

# ResearchOnline@JCU

This file is part of the following reference:

**Cashins, Scott David (2009) *Epidemiology of chytridiomycosis in rainforest stream tadpoles*. PhD thesis, James Cook University.**

Access to this file is available from:

<http://eprints.jcu.edu.au/23949/>

*The author has certified to JCU that they have made a reasonable effort to gain permission and acknowledge the owner of any third party copyright material included in this document. If you believe that this is not the case, please contact [ResearchOnline@jcu.edu.au](mailto:ResearchOnline@jcu.edu.au) and quote <http://eprints.jcu.edu.au/23949/>*

# **Epidemiology of Chytridiomycosis in Rainforest Stream Tadpoles**

**A thesis submitted by  
Scott David CASHINS BSc (ASU)  
September 2009**

**for the degree of Doctor of Philosophy  
within the School of Marine and Tropical Biology &  
the School of Public Health, Tropical Medicine and  
Rehabilitation Sciences**

**James Cook University**

## STATEMENT OF ACCESS

---

I, the undersigned, author of 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I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and I wish the following restrictions to be placed on this work:

*All users consulting this thesis will have to sign the following statement:  
In consulting this thesis I agree not to copy or closely paraphrase it in whole or in part without the written consent of the author; and to make proper public written acknowledgment for any assistance that I have obtained from it.*

I do not wish to place any further restriction on access to this work.

---

Signature

## **STATEMENT OF SOURCES**

---

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given. All research reported in this thesis received the approval of the ethics committees and the QPW.

---

Signature

---

Date

## **STATEMENT ON THE CONTRIBUTION OF OTHERS**

---

This thesis was co-supervised by Prof. Ross Alford and Dr. Lee Skerratt, but received valuable input from a number of other people. Ross Alford and Lee Skerratt contributed in the form of ideas, experimental design, editorial assistance, statistical advice and provided the majority of funding. Dr. Bryan Windmiller, Robert Puschendorf, Jamie Voyles and anonymous reviewers provided useful advice and suggestions on individual chapters. Dr. Stephen Garland and Ruth Campbell at the School of Public Health and Tropical Medicine, JCU and Veronica Olsen and Dr. Alex Hyatt at the Australian Animal Health Laboratory, CSIRO performed most of the diagnostic PCR tests for *Batrachochytrium dendrobatidis* and contributed experimental design and sampling advice. Bryan Windmiller provided valuable advice on the design of field work and together with Hayley Ricardo and 15 volunteers (listed by name in the acknowledgements) provided logistical and field assistance throughout this project.

This research was funded by The Australian Government Department of Environment and Heritage tenders 42/2004 and 43/2004, the US National Science Foundation Integrated Research Challenges in Environmental Biology grant DEB-0213851 and the School of Marine and Tropical Biology, the School of Veterinary and Biomedical Sciences, and the School of Public Health, Tropical Medicine & Rehabilitation Sciences at James Cook University, Townsville.

## **ACKNOWLEDGEMENTS**

---

I would like to thank my supervisors Ross Alford and Lee Skerratt who gave me the freedom to pursue my ideas, enough rope to hang myself with, and the guidance to do it properly. I have learned a great deal from them both. Thank you to the Amphibian Disease Ecology Group (ADEG) including Ross Alford, Sara Bell, Lee Berger, Nicole Kenyon, Keith McDonald, Diana Mendez, Andrea Phillott, Robert Puschendorf, Lee Skerratt, Rick Speare, Jamie Voyles, Rebecca Webb, Sam Young and many others. This group has been a great source for discussion and support to further understand amphibian disease and decline. I would like to thank Elizabeth Davidson who shared her love of science with me and started me on my path as a scientist while at Arizona State University.

