridae	*Craugastor podiciferus*	10.1778	-84.0972	2050	0
Craugastoridae	*Craugastor podiciferus*	10.1778	-84.0972	2050	0
Craugastoridae	*Craugastor ranoides*	10.8996	-85.7328	100	0
Craugastoridae	*Craugastor ranoides*	10.8996	-85.7328	100	0
Craugastoridae	*Craugastor ranoides*	10.8996	-85.7328	100	0
Craugastoridae	*Craugastor ranoides*	10.8996	-85.7328	100	0
Craugastoridae	*Craugastor ranoides*	10.8996	-85.7328	100	0
Craugastoridae	*Craugastor ranoides*	10.8996	-85.7328	100	0
Craugastoridae	*Craugastor ranoides*	10.8996	-85.7328	100	0
Craugastoridae	*Craugastor ranoides*	10.8996	-85.7328	100	0
Craugastoridae	*Craugastor ranoides*	10.8996	-85.7328	100	0
Craugastoridae	*Craugastor talamancae*	10.3566	-84.0674	280	0
Craugastoridae	*Craugastor talamancae*	10.2357	-83.5673	80	0
Craugastoridae	*Craugastor talamancae*	10.2357	-83.5673	80	0
Craugastoridae	*Craugastor talamancae*	10.2357	-83.5673	80	1
Craugastoridae	*Craugastor talamancae*	10.2760	-84.1695	114	1
Dendrobatidae	*Oophaga pumilio*	10.3556	-84.0556	280	0
Dendrobatidae	*Oophaga pumilio*	10.3350	-84.1158	700	0
Dendrobatidae	*Oophaga pumilio*	10.3350	-84.1158	700	0
Dendrobatidae	*Oophaga pumilio*	10.3045	-84.0883	119	1
Dendrobatidae	*Oophaga pumilio*	10.3556	-84.0556	280	0
Dendrobatidae	*Oophaga pumilio*	10.3556	-84.0556	280	0
Eleutherodactylidae	*Diasporus diastema*	10.3556	-84.0556	280	0
Eleutherodactylidae	*Diasporus diastema*	10.3350	-84.1158	700	0
Eleutherodactylidae	*Diasporus diastema*	10.2554	-84.1022	1300	0
Eleutherodactylidae	*Diasporus diastema*	10.2787	-84.0850	960	0
Eleutherodactylidae	*Diasporus diastema*	10.0431	-83.5486	700	0
Eleutherodactylidae	*Diasporus diastema*	10.0431	-83.5486	700	0
Eleutherodactylidae	*Diasporus diastema*	10.2785	-84.0853	960	0
Eleutherodactylidae	*Diasporus diastema*	10.2785	-84.0853	960	0
Eleutherodactylidae	*Diasporus diastema*	10.2785	-84.0853	960	0
Eleutherodactylidae	*Diasporus diastema*	10.2785	-84.0853	960	0
Eleutherodactylidae	*Diasporus diastema*	10.2720	-84.1346	108	0

Eleutherodactylidae	*Diasporus diastema*	10.2720	-84.1346	108	0
Eleutherodactylidae	*Diasporus hylaeformis*	10.2361	-84.1028	1500	0
Eleutherodactylidae	*Diasporus hylaeformis*	10.2361	-84.1028	1500	0
Eleutherodactylidae	*Diasporus hylaeformis*	10.2075	-84.1346	1700	0
Eleutherodactylidae	*Diasporus hylaeformis*	10.1778	-84.0972	2050	0
Eleutherodactylidae	*Diasporus hylaeformis*	10.2361	-84.1028	1500	0
Eleutherodactylidae	*Diasporus hylaeformis*	10.2361	-84.1028	1500	0
Eleutherodactylidae	*Diasporus hylaeformis*	10.2361	-84.1028	1500	0
Eleutherodactylidae	*Diasporus hylaeformis*	10.2075	-84.1346	1700	0
Hylidae	*Duellmanohyla rufioculis*	10.2787	-84.0850	960	0
Hylidae	*Duellmanohyla rufioculis*	10.2785	-84.0853	960	0
Hylidae	*Duellmanohyla uranochroa*	10.2075	-84.1346	1700	0
