Puschendorf, Robert (2009) ***Environmental effects on a host-pathogen system: frogs and Batrachochytrium dendrobatidis in wet and dry habitats.*** **PhD thesis, James Cook University.**

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

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September 2009

For the degree of Doctor of Philosophy
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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.

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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.

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