I owe a big thank you to Bryan Windmiller, who while on a self-styled sabbatical at JCU enthusiastically agreed to join me for months of hard labor in often very wet and difficult conditions. I benefited greatly from his assistance and our frequent discussions. I would like to thank Hayley Ricardo, an invaluable field partner through the long days of field work. I would like to thank Robert Puschendorf and Jamie Voyles for great discussions and support throughout our time at JCU. Thank you to Sean Conlan for assistance with mapping and building enclosures, and Sara Bell for her help in countless ways. I would like to thank Tawni Duran for the love and laughter invested and shared. I am truly grateful for all of my field volunteers not previously mentioned including Tawni Duran, Robert Puschendorf, Sean Conlan, Jamie Voyles, Sara Bell, Lee Skerratt, Rob Gegg, Becky Sears, Noriko Iwai, Sam Young, Hui Jin, Nicole Sutcliffe, Russell Stanford, Eric Russell and Erica Todd.

Finally, I would like to thank my family; my parents Mary and Robert, for their love, support and understanding through it all, and to my brothers Michael and Dennis and my sister Andrea for their love, support and inspiration across the ocean.

## ABSTRACT

---

Amphibians are declining at an alarming rate and approximately one third of species are currently threatened with extinction. A primary cause of this decline has been the emergence of the disease chytridiomycosis caused by the pathogen, *Batrachochytrium dendrobatidis* (*Bd*). Historically, the extinction of free-living species due to disease is exceedingly rare; however, dozens of amphibians in recent years are feared gone due to *Bd*. For disease to drive extinction, theory indicates a reservoir host is needed to maintain a positive force of infection on susceptible individuals to prevent pathogen “fade out” as the doomed species decline. Accordingly, understanding pathogen dynamics (e.g. prevalence, intensity, transmission, seasonality) within reservoir hosts is critical to properly understand and mitigate species declines and prevent extinction. In the case of chytridiomycosis, no non-amphibian hosts have been found, however, less susceptible adults and amphibian larvae can serve as reservoirs.

While most research has focused on infection in adults, tadpoles probably are important reservoirs; they carry the pathogen and are thought to suffer few negative effects, and most species that have declined are associated with aquatic habitats. To better understand the role tadpoles play in pathogen dynamics I investigated the epidemiology of *Bd* in a tadpole assemblage (consisting of five species) within two rainforest streams over two years. I studied changes in prevalence and intensity of infection over time and how their values were affected by abiotic factors such as temperature and water flow rate, as well as by biotic factors such as the ecology, behaviour and developmental rate of each species. In species with a high prevalence of infection, I studied the response of tadpoles to infection and the effects these responses had on the infection and on their physical condition. A saprobic or long-lived life stage of *Bd* could significantly alter pathogen dynamics among hosts. To investigate this possibility, I developed a method to detect *Bd* in the environment and I used this to sample the stream over the course of one year.

I found significant species-specific variation in space and resource use within the tadpole assemblage; these differences appear to affect susceptibility to infection. Torrent-adapted tadpoles were significantly more likely to be infected than pool-adapted

## ABSTRACT

---

tadpoles. This is likely due to differences in rates of development that affect duration of exposure to *Bd* and differences in behaviour that affect pathogen transmission.

Prevalence of infection in torrent tadpoles increased with body size (proxy for duration of exposure) indicating that transmission occurred throughout the year. Prevalence varied seasonally between ~ 25-100% and was driven by a combination of duration of exposure, recruitment of small tadpoles and metamorphosis of large tadpoles. Drivers of infection intensity are less clear, however, body size and water flow are important and in fast-flowing habitats repeat transmission from the external environment appears to be more important than self-reinfection in determining individual infection intensities.

After infection most torrent tadpoles suffered significant tooth loss. This loss severely decreased their ability to feed (in some cases causing apparent starvation), which led to significant decreases in body condition for many individuals. Most tadpoles, however, regrew mouthparts despite continued infection, resumed feeding, and metamorphosed. The relationships between infection intensity, prevalence, tooth loss and body condition indicate that these tadpoles have a measure of tolerance or increased resistance, which may be a result of strong selection pressure exerted by chytridiomycosis.

