

JCU ePrints

This file is part of the following reference:

Kenyon, Nicole (2008) *Variable susceptibility to an emerging infectious disease, chytridiomycosis, in anurans.* PhD thesis, James Cook University.

Access to this file is available from:

<http://eprints.jcu.edu.au/3252>



**Variable Susceptibility
to an Emerging Infectious Disease,
Chytridiomycosis, in Anurans**



Picture of *Nyctimystes dayi* by N. Kenyon

Thesis submitted by
Nicole KENYON BSc, JCU
In July 2008

For the degree of Doctor of Philosophy
in Zoology and Tropical Ecology
within the School of Marine and Tropical Biology
James Cook University
Townsville, QLD, Australia

STATEMENT OF SOURCES DECLARATION

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 Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.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 OF CONTRIBUTION OF OTHERS

Two publications have been submitted from Chapter 2. Dr. Andrea Phillott provided input on the manuscripts and generously provided her frog monitoring population to compare the true identity of the frogs via toe-tipping and photographic identification method. Professor Ross Alford gave valuable advice on statistical analysis and writing the manuscript.

Chapter 5 is in the process of being submitted to Journal of Zoology with the help of Professor Ross Alford. The design of this experiment was an ongoing development and Ross provided valuable input during that time, including the analysis.

Chapter 6 has been submitted to Journal of Herpetology with the help of Sara Bell and Professor Ross Alford. Sara Bell offered advice on culturing *Bd* and writing the manuscript. Professor Ross Alford gave valuable advice on the experimental design, statistical analysis and writing the manuscript, which is currently in revision.

The submission of several chapters to different journals required the use of different English styles and reference formats which were retained in this thesis.

Declaration of ethics

Animals were obtained and all data collected under Animal Ethics Approval A960 granted by James Cook University Animal Ethics Committee, and Scientific Purposes Permits WITK01932505 and WISP01764304 granted by Queensland Parks and Wildlife Service.

STATEMENT OF ACCESS

I, the undersigned, the 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 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

Date

ELECTRONIC COPY

I, the undersigned, the 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

ACKNOWLEDGEMENTS

It has been, with many up and downs, a fantastic journey. Not only did I learn about field surveys, experiments, statistics, etc., but also how to stay true to myself and find the scientist I want to be. I could not have done this journey without the help of you all.

Thank you Ross Alford, my main supervisor, for allowing me to work on the chytridiomycosis project and giving me the freedom to create my own study. I have learnt so much from you! I greatly appreciate the time and effort you have put into explaining the odd world called “statistics” to me, your endless patience with English grammar and for tolerating my nagging and panic emails... ☺. Thanks to my co-supervisors, Andrea Phillott and Chris Johnson. Andrea, I would have never submitted that many papers without you! Thanks for your “give me update” emails and letting me use your monitoring frog population to test the photographic identification method. Chris, thank you for your kind, positive and constructive input when I needed it the most.

A special thanks goes to Sara Bell, my supervisor’s research assistant, who has supported me in so many ways (the list is just too long) through out my PhD and Rob Gegg for helping me designing and building the terraria, but also for being there as a close friend, desperately trying to teach me diplomacy.... I had a great time with you and your patience and generosity is inspiring.

It has been a pleasure to work with the Amphibian Disease Ecology Group (ADEG) members and froggers, Andrea Phillott, Diana Mendez, Jamie Voyles, Keith McDonald, Lee Berger, Lee Skerratt, Rick Speare, Robert Puschendorf, Ross Alford, Sam Young, Sara Bell, Scott Cashins, Stephen Garland and many more! Special thanks to Stephen Garland for carrying out the PCR analysis, Scott Cashins for raising *L. genimaculata* eggs to tadpoles and Robert Puschendorf for creating the study site map.

I am grateful for the fantastic collaboration with Dr. Louise Rollins-Smith and Dr. Doug Woodhams at Vanderbilt University, Nashville, USA. Chapter 3 and 4 would not have been possible without their help. Also thank you to Dr. Kirsten Heimann at JCU for her

ACKNOWLEDGEMENTS

valuable input on Chapter 4 and Associate Professor Lin Schwarzkopf at JCU for her honest opinion on my conference presentations.

I have been lucky to have received numerous financial contributions. Firstly, thank you to the mayor of my home town (Stein am Rhein, Switzerland), Franz Hostettmann, for providing me with a one semester scholarship (without me having to fill out any forms!) to start my postgraduate study which enabled me to pursue my dream. Secondly, thank you to the Department of Education, Science and Training, for the international postgraduate scholarship (IPRS), the Marine and Tropical Biology at James Cook University for contributing towards the IPRS and the Department of Environment and Water Resources for supporting my study.