Hylidae	*Duellmanohyla uranochroa*	10.2361	-84.1028	1500	0
Hylidae	*Duellmanohyla uranochroa*	10.2785	-84.0853	960	0
Hylidae	*Duellmanohyla uranochroa*	10.2720	-84.1346	1000	0
Hylidae	*Duellmanohyla uranochroa*	10.2720	-84.1346	108	1
Hylidae	*Duellmanohyla uranochroa*	10.2720	-84.1346	1000	0
Hylidae	*Hylomantis lemur*	10.2785	-84.0853	960	0
Hylidae	*Isthmohyla angustilineata*	10.2075	-84.1346	1700	0
Hylidae	*Isthmohyla angustilineata*	10.2075	-84.1346	1700	0
Hylidae	*Isthmohyla angustilineata*	10.2361	-84.1028	1500	0
Hylidae	*Isthmohyla angustilineata*	10.2361	-84.1028	1500	0
Hylidae	*Isthmohyla angustilineata*	10.2361	-84.1028	1500	0
Hylidae	*Isthmohyla angustilineata*	10.2361	-84.1028	1500	0
Hylidae	*Isthmohyla angustilineata*	10.2075	-84.1346	1700	0
Hylidae	*Isthmohyla calypsa*	8.9456	-82.7574	1750	0
Hylidae	*Isthmohyla calypsa*	8.9456	-82.7574	1750	0
Hylidae	*Isthmohyla calypsa*	8.9456	-82.7574	1750	0
Hylidae	*Isthmohyla calypsa*	8.9456	-82.7574	1750	0
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla pseudopuma*	10.1773	-84.1194	2000	1
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla pseudopuma*	10.1778	-84.0972	2050	0
Hylidae	*Isthmohyla rivularis*	10.2361	-84.1028	1500	0
Hylidae	*Isthmohyla rivularis*	10.2361	-84.1028	1500	0
Hylidae	*Isthmohyla rivularis*	10.2075	-84.1346	1700	0
Hylidae	*Isthmohyla rivularis*	10.2075	-84.1346	1700	0
Hylidae	*Isthmohyla rivularis*	10.2075	-84.1346	1700	0
Hylidae	*Isthmohyla rivularis*	8.9456	-82.7574	1750	0
Hylidae	*Isthmohyla rivularis*	8.9456	-82.7574	1750	0
Hylidae	*Isthmohyla rivularis*	8.9456	-82.7574	1750	0
Hylidae	*Isthmohyla rivularis*	8.9456	-82.7574	1750	0
Hylidae	*Isthmohyla rivularis*	10.2075	-84.1346	1700	0
Hylidae	*Isthmohyla rivularis*	10.2075	-84.1346	1700	0

Hylidae	*Isthmohyla rivularis*	10.2075	-84.1346	1700	0
Hylidae	*Smilisca phaeota*	10.3350	-84.1158	700	0
Hylidae	*Smilisca sordida*	10.3556	-84.0556	280	0
Hylidae	*Smilisca sordida*	10.3556	-84.0556	280	0
Hylidae	*Smilisca sordida*	10.3556	-84.0556	280	0
Hylidae	*Smilisca sordida*	10.3556	-84.0556	280	0
Hylidae	*Smilisca sordida*	10.3556	-84.0556	280	0
Hylidae	*Smilisca sordida*	10.3556	-84.0556	280	0
Hylidae	*Smilisca sordida*	10.3556	-84.0556	280	0
Ranidae	*Lithobates vibicarius*	10.1592	-84.0978	2400	0
Ranidae	*Lithobates vibicarius*	10.1592	-84.0978	2400	0
Ranidae	*Lithobates vibicarius*	10.1773	-84.1194	2000	1
Ranidae	*Lithobates vibicarius*	10.1778	-84.0972	2050	0
Ranidae	*Lithobates vibicarius*	10.1778	-84.0972	2050	0
Ranidae	*Lithobates vibicarius*	10.1778	-84.0972	2050	0
Ranidae	*Lithobates vibicarius*	10.3466	-84.7450	1500	1
Ranidae	*Lithobates vibicarius*	10.3466	-84.7450	1500	1
Ranidae	*Lithobates vibicarius*	10.3466	-84.7450	1500	1
Ranidae	*Lithobates vibicarius*	10.3466	-84.7450	1500	1
Ranidae	*Lithobates vibicarius*	10.3466	-84.7450	1500	1