Environmental sampling for *Bd* revealed that environmental levels are low throughout the year, but may increase when prevalence in tadpoles is highest, suggesting that tadpoles are the major source of *Bd* zoospores in the environment.



# TABLE OF CONTENTS

---

STATEMENT OF ACCESS.....	i
STATEMENT OF SOURCES.....	ii
STATEMENT ON CONTRIBUTION OF OTHERS.....	iii
ACKNOWLEDGEMENTS.....	iv
ABSTRACT.....	v
TABLE OF CONTENTS.....	vii
LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
<b>CHAPTER 1 : GENERAL INTRODUCTION.....</b>	<b>1</b>
<b>CHAPTER 2 : SODIUM HYPOCHLORITE DENATURES THE DNA OF THE AMPHIBIAN CHYTRID FUNGUS <i>BATRACHOCHYTRIUM DENDROBATIDIS</i>.....</b>	<b>7</b>
ABSTRACT.....	7
INTRODUCTION.....	7
MATERIALS AND METHODS.....	9
RESULTS.....	11
DISCUSSION.....	12
<b>CHAPTER 3 : EFFECT OF SAMPLE COLLECTION TECHNIQUES ON SENSITIVITY OF A REAL TIME PCR ASSAY FOR DETECTING THE AMPHIBIAN PATHOGEN <i>BATRACHOCHYTRIUM DENDROBATIDIS</i>.....</b>	<b>15</b>
ABSTRACT.....	15
INTRODUCTION.....	15
MATERIALS AND METHODS.....	18
RESULTS.....	20
DISCUSSION.....	20
<b>CHAPTER 4 : LETHAL EFFECT OF LATEX, VINYL AND NITRILE GLOVES ON TADPOLES.....</b>	<b>24</b>
INTRODUCTION.....	24
CASE STUDY 1: LABORATORY.....	24
EXPERIMENT 1: GLOVE SOAK.....	27
EXPERIMENT 2: GLOVE CONTACT.....	27
CASE STUDY 2: FIELD.....	28
<b>CHAPTER 5 : SITES AND TADPOLE ECOLOGY.....</b>	<b>32</b>
INTRODUCTION.....	32
SITE AND SPECIES DESCRIPTIONS.....	33
MATERIALS AND METHODS.....	40
RESULTS.....	46
DISCUSSION.....	60
<b>CHAPTER 6 : EPIDEMIOLOGY OF <i>BATRACHOCHYTRIUM DENDROBATIDIS</i> IN RAINFOREST STREAM TADPOLES.....</b>	<b>65</b>
INTRODUCTION.....	65
MATERIALS AND METHODS.....	67
RESULTS.....	72
DISCUSSION.....	97
<b>CHAPTER 7 : EFFECTS OF INFECTION WITH <i>BATRACHOCHYTRIUM DENDROBATIDIS</i> ON TADPOLES.....</b>	<b>111</b>
INTRODUCTION.....	112

# TABLE OF CONTENTS

---

MATERIALS AND METHODS .....	116
RESULTS .....	117
DISCUSSION .....	144
<b>CHAPTER 8 : ENVIRONMENTAL SAMPLING FOR <i>BATRACHOCHYTRIUM</i></b>	
<b><i>DENDROBATIDIS</i> .....</b>	<b>157</b>
INTRODUCTION .....	157
METHODS .....	160
RESULTS .....	162
DISCUSSION .....	167
<b>CHAPTER 9 : GENERAL DISCUSSION.....</b>	<b>171</b>
INTRODUCTION .....	171
AIMS AND APPROACH.....	172
DEVELOPMENT OF TECHNIQUES .....	173
VARIATION IN SPACE AND RESOURCE USE.....	174
DIFFERENTIAL RISK OF INFECTION .....	175
DRIVERS OF SEASONAL <i>Bd</i> PREVALENCE AND INTENSITY IN TADPOLES .....	176
EFFECT OF INFECTION ON TADPOLES .....	177
IMPLICATIONS.....	178
<b>REFERENCES .....</b>	<b>181</b>

