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JAMES COOK UNIVERSITY
AUSTRALIA

Population and disease dynamics of the amphibian chytrid fungus in the stream-associated frog *Litoria rheocola*

Title page photos

Top left – A gravid female *Litoria rheocola* sitting on a leaf

Top right – *Litoria rheocola* tadpole captured in a net

Bottom – Frenchman Creek, northern Queensland, Australia

Photo credit: Sarah J. Sapsford

Statement of contribution of others

This thesis was co-supervised by Prof. Lin Schwarzkopf and Prof. Ross Alford. Both contributed in the form of ideas, experimental design, editorial assistance, and statistical advice, and also provided the majority of the funding. Ross Alford provided extra assistance with data analysis. Elizabeth Roznik collaborated on Chapter 2 and assisted with field work, data analysis, and editorial comments. David Pike also provided useful editorial comments for Chapter 2. Robert Puschendorf provided valuable editorial comments for Chapter 5. Maarten Voordouw provided assistance with Program MARK and advice on multistate models for Chapter 6. Ten volunteers (listed by name in the acknowledgements) provided field assistance throughout the project. All chapters will be published in collaboration with my supervisors Lin Schwarzkopf and Ross Alford. Chapter 2 will be published in collaboration with Elizabeth Roznik. Chapter 6 will be published in collaboration with Maarten Voordouw.

Declaration on ethics

All research procedures reported in this thesis received the appropriate approval of ethics: the project was carried out under permit WITK03070508 issued by the Queensland Department of Environment and Resource Management and I adhered to animal ethics protocols approved by the James Cook University Animal Ethics Committee (approval A1420).

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Abstract

Infectious diseases pose a major threat to global biodiversity. Chytridiomycosis is an amphibian disease that is caused by the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*). *Bd* has caused declines in hundreds of species of amphibians and extinctions of dozens. Some species that suffered local extinctions when the disease first emerged have reappeared and seem to be coexisting with the disease. Multiple processes can regulate changes in infection dynamics of a disease including physical and chemical characteristics of the environment and interactions among intra- and interspecific individuals. For example, the infection dynamics of *Bd* is heavily influenced by season and elevation and *Bd* can infect multiple hosts. Some hosts act as reservoirs, which are less susceptible to the pathogen, and enable the pathogen to persist in populations even when host populations are small. To fully understand the effects of a disease on a population, it is important to consider the interactions between multiple hosts, as well as the influences of environmental factors such as season and elevation.

Chytridiomycosis has many reservoir hosts including animals that are not amphibians, and adult and larval amphibians. The interactions between infection dynamics of *Bd* in adult amphibians and their larval life stage are poorly understood. To better understand infection dynamics of *Bd*, I examined six populations (adult and tadpole) of *Litoria rheocola*, the common mistfrog, in northern Queensland, Australia. I studied changes in prevalence of infection over time in adults and tadpoles and determined how prevalence of *Bd* was affected by season and elevation. To quantify elevational influences of infection I surveyed populations of adult and tadpole *L. rheocola* at three site types: 1) high elevation (> 400 m above sea level (ASL)), 2) low elevation (< 400 ASL) sites connected by stream flow to high elevations (i.e., contiguous low elevation sites), and 3) low elevation sites that lacked connectivity to high elevations (i.e., non-

contiguous low elevations). I tested all frogs and tadpoles captured to determine if they were infected with *Bd*. I marked adult frogs with visible implant elastomer tags to quantify population dynamics of frogs. I determined the probability of survival and recapture, and compared these estimates among seasons and site type.

Infection dynamics of *Bd* in contiguous low elevation sites could be influenced by two, non-exclusive processes: (i) the flow of cool water from higher elevations maintaining cooler water temperatures, making the site more hospitable to *Bd*, and (ii) downstream transport of Bd zoospores from high elevation.

Prevalence of *Bd* in tadpoles fluctuated seasonally, and was high in winter and low in summer. Prevalence of *Bd* was also influenced by site type: *L. rheocola* tadpoles at all low elevation sites had lower maximum prevalences than those at high elevation sites. There was a significant interaction between the effects of season and site type on the prevalence of *Bd* in tadpoles. Seasonal changes were more prominent at high elevation sites than at low elevation sites, and the patterns of seasonal change differed among site types. It is possible that being connected to a high elevation site greatly influenced the infection dynamics of *Bd* at contiguous low elevation sites due to the flow of cool water from high elevations and/or the flow of *Bd* zoospores downstream.

In adult *L. rheocola* populations, both season and site type influenced prevalence of *Bd*. Prevalence of *Bd* was highest in winter and lowest in summer. One population, each at both the contiguous and non-contiguous low elevation sites, had prevalences of zero in summer; however, infections reappeared in autumn, strongly suggesting that reservoirs maintain *Bd* in these sites. In comparison, infection persisted throughout summer and winter in populations at high elevations. In adult frogs, contiguous and non-contiguous low elevation populations had similar *Bd* infection dynamics, suggesting that connectivity to high elevation sites did not have a direct effect on infection dynamics of *Bd* in adults. This contrasts with the effect of site type on

the dynamics of *Bd* in tadpole populations, where site type had a strong influence on dynamics. With high prevalence of *Bd* in summer, tadpoles seem to be maintaining disease in adult populations at contiguous low elevation sites. In comparison, tadpoles at the non-contiguous low elevation site were not infected with *Bd* in summer. Therefore, tadpoles may not be an important reservoir for *Bd* at non-contiguous low elevation sites. Other species may be more effective reservoirs in these non-contiguous populations, as the disease persists in these areas, in spite of occasional apparent complete disappearance of *Bd* in adults.

The probability of survival of adult frogs, estimated using program MARK, was not influenced by chytrid infection, but did differ among seasons and site type. Recapture probabilities were influenced by site type only. Rates of incidence of infection were influenced by season. Recovery rate remained constant at 80.3% across all site types: high, contiguous low, and non-contiguous low elevations. These results suggest that instances of individual mortality caused by *Bd* do not translate into overall low survival probabilities at the population level; thus it appears that in the populations I studied, disease-induced mortality is compensatory rather than additive. Even high elevation *L. rheocola* populations are now coexisting with *Bd*, despite strong evidence that the fungus caused local extirpations in the past. The coexistence of *L. rheocola* and *Bd* suggests that either *L. rheocola* populations have evolved increased resistance to chytridiomycosis or that recent environmental conditions (or other factors operating more recently, e.g., population density) have not favoured the development of outbreaks of fatal disease.

I found that site type (elevation and contiguity with infected upland sites) influences disease dynamics of *Bd*, especially in tadpoles. In addition, my research strongly suggests that infection dynamics of tadpoles are influencing infection dynamics of *Bd* in co-occurring adult amphibian populations. My data on re-established populations suggests that the host-pathogen relationship has changed, either temporarily or more permanently, to favour the host. My study

emphasizes the importance of simultaneously investigating multiple processes that could affect infection dynamics of a disease (e.g., transmission among multiple hosts and environmental characteristics, such as topography and season). By looking at the effects of season, elevation, and multiple hosts I get a better understanding of disease dynamics and the effects of disease on populations; looking at multiple processes will become important in future studies in order to fully understand disease dynamics.

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Chapter 1 : General Introduction

The study of population dynamics examines how and why populations change over time. Population size is determined by gain and loss of individuals through reproduction, immigration, mortality, and emigration (Williams et al. 2002). These four processes drive changes in population size, and can be influenced by both abiotic and biotic factors (Williams et al. 2002). Abiotic factors, such as the physical and chemical characteristics of an individual's environment (e.g., temperature, water availability, salinity, and pH), can influence population dynamics *via* multiple pathways (Williams et al. 2002). Biotic factors influencing populations include intraspecific and interspecific interactions, such as predation, competition, parasitism, and disease (Williams et al. 2002). Biotic and abiotic factors may influence more than one population process (i.e., reproduction, immigration, mortality, or emigration).

Understanding interactions between biotic and abiotic factors and how they influence and regulate wildlife populations has been the basis of population ecology (e.g., Lachish et al. 2009). Surprisingly, especially given their potential negative effects on host populations, the influences of parasites and disease have not been a major focus of study until recently (Lachish et al. 2009, Tompkins et al. 2011). Understanding the influence of disease on populations is important because diseases can regulate populations by reducing survival and fecundity (Tompkins et al. 2002). At worst, disease can cause extinctions of populations and species (Daszak et al. 1999, McCallum and Dobson 1995, Pedersen et al. 2007). Diseases can also be transmitted among humans, wild animals, and domestic animals and can thus have detrimental effects not just on wildlife, but also on public health and our economy (Cleaveland et al. 2001, Daszak et al. 2000).

In single-host, single-pathogen associations, extinction of hosts is highly improbable, as horizontal transmission of the pathogen usually does not occur if the host population density declines below a critical threshold (McCallum and Dobson 1995, Pedersen et al. 2007). However, extinctions of the host population are more probable when pathogens have multiple hosts, because then the pathogen can persist and transmit at low host population densities (Daszak et al. 1999, McCallum and Dobson 1995, Pedersen et al. 2007). Hosts that are abundant, widespread, and usually less susceptible to the pathogen than the original host (i.e., reservoir hosts), can act as carriers and/or vectors for the pathogen (Blaustein et al. 2005, Brunner et al. 2004) and can allow it to persist in more susceptible host populations, even when the host population has reached very low densities (de Castro and Bolker 2005, McCallum and Dobson 1995). This facilitates continued infection in the susceptible host *via* reservoir-to-susceptible-host disease transmission (Brunner et al. 2004). Furthermore, if a host species population is fragmented, small, and has low genetic variability, it may be predisposed to extinction; these factors can increase host susceptibility and exposure to a pathogen if there is a reservoir (Pedersen et al. 2007). Therefore, the ability of a pathogen to infect small, susceptible host populations and persist in reservoir hosts becomes of great concern for conservation (Brunner et al. 2004, Cleaveland et al. 2001).

As mentioned above, diseases that influence wildlife can also affect human societies and industries. The introduction of rinderpest (*Morbilivirus sp.*) into sub-Saharan Africa in 1887 was a devastating example of a wildlife disease with significant economic effects (Daszak et al. 2000, McCallum and Dobson 1995, Morens et al. 2011). The virus caused the death of approximately 90% of individuals of several African ungulate species including gazelles, elands, and antelope (McCallum and Dobson 1995, Morens et al. 2011). In addition, domestic cattle were also infected and mass mortalities occurred; the farming industry suffered tremendously causing famine and disease in human populations. Thus, the rinderpest virus, indirectly, was

also responsible for countless human deaths during this time (Morens et al. 2011). Fortunately, a vaccine for rinderpest virus in cattle was developed in the 1950s; as the proportion of vaccinated cattle increased, outbreaks of the rinderpest virus declined (McCallum and Dobson 1995). Affected wildlife began to recover, suggesting that cattle acted as a reservoir for this disease (McCallum and Dobson 1995) and as of May 2011 the rinderpest virus was declared eradicated (Morens et al. 2011).

Even though rinderpest is no longer a problem, other wildlife diseases, such as *Brucellosis* (*Brucella sp.*; Corbel 1997), Mycoplasmal conjunctivitis (*Mycoplamsa gallisepticum*; Hartup et al. 2001), and chytridiomycosis, have become major concerns in recent decades. Chytridiomycosis, for example, is caused by the fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*; Daszak et al. 1999, North and Alford 2008). *Bd* is in the Phylum Chytridiomycota, Class Chytridiomycetes, and Order Chytridiales (Berger et al. 1998). Members of Phylum Chytridiomycota are heterotrophic, ubiquitous, and cosmopolitan and found mainly in soil and water where they act as primary degraders or saprobes using substrates such as chitin, plant detritus, and keratin (Berger et al. 1998). *Bd*, specifically, infects the keratinized cell layers of amphibian skin and mouthparts of tadpoles. The lifecycle of *Bd* begins as a flagellated, motile, zoospore, which encysts on the host, penetrates the outer layers of the host's tissue via a germ tube, and injects cytoplasm and its nucleus. Within the host's epidermis it grows into a thallus that produces a single sporangium containing multiple zoospores. This sporangium penetrates the surface of the skin and releases zoospores into the environment; some of these recolonise the host, causing persistence and build-up of infection (Berger et al. 1998, Berger et al. 2005). With severe infection, electrolyte transport across the epidermis is inhibited which eventually causes death by asystolic cardiac arrest (Voyles et al. 2009).

The growth of *Bd* is highly temperature dependent: optimal growth occurs between 17°C and 25°C (Piotrowski et al. 2004). Growth slows between 25°C and 28°C and *Bd* dies at 30°C

and higher (Piotrowski et al. 2004). The prevalence of *Bd*, therefore, is influenced by environmental temperatures and changes with season and elevation. In tropical regions, such as Australia and South America, prevalence of *Bd* is higher during cool winter months in comparison to warm summer months and is higher at high elevations than at low elevations (Brem and Lips 2008, Woodhams and Alford 2005). In addition, there are also potential influences of water flow and connectivity. High and low elevation areas are often connected by flowing water, and *Bd* zoospores may be carried from high to low elevations by that flow, influencing prevalence of *Bd* in populations at low elevations (sites so connected can be referred to as contiguous low elevation sites). The influx of relatively cool water at such sites may also moderate water temperature fluctuations at such sites, preventing elevated temperatures from slowing or killing *Bd.* Not all low elevation sites are connected to high elevations by flowing water. Unconnected sites, which I refer to as non-contiguous low elevation sites, may have different infection dynamics than contiguous low elevation sites because they lack the potential downstream flow of *Bd* zoospores. Season, elevation, and connectivity can interact to influence infection dynamics of *Bd* in different amphibian populations and, thus, are important in understanding the effects of this disease on susceptible populations.

Chytridiomycosis seems to have a greater detrimental effect on amphibian species that spend most of their time in or near water (Kriger and Hero 2007, McDonald and Alford 1999, Williams and Hero 1998). Since its emergence in the early 1990s (Berger et al. 1998, McDonald and Alford 1999, Richards et al. 1993), *Bd* has caused declines in hundreds of species (Skerratt et al. 2007, Stuart et al. 2004), and extinctions of dozens: for example, at least 30 species of *Atelopus* (La Marca et al. 2005), the gastric brooding frogs of Australia (McDonald and Alford 1999), and the sharp-snouted day frog of Australia (McDonald and Alford 1999, Schloegel et al. 2006).

In general, micro-parasites such as *Bd*, which can have a relatively short duration of infection and high death rates (Daszak et al. 1999), tend not to persist in populations. However, *Bd* can infect multiple hosts, some of which are not severely affected by infections, and thus are reservoir hosts that act as carriers for the pathogen and transmit the disease to more susceptible hosts (Blaustein et al. 2005, Brunner et al. 2004). Many organisms can act as effective reservoirs for *Bd*. In Panama, prevalence of *Bd* in reptiles was positively correlated with the prevalence of *Bd* in co-occurring amphibian populations (Kilburn et al. 2011). In Idaho, USA, *Rana luteiventris* is a potential reservoir for *Bd* for co-occurring frog species, because it is relatively *Bd*-resistant (Russell et al. 2010). Similarly, the resistant *Rana catesbeiana* farmed in Brazil are a potential reservoir for *Bd* in less-resistant native Brazilian frogs (Schloegel et al. 2010). *Bd* adheres to and proliferates on the keratinous toes of aquatic birds (Garmyn et al. 2012); this potential reservoir may spread infection to other populations.

Amphibians interact with each other and are, in most cases, in close association with their larval stages, but the interactions among species and life stages is poorly understood and in most cases, unknown. The ability of *Bd* to sub-clinically infect the keratinized cell layers of larval amphibian mouthparts implies that amphibian larvae could act as a reservoir for this disease; larval amphibians can carry the disease, but do not die (Blaustein et al. 2005, Daszak et al. 1999). Because larvae can be numerous, are closely associated with, and share some habitats with adults, larvae may act as effective carriers for the disease.

The existence of a diversity of hosts varying in susceptibilities facilitates for disease-driven declines in amphibians, however, very little is understood about interactions among hosts inhabiting the same area. To date, research on the effects of *Bd* on amphibians tends to focus on presence and absence of the disease in one or a few host populations, and on whether the disease negatively affects survival and other population parameters in these hosts. For example, Longo and Burrowes (2010) examined the effects of *Bd* on survival in two amphibian

populations at high elevations in Puerto Rico. Similarly, Murray et al. (2009) investigated a single population of amphibians in eastern Queensland, Australia. The question arises of whether other species co-occurring in the area with the host population of interest can act as reservoirs, causing persistence in the host species by transmission of the fungus. However, it is possible that other environmental factors are influencing the disease and this requires study.

Mark-recapture studies are necessary for understanding the infection dynamics of *Bd* and the population effects of the fungus on host populations. They allow estimation of many ecological parameters such as the probability of survival, and population size and density (Ferner 2010, Krebs 1999). Selecting an appropriate marking technique is important as the technique has to meet certain criteria: a mark should not affect survival or behaviour, should not cause pain or stress, and must last for at least the duration of the study (Campbell et al. 2009, Ferner 2010, Grant 2008, Heard et al. 2008). The technique also needs to be suitable for the study species. Toe-clipping is the most popular technique for marking amphibians, however, it may affect behaviour, survival and recapture rates (May 2004, McCarthy and Parris 2004, Parris and McCarthy 2001, Phillot et al. 2007, 2008). There are many other techniques available (e.g., passive integrated transponder tags (Ireland et al. 2003), toe and waist banding (Raney 1940), and tattooing (Kaplan 1958)), however, visible implant elastomer (VIE) tagging is a new technique that, in the laboratory, has proved to be long lasting with marks remaining readable throughout a study (Nauwelaerts et al. 2000). However, little is known on the effects of VIE on survival and behaviour of amphibians.

The general aims of my study were to 1) examine the influence of season, elevation, and connectedness on the population and infection dynamics of *Bd* in adult frog populations, 2) examine the influence of season, elevation, and connectedness on the infection dynamics of *Bd* in larval amphibians and 3) examine tadpoles` potential interactions with co-occurring adults. To achieve this, I surveyed populations of *Litoria rheocola* (the common mistfrog) for one year at

six rainforest streams at three different site types (mentioned above: high elevation, contiguous low elevation, and non-contiguous low elevation). I also conducted a mark-recapture study over one year to determine the population dynamics of adult amphibians using visible implant elastomer. The endangered common mistfrog (*Litoria rheocola*), which suffered local extinctions at high elevations in the 1990s, has since started to reappear at some high elevation sites in the past few years (McDonald & Alford 1999, McDonald et al. 2005). By addressing these aims, I provide a more comprehensive understanding of the dynamics of the amphibian chytrid fungus in larval and adult amphibians, and provide insight on the possible interactions occurring between different life-stages of this host.

This thesis comprises several stand-alone, but interrelated manuscripts. Chapter 2 investigates the effects of marking amphibians with visible implant elastomer (VIE) on survival and behaviour. This is an important aspect of mark-recapture studies as the marking technique should not change the behaviour of an animal nor have negative effects on survival. Chapter 3 looks more closely at using VIE in amphibians by determining the long-term effects of marking using this method. It expands on Chapter 2 by providing further evidence that VIE is an effective marking technique for tree frogs. In Chapter 4, I examine the infection dynamics of *Bd* in tadpoles and whether infection dynamics are influenced by season and three different site types. I demonstrate that the infection dynamics of *Bd* vary with site type and season, and that tadpoles act as potential reservoirs for this fungus. In Chapter 5, I examine the infection dynamics of *Bd* in adult *Litoria rheocola* frogs. I demonstrate that both contiguous low elevation and non-contiguous low elevation populations lose their infection in summer, but because of the presence of reservoir hosts, *Bd* persists in these populations (i.e., infection reappears). In Chapter 6, I investigate survival and recapture probabilities, incidence of infection and recovery from infection (transition from *Bd*-positive to *Bd*-negative) in adult *L. rheocola* frogs. I demonstrate that the chytrid infection does not influence survival or recapture rates, and that

these populations may, therefore, be persisting with infection. Lastly, in Chapter 7 I summarise

my findings, especially in relation to the infection dynamics of *Bd*, and discuss possible

directions for future research.