Ranidae	*Lithobates vibicarius*	10.3466	-84.7450	1500	1
Ranidae	*Lithobates vibicarius*	10.3466	-84.7450	1500	1
Ranidae	*Lithobates vibicarius*	10.3466	-84.7450	1500	1
Ranidae	*Lithobates vibicarius*	10.3466	-84.7450	1500	1
Ranidae	*Lithobates vibicarius*	8.9456	-82.7574	1750	0
Ranidae	*Lithobates vibicarius*	10.1509	-84.0968	2500	0
Ranidae	*Lithobates vibicarius*	10.1773	-84.1194	2000	1
Ranidae	*Lithobates vibicarius*	10.1592	-84.0978	2400	0
Ranidae	*Lithobates vibicarius*	10.1778	-84.0972	2050	0
Ranidae	*Lithobates vibicarius*	10.1778	-84.0972	2050	0
Ranidae	*Lithobates warszewitschii*	8.9456	-82.7574	1750	0
Ranidae	*Lithobates warszewitschii*	10.2554	-84.1022	1300	0
Ranidae	*Lithobates warszewitschii*	10.3350	-84.1158	700	0
Ranidae	*Lithobates warszewitschii*	10.3350	-84.1158	700	0
Strabomantidae	*Pristimantis altae*	10.2785	-84.0853	960	0
Strabomantidae	*Pristimantis altae*	10.2785	-84.0853	960	0
Strabomantidae	*Pristimantis caryophyllaceus*	10.3566	-84.0674	280	0
Strabomantidae	*Pristimantis caryophyllaceus*	10.2785	-84.0853	960	0
Strabomantidae	*Pristimantis caryophyllaceus*	10.2785	-84.0853	960	0
Strabomantidae	*Pristimantis caryophyllaceus*	10.2785	-84.0853	960	0
Strabomantidae	*Pristimantis caryophyllaceus*	10.2785	-84.0853	960	0
Strabomantidae	*Pristimantis caryophyllaceus*	10.2720	-84.1346	108	0
Strabomantidae	*Pristimantis caryophyllaceus*	10.2720	-84.1346	108	0
Strabomantidae	*Pristimantis caryophyllaceus*	10.3350	-84.1158	700	0
Strabomantidae	*Pristimantis cerasinus*	10.3566	-84.0674	280	0
Strabomantidae	*Pristimantis cerasinus*	10.3350	-84.1158	700	0
Strabomantidae	*Pristimantis cerasinus*	10.3368	-84.1135	500	0
Strabomantidae	*Pristimantis cruentus*	10.3350	-84.1158	700	0
Strabomantidae	*Pristimantis cruentus*	10.2785	-84.0853	960	0
Strabomantidae	*Pristimantis cruentus*	10.2785	-84.0853	960	0
Strabomantidae	*Pristimantis cruentus*	10.2785	-84.0853	960	0
Strabomantidae	*Pristimantis cruentus*	10.2361	-84.1028	1500	0
Strabomantidae	*Pristimantis cruentus*	10.2361	-84.1028	1500	0
Strabomantidae	*Pristimantis cruentus*	10.2361	-84.1028	1500	0

Strabomantidae	*Pristimantis cruentus*	10.2785	-84.0853	960	0
Strabomantidae	*Pristimantis cruentus*	10.3556	-84.0556	280	0
Strabomantidae	*Pristimantis cruentus*	10.3350	-84.1158	700	0
Strabomantidae	*Pristimantis cruentus*	10.2720	-84.1346	108	0
Strabomantidae	*Pristimantis cruentus*	10.2785	-84.0853	960	0
Strabomantidae	*Pristimantis cruentus*	10.3556	-84.0556	280	0
Strabomantidae	*Pristimantis cruentus*	10.3350	-84.1158	700	0
Strabomantidae	*Pristimantis cruentus*	10.2720	-84.1346	108	0
Strabomantidae	*Pristimantis ridens*	10.0431	-83.5486	700	0
Strabomantidae	*Pristimantis ridens*	10.0431	-83.5486	700	0
Strabomantidae	*Pristimantis ridens*	10.0431	-83.5486	700	0