## LIST OF TABLES

---

<b>Table 6-1.</b> Chi square comparisons of prevalence between tadpoles of all species and between sites within species .....	<b>73</b>
<b>Table 6-2.</b> Chi squares of <i>Bd</i> prevalence in <i>L. nannotis</i> by season and size class .....	<b>77</b>
<b>Table 6-3.</b> Logistic regression model of <i>Bd</i> prevalence.....	<b>83</b>
<b>Table 6-4.</b> Multiple regression model of <i>Bd</i> infection intensity in <i>L. nannotis</i> .....	<b>92</b>
<b>Table 7-1.</b> <i>Bd</i> infection status by degree of tooth loss and jaw loss, Tully Gorge NP (2007-2008).....	<b>119</b>
<b>Table 7-2.</b> Logistic regression model of the association of mouthpart loss and infection status in <i>L. nannotis</i> and <i>L. rheocola</i> .....	<b>121</b>
<b>Table 7-3.</b> Relationship of overall tooth loss and <i>Bd</i> infection in <i>L. genimaculata</i> and <i>L. xanthomera</i> .....	<b>123</b>
<b>Table 7-4.</b> Relationship of overall tooth loss and <i>Bd</i> infection in <i>L. nannotis</i> and <i>L. rheocola</i> .....	<b>124</b>
<b>Table 8-1.</b> Inhibition of environmental samples for PCR, Tully Gorge NP (2006-2007).....	<b>164</b>
<b>Table 8-2.</b> Environmental filtering PCR results, Tully Gorge NP, 2006-2007.....	<b>165</b>

## LIST OF FIGURES

---

<b>Figure 2.1.</b> Numbers of <i>Bd</i> zoospores detected in controls and at three concentrations of NaOCl after varying periods of exposure.....	12
<b>Figure 3.1.</b> Interaction diagram between sample material and <i>Bd</i> zoospore concentration.....	21
<b>Figure 3.2.</b> Mean PCR cycle threshold value (Ct) by sample material and <i>Bd</i> zoospore concentration.....	22
<b>Figure 4.1.</b> Tadpole mortality in response to disposable glove exposure.....	26
<b>Figure 5.1.</b> Location of study sites in the Wet Tropics, Queensland Australia .....	34
<b>Figure 5.2.</b> Close up of study site transects .....	35
<b>Figure 5.3.</b> Topography of Tully Gorge National Park transect.....	35
<b>Figure 5.4.</b> Tully Gorge National Park habitat photo. ....	36
<b>Figure 5.5.</b> Murray Upper National Park habitat photo.....	36
<b>Figure 5.6.</b> Tully Gorge National Park habitat photo following heavy rain. ....	37
<b>Figure 5.7.</b> Torrent adapted tadpole photos .....	39
<b>Figure 5.8.</b> Torrent tadpole sampling photo.....	42
<b>Figure 5.9.</b> <i>L. nannotis</i> mouthpart loss. A) no loss B) $\geq 75\%$ loss .....	42
<b>Figure 5.10.</b> <i>L. nannotis</i> marked with a visible implant elastomer.....	45
<b>Figure 5.11.</b> Boxplot of tadpole movements off substrate.....	47
<b>Figure 5.12.</b> Mean water flow rate profile of tadpoles .....	47
<b>Figure 5.13.</b> Boxplot of mean water flow rate by functional group .....	48
<b>Figure 5.14.</b> Mean water flow profile by site and species. ....	49
<b>Figure 5.15.</b> Mean water flow rate occupied by <i>L. nannotis</i> by size class .....	49
<b>Figure 5.16.</b> Relative abundance of <i>L. nannotis</i> tadpoles by size class, Tully Gorge NP .....	51
<b>Figure 5.17.</b> Size-frequency distributions of <i>L. nannotis</i> , Tully Gorge NP.....	52
<b>Figure 5.18.</b> Length (mm) vs mass (g) scatterplots for <i>L. nannotis</i> and <i>L. rheocola</i> .....	53
<b>Figure 5.19.</b> Size-frequency distributions of <i>L. rheocola</i> , Tully Gorge NP .....	54
<b>Figure 5.20.</b> Size-frequency distributions of <i>L. xanthomera</i> , Tully Gorge NP .....	55
<b>Figure 5.21.</b> Size-frequency distributions of <i>L. genimaculata</i> , Tully Gorge NP.....	56
<b>Figure 5.22.</b> Size-frequency distributions of <i>L. genimaculata</i> , Murray Upper NP .....	56
<b>Figure 5.23.</b> Population estimate of torrent tadpoles before and after peak mouthpart loss, Tully Gorge NP .....	59
<b>Figure 6.1.</b> Mean prevalence of <i>Bd</i> +/- 95% CI in tadpoles of five species over two years, Tully Gorge NP and Murray Upper NP .....	72
<b>Figure 6.2.</b> Mean prevalence +/- 95% CI of <i>Bd</i> infection in tadpoles by site.....	74
<b>Figure 6.3.</b> Mean prevalence +/- 95% CI of <i>Bd</i> in <i>L. nannotis</i> and <i>L. rheocola</i> by size class, Tully Gorge NP and Murray Upper NP.....	75
<b>Figure 6.4.</b> Mean prevalence +/- 95% CI of <i>Bd</i> in <i>L. genimaculata</i> and <i>L. xanthomera</i> by size class, Tully Gorge NP and Murray Upper NP.....	75
<b>Figure 6.5.</b> Mean body length of infected and uninfected <i>L. genimaculata</i> .....	76
<b>Figure 6.6.</b> Mean prevalence +/- 95% CI of <i>Bd</i> in <i>L. nannotis</i> by size class and season .....	78
<b>Figure 6.7.</b> Seven-day mean water temperature +/- 95% CI for infected and uninfected tadpoles .....	78
<b>Figure 6.8.</b> Seasonal prevalence of <i>Bd</i> in torrent-	