Chapter 2 : Visible implant elastomer marking does not appear to affect short-term movements and survival rates of treefrogs

Abstract

Many recent amphibian studies have involved marking individuals using visible implant elastomer (VIE), but whether VIE affects the movements or survival of amphibians in the wild is unknown. My aim was to determine whether VIE marking influenced the movement distances and survival rates of common mistfrogs (*Litoria rheocola*) in the wild over a three-week period immediately after marks were applied. The period after marking is the time when effects might be expected to be strongest because frogs are recovering from handling and the physical effects of being marked. I used harmonic direction finding to track adult frogs that were either unmarked or marked with VIE (N = 20 in each treatment), and I compared their movement distances, probability of movement, and survival estimates. To account for any effects of the external tracking devices on frog survival, I also estimated survival rates for frogs that were not tracked, but were marked and recaptured at the same site during the same time period (N = 106). As the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) can infect *L. rheocola* at my study site, I had to test for presence and absence of the disease to control for any effects of the pathogen on frog behaviour and survival. I found no significant effects of VIE marking on distances moved by frogs or the probability of movement. I found only small, non-significant differences in estimated daily survival rates between marked and unmarked frogs that were tracked; the 95% confidence intervals for all differences overlapped significantly and estimates for marked frogs were slightly higher than those for unmarked frogs. Estimated survival rates were also very similar between marked frogs that were tracked, and marked frogs that were not tracked, strongly suggesting that tracking devices did not influence survival during my study.

The only potential effect of VIE marking that I found was that marking may influence recapture rates of frogs; of tracked frogs, individuals that were marked had 12.4% higher recapture rates than unmarked individuals. Overall, I found that VIE had minimal effects on movements and survival of frogs over the three-week period immediately after marking.

Introduction

In many ecological field studies, it is necessary to identify individuals (Ferner 2010). Recognizing individuals over time can allow measurement of basic biological and ecological attributes, such as individual growth rates and movement patterns (Donnelly et al. 1994, Ferner, 2010, Kenward et al. 2002). By identifying individual animals, researchers can estimate the size and density of populations (Pellet et al. 2007, Williams et al. 2002), and can calculate demographic parameters, including rates of population growth, survival, recruitment, and dispersal (Lebreton et al. 1992, Lebreton et al. 2003, Pradel 1996). Studying these attributes is important when assessing the status and trends of populations (Bradshaw et al., 2007), and can lead to more powerful analyses that incorporate the effects of external factors such as habitat quality (Lin et al., 2001), contaminants (Frick et al., 2007), introduced species (Pope, 2008), and disease (Pilliod et al., 2010).

To identify individuals, it is essential to apply tags or marks. To meet the assumptions of mark-recapture studies, marks must be unique, easily read, and permanent, and must not affect the behaviour or survival of the animal (Ferner 2010, Pollock et al. 1990). For ethical reasons, marks should inflict the minimum possible pain, and impose the smallest possible reduction in fitness. Numerous techniques have been used to individually mark amphibians (reviewed in Ferner 2010); these include toe-clipping (Martof 1953), tattooing (Kaplan 1958), heat or freeze branding (Clark 1971, Daugherty 1976), tagging (e.g., numbered toe bands: Kaplan 1958; coloured waist bands: Emlen 1968), and implanting passive integrated transponder (PIT) tags

(Ireland et al. 2003), visible implant alphanumeric (VIA) tags (Gower et al. 2006), and visible

implant elastomer (VIE) tags (Nauwelaerts et al. 2000). In addition, natural features of an

individual, such as markings and colour patterns, can be photographed or sketched and used

for individual identification (Doody 1995, Durban et al. 2010, Kenyon et al. 2009).

There are advantages and disadvantages to each marking technique. Toe-clipping,

which involves removing toes in unique combinations, is one of the most widely used and least

expensive marking methods (Donnelly et al. 1994, Ferner 2010). However, because many

amphibians can regenerate toes and therefore lose their marks over time, this method may not

be appropriate for all studies (Ferner, 2010). Furthermore, toe-clipping amphibians is

controversial because of ethical concerns and potential impacts on behaviour, survival, and

recapture rates (May 2004, McCarthy and Parris 2004, Parris and McCarthy 2001, Phillott et al.

2007, 2008). One of the most promising alternatives to toe-clipping for marking amphibians is

visible implant elastomer (VIE, Northwest Marine Technology, Inc., Shaw Island, WA USA;

Nauwelaerts et al. 2000). VIE is especially useful in small animals whose size precludes using

other types of marks. VIE is a fluorescent silicone-based material that is injected beneath

relatively transparent areas of skin and remains externally visible; it can be used in different

colour combinations and/or in different body locations to create a large number of unique marks.

VIE is a two-part material that consists of elastomer and a clear curing agent; it is mixed before

use and then injected as a liquid that cures to a pliable solid. VIE was originally developed to

mark fishes, but it has been used successfully in other taxa, including amphibians (Bailey 2004,

Davis and Ovaska 2001, Grant 2008, Hoffmann et al. 2008, Nauwelaerts et al. 2000).

Before using any marking technique in a long-term study, it is important to ensure that

the marks are permanent and easily read, and that they do not influence the behaviour or

survival of the study animals (Donnelly et al. 1994, Ferner 2010). Researchers have reported

that VIE marks are easy to apply, visible, and long-lasting (Campbell et al. 2009, Heemeyer et

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al. 2007, Hoffmann et al. 2008, Measey et al. 2001), but little is known about their potential effects on behaviour and survival. Phillips and Fries (2009) reported that VIE marking did not affect growth or survival of captive salamanders, and Kinkead et al. (2006) found no effects of VIE marking on stress hormone levels or behaviour of captive salamanders. However, Schmidt and Schwarzkopf (2010) found that the maximum jumping distance of captive frogs decreased immediately after VIE marking and remained lower two weeks after marking. Whether VIE marking affects the survival or movements of amphibians in the wild is unknown.

The goal of my study was to determine whether VIE marking affected the movements and survival of common mistfrogs (*Litoria rheocola*) in the wild over a three-week period immediately following marking. Any effects of marking are most likely to occur immediately after application of marks. The period immediately after marking is when animals are recovering from handling and from the minor injury involved in marking, are likely to be experiencing any post-marking infection, and may be adjusting to any limitations imposed by the marking on their movements. I would also expect effects of marking to be most pronounced in a small amphibian; thus, I chose *L. rheocola* (adult male body mass: 2 g) as my study species. I used harmonic direction finding (Rowley and Alford 2007) to track adult frogs; all frogs carried individually identifiable tracking devices, but some frogs were marked with VIE, whereas others were not. I used these data to compare daily movement distances, movement probabilities, and survival rates between frogs that were and were not VIE-marked. To account for any effects of the external tracking devices on frog survival, I also compared these survival estimates with estimates obtained from frogs that were VIE-marked and recaptured (but not tracked) at the same site and during the same time period that tracking took place.

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Materials and methods

Study species

The common mistfrog (*Litoria rheocola*) is a small (average male body size: 2 g, 32 mm snout-urostyle length; S.J. Sapsford, personal observation), endangered (IUCN 2012), hylid frog that occurs at rocky, fast-flowing, rainforest streams in northern Queensland, Australia (Dennis 2012, Hoskin and Hero 2008). On rainy days and at night, these nocturnal frogs perch on rocks, logs, and vegetation near riffles, and on dry days they shelter between moist rocks and leaf litter in the stream (Dennis 2012, Hodgkison and Hero 2002, Hoskin and Hero 2008).

Study site

My study was conducted at Windin Creek (750 m elevation) in Wooroonooran National Park, Queensland, Australia (17°21'56.7"S, 145°42'53.7"E). Windin Creek was surrounded by tropical rainforest and was characterized by pools, riffles, and waterfalls. The stream bed was composed mainly of small rocks (1-20 cm diameter). I captured and observed frogs along a 400 m section of stream over a three-week period during the cool/dry season (18 August – 9 September 2009). Prior to marking frogs, I placed flags at 10 m intervals along the stream transect so that I could record the location of each frog each time it was captured.

Overview of field methods

I evaluated the effects of visible implant elastomer (VIE) marking on frog behaviour and survival using two different methods. I attached diode tags to 40 frogs to track them using harmonic direction finding (Gourret et al. 2011). These tracking devices have little or no effect on the behaviour of frogs after the first 24 h following attachment (Rowley and Alford 2007), and can be located at a distance using a special transceiver. I individually marked the antennas of the tracking devices with unique colour combinations so that tracked frogs could always be

identified when they were relocated. Twenty of the frogs I tracked were also marked using VIE, and 20 were not VIE-marked. The tracking devices allowed us to regularly locate and individually identify all frogs, whether or not they carried VIE marks. I could therefore estimate and compare the survival rates of VIE-marked and non-VIE-marked frogs over the three-week study period. Because it is unknown whether tracking devices affect short-term survival rates, I also marked an additional 106 frogs with VIE only and carried out a standard mark-recapture study concurrent with the tracking study, which allowed us to obtain survival estimates for frogs that carried VIE marks but no tracking device, for comparison with tracked frogs.

VIE marking

To prevent disease transmission among captured frogs, each frog was captured in an unused plastic bag worn as a glove, and handled with disposable gloves. Following capture, frogs were sexed (using presence/absence of nuptial pads), mass determined to the nearest 0.1 g, measured (snout-urostyle length), and marked with VIE (Northwest Marine Technology Inc., Shaw Island, Washington, USA). Five colours of VIE were used to create a marking scheme: pink, orange, yellow, green, and blue. VIE was injected subcutaneously into the inner thigh of the frog (Schmidt and Schwarzkopf 2010) using a 29-gauge insulin needle (Terumo Medical Corporation, Elkton, Maryland, USA). Individual frogs received up to three marks in total, which were injected into the left leg, the right leg, or distributed between both legs. Once marking and processing were completed, frogs were released at their capture locations. Needles were sterilized between uses by placing them in 70% ethanol for at least 20 seconds (Australian Department of Environment and Heritage 2006, Johnson et al. 2003).

Tracking of frogs with and without VIE marks

I tracked 40 frogs using harmonic direction finding (Rowley and Alford 2007). Of these individuals, 20 (14 males, 6 females) were marked with VIE, and 20 (15 males, 5 females) were not marked with VIE. I tracked frogs using RECCO detectors (models R4 and R8, RECCO Avalanche Rescue System, Lidingö, Sweden). These hand-held devices act as both transmitters and receivers; they emit a continuous radio-frequency signal that is absorbed and re-emitted at a higher harmonic frequency by diodes. I built tracking devices using SOT-323 surface-mount zero-bias Schottky detector diodes (Agilent Technologies, Forest Hill, VIC, Australia) attached to a belt made of silicone tubing (Gourret et al. 2011). The tubing was cut to length so it just encircled the frog's inguinal region (waist). A length of cotton thread ran through the tubing and was tied to secure the tubing around the frog's inguinal region. The antenna of each tracking device was uniquely colour-coded so each individual could be identified when it was sighted. The combined mass of the tracking device and belt did not exceed 8% of any tracked frog's body mass, which is below the recommended maximum 10% transmitter-to-body-mass ratio for amphibians (Richards et al. 1994). I attempted to locate frogs once during each day (1000 h -1700 h) and once each night (2000 h - 0300 h) throughout the tracking period. To track frogs, I walked slowly along the centre and edges of the stream and scanned all areas potentially used by frogs, including on and around rocks in the stream and on vegetation along the stream edge. Each time I located a frog, I recorded whether the frog had moved from its previous location, and if it had, I recorded its new location and used that to determine its horizontal and vertical displacement (to the nearest 0.10 m). At the end of the tracking period, I removed the tracking devices from all recaptured frogs.

When using harmonic direction finding, frogs cannot always be located during each tracking session; the tracking detector typically has a maximum detection range of 15 m around rainforest streams, and cannot penetrate rocks (Rowley and Alford 2007). However, the

difficulty of locating frogs was unlikely to bias my movement estimates in this study. *Litoria*

rheocola has high site fidelity, so when a frog was not found on a particular survey (or surveys),

it was almost always eventually relocated less than 2 m from its last known location. The high

site fidelity of frogs suggests that these frogs move short distances, and were sheltering

beneath rocks when I could not detect them. Because my knowledge of tracked frogs was

imperfect, I also searched for and recorded the identities and locations of frogs carrying tracking

devices during mark-recapture surveys (described below), and I used the Cormack-Jolly-Seber

model implemented in program MARK (version 5.1; White and Burnham 1999) to model the

survival of tracked frogs (as detailed below). My survival estimates incorporated the probabilities

of mortality and emigration (termed 'apparent survival'; White and Burnham 1999), which in my

study equated to persistence in the study area within detection range. Therefore, any

differences in estimated survival rates between marked and unmarked frogs could be caused by

differences in mortality rates or by differences in behaviour that influenced detectability.

Mark-recapture surveys of frogs marked only with VIE

       I marked a total of 106 frogs (n = 83 males, 10 females, 13 juveniles) in the 400 m

stretch of stream along which frogs were tracked, and I recaptured them during nightly surveys

conducted on the same nights as tracking. During recapture surveys, I walked slowly along the

centre and edges of the stream and visually searched for frogs perched on rocks in the stream

and on vegetation along the stream edge. I also attempted to locate each male frog that I heard

calling. When I recaptured a frog (by hand), I recorded its identity and its location along the

stream transect (to the nearest m). Each recaptured frog was released immediately at its point

of capture.

Assessing disease status

The amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), which causes the disease chytridiomycosis, can infect *Litoria rheocola* at my study site (Woodhams and Alford 2005). Therefore, to control for any effects of the pathogen on frog behaviour and survival, I tested each frog for the presence of the pathogen at first capture, and also at the end of the study period if the frog was recaptured at that time. To measure presence of infection, I swabbed the ventral surface and all four feet of each frog using a sterile rayon swab (covering these areas twice with the swab). These samples were analysed using real-time quantitative PCR assays (Boyle et al. 2004). For all analyses, a frog was considered infected if it tested positive for the amphibian chytrid fungus at either the start or end of the study period. The present paper does not focus on the effects of disease on frog movements or survival; however, I used infection status (i.e., infected or uninfected) as a factor in all analyses to examine the possibility that infection by the amphibian chytrid might interact with any effects of marking.

Analysis of Movements

I excluded data collected during the 24-hour period following attachment of tracking devices because of potential short-term behavioural effects of handling (Langkilde and Alford 2002) that are unlikely to persist after the first night following tag attachment (Rowley and Alford 2007). *Litoria rheocola* is a treefrog, and individuals move along and at right angles to the stream and also climb up and down vegetation. Thus, they use all three dimensions of space, with their directions of movement generally unconstrained in the horizontal plane, but restricted to movements up and down individual plants in the vertical direction. Therefore, I measured their movements as total displacement (the sum of horizontal and vertical displacement) between consecutive surveys; total displacement includes the displacement from a nocturnal perch site to the following diurnal shelter site or from a diurnal shelter site to the subsequent

nocturnal perch site. Movement distances were calculated only when individuals were located on consecutive surveys (i.e., from a day survey to the following night survey, or from a night survey to the following day survey). When frogs were not located on consecutive surveys, movement distances for the time interval concerned were recorded as missing values and were not included in any analyses. Because movement distances often follow asymmetrical distributions, the median is a better representation of central tendency than the mean. I therefore examined the possible effects of VIE mark status (marked or unmarked) and infection status (infected or uninfected) and their interaction on the median of movement distance for each individual frog using a two-way ANOVA.

To determine whether marked frogs moved more or less often than unmarked frogs, I calculated the movement probability for each individual as the number of times it moved between consecutive locations divided by the total number of times it was located. As with my distance data, when frogs were not located on successive surveys, the sequence of locations was restarted when the individual was first relocated following the gap in the data. I used a two-way ANOVA to examine how individual movement probability was affected by VIE mark status, infection status, and their interaction. Small sample sizes for females (total $n = 11$; marked infected = 4, marked uninfected = 2, unmarked infected = 4, unmarked uninfected = 1) prevented us comparing the effects of marking between sexes. Because the distribution of females among treatments was reasonably balanced, any gender effects should not be confounded with treatment effects.

Survival Analysis

To determine whether marking frogs with VIE affected their survival, I used the Cormack-Jolly-Seber (CJS) model implemented in program MARK (White and Burnham 1999). My encounter history included the presence/absence of each frog for each night that I sampled

the population (i.e., 20 surveys); therefore, my estimates were daily survival probabilities. Even though individuals with tracking devices were tracked day and night, I only used encounters at night for the survival analysis to keep the encounter history comparable to marked-only (not tracked) individuals (which were only located on night surveys). I included six groups in my model: 1) marked frogs that were not tracked and were infected with the chytrid fungus ($n = 56$), 2) marked frogs that were not tracked and were not infected with the chytrid fungus ($n = 50$), 3) infected marked frogs that were tracked ($n = 15$), 4) uninfected marked frogs that were tracked ($n = 5$), 5) infected unmarked frogs that were tracked ($n = 13$), and 6) uninfected unmarked frogs that were tracked ($n = 7$). I developed a set of candidate models that tested whether frog survival was influenced by VIE marks, tracking devices, chytrid infection, or combinations of these factors (Table 2.1). I excluded time as a model parameter to prevent over-parameterization of my model (i.e., including more parameters than can be estimated for the data). I derived an estimate of lack of fit for the global (i.e., most parameterized) model in my candidate set, using program RELEASE, implemented in MARK. The global model fit the data well ($\chi^2 = 63.14$, df = 122, $P = 1.000$). In addition, to quantify the amount of over-dispersion, I calculated the value of the variance inflation factor ($\hat{c} = \chi^2/\text{df}$). Because $\hat{c} < 1$, and $\hat{c}$ was set to 1, no adjustment of the data was necessary.

The best-supported models were selected using Akaike's Information Criterion adjusted for small sample size (AIC$_c$; Burnham and Anderson 2002); the best-supported models are those that make up the top 90% of Akaike weights and with relative deviations from the best model of less than 2 (ΔAIC$_c < 2$; Burnham and Anderson 2002). My survival estimates (termed 'apparent survival'; White and Burnham, 1999) incorporated both mortality and emigration; survival thus equated to persistence in the study area within detection range. If the best-supported models suggested that there were differences between groups, I compared the 95% confidence intervals for the means of the groups concerned to determine whether they differed

significantly, following the approach described by Cumming et al. (2007). When the best-supported models suggested that there were no significant differences, I examined the magnitude of non-significant differences and their variability to determine whether the differences appeared genuinely unimportant or might be biologically meaningful.

Results

Movements of tracked frogs

Of the 40 frogs fitted with tracking devices, I relocated all of the 20 frogs that were marked using VIE and 17 of the 20 frogs that were not marked with VIE. I located 14 VIE-marked frogs and 7 unmarked frogs on at least one consecutive pair of surveys. The mean of the median individual movement distances did not differ significantly between VIE-marked and unmarked frogs ($F_{1,17}$ = 1.071, P = 0.315), and there was no significant interaction between the effects of marking and infection status ($F_{1,17}$ = 2.500, P = 0.132). The mean of the median individual movement distance between locations (± 1 SD) was 2.8 ± 0.9 m for marked frogs and 2.1 ± 1.1 m for unmarked frogs. The mean of the median individual probability of movement was not significantly affected by VIE marking ($F_{1,33}$ = 0.541, P = 0.467), and there was no significant interaction between the effects of marking and infection status ($F_{1,33}$ = 1.734, P = 0.197). The mean of the median movement probability (± SD) was 0.970 ± 0.294 for marked frogs and 1.000 ± 0.163 for unmarked frogs.

Survival Rates

There were three well-supported models in my survival analysis (i.e., ΔAIC_c < 2; Table 2.1). Because these three models all had ΔAIC_c values less than 2, it was not possible to determine which model fit the data the best (Burnham and Anderson 2002). Therefore, I model-averaged the best-supported models to derive estimates of daily survival and recapture

(Burnham and Anderson 2002). The other candidate models carried very little or no weight (Table 2.1). The mark-dependent model takes into account the effect of VIE marks, irrespective of being tracked or not. The track-dependent model takes into account the effect of being tracked, irrespective of being VIE marked or not. The group-dependent model takes into account the effects of VIE marking and tracking by testing for differences among the three groups (1) marked and not tracked, 2) tracked and marked, and 3) tracked and not marked).Infection status did not influence survival rates in any of the three top models. The survival estimates for marked frogs (marked and tracked estimated mean survival: 94.5%, range of 95% CIs: 89.5-97.2%; marked and not tracked mean survival: 96.1%, range of 95% CIs: 92.6-97.9%) were actually slightly higher than that for unmarked frogs (estimated mean survival: 92.2%, range of 95% CI: 85.7-95.9%).