I would not have been able to conduct my field work without the help of many volunteers. Special thanks to Ashley Percy, Dane Trembath, Jason Schaeffer and Sam Forbes for looking after my frogs. Thank you Bettina Mueller, Claire Bisseling, Joe Wilkinson, Martha Velasco, Max Sargeson, Peter Roth, Philip Smith, Sam Noonan, Susan North, Tom Jackson (thanks for the blog), Vickie Hickin and any others that I may have forgotten (sorry) for being such a great help in the field and putting up with leeches (even in the eye), wait a while, stinging trees, mosquitoes, long cold rainy nights and slippery rocks!

During this study I have gained so many new and wonderful friends. Thank you to Mahanta my dearest friend and role model, Bettina Mueller for all the laughter, tears and Sunday morning Eggs Benedict, Brian King for all the flowers, emails, SMS's and great hugs at the right times, Jacquie Shields for being such a great friend even though we live miles apart, Katrin Schmidt for chocolate and coffee breaks, Leonie Valentine for taking me into the field and introducing me to the most wonderful, warm and generous family – the Dreghorn Clan ☺, Noriko Iwai for saving my frogs when the air-conditioner broke down on a Sunday morning, Peter Roth who is even more stubborn than me for all the dances and Swiss chats, to all the salsa dancers, the Dreghorn Clan for believing in me and many wonderful nights on the veranda with blue cheese and

ACKNOWLEDGEMENTS

jazz music, Kay Bradfield for all your constructive comments and of course to all the
frogs.

My final thanks and deepest gratitude goes to my mom and dad. Thank you for being
with me from step one, visualizing something I thought was impossible to achieve,
believing in me and supporting me in all levels, financially, emotionally and spiritually.
Thanks for the trust, love and encouragement – you guys are inspiring and just the best!

ABSTRACT

Chytridiomycosis is an emerging infectious amphibian disease, caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), and has caused numerous amphibian declines around the globe. Chytridiomycosis can be lethal in many amphibian species but not in others, leading to three different responses to exposure, 1) the amphibian becomes infected with *Bd* and dies, 2) the amphibian becomes infected with *Bd* and survives and 3) the amphibian does not become infected even though it occurs in a habitat where *Bd* exists. My project aimed to increase our understanding of the causes of these interspecific differences. I investigated the hypotheses that they could be caused by innate immune defences (antimicrobial peptides) against *Bd*, innate or adaptive responses of individuals through microenvironment selection, or behavioural avoidance of infective water.

I found evidence for all three mechanisms. *In vitro*, antimicrobial peptides (AMPs) of *Litoria genimaculata* (vulnerable to infection with *Bd* with highland population declines followed by recovery) and *L. rheocola* (vulnerable to infection with *Bd* with severe declines at higher elevations with little or slow recolonisation) can completely inhibit *Bd* growth. I also found large seasonal variation in antimicrobial peptide defences in both species. This may result from physiological shifts driven by temperature, or may reflect adaptation to seasonal fluctuations in the risk of infection. The proportion of *L. genimaculata* from high elevation populations, which have experienced strong viability selection pressure imposed by chytridiomycosis outbreaks, that produced AMPs that effectively inhibited *Bd in vitro*, was significantly higher than in low elevation populations, which have been protected from chytridiomycosis by environmental factors. There was also evidence that high elevation populations produced AMPs that differed slightly in chemical composition from those produced by low elevation populations. However, when individuals of either frog species produced AMPs that inhibited the growth of *Bd*, the effectiveness of AMPs from high and low elevation populations did not differ significantly. This suggests that any responses to selection may have occurred through an increase in the proportion of individuals producing effective AMPs, with no change in the types of AMPs produced. Antimicrobial peptide defences did not differ significantly between high and low elevation population of *L. rheocola*, suggesting that this species may have recolonised

ABSTRACT

upland areas. On the other hand, *L. rheocola* had more effective antimicrobial peptide defences against *Bd* than *L. genimaculata* and may have experienced stronger selection pressure after the appearance of chytridiomycosis.

Thermal microenvironments selected in the laboratory corresponded to those expected from decline patterns observed in the wild. *Litoria caerulea* (vulnerable to infection with *Bd* but no population declines due to chytridiomycosis have been detected) selected warm and hot environments significantly more often than *L. genimaculata*. Additionally, although not significant, there was a trend that intensity of *Bd* infection in all three species was more likely to decrease over time in individuals that had a choice of hydric and thermal microenvironments than in frogs that were housed under standard environmental conditions. There was also evidence of disease avoidance behaviour; some *L. caerulea* and *L. genimaculata* chose uncontaminated water significantly more often than water that contained *Bd* zoospores. None of the frog species were able to completely avoid water containing *Bd* zoospores, possibly in part because their pond selection was also influenced by site fidelity.