## LIST OF FIGURES

---

adapted and pool-adapted tadpoles, Tully Gorge NP, 2006 – 2008 .....	80
<b>Figure 6.9.</b> Seasonal prevalence of <i>Bd</i> in torrent-adapted and pool-adapted tadpoles, Murray Upper NP, 2006 – 2007 .....	81
<b>Figure 6.10.</b> Seasonal prevalence of <i>Bd</i> in <i>L. nannotis</i> tadpoles by size-group, Tully Gorge NP (2006-2008).....	82
<b>Figure 6.11.</b> Mean intensity of <i>Bd</i> infection in tadpoles, Tully Gorge NP and Murray Upper NP (2006-2008) .....	85
<b>Figure 6.12.</b> Mean intensity of <i>Bd</i> infection by size class in <i>L. nannotis</i> , Tully Gorge NP and Murray Upper NP (2006-2008) .....	86
<b>Figure 6.13.</b> Mean intensity of <i>Bd</i> infection by size class in torrent-adapted tadpoles, Tully Gorge NP and Murray Upper NP (2006-2008).....	86
<b>Figure 6.14.</b> Mean intensity of <i>Bd</i> by species and site (2006-2007).....	88
<b>Figure 6.15.</b> Mean intensity of <i>Bd</i> in <i>L. nannotis</i> by size class and site (2006-2007) .....	88
<b>Figure 6.16.</b> Mean intensity of <i>Bd</i> in <i>L. rheocola</i> by size class and site (2006-2007) .....	89
<b>Figure 6.17.</b> Mean intensity of <i>Bd</i> in <i>L. nannotis</i> by sampling period and site (2006-2007) .....	89
<b>Figure 6.18.</b> Mean intensity of <i>Bd</i> in <i>L. rheocola</i> by sampling period and site (2006-2007).....	90
<b>Figure 6.19.</b> Mean intensity of <i>Bd</i> in <i>L. nannotis</i> , <i>L. rheocola</i> and <i>L. genimaculata</i> over time at Tully Gorge NP (2006-2008).....	93
<b>Figure 6.20.</b> Prevalence and intensity of <i>Bd</i> over time in <i>L. nannotis</i> and <i>L. rheocola</i> , Tully Gorge NP (2006-2008) with water temperature, mouth-part loss and tadpole demographic patterns .....	94
<b>Figure 6.21.</b> Host relative abundance and prevalence of <i>Bd</i> in medium size-group <i>L. nannotis</i> tadpoles, Tully Gorge NP (2006-2007).....	96
<b>Figure 6.22.</b> Intensity of <i>Bd</i> in large size-group <i>L. nannotis</i> tadpoles and relative density of infected torrent adapted tadpoles, Tully Gorge NP (2006-2007). .....	96
<b>Figure 7.1.</b> <i>L. nannotis</i> mouthparts with labeled tooth rows and jaw sheath.....	116
<b>Figure 7.2.</b> Mean prevalence of <i>Bd</i> in <i>L. nannotis</i> and <i>L. rheocola</i> by degree of overall mouthpart loss, Tully Gorge NP (2007-2008) .....	119