Daily survival estimates for frogs that were VIE-marked and not tracked, VIE-marked and tracked, and non-VIE-marked and tracked were similar (92.2-96.1%; Fig. 2.1) and did not differ significantly when compared using the criteria of Cumming et al. (2007). To further examine the difference between frogs marked with VIE and frogs not marked with VIE I took the difference between the two groups of tracked frogs. The difference was relatively small (marked – not marked = 2.3%), confidence intervals for these two groups were reasonably tight, confidence intervals overlapped such that the estimates did not differ significantly, and the difference was in a direction that parsimony suggests is unlikely (i.e., that marked and tracked frogs survived at higher rates than tracked frogs not marked with VIE).

Survival and Tracking Devices

To account for any effects of the external tracking devices on frog survival, I compared survival estimates for VIE-marked frogs that were tracked with VIE-marked frogs that were not tracked. I found that the maximum difference in survival rates between groups was 1.6%.The

confidence intervals for survival rates were small (< 8%), and overlapped such that the

estimates did not differ significantly.

Table 2.1 Candidate models used to estimate daily survival rates and recapture probabilities for common mistfrogs (*Litoria rheocola*) that were marked with visible implant elastomer (VIE) only, VIE-marked and tracked, and non-VIE marked and tracked.

Model description		AIC_c	ΔAIC_c	Model weight	Parameters	Deviance
Survival rates	Recapture rates					
Track-dependent	Group × infection	1335.89	0.00	0.36	8	1066.84
Mark-dependent	Group × infection	1336.07	0.18	0.33	8	1067.02
Group-dependent	Group × infection	1337.16	1.27	0.19	9	1066.02
Mark × infection	Group × infection	1339.57	3.68	0.06	10	1066.32
Track × infection	Group × infection	1339.74	3.85	0.05	10	1066.49
Group-dependent	Group-dependent	1341.58	5.69	0.02	12	1064.09
Mark-dependent	Group-dependent	1354.31	18.42	0.00	5	1091.47
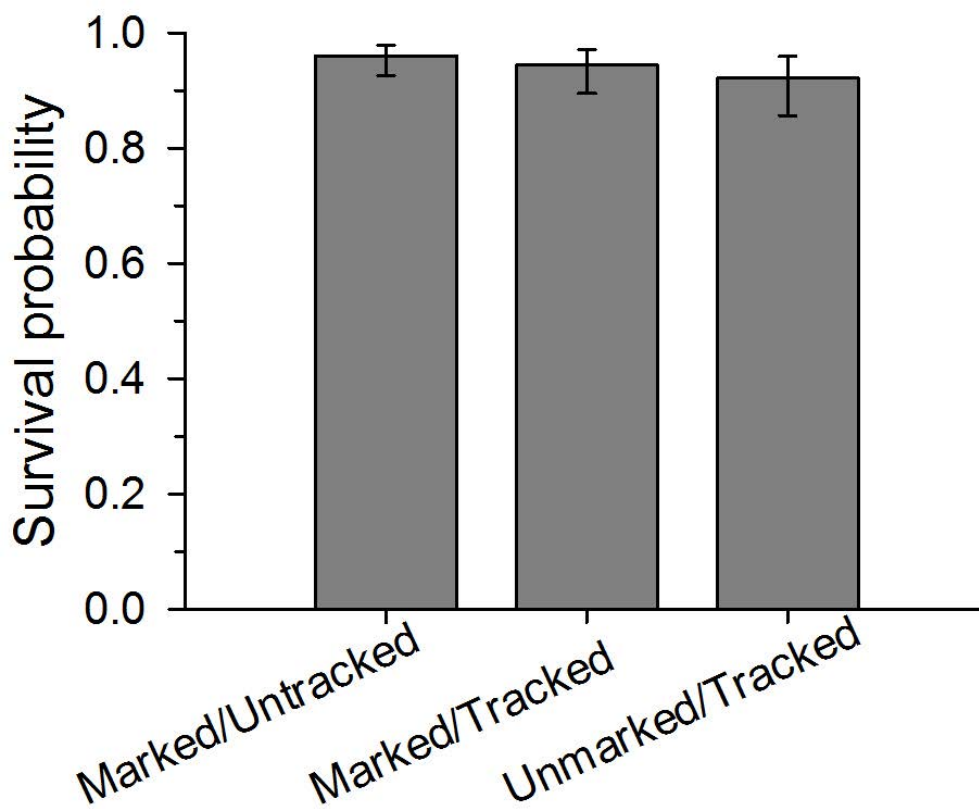
Figure 2.1 Mean daily survival probabilities (with 95% confidence intervals) for common mistfrogs (*Litoria rheocola*) in northern Queensland, Australia over the three-week period immediately after some individuals were marked using visible implant elastomer (VIE) and tracked using harmonic direction finding.

Recapture Rates

The three supported models in my analysis included a group × infection effect, which suggested that recapture rates differed among six classes of frogs, classified both by group (marked and untracked, marked and tracked, unmarked and tracked) and by infection status

(infected, uninfected; Fig. 2.2). Tracked frogs were recaptured at higher rates than marked frogs that were not tracked. Of tracked frogs, frogs infected with the amphibian chytrid fungus were recaptured more often than uninfected individuals. The opposite pattern was found with marked frogs that were not tracked: infected frogs were recaptured less often than uninfected frogs.
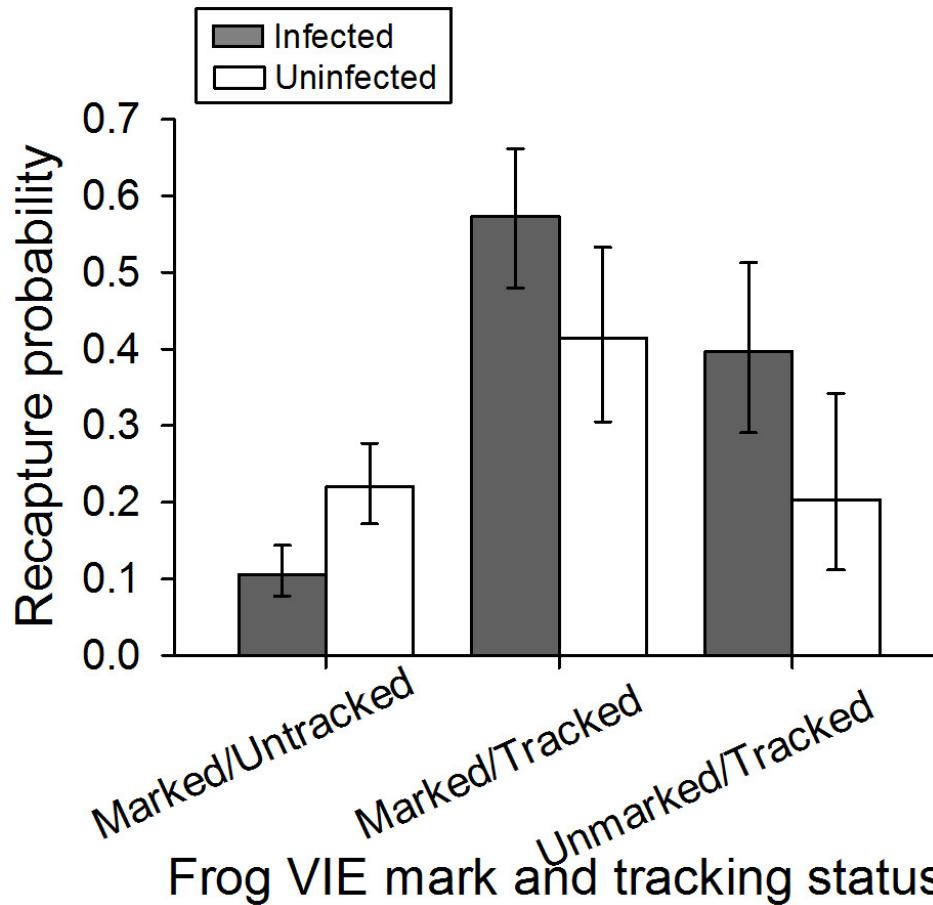
Figure 2.2 Mean daily recapture probabilities (with 95% confidence intervals) for common mistfrogs (*Litoria rheocola*) in northern Queensland, Australia over the three-week period immediately after some individuals were marked using visible implant elastomer (VIE) and tracked using harmonic direction finding.

Discussion

The primary aim of my study was to determine whether visible implant elastomer (VIE) affects the behaviour and short-term survival rates of *Litoria rheocola*; I compared movement distances and survival rates between frogs that were marked or not marked with VIE during the three weeks following marking. To carry out the comparison between VIE-marked and non-VIE marked frogs, I needed some other means of identifying and locating individuals, so I tracked them using harmonic direction finding. Because attachment of a tracking device might, in itself, affect behaviour or survival rates, I also carried out a mark-recapture study using individuals that received VIE marks, enabling comparisons with marked and unmarked individuals that were tracked.

Because the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), which causes the disease chytridiomycosis, can affect survival rates of frogs even where it is endemic (Murray et al. 2009), I also needed to consider possible effects of infection on *L. rheocola* at my study site (Woodhams and Alford 2005). If infections were altering frog behavior or decreasing survival rates, failing to account for infection status could have inflated the variance in my results or biased my estimates of effects. I found no significant effects of infection on the distances moved or the probability of movement of individuals. I also did not find biologically meaningful effects of infection status in my analyses of daily survival rates of tracked (marked and unmarked) and untracked frogs during my three-week study period. The lack of increased mortality of infected individuals is not surprising; numerous studies have shown that individuals and populations of amphibians can survive for extended periods with little mortality, despite high prevalence of infection by the amphibian chytrid fungus (e.g., review by Alford 2010, Puschendorf et al. 2011, Retallick et al. 2004). I did find that recapture rates were affected by the interaction of marking status and infection status (Table 2.1; Fig. 2.2). Tracked individuals that were infected were more likely to be recaptured than those that were not infected. This

suggests that although I did not find significant effects of infection status in my analysis of movement parameters, small, non-significant differences that existed in the data may have been sufficient to affect recapture rates.

I did not find any significant effects of VIE marking on the distances moved in frogs or on the probability of movement of my tracked frogs. I found that marked and unmarked frogs moved similar distances between day and night locations, and that marked frogs were not significantly more or less likely to change locations than unmarked frogs. In addition to lacking statistical significance, the differences in movement parameters between marked and unmarked frogs were very small relative to the means of those parameters, and to the standard deviations of their distributions, suggesting that they were not biologically relevant. Other studies that have investigated the effects of VIE marking on amphibian movement behaviour have had mixed results. Kinkead et al. (2006) observed no obvious differences in locomotion, limb placement, or feeding behaviour in captive salamanders marked with VIE. However, Schmidt and Schwarzkopf (2010) found that the maximum jumping distance in captive frogs decreased immediately after VIE marking and remained lower two weeks after marking. Schmidt and Schwarzkopf (2010) commented that their frogs did not respond well to captivity; possibly the stress of being held in captivity coupled with marking produced measurable effects in the laboratory.

In addition to having minimal effects on frog behaviour, my data suggest that VIE marking had little or no biologically meaningful effect on daily survival rates of frogs in the three weeks immediately after marking. The best-supported models in my survival analysis suggested that survival differed among the three groups of frogs (tracked and marked, tracked and unmarked, untracked and marked); however, mean daily survival estimates were very similar among all groups (ranging from 92.2-96.1%; Fig. 2.1), and the differences that existed suggest that marking increased survival. The apparent increase in survival estimates for marked frogs

could be explained by differences in sample sizes or recapture rates, both of which could have altered the precision of survival estimates. Of frogs that were tracked, marked frogs had 12.4% higher recapture rates than unmarked frogs (Fig. 2.2), suggesting that marked frogs may have behaved in ways that made them easier to relocate (e.g., by using more exposed microhabitats). Although I did not find any statistically significant differences between movements of marked and unmarked frogs that were tracked, it is possible that other aspects of behaviour that I did not measure were affected by VIE marking. Because it is unlikely that marking actually increased daily survival rates in the three weeks immediately following marking, my results suggest that any real differences in survival were not large enough to be biologically meaningful. My data also suggest that carrying tracking devices did not affect daily survival of frogs over a three-week period. Estimates of daily survival rates of tracked frogs were very similar to survival estimates for frogs marked using VIE, but not tracked (Fig. 2.1). The estimated difference between untracked frogs and tracked frogs was 1.6%, and this difference was not significant when using the criteria of Cumming et al (2007). My results thus suggest that carrying a tracking device did not decrease the daily survival rates of frogs, and the small difference I did observe suggests that at least in the short term, any real difference that may occur is very small.

In addition, the daily survival estimates provided by MARK for these data seem very low when estimated over the long-term. With an actual individual survival rate equivalent to my estimates, the population of frogs would disappear after approximately four months, which is highly unlikely. Data from a long-term study demonstrate that this population is persisting (Chapter 6). Fourteen months after this three-week study, at least 18% of the individuals that were originally marked in this study were recaptured (S.J. Sapsford, personal observation). Thus, rather than indicating low survival rates, my short-term data suggest that there might be

subtle, short-term effects of VIE on behavior (which may alter probability of encountering an individual).

Recapture rates were higher for tracked frogs than untracked frogs. The differences in recapture rates are likely due to the difference in recapture methods (visual surveys vs. harmonic direction finding). During my three-week study, only 61 out of 106 marked frogs were recaptured (58%) when using visual surveys, whereas when frogs were tracked, 20 out of 20 marked frogs were recaptured, and 17 out of 20 unmarked frogs were recaptured. Of tracked frogs, marked frogs were recaptured more often than unmarked frogs, suggesting that marking may influence some aspects of frog behavior, as discussed above. In addition, recapture rates also varied with chytrid infection status. Although I found no significant effects of chytrid infections on short-term survival, there is evidence that this pathogen may influence frog behavior in this species (E.A. Roznik, personal observation), potentially in ways that could influence the likelihood of detection when using visual surveys and tracking techniques.

In conclusion, my data suggest that both marking frogs with VIE, and tracking them using external tracking devices appear to have little to no negative effect on daily survival rates of *Litoria rheocola* during the three weeks immediately after marking, when negative effects might be particularly pronounced. All differences between VIE-marked and unmarked frogs were small relative to the magnitude of estimates, and no differences were statistically significant; therefore, it appears that differences between marked and unmarked groups were minimal. VIE also had minimal and non-significant effects on movement of *L. rheocola* in nature over the three-week period immediately following marking. The only potential effect of VIE marking that I found was that marking may influence recapture rates of frogs. While VIE appears to be a safe and effective marking technique, further studies on additional species and over longer time periods under both laboratory and natural settings are necessary to fully understand any impacts of this relatively new marking technique on amphibians.

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Chapter 3 : Visible implant elastomer as a viable marking technique for long-term amphibian studies

Abstract

Mark-recapture studies are often used to determine population demographic parameters such as rates of recruitment and survival, individual parameters such as growth rate and pattern, and other ecological information, such as individual movement patterns. The marking techniques used must satisfy several criteria: marks must not affect the behaviour or survival of individuals, marks must not cause pain or stress, marks should be long-lasting or permanent, and marks should be easily identifiable. A relatively new technique, visible implant elastomer (VIE), is being used in mark-recapture studies of fishes, amphibians and other vertebrates. I examined its effectiveness during a 1-year study of common mistfrogs (*Litoria rheocola*). I marked 1392 frogs and recaptured 255 at least once. VIE tags can move under the skin of amphibians, potentially affecting their readability, so I determined the readability and retention of marks over time. I marked animals under the skin of the thighs. I did not detect any loss of marks over the study period (i.e., no individual marked initially in two legs lost the marks in one leg); however, 16% of marks became impossible to read to determine individual identity. Movement parameters did not differ significantly, and were very similar, between frogs marked in one leg and frogs marked in two legs. I conclude that VIE is a safe and effective marking technique for long-term amphibian studies, especially in stream-associated hylid frogs.

Introduction

Mark-recapture studies allow estimation of many ecological and behavioural parameters that otherwise would be difficult or impossible to estimate, e.g., rates of growth and survival,

movement patterns, habitat selection and home range, and population size and density (Campbell et al. 2009, Ferner 2010, Hoffman et al. 2008, Krebs 1999). Marking techniques must satisfy certain requirements: marks should not affect survival or behaviour, should not cause pain or stress, and must last for at least the duration of the study (Campbell et al. 2009, Ferner 2010, Grant 2008, Heard et al. 2008). Marks often must be unique to individuals or cohorts (Ferner 2010).

It is important to select a marking technique that meets these criteria, but it is also important to choose a technique that is practical to apply and is easily and accurately read (Ferner 2010). Choosing among techniques inevitably involves tradeoffs. Toe-clipping is the most common marking technique for amphibians. Toe-clipping produces many unique marks and is inexpensive, but many amphibians can regenerate their toes, some more quickly than others, and thus toe-clipping may not be suitable for long-term studies (Ferner 2010). Toe clipping could affect behaviour, survival, and recapture rates, and is controversial from an ethical perspective as it may cause pain and suffering (May 2004, McCarthy and Parris 2004, Parris and McCarthy 2001, Phillot et al. 2007, 2008). Passive integrated transponder (PIT) tags are also quite popular in amphibian studies (Ireland et al. 2003). However, both tags and readers are relatively expensive, even the smallest tags may not be suitable for smaller species, and the effects of tags on behaviour, survival, and recapture rates have not been well studied (Phillot et al. 2007, 2008).

Many techniques (reviewed in Ferner 2010) are available in addition to toe-clipping and PIT tags; these include tattooing (Kaplan 1958), toe and waist banding (Brown 1997, Raney 1940, Woodbury 1956), heat or freeze branding (Clark 1971, Daugherty 1976), visible implant elastomer (VIE), and visible implant alphanumeric (VIA) tags (Gower et al. 2006). In addition, natural features and patterns of individuals can be used for identification if they are recorded (e.g., by photographs; Doody 1995, Kenyon et al. 2009).

Visible implant elastomer (VIE) has been used extensively to mark fish, and has only recently become a popular technique for marking amphibians (Campbell et al. 2009). It consists of a coloured elastomer which is mixed with a curing agent before being injected under the skin (Northwest Marine Technology Inc., Shaw Island, Washington, USA). Using small amounts of elastomer at a time, it is a relatively inexpensive technique which can be used to mark large numbers of animals (Hoffman et al. 2008, Marold 2001).

The effectiveness of VIE as a marking technique for fishes and crustaceans has been thoroughly evaluated (Astorga et al. 2005, Clark and Kershner 2006, Claverie and Smith 2007, Uglem et al. 1996, Younk et al. 2010). For example, there were no negative effects of marking with VIE on growth or survival in the fish *Fundulus heteroclitus* (Skinner et al. 2006) and VIE had a 96% retention rate over 6 months in rainbow trout, *Oncorhynchus mykiss* (Walsh and Winkelman 2004). Similar results have been found in laboratory studies of amphibians; salamanders retained clear marks 64 weeks after marking (Davis and Ovaska 2001) and 100% of VIE marks were retained with little change over eight months in *Rana esculanta* (Nauwelaerts et al. 2000). VIE marking had no influence on movement or survival of common mistfrogs (*Litoria rheocola*; Chapter 2). The effects of VIE on other aspects of amphibian biology have also been tested in captivity. The maximum jumping distance of frogs in the laboratory decreased immediately after VIE marking and remained lower two weeks after marking (Schmidt and Schwarzkopf 2010). In comparison, no differences in stress hormone levels or behaviour were reported in captive salamanders marked with VIE (Kinkead et al. 2006) and VIE marking did not affect growth or survival of captive salamanders (Phillips and Fries 2009). The results of laboratory studies thus suggest that VIE may produce long-lasting marks with little or no effect on most aspects of amphibian behaviour and ecology.

Very little is known regarding the long-term readability or effects of VIE marks on amphibians in the field. The goal of my study was to determine whether VIE is a viable marking

technique for long-term studies of the common mistfrog, *Litoria rheocola*, in nature. I marked

frogs over twelve months, examined changes in readability of marks, and evaluated effects of

marking on long-term measurements of frog movement. Marks were placed in either the right

leg, the left leg, or both legs. If marking affected mobility of the marked limb, I expected that

individuals marked in both legs should be more strongly affected and thus should move less

often, or should move shorter distances than individuals marked in only one leg.

Material and methods

Study species

The common mistfrog, *Litoria rheocola*, is a small (average body size: 2 g, 32 mm snout-

vent length; S. J. Sapsford, personal observation) endangered hylid frog (IUCN, 2012) that

occurs near rocky, fast-flowing, rainforest streams in northern Queensland, Australia (Dennis

2012, Hoskin and Hero 2008). At night, they typically perch on rocks, logs, and stream-side

vegetation near riffles (Dennis 2012, Hodgkison and Hero 2002, Hoskin and Hero 2008), and

during the day they usually shelter between moist rocks in the stream bed (E. A. Roznik,

personal observation).