My study demonstrates the complexity of host-pathogen interactions and that multiple factors, including innate immune defence, microenvironment selection and disease avoidance behaviour, can influence the progress of chytridiomycosis and should be considered when establishing species specific management plans.

TABLE OF CONTENTS

TITLE PAGE	I
STATEMENT OF SOURCES DECLARATION	II
STATEMENT OF CONTRIBUTION OF OTHERS	III
STATEMENT OF ACCESS	IV
ELECTRONIC COPY	V
ACKNOWLEDGEMENTS	VI
ABSTRACT	IX
CHAPTER 1: GENERAL INTRODUCTION	
Diseases	1
Amphibians	5
Introduction	5
Chytridiomycosis	6
Aims of this study	15
CHAPTER 2: PHOTOGRAPHIC IDENTIFICATION METHOD (PIM) AS A MEANS OF RECOGNISING INDIVIDUAL ADULT AND JUVENILE GREEN-EYED TREE FROGS, <i>LITORIA GENIMACULATA</i> (ANURA: HYLIDAE).	
Abstract	17
Introduction	18
Materials and Methods	19
Results	23
Discussion	34
CHAPTER 3: HAVE ANURAN SKIN PEPTIDE DEFENCES AGAINST THE EMERGING AMPHIBIAN PATHOGEN <i>BATRACHOCHYTRIUM DENDROBATIDIS</i> RESPONDED TO NATURAL SELECTION?	
Abstract	36
Introduction	37
Materials and Methods	39
Results	49
Discussion	63
CHAPTER 4: ANTIMICROBIAL PEPTIDE DEFENCE AND PROFILE IN <i>LITORIA GENIMACULATA</i> (ANURA: HYLIDAE): COMPARISON AMONGST FIVE DIFFERENT GEOGRAPHICAL LOCATIONS.	
Abstract	66
Introduction	68
Materials and Methods	70
Results	74
Discussion	89

XI

TABLE OF CONTENTS

CHAPTER 5: A LABORATORY BEHAVIOUR STUDY ON THE EFFECT OF MICROENVIRONMENT SELECTION BY ANURANS ON CHYTRIDIOMYCOSIS.

Abstract	91
Introduction	92
Materials and Methods	94
Results	100
Discussion	115

CHAPTER 6: DO FROGS AVOID WATER THAT CONTAINS *BATRACHOCHYTRIUM DENDROBATIDIS* ZOOSPORES?

Abstract	118
Introduction	119
Materials and Methods	121
Results	125
Discussion	131

CHAPTER 7: SUMMARY AND CONCLUSION

Innate immune defences – Antimicrobial peptides	133
Microenvironment selection	134
Disease avoidance behaviour	135
Future research	136

LITERATURE SITED 138

APPENDIX

Appendix A. Wild amphibian species known to be infected with <i>Batrachochytrium dendrobatidis</i> as of 30 June 2008.	161
--	-----