<b>Figure 7.3.</b> Mean prevalence of <i>Bd</i> infection in <i>L. nannotis</i> and <i>L. rheocola</i> by degree of jaw sheath loss, Tully Gorge NP (2007-2008). .....	120
<b>Figure 7.4.</b> Mean intensity of <i>Bd</i> in <i>L. nannotis</i> and <i>L. rheocola</i> by degree of overall tooth loss, Tully Gorge NP (2007-2008). .....	126
<b>Figure 7.5.</b> Mean overall tooth loss score of <i>L. nannotis</i> and <i>L. rheocola</i> with varying degrees of jaw sheath loss, Tully Gorge NP (2007-2008) .....	130
<b>Figure 7.6.</b> Mean individual tooth row loss by overall tooth loss in <i>L. nannotis</i> and <i>L. rheocola</i> , Tully Gorge NP (2007-2008) .....	130
<b>Figure 7.7.</b> Mean water flow rate by tooth loss in <i>L. nannotis</i> , Tully Gorge NP (July 4-August 1, 2007). .....	131
<b>Figure 7.8.</b> Mean individual tooth loss in <i>L. nannotis</i> over time, Tully Gorge NP.....	132

## LIST OF FIGURES

---

<b>Figure 7.9.</b> Number of infected and uninfected <i>L. nannotis</i> by overall tooth loss and size class, Tully Gorge NP (2007-2008) .....	133
<b>Figure 7.10.</b> Number of infected and uninfected <i>L. nannotis</i> tadpoles by size class over time, Tully Gorge NP (2007-2008). .....	134
<b>Figure 7.11.</b> Overall tooth loss in <i>L. nannotis</i> by size class over time, Tully Gorge NP (2007-2008).....	135
<b>Figure 7.12.</b> Intensity of <i>Bd</i> infection in <i>L. nannotis</i> by size class over time, Tully Gorge NP (2007-2008).....	136
<b>Figure 7.13.</b> Scatter plot and linear regression of $\log_{10}$ mass and $\log_{10}$ body length of <i>L. rheocola</i> and <i>L. nannotis</i> , Tully Gorge NP (2007-2008) .....	138
<b>Figure 7.14.</b> Mean mass residuals overlaid on stacked bar graphs representing degrees of tooth loss over time. A) <i>Litoria rheocola</i> , B) <i>L. nannotis</i> , Tully Gorge NP (2007-2008).....	139
<b>Figure 7.15.</b> Comparison of a healthy <i>L. nannotis</i> and an unhealthy, infected <i>L. nannotis</i> .....	140
<b>Figure 7.16.</b> Box Plot of body lengths of large <i>L. nannotis</i> tadpoles at Tully Gorge NP and McLeod Creek .....	142
<b>Figure 7.17.</b> Population estimates of torrent adapted tadpoles before and after peak mouthpart loss.....	143
<b>Figure 8.1.</b> Comparison of mean <i>Bd</i> zoospore equivalents among filter treatments.....	163