Study sites

This study was conducted at six different rainforest streams in the Wet Tropics Bioregion

in northern Queensland, Australia: Mena Creek (17°38' 59.6"S, 145°59' 11.2"E; 59 m above sea

level (ASL)), Stoney Creek (17°55' 17.9"S, 146° 04' 07.2"E; 24 m ASL), Tully Creek (17°46'

29.5"S, 145°38' 38.2"E; 114 m ASL), Frenchman Creek (17°18' 32.8"S, 145°55' 04.2"E; 59 m

ASL), Windin Creek (17°22'04.2"S 145°42'52.1"E; 718 m ASL), and Bobbin Bobbin Falls (17°22'

43.5"S, 145°46' 21.7"E; 586 m ASL). These streams were surrounded by tropical rainforest and

had pools, riffles, and waterfalls. The stream beds were composed mainly of small rocks (1-10 cm diameter).

Overview of field methods

Frogs were captured by hand. I captured, marked, recaptured, and observed frogs along a 400 m transect at each stream on five consecutive nights every three months from June 2010 to July 2011. I placed numbered flags at 10 m intervals along the transect so that I could record the exact capture location of each frog along the transect, as well as its position at any future recapture events.

Following capture, frogs were sexed (presence/absence of nuptial pads), mass determined to the nearest 0.1 g, measured to the nearest 0.1 mm (snout-urostyle length), marked with VIE, and released at the point of capture. The height above the stream, distance from the centre of the stream, and position along the transect of each capture were recorded. To prevent disease transmission between individuals, each frog was captured using an unused plastic bag worn as a glove and handled with new powder-free latex gloves during processing.

Marking

Five colours of VIE were used to create a marking scheme: pink, orange, yellow, green, and blue. VIE was injected just below the skin of the inner thigh of the frog (Schmidt and Schwarzkopf 2010) using a 29-gauge insulin needle (Terumo Medical Corporation, Elkton, Maryland, USA). Marks of one of the five colours were placed at one or more of three positions (at approximately one third, one half, and two thirds of the distance from the waist to the knee) on one or both of the legs of each frog, allowing for 46,656 (6^6 given six positions: five colours and no mark) potential marks (Fig. 3.1). I constrained this scheme for the purpose of this study so that I could be certain whether marks moved: two marks of the same colour were never

placed beside each other but could be separated by a different colour (i.e., blue in position 1, green in position 2, and blue in position 3; Fig. 3.1). Each colour was injected using a different syringe and needle. Once marking was completed, the mark was photographed using a digital camera (Pentax Optio 33WR, 3.2 Megapixels). Frogs were then released at their points of capture. Needles were sterilized between individuals by placing them in 70% ethanol for at least 20 seconds (Johnson et al. 2003, Australian Department of Environment and Heritage 2006).
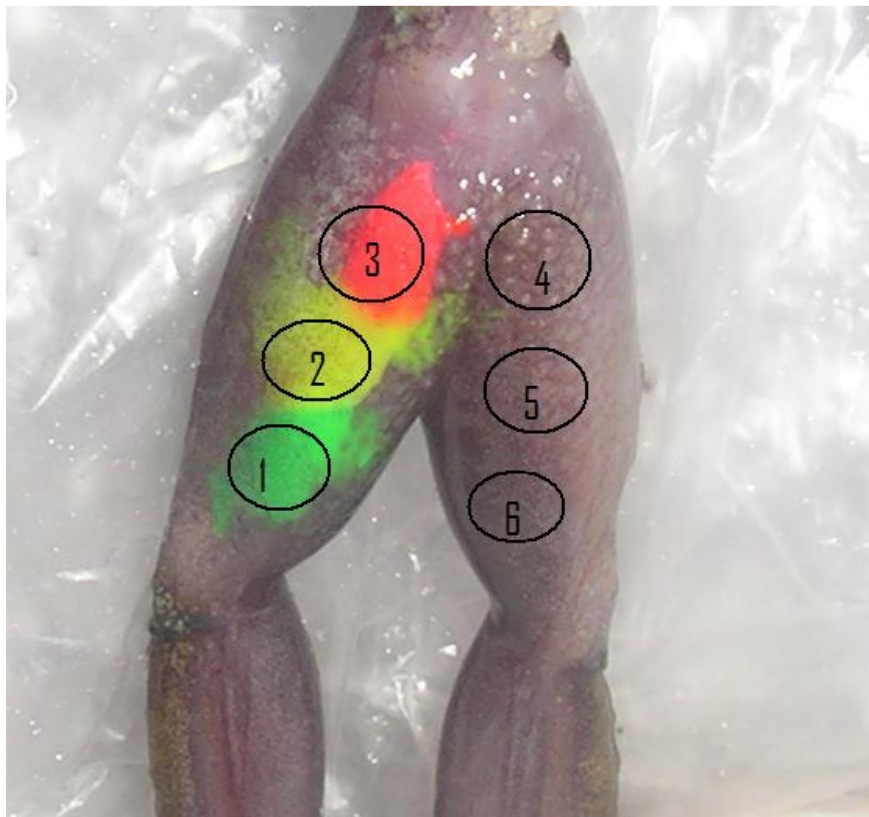
Figure 3.1 Six different possible locations where visible implant elastomer marks could be injected using one of five colours: pink, orange, yellow, green, and blue. VIE was injected just below the skin of the inner thigh using a 29-gauge insulin needle.

Recapturing

During surveys, I walked slowly along the centre and edges of the stream and visually searched all rocks in the stream and all vegetation along the stream edge. I also attempted to locate each male that I heard calling. When I recaptured a frog, I recorded its identity, its height above the stream, its distance from the centre of the stream, its location along the stream transect (to the nearest m), and whether the frog was calling. Whenever a marked frog was recaptured, its mark was photographed (Pentax Optio 33WR 3.2 Megapixels).

Analysis of marks

I compared the photographs of recaptured frogs to those taken when they were initially marked, and categorized their marks into five states: 0-no change, 1-migration to body, 2-colours touching but distinct, 3-colours mixed, and 4-colour(s) fragmented (Fig. 3.2). Since some marks only had one VIE injection (1 colour in the leg) they could not be categorized into state 2 or 3. Some marks were made with two or more injections (2+ colours in the leg) and therefore it was possible for marks to be in more than one state. In these cases, I picked the state that best described the change in the mark. Therefore, marks made with one colour and two or more colours were categorized separately. All new marks were designated as state 0, as no change could have occurred at initial marking.

Analysis of movement

One of the assumptions of a mark-recapture study is that marks should not affect movement. Therefore, I examined the extent to which marking with VIE affected frog movement. I hypothesized that if marking affected movement by altering the performance characteristics of the marked limb, frogs marked in both legs should move differently than those marked in a single limb. I therefore compared movement parameters between these classes of individuals. I

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marked few females, and thus could not compare the effects of marking between sexes. *Litoria rheocola* often climb vegetation at night, and thus use three-dimensional space, but movements between locations are largely on the horizontal plane or up and down vegetation. Therefore, to examine effects on movement I used a two-way ANOVA to analyse the median three-dimensional distance moved (measured as the sum of the horizontal and vertical displacements) of individual frogs between successive recaptures. Factors included in the ANOVA were location of marks (one leg or both legs), the survey site at which each individual was captured, and the survey trip on which the individual was recaptured. Frogs could be recaptured on five different trips during different seasons. Movement values were missing in some sites during trips 3 and 4, so I compared median three-dimensional displacement on trips 1, 2 and 5. By using trip as a factor I accounted for differences in movement of frogs during different seasons: *L. rheocola* tends to spend more time in arboreal habitats during the warm summer months and more time in aquatic habitats when it is cold (Retallick 2002). By taking trip into consideration I was also allowed to account for the inequality of the location of marks on each trip (i.e., differences in proportion of marks in one vs. two legs for each trip). However, this paper focuses on the effects of VIE on movement and I will not present results related to differences in movement among seasons.
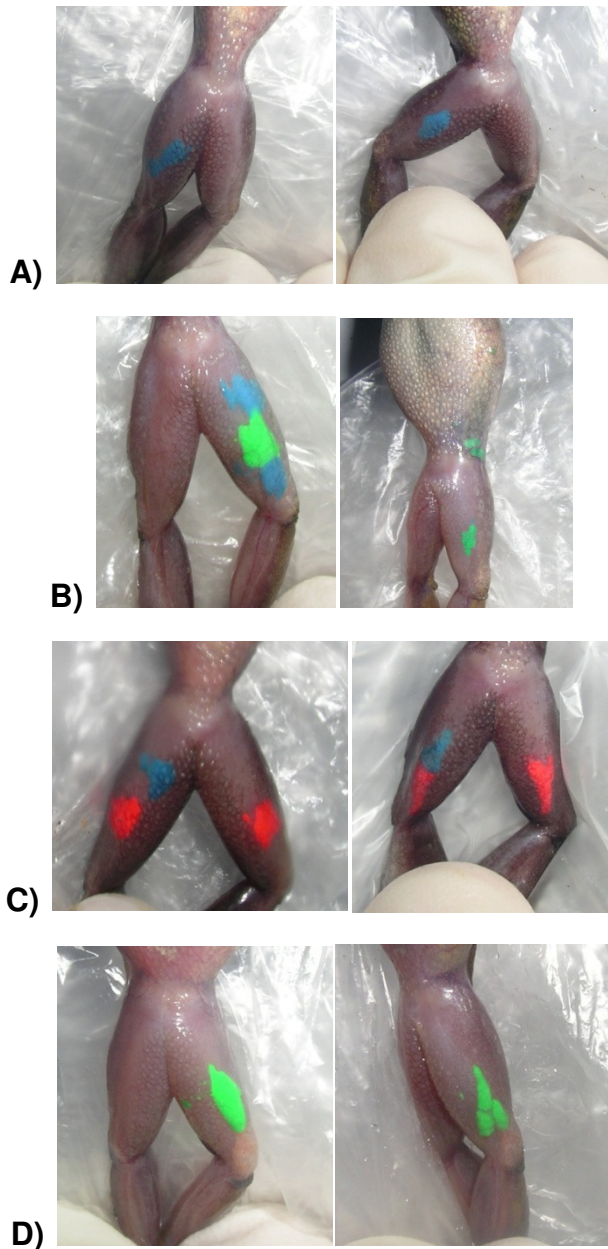
Figure 3.2 Over time marks may change from when they were initially made. Pictures on the left show the initial mark state, while pictures on the right show possible changes. A) no change after 3 months. B) mark migrated into the body, colours mixed. C) mark transitioned to a state with colours touching but distinct (in the leg with two marks). D) mark fragmented.

Results

Marks

I marked 1392 individuals with a total of 2141 VIE marks. Individuals were marked in the right, left, or both legs. Two hundred fifty-five individuals were recaptured at least once during the study; these individuals carried a total of 296 separate VIE marks. These marks (not individuals) were considered in the analysis to determine how the properties of marks changed over time. Marks with only a single VIE injection (1 colour in the leg) were examined separately from marks made with two or more injections (2+ colours in the leg) because the possible state transitions differed depending on how many colours were used.

If marks changed, almost without exception this occurred between the time when a mark was first made (time 0) and 3 months after initial mark (time 1) and the mark remained constant after this initial change. No marks were actually lost at any time (i.e., no individual marked initially in two legs lost the marks in one leg). Approximately, 42% of marks did not change; when changes occurred, the most common was that colours touched but remained distinct (Table 3.1). All marks seen more than once between 6 and 12 months, after they were initially made, remained unchanged between observations.

Movement

Median three-dimensional displacement did not differ significantly between frogs marked in one leg or both legs ($F_{1,1}$ = 2.422, P = 0.121; Fig. 3.3). The mean of the median individual movement distance between successive recaptures (± SD) was 1.41 ± 7.69 m for frogs marked in one leg and 1.25 ± 5.39 m for frogs marked in both legs. Site ($F_{1,5}$ = 1.500, P = 0. 189) and the interaction between site and number of marks ($F_{1,5}$ = 1.684, P = 0.137) also did not significantly affect movement of frogs.

Table 3.1 Number of transitions of visible implant elastomer (VIE) marks from first capture to successive recapture three months later. Marks could change from state 0 to any of the five states: 0-no change, 1-migration to body, 2-colours touching but distinct, 3-colours mixed, and 4-colour(s) fragmented. When only one colour was used to mark a leg changes could only occur into categories 1 and 4. When 2 colours were used, it was possible for marks to be in more than one state. In these cases, I picked the state that best described the final mark.

	Left Leg		Right Leg	
Mark State	1 VIE mark	2 VIE marks	1 VIE mark	2 VIE mark
0: No Change	56	2	64	2
1: Migration to body	1	1	1	0
2: Colours touching but distinct	0	50	0	58
3: Colours mixed	0	26	0	19
4: Colour(s) fragmented	8	1	7	0
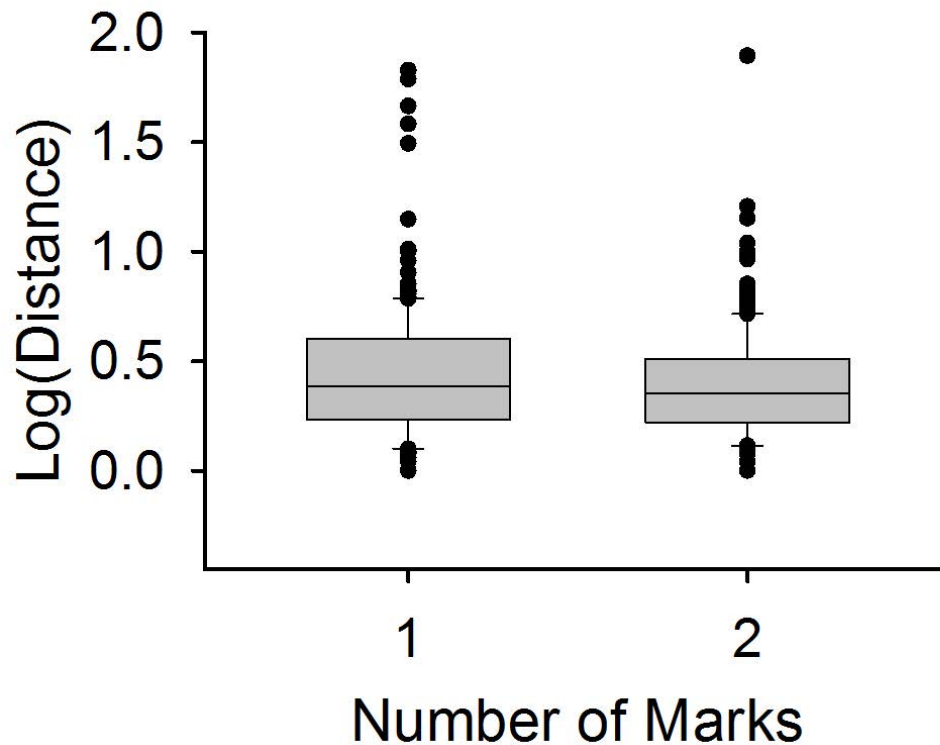
Figure 3.3 The median three-dimensional displacement (measured as the sum of the horizontal and vertical displacements) of individual frogs between successive recaptures comparing frogs marked with VIE in one leg and frogs marked in both legs.

Discussion

VIE is a viable marking scheme for amphibians in a laboratory setting (Davis and Ovaska 2001, Heemeyer et al. 2007, Nauwelaerts et al. 2000). However, very few studies have reported the effects of VIE in the wild. The results of this field study demonstrate that VIE is an appropriate marking scheme for common mistfrogs, *L. rheocola*, especially for a long-term study. Marks were long-lasting, and they changed relatively little, usually within the first 3

months after application, subsequently remaining unchanged up to 12 months after initial marking. My results demonstrated that 84% of marks were readable allowing individuals to be identified to the end of the study. Movements were very similar and did not differ significantly between individuals marked in one or two hind legs, suggesting that marking *L. rheocola* is unlikely to affect the movement of individuals of this species.

Marks

Throughout the 12 months, the majority of marks were easily read and identifiable. At 3 months after initial marking, 37% of marks moved such that colours were touching but distinct. Only 1.0% of marks migrated into the body (state 1) and in 15% of cases, the colours of marks mixed (state 3). When marks migrated or colours mixed, individuals could be difficult to identify based on marks alone. However, even if a mark was difficult to read, the presence and colours of the mixed VIE, in addition to the morphology and behaviour of individuals allowed identification (because of high site fidelity and repeated retreat site used by individuals; E. A. Roznik, unpublished, Rowley et al. 2007).

In addition, although some became unreadable, all marks were retained (i.e., no individual marked initially in two legs lost the marks in one leg). Some VIE colours are visible under a UV light (Northwest Marine Technology Inc., Shaw Island, Washington, USA), so even if a mark migrated from a translucent leg to an opaque body area, the mark was still visible. This reduces the rate at which a researcher may miss marked individuals.

In general, my results concur with previous laboratory studies. In salamanders, marks were retained over a year and were as easily read at 6 months as at 12 months after the initial mark (Heemeyer et al. 2007). In another study, VIE marks had high retention and readability over 15 weeks in salamanders (Marold 2001). My results demonstrate easy readability and high

retention over a year in the wild, which is important for researchers conducting field studies, and only emphasizes the success of VIE marking.

Movement

If marking had affected movement I expected to see differences between frogs marked in one leg (right or left leg) and those marked in two legs. I found that three-dimensional displacement was very similar between these classes of individuals and did not differ significantly, suggesting that movement is not influenced by VIE marks. This lack of effect is important because marking should have no effect on behaviour and movement (Donnelly et al. 1994, Ferner 2010). Schmidt and Schwarzkopf (2010) found that marking had a negative influence on locomotion as measured by jump length in frogs in captivity, but this was only in the initial 2 weeks after marking. Perhaps frogs recover from marking quickly, or the actual length of individual jumps is not a critical variable influencing overall daily field movement. Alternatively, it is possible that *Litoria nasuta*, examined by Schmidt and Schwarzkopf (2010), was more strongly influenced by marks. Movement of *L. rheocola* was not significantly affected by marking with VIE in a 3-week field study (Chapter 2). In a different study, marking with VIE had no effect on movement patterns of salamanders (Davis and Ovaska 2001). There have also been no observed differences in locomotion, limb placement, or feeding behaviour in captive salamanders marked with VIE (Kinkead et al. 2006).

In conclusion, my study is one of the few examinations on the effects of VIE on movement over a long-term period in amphibians in the wild. I demonstrated that over a long-term period VIE had a high rate of retention and readability. I also demonstrated that VIE does not significantly affect long-term movement in *Litoria rheocola*. It thus appears that VIE is a safe and effective marking technique for long-term studies in amphibians.

Chapter 4 : Dynamics of infection by *Batrachochytrium dendrobatidis* in Australian rainforest stream tadpoles

Abstract

The prevalence of parasitic infection and diseases increases with host age; older individuals are more likely to be infected because they have greater cumulative exposure to the risk of infection. The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), infects the mouthparts of tadpoles, usually without fatal effects. If individuals tend to retain infections for extended periods, then older, larger tadpoles, with greater cumulative exposure to the risk of transmission of infection, should have a higher prevalence of infection by *Bd* than younger, smaller tadpoles. Larval amphibians can take a year or more to reach metamorphosis in tropical streams and in many tropical amphibian species, breeding peaks in summer. An annual cycle of *Bd* prevalence could be driven entirely by tadpole demography. For example, prevalence of *Bd* in tadpoles would reach a minimum in summer when the tadpole population is composed of young individuals with initially low cumulative risk of transmission. As they remain in aquatic habitats through winter, prevalence would rise as cumulative risk increases, would peak in spring, and would decrease rapidly in early summer as older individuals metamorphose and leave the larval population and young individuals enter it. Levels of *Bd* prevalence in tadpoles should affect levels of infectious zoospores in the water they inhabit and thus should affect rates of transmission from tadpoles to adult and juvenile frogs of species that enter that water. Tadpole demography could thus play a key role in driving seasonal patterns of prevalence of *Bd* in both tadpoles themselves and in terrestrial frogs. The prevalence of *Bd* in stream-dwelling tadpoles may also be affected by water temperature and by exposure to zoospores carried by water flow. I collected information on tadpole demography and the prevalence of *Bd* infection

over a year in six rainforest streams: two upland sites, two lowland sites connected to upland sites by stream flow (contiguous sites) and thus subject to an influx of cool water that might carry *Bd* zoospores, and two lowland sites not connected to upland sites by stream flow (non-contiguous sites). My results demonstrate that prevalence of *Bd* infection was higher in older, larger tadpoles than it was in younger, smaller tadpoles, even within the same season. Prevalence of *Bd* in tadpoles was also higher in winter than summer and was affected by elevation and connectedness. Simple models indicated that the observed patterns of prevalence were probably driven by both demographic and environmental effects.