LIST OF FIGURES

Figure 1.1	Life cycle of <i>Batrachochytrium dendrobatidis</i>	7
Figure 1.2	Global distribution of amphibian species known to be infected with <i>Batrachochytrium dendrobatidis</i> as of 30 June 2008	9
Figure 2.1	Stage used to take digital images of <i>Litoria genimaculata</i>	20
Figure 2.2	Measured aspects of the dorsal pattern in <i>Litoria genimaculata</i> used to create binary state variables analysed in Figure 2.3	21
Figure 2.3	Non-metric multidimensional scaling ordination of Euclidean distances among individuals for dichotomised measurements of <i>Litoria genimaculata</i> juvenile dorsal pattern	24
Figure 2.4	Photographs of two juvenile <i>Litoria genimaculata</i> and their distinctive dorsal patterns	25
Figure 2.5	Persistence of the dorsal pattern in a juvenile <i>Litoria genimaculata</i> at week one and week nine	26
Figure 2.6	Constant dorsal pattern, with temporal variation in colour, of a juvenile <i>Litoria genimaculata</i>	27
Figure 2.7	The lack of a distinct dorsal hourglass and similar blotches in two adult <i>Litoria genimaculata</i>	31
Figure 2.8	Dorsal pattern change of an adult <i>Litoria genimaculata</i> individual within two months	32
Figure 2.9	Time taken to apply toe-tipping and photographic identification method to adult frogs	33
Figure 3.1	Map of the location of three National Park areas (encompassing 5 study sites) in northern Queensland where <i>Litoria genimaculata</i> and <i>L. rheocola</i> were captured to sample skin peptides	40
Figure 3.2	Prevalence of <i>Batrachochytrium dendrobatidis</i> in <i>Litoria genimaculata</i> and <i>L. rheocola</i> individuals, at each site, during summer and winter	50
Figure 3.3	<i>Batrachochytrium dendrobatidis</i> infection intensity in <i>Litoria genimaculata</i> at high and low elevation, in winter and summer	51
Figure 3.4	<i>Batrachochytrium dendrobatidis</i> infection intensity in <i>Litoria rheocola</i> at high and low elevation, in winter and summer	52
Figure 3.5	Relationship between total peptide secretion (μg) and body mass (g) in <i>Litoria genimaculata</i> and <i>L. rheocola</i>	53
Figure 3.6	Total peptides secreted (μg) per surface area (cm^2) by <i>Litoria genimaculata</i> at each site	54
Figure 3.7	Total peptides secreted (μg) per surface area (cm^2) by <i>Litoria rheocola</i> at each site	55
Figure 3.8	Total peptides secreted (μg) per surface area (cm^2) by <i>Litoria genimaculata</i> and <i>L. rheocola</i> in summer and winter	56
Figure 3.9	Proportion of peptide samples from <i>Litoria genimaculata</i> and <i>L. rheocola</i> that did and did not inhibit <i>Batrachochytrium dendrobatidis</i> during challenge assays	58
Figure 3.10	Proportion of peptide samples from <i>Litoria genimaculata</i> and <i>L. rheocola</i> , in summer and winter, at four different inhibitory concentrations	59
Figure 3.11	Overall protection afforded by antimicrobial peptides in <i>Litoria genimaculata</i> and <i>L. rheocola</i>	60

LIST OF FIGURES

Figure 3.12	Overall protection afforded by antimicrobial peptides in infected and uninfected <i>Litoria genimaculata</i> at high and low elevation	61
Figure 3.13	Overall protection afforded by antimicrobial peptides in <i>Litoria rheocola</i> at high and low elevation	62
Figure 4.1	Matrix-assisted laser desorption/ionization mass spectrometry reading of skin peptides of a <i>Litoria genimaculata</i> individual	75
Figure 4.2	Prevalence of <i>Batrachochytrium dendrobatidis</i> in <i>Litoria genimaculata</i> at five sites	76
Figure 4.3	<i>Batrachochytrium dendrobatidis</i> infection intensity in <i>Litoria genimaculata</i> at five sites	77
Figure 4.4	Total peptides secreted (μg) per surface area (cm^2) by infected and uninfected <i>Litoria genimaculata</i> at five sites	78
Figure 4.5	Correlation between <i>Batrachochytrium dendrobatidis</i> infection intensity and peptide secretion (μg) per surface area (cm^2), in <i>Litoria genimaculata</i>	80
Figure 4.6	Proportion of peptide samples from infected and uninfected <i>Litoria genimaculata</i> at five different inhibitory concentrations	81
Figure 4.7	Overall protection afforded by antimicrobial peptides in <i>Litoria genimaculata</i> at five sites	83
Figure 4.8	Correlation between <i>Batrachochytrium dendrobatidis</i> infection intensity and overall protection afforded by antimicrobial peptides in <i>Litoria genimaculata</i>	85
Figure 4.9	Number of peaks counted by matrix-assisted laser desorption/ionization mass spectrometry of skin peptides from <i>Litoria genimaculata</i>	86
Figure 4.10	Multidimensional scaling ordination on matrix-assisted laser desorption/ionization mass spectrometry of skin peptide samples from <i>Litoria genimaculata</i> at five sites	87
Figure 5.1	The design of the variable microenvironment terraria in which frogs could choose between two hydric (low and high relative humidity) and three thermal (cold, warm and hot) microenvironments	96
Figure 5.2	Relative humidity in three different terraria designs. 1= larger variable microenvironment terraria, 2= smaller variable microenvironment terraria, 3= constant microenvironment terraria	102
Figure 5.3	Thermal microenvironments (cold, warm and hot) in the three different terraria designs. 1= larger variable microenvironment terraria, 2= smaller variable microenvironment terraria, 3= constant microenvironment terraria	103
Figure 5.4	Proportion of digital images in which <i>Litoria caerulea</i> were observed during the day in each hydric environment (high and low) and substrate selection (pond, wall and ground)	106
Figure 5.5	Proportion of digital images in which <i>Litoria caerulea</i> , <i>L. wilcoxii</i> and <i>L. genimaculata</i> were observed during the day in each thermal environment (cold, warm and hot)	107
Figure 5.6	Proportion of digital images in which <i>Litoria caerulea</i> , <i>L. wilcoxii</i> and <i>L. genimaculata</i> were observed during the day in each combination of thermal and hydric environments (cold, warm and hot at low and high humidity)	108