Introduction

Many factors affect the prevalence of disease in wildlife populations. These include, but are not limited to, season, host age, and host sex (Hosseini et al. 2004, MacIntosh et al. 2010, Muhldorfer et al. 2011, Pathak et al. 2011, Wilson et al. 2002). Seasonality can cause a variety of changes in host-pathogen interactions including changes in host behaviour and contact rates, variation in the probability of encountering parasite or pathogen propagules in the environment, pulses in host birth and death rates, and changes in host immune defences (Altizer et al. 2006). Rates of pathogen transmission and mortality can be calculated by analysing age-specific patterns of prevalence and intensity (MacIntosh et al. 2010, Wilson et al. 2002). Differences in physiology and behaviour between classes of individuals, such as males and females, can also affect the prevalence of disease in populations (MacIntosh et al. 2010, Wilson et al. 2002). Although many factors can interact to influence the prevalence of disease, few studies have looked at these interactions.

The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), has caused population declines and extinctions in many amphibian species around the world (La Marca et al. 2005, Lips et al. 2006, Stuart et al. 2004). The pathogen has infected species over a large

geographic range, including areas that are relatively undisturbed by humans. In many areas, the greatest declines have occurred in stream-associated species (Alford 2010, McDonald and Alford 1999, Woodhams and Alford 2005). The fungus infects and replicates within the keratinized epidermis of adult amphibians and the keratinized cell layers of tadpole mouthparts (Berger et al. 1998, Longcore et al. 1999, Simoncelli et al. 2005).

The dynamics of *Bd* and its interactions with amphibians have been extensively studied *in vitro* and in adult amphibians. *Bd* growth is highly temperature-dependent; optimal growth occurs between 17°C and 25°C and death occurs at temperatures above 30°C (Piotrowski et al. 2004). In tropical rainforests in Australia and South America, *Bd* reaches higher prevalence at high elevations (in Australia, above approximately 400 m above sea level), where temperatures are cooler, than at low elevations (< 400 m in Australia), where temperatures are warmer (Brem and Lips 2008, Pullen et al. 2010, Retallick 2002, Woodhams and Alford 2005). In addition, the prevalence of *Bd* is typically higher during cooler, winter months than in warmer, summer months; this seasonal pattern occurs at both high and low elevations (Brem and Lips 2008, Retallick 2002, Woodhams and Alford 2005).

In addition to season and elevation, there are also potential effects of water flow and connectivity. High and low elevation areas are often connected by flowing water. When this occurs, *Bd* zoospores may be carried from high to low elevation sites, influencing prevalence of *Bd* in the populations at lower elevations through a process known to stream ecologists as drift (Brittain and Eikeland 1988). In addition, streams carry cooler water from high elevations to lower elevations, potentially moderating water temperatures and maintaining them within the range at which *Bd* grows well. However, these effects should only occur at low elevation sites that are connected to higher elevation sites by water flow, which I refer to as contiguous low elevation sites. In the Australian Wet Tropics Bioregion, for example, some catchments have no terrain with elevations greater than 400 m. I refer to streams within these catchments as non-

contiguous low elevation sites. Because stream flow cannot partially couple their thermal or *Bd* dynamics to upstream high elevation sites, non-contiguous sites may have different infection dynamics than contiguous low elevation sites. Comparing infection dynamics between contiguous and non-contiguous low elevation sites may provide insight into how these sites are affected by aquatic transport of zoospores and by water temperatures.

A major difference between tadpoles and adult amphibians is that larvae of many species, in which terrestrial juveniles and adults are highly vulnerable to the effects of chytridiomycosis, can carry *Bd* infections without fatal effects (Blaustein et al. 2005, Lamirande and Nichols 2002). Infections by *Bd* usually appear not to kill tadpoles, possibly because Bd only infects their mouthparts: as *Bd* infection in tadpoles' increases in severity, mouthparts (keratin) are lost, reducing the intensity of infections and potentially clearing individuals of infection (Cashins 2009, Venesky et al. 2009). Once infection is cleared, the mouthparts can be regenerated, increasing feeding efficiency and reducing the chance of mortality (Cashins 2009). Tadpoles can carry *Bd* infections at high prevalences for extended periods. Infected tadpoles release infectious zoospores into the surrounding environment (Rachowicz and Vredenburg 2004), and can thus transmit the pathogen to terrestrial juveniles and adults when uninfected individuals in those life-history stages contact water containing infected tadpoles. In some species, infected tadpoles can maintain their infections through metamorphosis (Marantelli et al. 2004). Tadpoles can therefore act as intraspecific reservoir hosts for *Bd* (Blaustein et al. 2004, Brunner et al. 2004, Cashins 2009, Daszak et al. 1999, Rachowicz and Vredenberg, 2004).

The prevalence of infection by many parasites and diseases increases with host age; older individuals are more likely to be infected because they have greater cumulative exposure to the risk of infection (MacIntosh et al. 2010, Wilson et al. 2002). If individuals tend to retain infections for extended periods, I would expect that in tropical stream systems, where many tadpoles require months or even years to reach metamorphosis (Alford 1999), older, larger

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tadpoles, with greater cumulative exposure to the risk of transmission of infection, should have a higher prevalence of infection by *Bd* than younger, smaller tadpoles. In some species, larger tadpoles are significantly more likely to be infected with *Bd* than are smaller tadpoles (Cashins 2009, Smith et al. 2007). In many tropical amphibian species, breeding peaks in summer. An annual cycle of *Bd* prevalence could be driven entirely by tadpole demography; prevalence would be low in summer when there is an abundance of small tadpoles. It would then increase through winter as tadpoles grow and accumulate infection, and would decrease in spring as overwintering individuals metamorphose and a new cohort of young individuals enters the system. Because water temperatures are often substantially cooler than air temperatures (Mohseni and Stefan 1999), *Bd* infections may persist in populations of tadpoles through summer, even where air temperatures cause prevalence in terrestrial amphibians to reach zero (Bosch et al. 2001, Rachowicz and Vredenburg 2004). Tadpole demography could thus play a key role in driving seasonal patterns of prevalence of *Bd* in both tadpoles themselves and in terrestrial frogs.

The aim of my study was to examine the infection dynamics of *Bd* in tadpoles in tropical rainforest streams and determine whether prevalence of *Bd* was influenced by season and elevation (as seen in adult populations). I also determined whether demography influenced the prevalence of *Bd* in tadpoles. I conducted surveys at different seasons and at sites of three different types: high elevations (above 400 m), contiguous low elevations (below 400 m), and non-contiguous low elevations. This distribution of sites allowed me to examine how both elevation and connectivity influenced the prevalence of *Bd* in tadpoles. This is the first comprehensive study to investigate the dynamics of the amphibian chytrid fungus in populations of tadpoles over seasonal and elevational gradients. This study will contribute greatly to the understanding of chytrid disease dynamics and shed light on potential new processes occurring in these amphibian stream communities.

Materials and methods

Study species

        I studied the larvae of frogs of four species endemic to the Wet Tropics Bioregion of Australia: *Litoria rheocola* (the common mistfrog), *Litoria nannotis* (the waterfall frog), *Nyctimystes dayi* (the Australian lace-lid frog), and *Litoria serrata* (the green-eyed tree frog). All four species suffered severe population declines due to chytridiomycosis in the early to mid 1990s (Berger et al. 1998, McDonald and Alford 1999, McDonald et al. 2005). *Litoria rheocola*, *L. nannotis*, and *N. dayi* became locally extinct at high elevation (> 400m) sites after the emergence of the disease; however, both *L. rheocola* and *L. nannotis* have since started to reappear at high elevations (McDonald & Alford 1999, McDonald et al. 2005). Populations of *L. serrata* maintained their original ranges, but underwent large declines at high elevations. All four species breed all year with peak breeding in summer; their tadpoles are present throughout the year and can take up to a year to reach metamorphosis (Alford 1999, Cashins 2009, Richards 1992).

        Tadpoles of *L. rheocola, L. nannotis*, and *N. dayi* are members of the "lotic-suctorial" ecomorphological guild and are adapted to fast-flowing (torrent) sections of streams (Altig and Johnston 1989, Liem and Hosmer 1973, Richards 1992). Their large ventral oral disc, depressed body shape and muscular tail allow them to adhere to rock surfaces (Richards 2002). Tadpoles of *L. serrata* belong to the "clasping" ecomorphological guild and are adapted to slow-flowing sections of streams (Altig and Johnston 1989, Davies 1989, Richards 1992). In comparison to torrent-adapted tadpoles, they are less streamlined, have a much smaller oral disc, and have a deeper tail fin. These "clasping" tadpoles have little to no ability to adhere to surfaces with their oral discs (Richards 2002).

Study sites

Sampling of tadpoles took place over one full annual cycle, from austral winter (June/July 2010), through spring (October 2010), summer (January 2011), autumn (March/April 2011) and the following winter (June/July 2011). Tadpoles were sampled at six sites in the Wet Tropics Bioregion in northern Queensland, Australia: two at high elevations (Bobbin Bobbin Falls, 17°22'43.5"S 145°46'21.7"E; 700 m ASL, and Windin Creek, 17°22'04.2"S 145°42'52.1"E; 718 m ASL, both in Wooroonooran National Park), two at low elevations that are contiguous with (downstream from) high elevations sites (Frenchman Creek, at 17°18'29.2"S 145°55'16.2"E; 59 m ASL, in Wooroonooran National Park, and Tully Creek at 17°46'29.5"S 145°38'38.2"E; 114 m ASL, in Tully Gorge National Park) and two at low elevations that were not downstream from high elevation sites (non-contiguous; Mena Creek, at 17°38'59.6"S 145°59'13.5"E; 59 m ASL, and Stoney Creek, at 17°55'17.9"S 146°4'7.2"E; 18 m ASL, in Hull River National Park). All of these creeks were surrounded by tropical rainforest and all contained pools and riffles; some also had waterfalls. The nature of the creek beds varied among sites: those of Frenchman Creek, Tully Creek, and Bobbin Bobbin Falls were composed of large boulders interspersed with sections composed of small rocks (1-10 cm in diameter). The beds of Windin, Mena, and Stoney Creeks were primarily composed of small rocks.

Field methods

Prior to surveys, I placed flags at 10 m intervals along a 400 m transect at each site so I could record the location of each tadpole that was captured. Sampling at each site in each season took place during four days. Tadpoles were sampled during the day using a dip net pressed against and slowly moved along the stream substrate. Larger rocks upstream from the net were displaced manually to dislodge tadpoles attached to rock surfaces. Once captured, tadpoles were held in individual 50 mL vials filled with stream water until they were processed.

During processing each tadpole was measured and swabbed for *Bd*. A new pair of well-rinsed vinyl gloves was worn while processing each tadpole (Cashins et al. 2008). Body length was measured to the nearest 0.1 mm from the tip of the snout to the base of the tail where the axis of the tail myotomes contacts the body wall (Altig 2007). For all species, body lengths were categorized into six body-size classes: 1) 5 – 6.9 mm, 2) 7 – 8.9 mm, 3) 9 – 10.9 mm, 4) 11 – 12.9 mm, 5) 13 – 14.9 mm, 6) 15- 16.9 mm (Cashins 2009). Developmental stage (Gosner 1960) was not recorded because the hindlimbs of *L. nannotis*, *L. rheocola*, and *N. dayi* develop in sheaths beneath the epidermis until late in development (Cashins 2009), preventing determination of developmental stage using hindlimb characteristics. After processing, tadpoles were released where they were captured. To reduce the likelihood of capture of tadpoles already processed during the sampling period, sampling always resumed upstream from locations at which tadpoles were released.

Assessing disease status

I swabbed each tadpole's mouthparts because they are the only body part of tadpoles known to carry amphibian chytrid fungus infections (Berger et al. 1998, Longcore et al. 1999). The swabbing technique followed Cashins (2009). Tadpoles were held (mouthparts facing up) between the fingers and thumb. A sterile fine-tip dry rayon swab (#113 Dry swabs, Medical Wire and Equipment, Corsham, Wiltshire U.K.) was gently passed along the mouthparts in a regular pattern: eight times horizontally across both the lower and upper tooth rows and jaw sheath, and eight times vertically across all tooth rows and jaw sheath for a total of sixteen strokes.

After sample collection, all tadpole swabs were placed in separate labelled vials and refrigerated until the end of sampling. All swab samples were processed using real-time quantitative PCR (Boyle et al. 2004). Samples were run in triplicate and considered positive for

Bd if at least two of the three PCR reactions had numbers of zoospore equivalents that were greater than zero.

Environmental temperatures

Air temperature was recorded using three Thermochron iButton dataloggers (model DS1921G, precision: 0.5 °C, accuracy: 1°C) at each site. They were placed in shaded locations at 0 m, 200 m, and 400 m along the transect, approximately 1 m from the creek's edge and 2 m above the ground. The dataloggers were coated in transparent plastic (Plasti Dip, Plasti Dip International, Blaine, MN, USA) to prevent water damage (Roznik and Alford 2012). Each iButton recorded air temperature at 90 min intervals over the entire study period (June 2010 to June 2011). Water temperatures were not recorded over the entire study period, but only when tadpoles were sampled (i.e., 4 days during each season). Water temperature was recorded where each tadpole was captured using a glass spirit thermometer with a range of -10°C-50°C. Between 30 and 60 tadpoles (including all four species) were captured at each site during each sampling trip; thus 30-60 water temperatures were recorded for each site during each trip.

Relationships between water and air temperatures

I examined the relationship between water and air temperature using a reduced major axis regression of mean water temperature at tadpole collection locations for each site during each trip and mean air temperature at each site across the days on which water temperature was measured in each trip.

Effects of site type, season, and temperature on prevalence

The only species that was present at all sites was *L. rheocola*. I have summarised data on the prevalence of *Bd* infection for the other three species where they occurred in Appendix 1. All further analyses were performed only on *L. rheocola*.

Because the growth of *Bd* is temperature-dependent (*in vitro* growth rate co-varies positively with temperature; Piotrowski et al. 2004), I hypothesized that the prevalence of *Bd* should be positively correlated with water temperature. I used a linear regression to examine whether prevalence of *Bd* in *L. rheocola* tadpoles in each site and season was influenced by water temperature (only temperatures at which *L. rheocola* were caught were used). Because prevalence is a proportion, and is non-normally distributed, I arcsine square-root transformed prevalence before analysis. Water temperature was used as the independent variable.

Because mean temperature and other aspects of the physical environment differ among sites and seasons, I wanted to determine whether season, site type, or their interactions were related to prevalence in my populations, as occurs in other amphibian populations in Australia and other regions (whether *Bd* prevalence was higher in winter than summer and whether prevalence was higher at high elevations than at low elevations; Brem and Lips 2008, Pullen et al. 2010, Retallick 2002, Russell et al. 2010, Savage et al. 2011). To determine whether season and site type had effects on the probability of *Bd* infection in tadpoles that were independent of the effect of water temperature, I used a generalized linear model with a binomial link function. Season, site type, and their interaction were categorical predictor variables, the temperature at each tadpole's capture site was included as a covariate, and disease status (infected or not infected) was the response variable. I used the two sites of each site type (high, contiguous low, non-contiguous low) as replicates.

Due to significant damage caused by Tropical Cyclone Yasi (a category 5 storm), which hit the northern Queensland, Australia, coast in February 2011, one of the high sites (Bobbin

Bobbin Falls) was not accessible in autumn 2011. Therefore, to keep my examination of effects orthogonal, I ran the above model on all data, for all sites, excluding the autumn season.

Examining the potential effects of tadpole demography

In some amphibian populations, older, larger tadpoles have higher prevalences of *Bd* infection than younger, smaller tadpoles (Cashins 2009, Smith et al. 2007). Since *L. rheocola* tadpoles take up to a year to metamorphose and breeding occurs year round with peak breeding in summer, I hypothesized that small, uninfected tadpoles would be more abundant in summer and that these individuals would accumulate infections over time, resulting in higher prevalences of *Bd* in the larger tadpoles usually found in winter. Thus, it is possible that the demography of tadpoles might drive prevalence of *Bd* in *L. rheocola* populations. Because larger tadpoles should, on average, be older, this should be reflected in a relationship between size-frequency distributions in populations and prevalence, and in differences in prevalence among tadpoles of different size classes. To determine whether the proportion of tadpoles in each size class differed among seasons and site types, I used a multinomial generalized linear model: season and site type were the predictor variables, and the size class of each *L. rheocola* tadpole was the response variable.

The possible influence of size structure on *Bd* prevalence could interact with the influence of season on *Bd* prevalence. I developed five models of how *Bd* prevalence should change seasonally. I parameterized each model using data from all sites but one, and compared their predictions to the actual patterns of prevalence at the site not used to parameterize the model. I repeated this procedure for all 6 sites. My models were:

1) a null model: prevalence of *Bd* is not influenced by site type, season, or size class. To build this model I ignored site type, season, and size class and I calculated the mean prevalence of *Bd* infection across all sites except the site of interest.

2) a null model for mean effects of size class distribution: prevalence of *Bd* is influenced by mean size class. To build this model I ignored site type and season and calculated average prevalence across all seasons in each size class at sites other than the one I was examining. I then used these prevalences with the mean size class distribution across all seasons for the site I was examining to calculate the expected mean prevalence of infection.

3) a seasonal, temperature only model: prevalence of *Bd* increases in winter and decreases in summer. To build this model I calculated the mean prevalence of *Bd* in each season across all sites except the site of interest, and used this as the expected value for that site.

4) a seasonal, size class only model: prevalence of *Bd* increases when the proportion of larger tadpoles is increases. As with model type (2), I ignored site type and season and calculated average prevalence across all seasons in each size class at sites other than the one I was examining. I then estimated the expected prevalence of *Bd* infection for the site of interest using the size-frequency distribution for that site and season and the mean prevalence in each size class.

5) a seasonal size class and temperature model: prevalence of *Bd* increases in winter and when the highest proportion of larger tadpoles. To build this model, calculations were similar to model type (4) except that for the expected prevalence in each size class I used the mean for each season across all sites other than the site of interest. The effects of temperature were thus included.

To determine which model best fit my observed data, I 1) calculated the magnitude of change in prevalence of *Bd* between each season (Winter-Spring, Spring-Summer, etc.) of each model and the observed data, 2) calculated the absolute difference between the magnitude of change between each model and the observed data, 3) calculated the sum of these changes

and 4) divided by four (4 seasonal changes). The model with the value closest to zero best described the changes in prevalence of *Bd* in the observed data.

Results

I swabbed a total of 1144 tadpoles: 300 *L. nannotis,* 744 *L. rheocola,* 99 *L. serrata, and* 5 *N. dayi.* Prevalences of *Bd* infection for all species except *L. rheocola* appear in Appendix 1. Only *L. rheocola* occurred at all six sampling sites. Of the 744 *L. rheocola* tadpoles swabbed over the sampling period, 192 were sampled in the first winter, 147 in spring, 123 in summer, 103 in autumn, and 179 in the second winter.

Temperature

I determined the proportions of the water temperatures that fell into four ranges relevant to *Bd* growth. The distributions of the proportions differed among site types, but the sites within each type had similar patterns (Fig. 4.1). Water temperatures in the high elevation sites were never above 23 °C. High elevation sites had lower water temperatures throughout the year than both low elevation sites. Both the contiguous low elevation sites and non-contiguous sites were often warmer than high elevation sites in summer and autumn. Non-contiguous low elevation sites were warmer than contiguous low elevation sites. Temperatures at non-contiguous low elevations sites were between 25 °C and 28 °C substantially more often than contiguous low elevation sites. Temperatures were between 25 °C and 28 °C 30% and 50% of the time in summer and autumn at Mena Creek (non-contiguous) and 100% of the time during summer in Stoney Creek (non-contiguous).

There was a positive linear correlation between water temperature and air temperature (t_{27} = 8.6041, p = 0.0000, r^2 = 0.7328; Fig. 4.2).
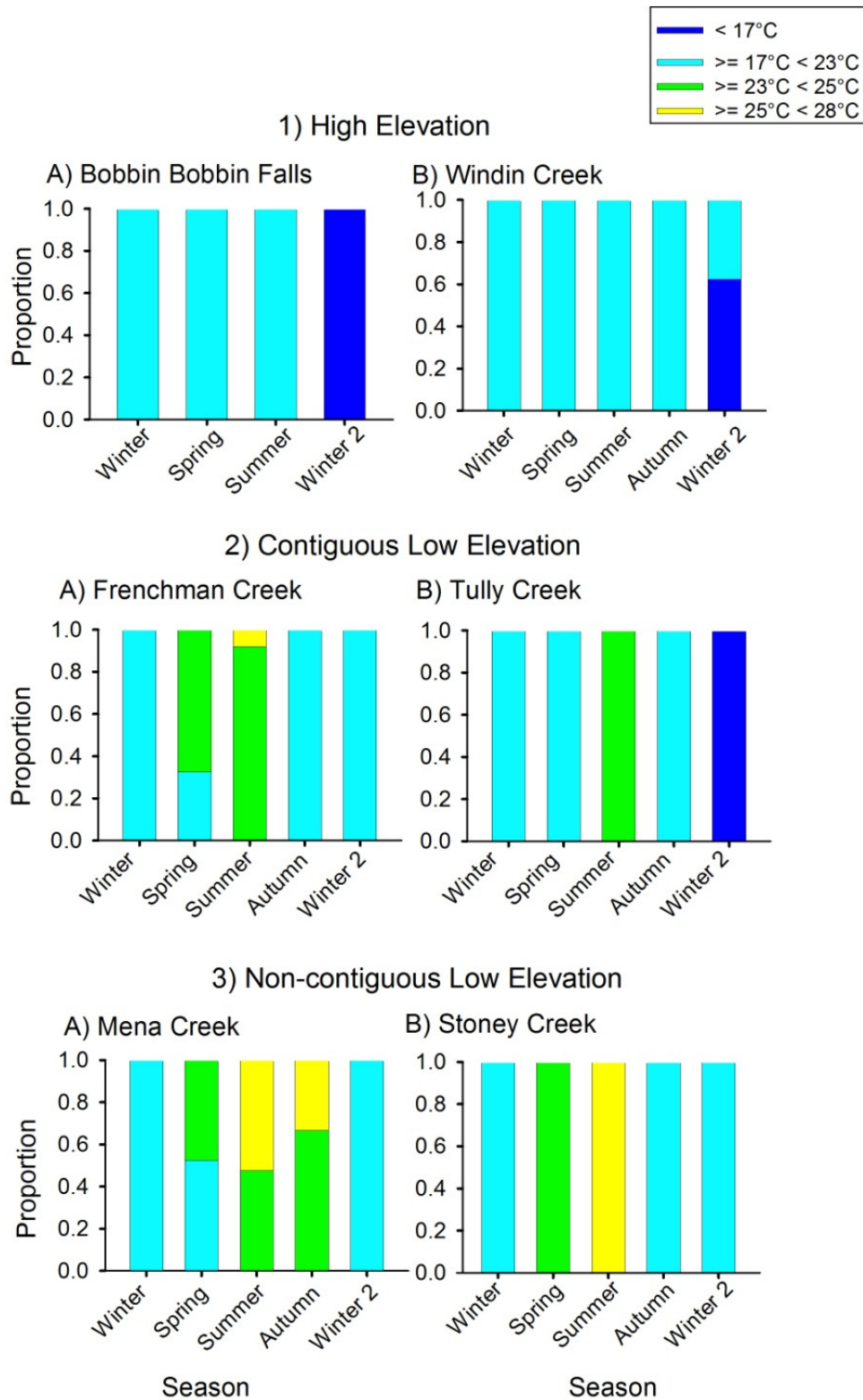
Figure 4.1 Proportion of water temperatures in ranges relevant to *Batrachochytrium dendrobatidis* growth at different site types in northern Queensland, Australia.
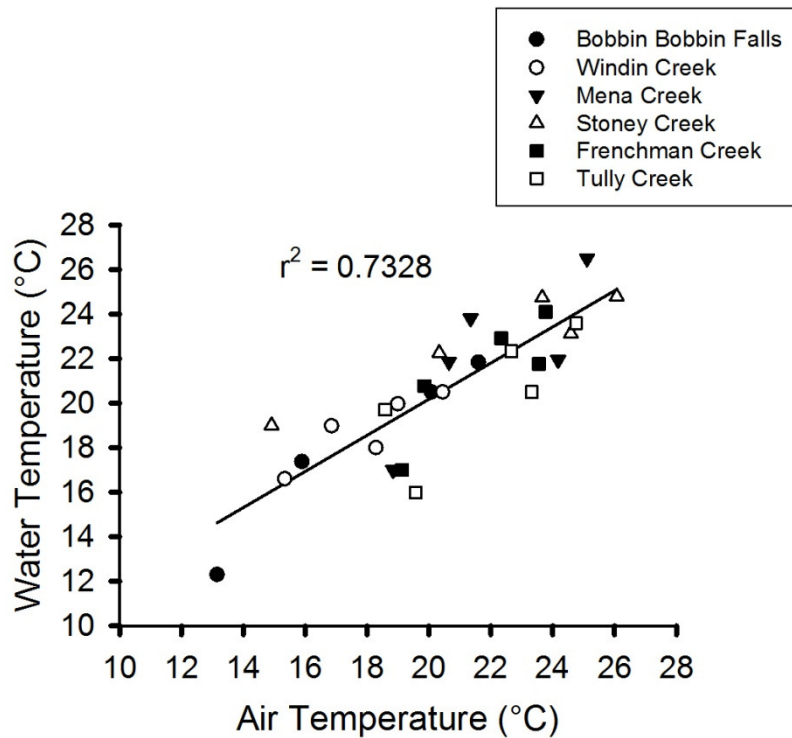
Figure 4.2 Relationship between water temperature and air temperature in streams in northern Queensland, Australia.

Temperature and prevalence

Because *Bd* growth is temperature dependent, I determined whether the prevalence of *Bd* in *L. rheocola* tadpoles was related to mean water temperature at each site on each sampling trip. There was a negative correlation between water temperature and prevalence of *Bd* in tadpoles (r^2 = 0.2784, n = 29, p = 0.003; Fig. 4.3); the proportion of infected individuals decreased with increases in water temperature.
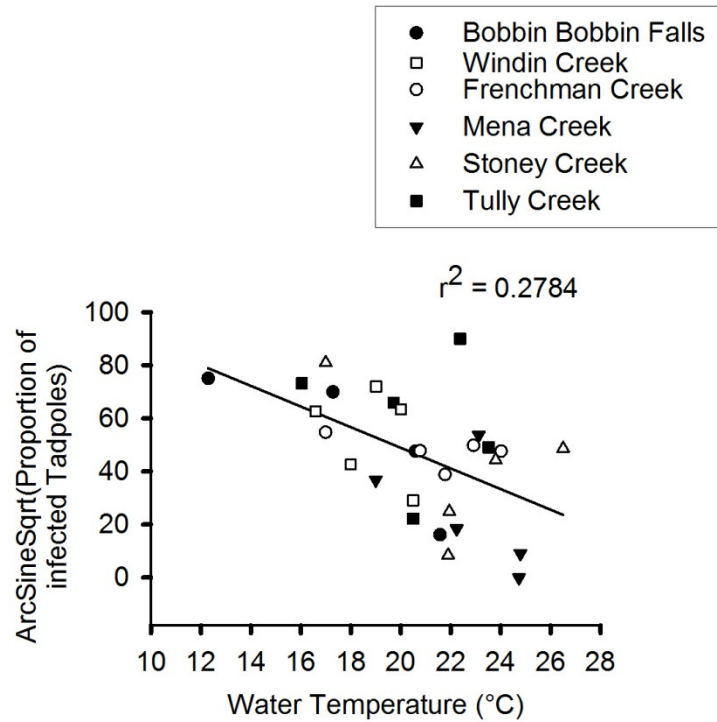
Figure 4.3 Relationship between water temperature and proportion of *Litoria rheocola* individuals that were infected with *Batrachochytrium dendrobatidis* across sites and seasons in northern Queensland, Australia.

Effects of season and site type on prevalence

There was a highly significant effect of temperature on individual probability of infection (Table 4.1), but it did not eliminate the effects of the categorical variables. Infection probability was significantly affected by season (Table 4.1): all site types had higher prevalence of *Bd* in winter than in summer (although CIs overlapped the mean prevalence demonstrated this pattern; Fig 4.4). Infection probability was also affected by site type (Table 4.1). *Litoria rheocola* tadpoles at all low elevation sites had lower maximum prevalences than those at high elevation sites (Fig. 4.4). There was a significant interaction between the effects of season and site type on the prevalence of *Bd* in tadpoles (Table 4.1). Seasonal changes were more dramatic (dropped from 90% to 10% over two seasons) at high elevation sites than at low elevation sites

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(Fig. 4.4), and the exact patterns of seasonal change differed among sites of all three types. At

low elevations, tadpoles at Frenchman Creek had more consistent prevalences throughout the

year and prevalences at both contiguous low sites were usually higher than those at non-

contiguous low-elevation sites, where prevalence was usually lower and fell to zero at Mena

Creek (non-contiguous) during autumn (Fig. 4.4). These results show that although water

temperature is important in determining the effects of sites and seasons on prevalence of *Bd*,

there are additional effects not accounted for by temperature.

Table 4.1 Results of generalised linear model analysis of effects of site type, season, and mean site water temperature on the *Bd* infection status of individual tadpoles.

Source	Wald Chi-square	d.f.	P
Site type	18.068	2	<0.0001
Season	24.193	3	<0.0001
Site type * season	56.640	6	<0.0001
Temperature	17.077	1	<0.0001
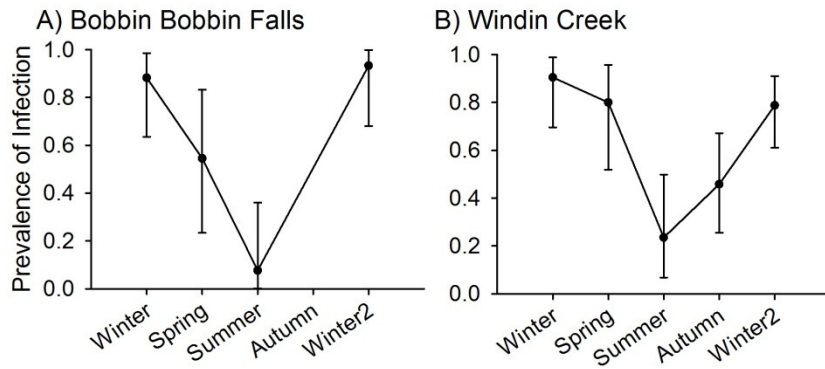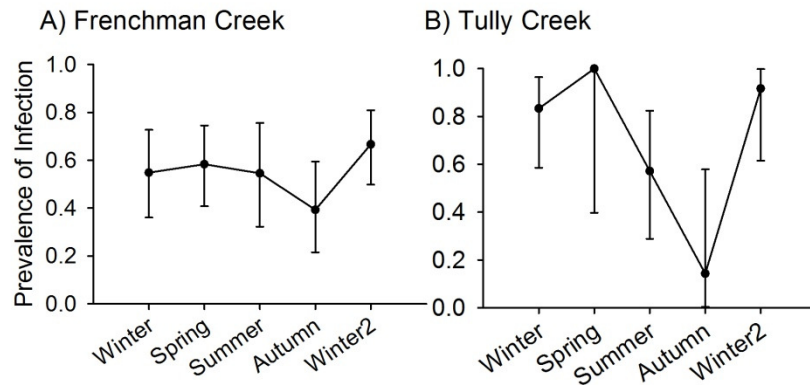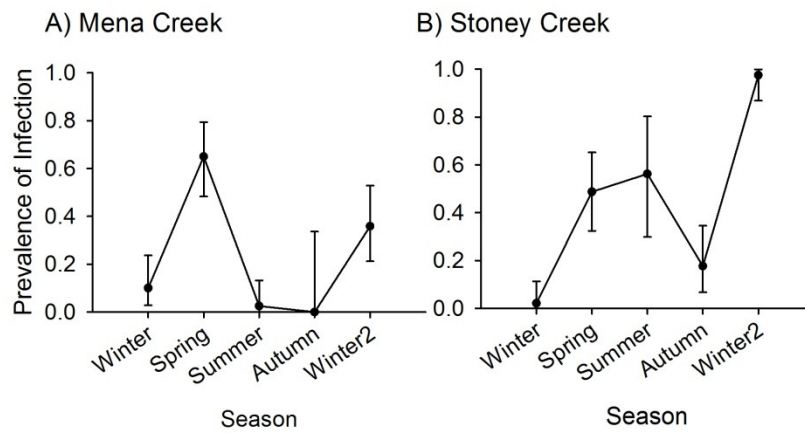
Figure 4.4 Prevalence of *Batrachochytrium dendrobatidis* in *Litoria rheocola* tadpoles at difference site types in northern Queensland, Australia.

Tadpole size-structure and prevalence

The prevalence of *Bd* infection increased consistently with increasing *L. rheocola* tadpole body size (Fig. 4.5). The size-frequency distribution of tadpoles differed significantly among seasons (χ^2_4 = 50.808, p < 0.001) and site types (χ^2_3 = 42.156, p < 0.001; Fig. 4.6). Across all site types, there were relatively more tadpoles in smaller size classes in summer. The size-frequency distribution shifted towards greater proportions of larger tadpoles in winter at high and non-contiguous low sites, but this trend wasn't as clear at contiguous low sites. Predictions derived using the models relating tadpole demography to *Bd* prevalence described above appear in Figure 4.7. The overall null model (model 1) was the worst fit for most of the sites (Table 4.2). The seasonal size class and temperature model (model 5) best predicted the patterns I found at both high elevation sites, Frenchman Creek (contiguous low site), and Mena Creek (non-contiguous site). The seasonal temperature only model (3) best predicted the pattern I found at Tully Creek (contiguous low site) and the seasonal size class only model (4) best described the pattern I found at Stoney Creek (non-contiguous site).
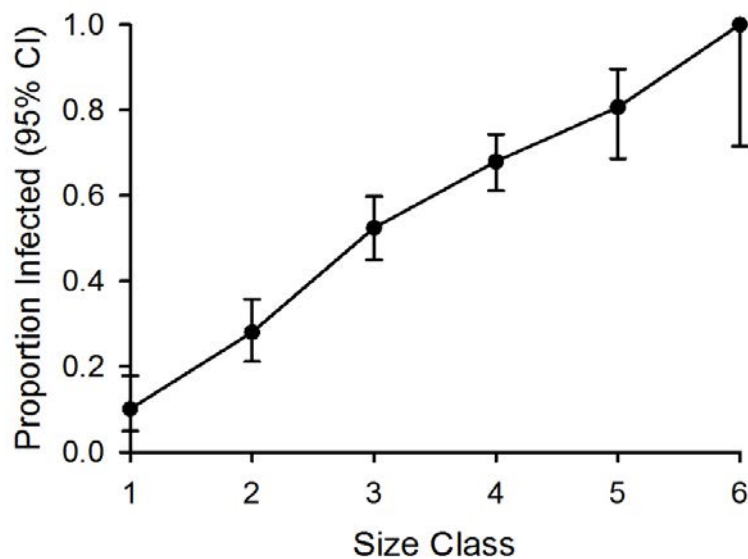
Figure 4.5 Prevalence of *Bd* in each size class of *Litoria rheocola* tadpoles.
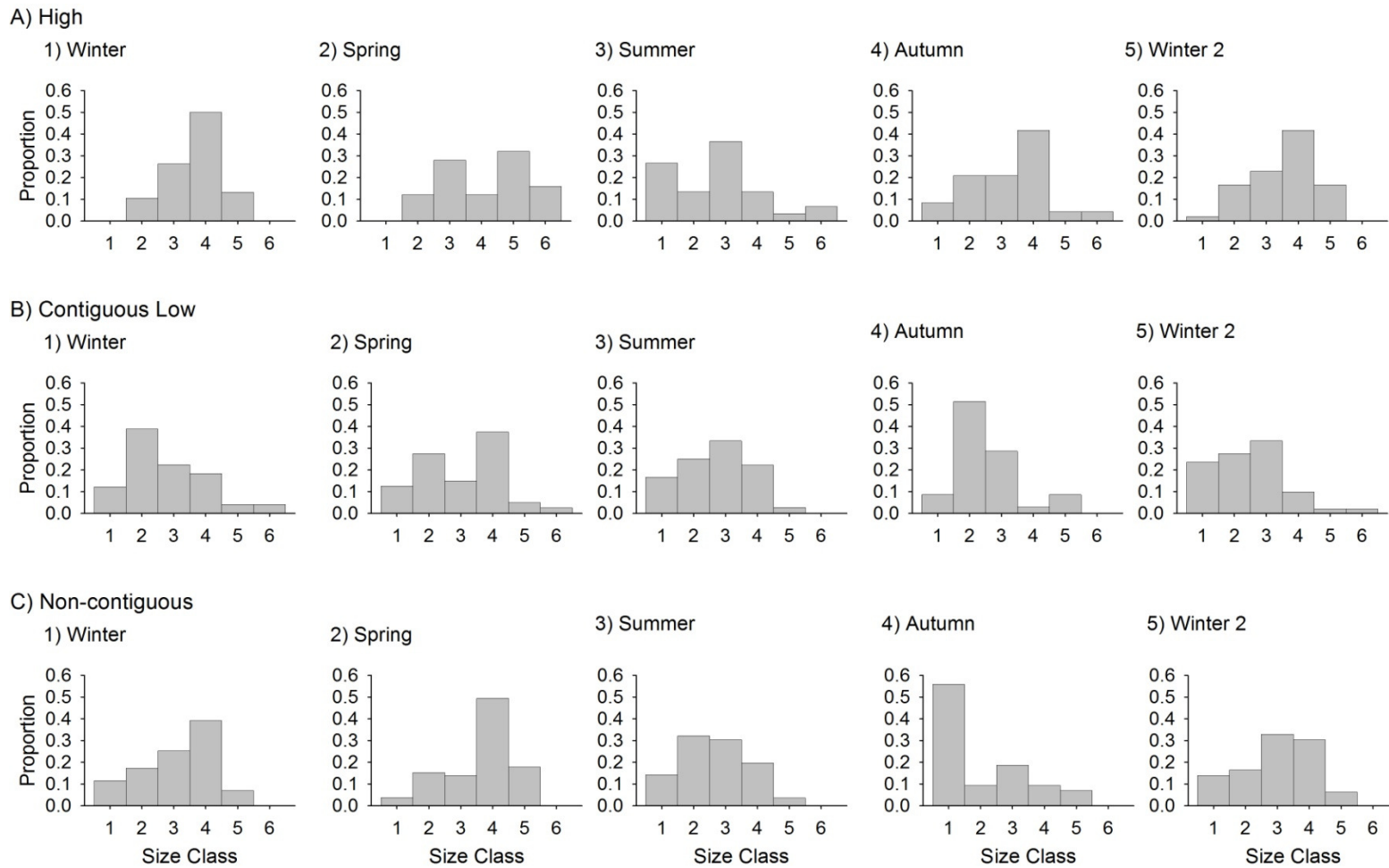
Figure 4.6 Distribution of size classes of *Litoria rheocola* tadpoles at different seasons and site types in northern Queensland, Australia.
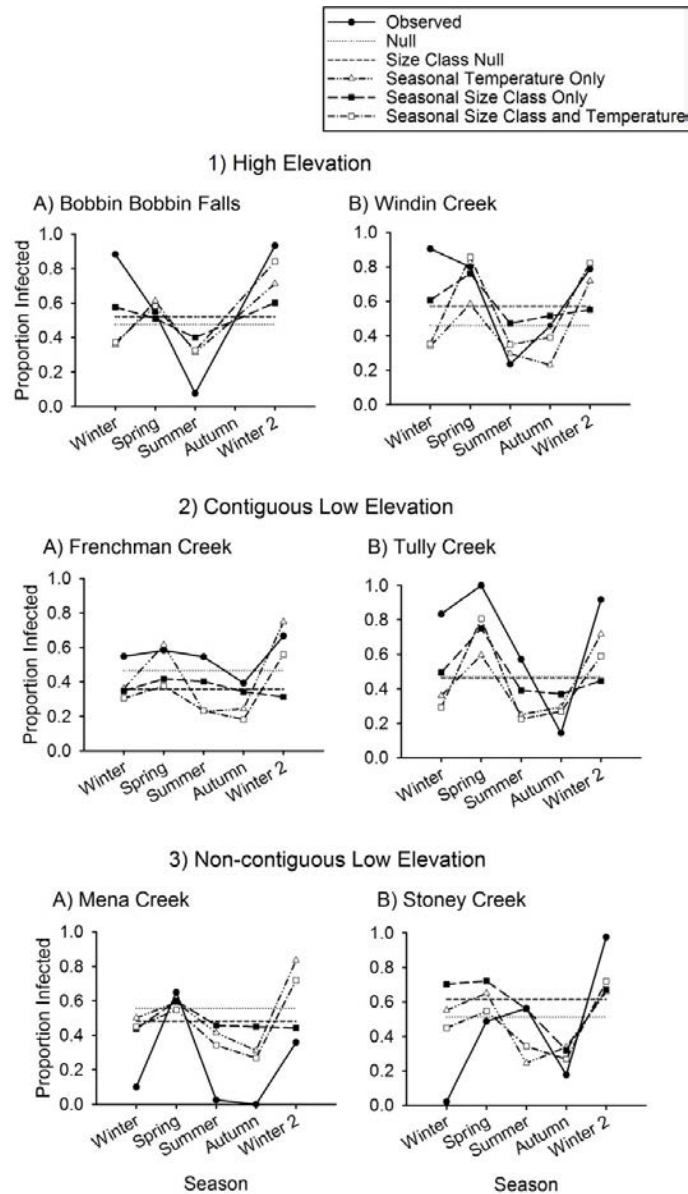
Figure 4.7 Predicted and observed prevalence of *Batrachochytrium dendrobatidis* changes in *Litoria rheocola* (common mistfrog) tadpoles at different site types in northern Queensland, Australia. Five models were predicted to compare to the observed data: 1) null model, 2) size class null model, 3) seasonal temperature only model, 4) seasonal size class model, and 5) seasonal size class and temperature model.