XIV

LIST OF FIGURES

Figure 5.7	Proportion of digital images in which <i>Litoria caerulea</i> , <i>L. wilcoxii</i> and <i>L. genimaculata</i> were located during the day on each substrate (pond, wall and ground)	109
Figure 5.8	Proportion of digital images in which <i>Litoria caerulea</i> , <i>L. wilcoxii</i> and <i>L. genimaculata</i> had moved (< body length, > body length or no movement) during the day within the terraria	110
Figure 5.9	Proportion of digital images in which infected and uninfected <i>Litoria wilcoxii</i> movement pattern (< body length, > body length or no movement), during the night, were observed	111
Figure 5.10	Proportion of digital images in which infected and uninfected <i>Litoria genimaculata</i> were observed during the day on each substrate (pond, wall and ground)	112
Figure 5.11	Proportion of digital images in which uninfected <i>Litoria caerulea</i> , <i>L. wilcoxii</i> and <i>L. genimaculata</i> were observed during the night on each substrate (pond, wall and ground)	113
Figure 5.12	Proportion of digital images in which infected <i>Litoria caerulea</i> , <i>L. wilcoxii</i> and <i>L. genimaculata</i> were observed during the night on each substrate (pond, wall and ground)	114
Figure 6.1	Number of digital images of <i>Litoria caerulea</i> and <i>L. genimaculata</i> that chose a pond more than five times and their choice of preferred pond (pond that was chosen by an individual more frequently compared to the other pond) and not preferred pond	126
Figure 6.2	Distributions of the differences between the numbers of times individual <i>Litoria caerulea</i> and <i>L. genimaculata</i> chose ponds without and with <i>Batrachochytrium dendrobatidis</i> zoospores present	127
Figure 6.3	Differences between the number of images where a frog was submerged in water with <i>Batrachochytrium dendrobatidis</i> minus number of images where a frog was submerged in water without <i>Batrachochytrium dendrobatidis</i> zoospores present	129
Figure 6.4	Number of images of <i>Litoria caerulea</i> , <i>L. wilcoxii</i> and <i>L. genimaculata</i> found submerged in water with and without <i>Batrachochytrium dendrobatidis</i> zoospores present	130

LIST OF TABLES

Table 1.1	Emerging infectious diseases and their impact on wildlife	3
Table 2.1	Temporal variation in dorsal pattern background colour of 20 <i>Litoria genimaculata</i> juveniles during the nine-week study period	28
Table 2.2	Recognition of new and recaptured <i>Litoria genimaculata</i> in Murray Upper National Park using photo identification method	30
Table 3.1	Locations and descriptions of the five study sites where <i>Litoria genimaculata</i> and <i>L. rheocola</i> skin peptides were collected	41
Table 3.2	96-well plate layout for <i>Batrachochytrium dendrobatidis</i> growth inhibition assay	46
Table 4.1	<i>Posthoc</i> analysis (unequal N HSD) of total amount of peptides (μg) secreted per surface area (cm^2) by <i>Litoria genimaculata</i> at five sites	79
Table 4.2	<i>Posthoc</i> analysis (unequal N HSD) of overall protection afforded by antimicrobial peptides in <i>Litoria genimaculata</i> at five sites.	84
Table 4.3	Number of <i>Litoria genimaculata</i> skin peptide samples on which matrix-assisted laser desorption/ionization mass spectrometry analysis were conducted	86
Table 4.4	The sum of Maculatin peptides found in the skin secretion of 69 male <i>Litoria genimaculata</i> at five sites	88
Table 5.1	Terraria size, frog species and sample size used during the six trials of the microenvironment experiment.	96
Table 5.2	Infection status of <i>Litoria caerulea</i> , <i>L. wilcoxii</i> and <i>L. genimaculata</i> individuals, at the beginning of each trial,	104
Table 5.3	Changes in intensity of <i>Batrachochytrium dendrobatidis</i> infection of <i>Litoria caerulea</i> , <i>L. wilcoxii</i> and <i>L. genimaculata</i> individuals, before and after each trial	104
Table 6.1	<i>Batrachochytrium dendrobatidis</i> infection status of <i>Litoria genimaculata</i> , <i>L. wilcoxii</i> and <i>L. caerulea</i> individuals, before and after each trial	126