Table 4.2 Model fit of predicted models used to assess the influence of demography and season on changing prevalence of

Batrachochytrium dendrobatidis **in common mistfrog (***Litoria rheocola***) tadpoles over time. Using my own data I built five**

models to determine how prevalence of *Bd* in tadpoles changed over time: 1) null model, 2) size class null model, 3)

seasonal temperature only model, 4) seasonal size class only model, and 5) seasonal size class and temperature model.

Best fit was determined by 1) calculating the magnitude of change in prevalence between each season of each model and

the observed data, 2) taking the absolute difference between each seasonal change of the model and the observed data, 3)

taking the sum of these changes and dividing by four (4 seasonal changes). Best fit models are presented in bold for each

site.

Site	Null	Size Class Null	Seasonal Temperature Only	Seasonal Size Class Only	Seasonal Size Class and Temperature
Bobbin Bobbin Falls (High)	0.5539	0.5539	0.4058	0.4274	**0.3661**
Windin Creek (High)	0.3055	0.3055	0.2673	0.2525	**0.2366**
Frenchman Creek (Contiguous)	0.1248	0.1248	0.2407	0.1132	**0.0863**
Tully Creek (Contiguous)	0.4494	0.4494	**0.2445**	0.3158	0.3558
Mena Creek (Non-contiguous)	0.3897	0.3897	0.2876	0.3138	**0.2541**
Stoney Creek (Non-contiguous)	0.4314	0.4314	0.4509	**0.3207**	0.3264

Discussion

The infection dynamics of *Batrachochytrium dendrobatidis* (*Bd*) in tadpoles were influenced by season. Prevalence fluctuated seasonally in similar patterns in *L. nannotis* tadpoles; however, seasonal patterns were weaker in *L. serrata* tadpoles (Appendix 1).

In *L. rheocola,* prevalence was dramatically and clearly related to season at the high elevation sites, reaching a maximum of nearly 100% in winter and dropping to low levels in summer (Fig. 4.4). Seasonal patterns were weaker at low elevation sites; although prevalence reached a minimum in summer or autumn, winter peaks were sometimes lower. Cashins (2009) found similar seasonal effects on prevalence of *Bd* in torrent-adapted tadpoles in northern Queensland in a low elevation rainforest stream contiguous with high elevation streams. This seasonal pattern is also similar to patterns that are well known for infected populations of adult amphibians (Brem and Lips 2008, MacDonald et al. 2005, Pullen et al. 2010, Retallick 2002, Russell et al. 2010, Savage et al. 2011, Woodhams and Alford 2005).

In addition to season, the infection dynamics of *Bd* were influenced by site type. Prevalences at the non-contiguous low elevation sites tended to be lower than contiguous low sites. Prevalences approached or reached zero in at least one season in both non-contiguous low elevation sites. Seasonal fluctuations in prevalence at Frenchman Creek (contiguous low elevation site) were weak (consistent throughout the year), while at Tully Creek (contiguous low elevation site) they resembled patterns at high elevation sites. The difference between the contiguous low and non-contiguous sites suggests that being connected by water flow to high elevation sites may influence the dynamics of *Bd* at lower elevations. Stream flow from higher elevations that contains *Bd* zoospores may keep tadpoles consistently infected in contiguous streams at lower elevations; *Bd* zoospores could be transported downstream similar to the process of transporting aquatic invertebrates, known to stream ecologists as drift (Brittain and Eikeland 1988). There also may be thermal effects caused by the flow of cold water from high

elevation to contiguous low elevation sites. Water temperatures at all low elevation sites were warmer than at high elevation sites, but there was a crucial difference between contiguous and non-contiguous low elevation sites. At contiguous sites, water temperatures almost never exceeded 25°C (maximum of 25°C reached in summer). Water temperatures reached above 25°C at least 50% of the time during at least one sampling trip at both non-contiguous sites (maximum of 26.5°C reached in summer and 25.3°C in autumn). This is crucial, because at temperatures greater than 25°C the growth rate of *Bd* declines rapidly, reaching zero at approximately 28°C (Piotrowski et al. 2004). Rowley (2006) demonstrated that the probability of infection by *Bd* of radio-tracked frogs in the field decreased rapidly as the proportion of time they spent above 25°C increased. It thus seems likely that higher water temperatures, as well as lack of drift of *Bd* zoospores from higher elevation sites, are responsible for the very low *Bd* prevalence, sometimes even reaching zero, in non-contiguous low elevation sites over summer.

The predictions of my models (Fig. 4.7) also suggest that there are effects of connectivity between high elevation and low elevation sites on seasonal patterns of prevalence. Predictions were derived using data for all sites except the one for which predictions were made. Prevalence at contiguous low elevation sites tended to be higher than predicted, while prevalence at non-contiguous low elevation sites was lower than predicted. These patterns emerged because when predictions were made for non-contiguous sites, for example, most of the data used in making those predictions came from contiguous sites and vice-versa.

Along with the influences of season, site type, and connectivity, my results indicate there is a substantial influence of demography on the prevalence of *Bd* in tadpoles. My results (Fig. 4.5) indicated that infection probability increased approximately linearly with tadpole body length. It thus appears that the probability that *L. rheocola* tadpoles carry *Bd* infections is cumulative, so that older, larger individuals are more likely to be infected (Bosch et al. 2001, Briggs et al. 2010, Cashins 2009, Smith et al. 2007). For five of my six sites, predictions of *Bd*

prevalence that incorporated the effects of tadpole size structure more closely matched observed patterns than predictions that did not incorporate size structure.

In my study, tadpoles acted as a reservoir for *Bd* that might affect its persistence and prevalence in co-occurring terrestrial stages of frogs. Infected tadpoles with long larval stages release infectious zoospores into the environment over extended periods and may increase the likelihood of outbreaks of disease and allow the pathogen to persist in adult populations (Bosch et al. 2001, Rachowicz and Vredenburg 2004). If disease prevalence decreases to very low levels or zero in terrestrial stages over summer months, propagules released by tadpoles may allow the pathogen to remain in the system. The degree of connection of low elevation sites to higher elevation sites may alter disease dynamics in terrestrial stage host through its effects on host larvae. At non-contiguous low elevation sites tadpoles may be less effective reservoirs; at one site prevalence in tadpoles decreased to zero, while it was at a very low level in the other site during the first winter sampling period, suggesting it had reached very low levels in the preceding autumn.

This study is a stepping stone in understanding the infection dynamics of *Bd* in frog and tadpole assemblages. My results demonstrate that infection dynamics of *Bd* are influenced by many interactions among environment, pathogen, host, and reservoirs. My finding that stream connectivity between high and low elevations influenced the infection dynamics of *Bd* in tadpoles suggests that this may be important in other regions as well, and could explain, for example, why frogs persist at lowland sites in some regions of Costa Rica while they have been extirpated from others (Puschendorf et al. 2009).

Chapter 5 : Site type, temperature, and habitat connectivity affect the dynamics of prevalence of the amphibian chytrid fungus

Abstract

Infectious diseases can cause population declines and even the extinction of species. The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), has caused population declines and extinctions in amphibians on most continents. In the tropics, research on the dynamics of this disease has focused on amphibian populations in mountainous areas. In most of these areas, high and low elevation sites are connected by an assemblage of streams that may transport the infectious stage of the pathogen from high to low elevations. In these systems, flow from high elevations may also reduce maximum water temperatures at lower elevations. I refer to the low elevation sites in such areas as contiguous low sites. Disease dynamics at low elevation sites without aquatic connections to higher elevation sites, which I call non-contiguous low sites, are not well studied and may differ from those at contiguous low sites. I sampled adult common mistfrogs (*Litoria rheocola*) at six sites: two at high (> 400m) elevations, two at contiguous low elevations, and two at non-contiguous low elevations. Adults were swabbed for *Bd* over one full annual cycle, commencing and finishing in winter. The prevalence of *Bd* fluctuated seasonally and was highest in winter across all site types. Site type also significantly affected prevalence of *Bd*. Prevalence declined to zero over summer in low elevation sites of both types, but infections re-appeared in autumn. Prevalence remained above zero throughout the year at the high elevation sites. My results suggest that both contiguous low and non-contiguous sites have similar infection dynamics of *Bd* in adult frogs (connectivity does not have a direct affect on adults) and that reservoir hosts, such as tadpoles or other frog species, play a role in the persistence of *Bd* in both types of low elevation populations.

However, tadpoles appear to act as an intraspecific reservoir in contiguous low elevations whereas interspecific species act as reservoirs in non-contiguous low elevations. My results suggest that non-contiguous low elevation sites may be more effective refugia than contiguous low elevation sites, which could be important in managing the impact of future outbreaks of chytridiomycosis.

Introduction

Emerging infectious diseases (EIDs) can pose major threats to wildlife species, especially in those whose population size, range, or habitat has been reduced (Daszak et al. 1999). At the individual level, disease can cause illness and may cause death and, at the species level, disease can cause population declines and extirpations and species extinctions (Aguirre and Tabor 2008, Berger et al. 2004, Blaustein et al. 2005, La Marca et al. 2005, Rowley et al. 2007). Infection prevalence, rates of transmission to susceptible hosts, and pathogen load within hosts are the driving forces behind the dynamics of infectious diseases (Begon et al. 2002, Briggs et al. 2010). Diseases do not usually cause extinctions because population decline caused by pathogens are typically self-limiting: as pathogens cause host populations to decline, host population density falls below a threshold level required to maintain transmission, and the pathogen dies out (Daszak et al. 1999). Local extirpations and even global extinctions are possible, however, when a disease has a reservoir host or hosts. Reservoir hosts are typically less susceptible species that can sustain the pathogen and allow the pathogen to persist in the environment, even when susceptible species decline and disappear from the system (Blaustein et al. 2005, Brunner et al. 2004, de Castro and Bolker 2005, McCallum and Dobson 1995). Determining the dynamics of diseases is important in understanding their effects on host populations, and also in providing possible ways of mitigating host declines (e.g., by reducing transmission rates).

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The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), has caused many population declines, and both local and global extinctions of frogs around the world (La Marca et al. 2005, Lips et al. 2006, Stuart et al. 2004). *Bd* infects species over large geographic areas and even in areas that are not highly disturbed by humans; the greatest declines have occurred in stream-associated species (McDonald and Alford 1999, Woodhams and Alford 2005). The fungus infects and reproduces within the keratinized cell layers of the epidermis of amphibians, with disease signs not becoming obvious until the later stages of the process: loss of righting reflex, abnormal posture, lethargy, sloughing of the epidermis and, eventually, death (Simoncelli et al. 2005). Death is caused by inhibition of electrolyte transport across the epidermis, which causes asystolic cardiac arrest (Voyles et al. 2009).

The population dynamics of *Bd* are temperature dependent. *Bd* can grow and reproduce between 4°C and 25°C and grows optimally between 17°C and 25°C; *Bd* growth slows above 25°C and *Bd* dies above 30°C (Piotrowski et al. 2004). Thus, the prevalence of the pathogen can be affected by environmental temperatures (Brem and Lips 2008, Pullen et al. 2010, Retallick 2002, Woodhams and Alford 2005). In Australia, *Bd* prevalence tends to be higher at high elevations, which I define as those above 400 m, for the approximate elevational cut-off above which chytridiomycosis has caused severe frog population declines in the Australian Wet Tropics (McDonald and Alford 1999, Retallick 2002). At high elevation sites temperatures are cooler on average than at low elevation sites (Retallick 2002, Woodhams and Alford 2005). Prevalence of *Bd* is also higher during colder, winter months than in warmer, summer months; this seasonal pattern occurs at both high and low elevations (Retallick 2002, Woodhams and Alford 2005).

High and low elevation areas are often connected by streams, making them contiguous, such that water can flow from high to low elevation sites. In such sites, *Bd* zoospores may be

carried from high to low elevation, influencing prevalence of *Bd* in the populations at lower

elevations through drift (Brittain and Eikeland 1988). Cooler water will flow from high elevations

to low elevations, potentially reducing maximum water temperatures. Not all low elevation sites,

however, are connected to high elevation sites. Some low elevation streams have no adjacent

regions higher than 400 m. Differences in drift of pathogen propagules and moderation of

maximum temperatures could cause non-contiguous areas to have different infection dynamics

than contiguous low areas. Cooler water temperatures and the flow of infectious propagules

may affect adult populations indirectly when adult frogs come into contact with water. In

addition, differences in air temperature of different elevations may also be responsible for

different infection dynamics. The question arises, therefore, whether the dynamics of *Bd*

infection prevalence differ between the two kinds of low elevation areas and the high elevation

sites.

The aim of my study was to determine and describe the infection dynamics of *Bd* in one

frog host species across high elevation, contiguous low elevation, and non-contiguous low

elevation sites. I surveyed six populations of the adult common mistfrog, *Litoria rheocola*, two at

each site type, over a one year period from midwinter to the succeeding midwinter. This is the

first comprehensive study of the seasonal dynamics of the amphibian chytrid fungus in multiple

populations of adult frogs that incorporates replicate sites in contiguous and non-contiguous

lowlands and uplands.

Materials and Methods

Study species

The adult common mistfrog (*Litoria rheocola*) is a small (average male body size: 2 g, 32

mm snout-urostyle length; S. J. Sapsford, personal observation), endangered, hylid frog (IUCN

2012). *Litoria rheocola* occurs in rocky, fast-flowing, rainforest streams in northern Queensland,

Australia (Dennis 2012, Hoskin and Hero 2008). *Litoria rheocola* declined and disappeared from high elevation sites in the Australian Wet Tropics Bioregion in the 1990s (McDonald and Alford 1999, Richards et al. 1993), but has since reappeared at some high elevation sites (McDonald & Alford 1999, McDonald et al. 2005). At night, and on rainy days, individuals typically perch on rocks, logs, and stream-side vegetation near riffles (Dennis 2012, Hodgkison and Hero 2002, Hoskin and Hero 2008), and on dry days they typically shelter between moist rocks in the stream bed (E.A. Roznik, unpublished).

Study sites

Sampling of adults took place over one full annual cycle, from austral winter (June/July 2010), through spring (October 2010), summer (January 2011), autumn (March/April 2011) and the following winter (June/July 2011). Adults were sampled at six sites in the Wet Tropics Bioregion in northern Queensland, Australia. Two sites were at high (>400 m) elevations, two sites were at low elevations and were contiguous with (downstream from) high elevation sites and two sites at low elevations that were not downstream from high elevation sites (non-contiguous sites; Table 5.1). All of the creeks were surrounded by tropical rainforest and all contained pools and riffles; some also had waterfalls. The nature of the creek beds varied among sites: those of Frenchman Creek, Tully Creek, and Bobbin Bobbin Falls were comprised of large boulders interspersed with sections composed of small rocks (1-10 cm in diameter). In comparison, the beds of Windin, Mena, and Stoney Creeks were primarily comprised of small rocks.

Table 5.1 The site descriptions of creeks where six *Litoria rheocola* populations were located in the Wet Tropics Bioregion in northern Queensland, Australia.

Site	National Park	Site Type	Location	Elevation (m)
Windin Creek	Wooroonooran	High	17°22'04.2"S 145°42'52.1"E	718
Bobbin Bobbin Falls	Wooroonooran	High	17°22'43.5"S 145°46'21.7"E	700
Frenchman Creek	Wooroonooran	Contiguous low	17°18'29.2"S 145°55'16.2"E	59
Tully Creek	Tully Gorge	Contiguous low	17°46'29.5"S 145°38'38.2"E	114
Mena Creek	Private land	Non-contiguous low	17°38'59.6"S 145°59'13.5"E	59
Stoney Creek	Hull River	Non-contiguous low	17°55'17.9"S 146°4'7.2"E	18

Field methods

Prior to surveys, I placed flags at 10 m intervals along a 400 m transect at each site so I could record the location of each frog that was captured. Sampling was conducted over five nights at each site during each season. Adult frogs were captured at night, using clean plastic sandwich bags worn as a glove, by using visual and auditory cues. Following capture, all frogs were swabbed for *Bd*, sexed (using presence/absence of nuptial pads), measured to the nearest 0.1 mm (snout-urostyle length), and mass determined to the nearest 0.1 g. To prevent disease transmission among frogs, a new pair of powder-free latex gloves was used to handle each individual. Once processing was complete, frogs were released at their capture location.

Assessing disease status

Frogs were swabbed using a sterile, fine-tip, dry rayon swab (#113, Dry swabs, Medical Wire and Equipment, Corsham, Wiltshire U.K.). The ventral side of each foot, inner thigh area,

both lateral sides of the stomach, medial section of the stomach, and ventral side of each hand were swabbed three times because the fungus is mainly found on these areas of the body (North and Alford 2008, Skerratt et al. 2008).

After processing, all swabs were placed in separate, labelled vials and refrigerated until the end of sampling. All swab samples were processed using real-time quantitative PCR (Boyle et al. 2004). Samples were run in triplicate and considered positive for *Bd* if at least two of the three PCR reactions had numbers of zoospore equivalents that were greater than zero.

Environmental temperatures

Air temperature was recorded using three Thermochron iButton dataloggers (model DS1921G, precision: 0.5 °C, accuracy: 1°C) at each site. They were placed in shaded locations at 0 m, 200 m, and 400 m along the transect, approximately 1 m from the creek edge and 2 m above the ground. These dataloggers were coated in transparent plastic (Plasti Dip, Plasti Dip International, Blaine, MN, USA) to prevent water damage (Roznik and Alford 2012). Each iButton recorded air temperature at 90 min intervals over the entire study period (June 2010 to June 2011).

Analysis of prevalence

Because the growth of *Bd* is temperature-dependent, I used a linear regression to examine whether prevalence of *Bd* in adult frogs in each site and season was influenced by air temperature. Because prevalence is a proportion, and is non-normally distributed, I arcsine square-root transformed prevalence before analysis. Air temperature was used as the independent variable.

I wanted to determine whether season, site type, and their interactions were related to prevalence in my populations, as occurs in other amphibian populations in Australia and other

regions (Savage et al. 2011, Woodhams and Alford 2005). To determine whether season and site type had effects on the probability of *Bd* infection in adult frogs that were independent of the effect of temperature, I used a generalized linear model with a binomial link function. Season, site type, and their interaction were categorical predictor variables, mean temperature was included as a covariate, and disease status (infected or not infected) was the response variable. I used the two sites at each site type (high, contiguous low, non-contiguous low) as replicates.

Due to significant damage caused by Tropical Cyclone Yasi (a category 5 storm), which hit the northern Queensland, Australia, coast in February 2011, one of the high sites (Bobbin Bobbin Falls) was not accessible in autumn 2011. Therefore, to keep my examination of effects orthogonal, I ran the above model on all data, for all sites, excluding the autumn season.

Results

I swabbed 1877 adult frogs: winter (N = 417), spring (N = 560), summer (N = 333), autumn (N = 388) and winter the following year (N = 179).

Temperature

I measured air temperature at each site and determined the proportion of air temperatures in ranges relevant to *Bd* growth. The proportions differed among site types, but the sites within each type had similar temperature patterns (Fig. 5.1). The high sites were much cooler than both the contiguous low and non-contiguous sites, even in the summer months. Air temperatures at the high sites were almost entirely either less than 17°C (20.9%), which might slow *Bd* growth slightly, or between 17°C and 23°C (74.5%), which is within the range for optimal *Bd* growth (Piotrowski et al. 2004). Air temperatures were never above 28°C, and were only between 23°C and 28°C 4.5% of the time. Air temperatures at both types of low elevation sites were similar to one another, and substantially higher than those at high elevation sites:

low elevation sites spent more time between 23°C and 28°C (38.8%) and during summer temperatures were frequently above 25°C, which substantially slows *Bd* growth (Piotrowski et al. 2004). Temperature at both low elevation sites were sometimes above 30°C (1.2%), which is within the range that kills *Bd* (Piotrowski et al. 2004).

Temperature and prevalence

Because the growth of *Bd* is temperature dependent, I examined whether air temperatures influenced prevalence of *Bd* in adults. Across all combinations of site and season there was a significant negative correlation between air temperature and *Bd* prevalence in terrestrial frogs (r^2 = 0.5480; n = 29; P < 0.001; Fig. 5.2) indicating that prevalence was significantly lower at higher air temperatures.

Site type, infection status, and probability of infection

In addition to the effects of air temperature, which were significant, there were other effects (Table 5.2) on the probability that individual frogs were infected by *Bd*. Infection probability was significantly affected by both site type and season (Table 5.2), and their interaction, although not significant, approached significance (Table 5.2). Examining prevalence by site and season (Fig. 5.3) reveals that there were differences among site types; prevalence never reached zero in high elevation sites, whereas prevalence in non-contiguous sites reached lower and higher values than in contiguous low sites. These results show that although air temperature is important in determining the effects of sites and seasons on prevalence of *Bd*, there are additional effects not accounted for by temperature.
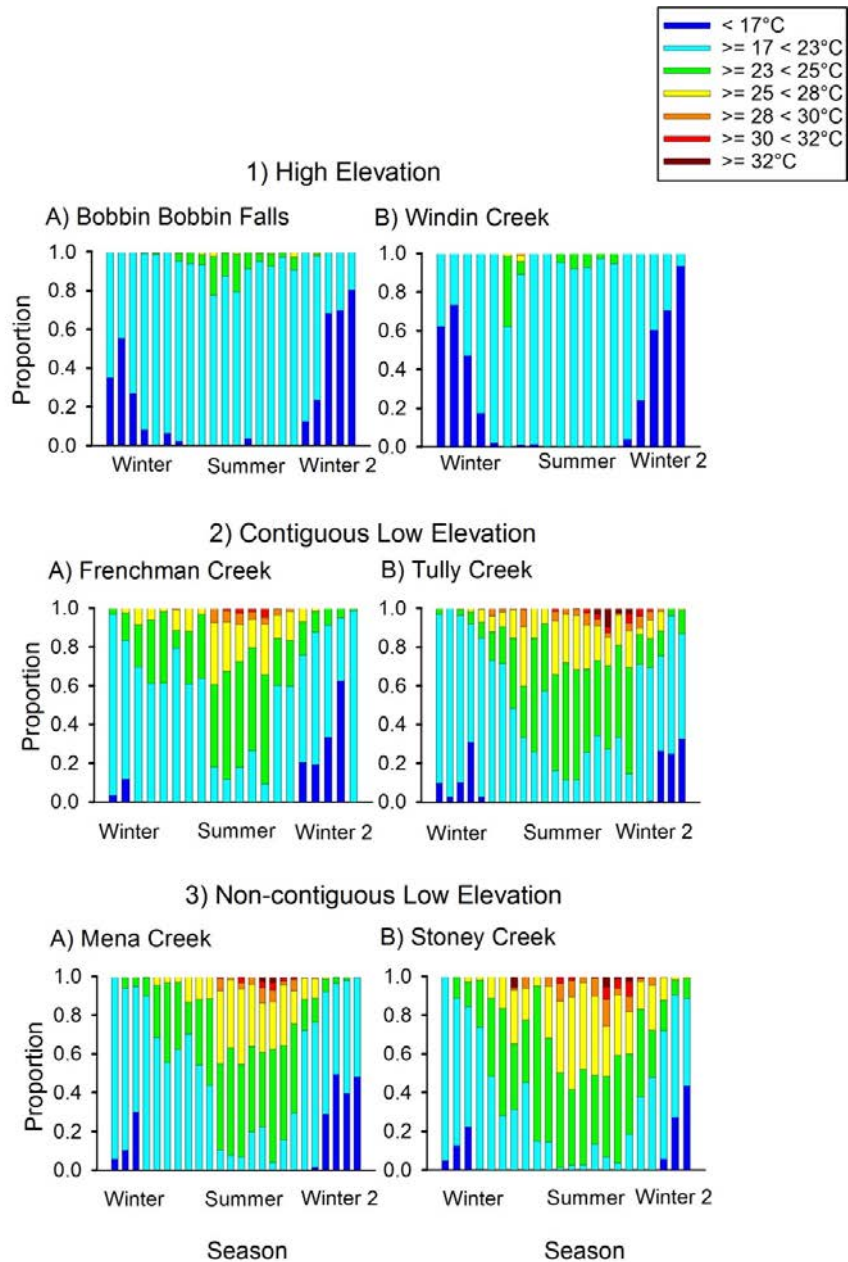
Figure 5.1 Proportion of environmental air temperatures in ranges relevant to *Batrachochytrium dendrobatidis* **growth. Each bar represents the proportion of air temperature in each range of** *Bd* **temperature during five two week periods from June 2011 to June 2012. These coincide with my sampling periods; because they include two winter periods, the proportions of time at lower temperatures are probably over-represented compared to a simple annual cycle.**
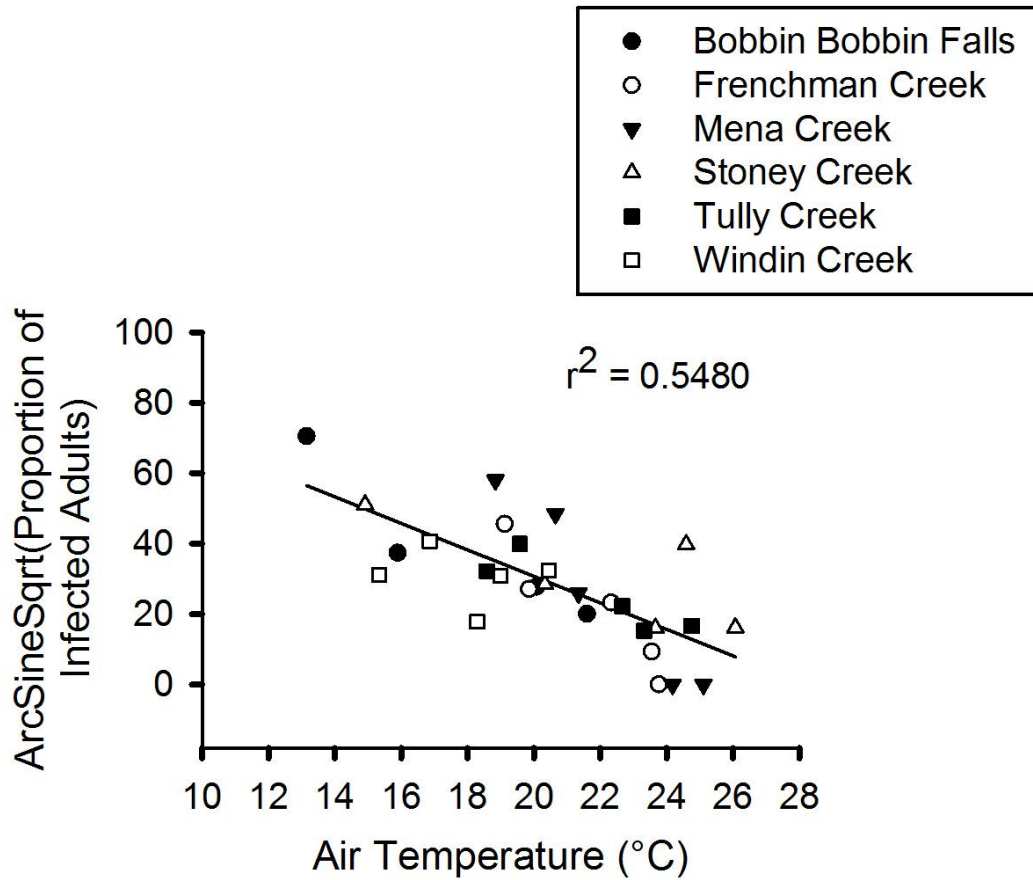
Figure 5.2 Relationship between air temperature and prevalence of *Batrachochytrium dendrobatidis* in *Litoria rheocola* across sites and seasons in northern Queensland, Australia.

Table 5.2 Results of generalised linear model analysis of effects of site type, season, and mean site air temperature on the infection status of individual frogs.

Source	Wald Chi-square	d.f.	P
Site type	19.521	2	<0.0001
Season	45.511	3	<0.0001
Site type * season	4.348	1	0.076
Temperature	11.447	6	0.037

1) High Elevation

A) Bobbin Bobbin Falls

B) Windin Creek

2) Contiguous Low Elevation

A) Frenchman Creek

B) Tully Creek

3) Non-contiguous Low Elevation

A) Mena Creek

B) Stoney Creek
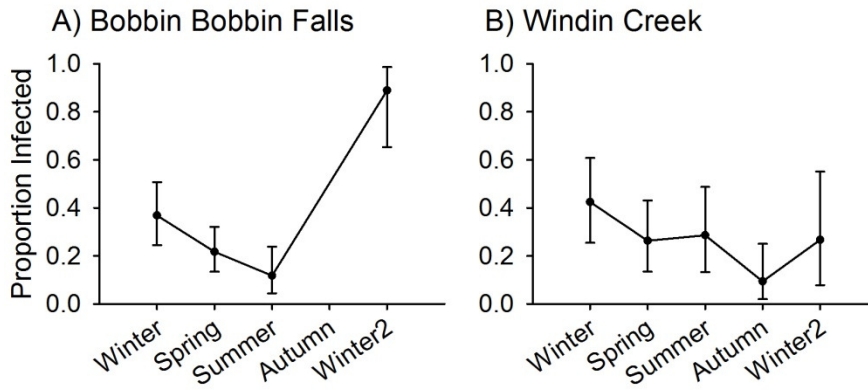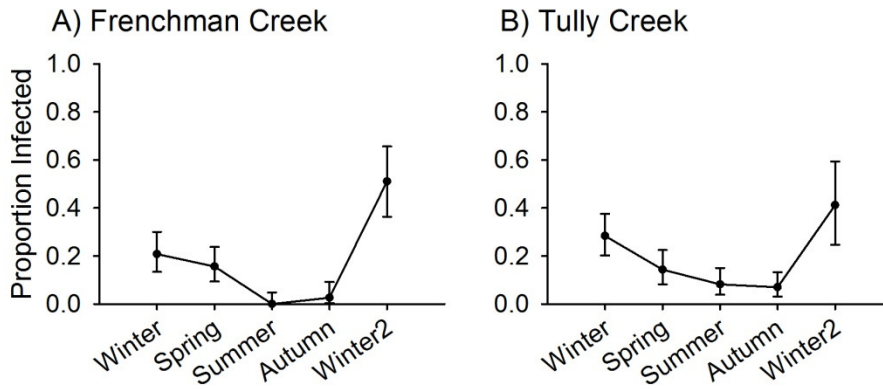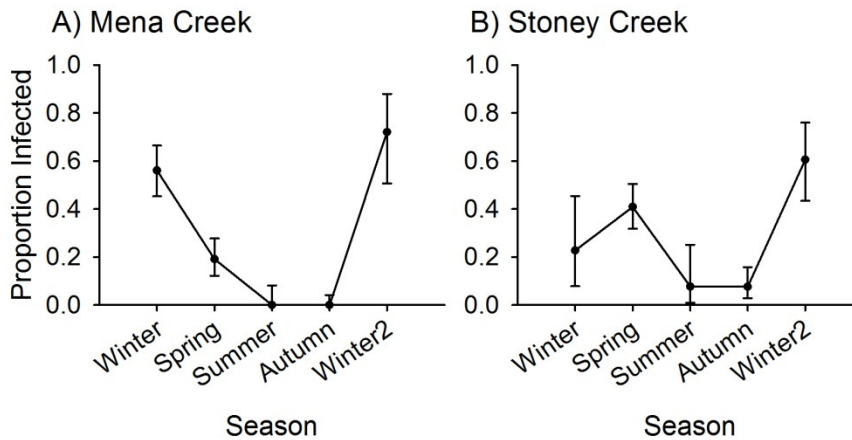
Figure 5.3 Prevalence of *Batrachochytrium dendrobatidis* in adult *Litoria rheocola* frogs at different site types in northern Queensland, Australia.

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Discussion

      My results demonstrate that the dynamics of *Batrachochytrium dendrobatidis* (*Bd*) infections on *L. rheocola* are influenced by temperature, season and site type. As has been seen in previous studies in Australia and elsewhere (Brem and Lips 2008, Pullen et al. 2010, Retallick 2002, Russell et al. 2010, Savage et al. 2011, Woodhams and Alford 2005), prevalence of *Bd* in adult frogs was higher in cooler than in warmer months. In addition, at least partially because of the effects of elevation on temperature, *Bd* was also influenced by elevation in my study, as it has been in others (Brem and Lips 2008, Kriger and Hero 2008, Pearl et al. 2009). I found that the prevalence of infection dropped to zero in populations in both types of low elevation sites (contiguous and non-contiguous) in summer, but increased again in autumn. Prevalence in populations at high elevation sites, by comparison, remained above zero throughout summer.

      My results suggest that contiguous low and non-contiguous low elevation sites are not directly affected by drift as their disease dynamics are similar to each other. However, there may be an indirect effect through reservoir hosts. My study suggests that both intraspecific and interspecific reservoir hosts are probably an important factor causing persistence of *Bd* at low elevation sites (both contiguous and non-contiguous), where prevalences in *L. rheocola* fell to zero in summer, but increased again in autumn. Elsewhere (Chapter 4) I have shown that at contiguous low elevation sites, *L. rheocola* tadpoles had a high prevalence of *Bd* infection (58.0 %), suggesting that they may be acting as an intraspecific reservoir at these sites. However, I also showed that the prevalence of infection in tadpoles in non-contiguous low elevation sites fell to zero in summer (Chapter 4), indicating that in non-contiguous low elevation sites there must be an interspecific reservoir for *Bd*. Adults of other frog species (e.g., *Litoria wilcoxii*; Retallick et al. 2004, Woodhams and Alford 2005), other higher taxa (e.g., Garmyn et al. 2012, Kilburn et al. 2011), or the environment (water, soil, etc., Johnson and Speare 2003, 2005,

Longcore et al. 1999, Rowley et al. 2007) may act as reservoirs at non-contiguous sites. There is some evidence that terrestrial stages of other frog species in non-contiguous sites were also uninfected in summer (*Nyctimystes dayi*, *Litoria serrata*, *Litoria infrafrenata*, *Litoria leseuri* (complex); A. McNab, unpublished). It is not clear at present whether *Bd* can persist for long periods as a saprobe or in a resting stage (Di Rosa et al. 2007). Recently, it was demonstrated that crayfish can harbour *Bd* infections and transmit infection to amphibians in the USA (McMahon et al. 2013); however, *Bd* infection has not been found in crustaceans in northern Queensland, Australia where *Litoria rheocola* populations inhabit (Rowley et al. 2007). It seems most likely that adults or tadpoles of other frog species or other animal species act as reservoirs for the disease at non-contiguous sites. The presence of infected tadpoles at contiguous low sites in summer, while they are absent from non-contiguous sites in summer, where air temperatures are similar, strongly suggests that either drift (Brittain and Eikeland 1988) or moderation of water temperatures *via* flow from cooler upland areas may be important in maintaining infections in tadpoles in contiguous low habitats (Chapter 4).

This study was the first to examine whether stream connectivity between high and low elevations influences the infection dynamics of *Bd*. I found no effects on dynamics in adult frogs; my results demonstrated that both contiguous low and non-contiguous sites have similar infection dynamics of *Bd*. However, in other systems or for other species, connectivity may remain important. My results indicate that reservoir hosts may be necessary for *Bd* to persist in low elevation areas. They also suggest that at contiguous low elevation populations, tadpoles of *L. rheocola* may serve as intraspecific reservoir hosts, since their prevalences of *Bd* infection remained high over summer (Chapter 4). At non-contiguous low elevation sites, prevalence of *Bd* in larval *L. rheocola* reached zero over summer, indicating that interspecific reservoirs are necessary to maintain *Bd* at these sites. Fully understanding the factors that determine patterns of infection prevalence in frog populations is an important step towards developing strategies to

reduce the impact of chytridiomycosis in natural populations. My results suggest that non-contiguous low elevation sites may be more effective refugia than contiguous low elevation sites, which could be important in managing the impact of future outbreaks of chytridiomycosis.

Chapter 6 : Population dynamics and a deadly disease: infection with the amphibian chytrid fungus does not influence survival or recapture rates of a rainforest frog

Abstract

Pathogens and parasites can be key drivers of host population dynamics. The chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), has caused declines and extinctions in some amphibian species, whereas other hosts can persist with the disease without suffering declines. In the 1990s, the endangered common mistfrog, *Litoria rheocola*, was locally extirpated by *Bd* at numerous sites in the Australian Wet Tropics; today, populations of this frog species have reappeared and co-exist with the fungus at many of these sites. *Litoria rheocola* and *Bd* provide an excellent model system to study the host-pathogen dynamics of an emerging disease in populations with different histories and ecologies. I conducted a one-year capture-mark-recapture study to quantify the effects of *Bd* on frog survival in six different populations of *L. rheocola* that differed in elevation and connectivity (site type). I used multistate models in program MARK to test which ecological factors (site type, population identity, season, and *Bd* infection status) influenced survival, recapture, infection, and recovery rates. Survival rates were influenced by both site type and season whereas recapture rates were influenced by site type only. Although I observed some disease-induced mortality in the field, infection status did not affect either survival or recapture estimates. Incidence of infection was highest in high elevation populations and between autumn and winter. Recovery rate was high (80.3%) and constant over time. In this study, occasional instances of *Bd*-induced mortality at the individual level did not reduce survival rates at the population level, suggesting that mortality caused by chytridiomycosis is compensatory rather than additive in these frog populations. The

coexistence of *L. rheocola* with *Bd* suggests that *L. rheocola* populations have evolved

resistance to chytridiomycosis, or that current environmental conditions inhibit outbreaks of

chytridiomycosis.

Introduction

Pathogens and parasites can reduce host survival and fecundity and cause declines in

their host populations (de Castro and Bolker 2005, Jolles et al. 2006, McCallum and Dobson

1995, Tompkins and Begon 1999). However, disease-induced mortality should only cause host

population declines if it is additive to other sources of host mortality (i.e., competition or

predation; Anderson and May 1979, Burnham and Anderson 1984, Jolles et al. 2006, Tompkins

et al. 2002). When mortality is compensatory, increases in disease-induced deaths will be

balanced by a reduction in natural mortality from other causes (Jolles et al. 2006, Lebreton

2005, Tompkins and Begon 1999), and populations will remain stable. Despite great interest in

the ecology of infectious diseases, the effects of parasites and pathogens on the dynamics of

wildlife populations remain poorly understood (Jolles et al. 2006, Tompkins and Begon 1999).

All well-studied species of wildlife suffer from a range of diseases and parasites but many

populations are relatively stable, suggesting that mortality is often compensatory (Albon et al.

2002, Krkosek et al. 2011, Caley et al. 2002, Tobler et al. 2012).

Chytridiomycosis, caused by the amphibian chytrid fungus, *Batrachochytrium*

dendrobatidis (*Bd*), has contributed to declines and extinctions of amphibian populations on

most continents (Berger et al. 1998, Daszak et al. 1999, 2003). In lethal chytridiomycosis,

severe *Bd* infections disrupt cutaneous function, causing an imbalance in electrolyte transport

across the epidermis, which leads to asystolic cardiac arrest (Voyles et al. 2009).

Chytridiomycosis can cause extinctions because *Bd* has a broad host range, including both

larval and adult amphibians, some of which are resistant to its effects and act as reservoirs for *Bd* (Brunner et al. 2004, Lips et al. 2006, Murray et al. 2009, Retallick et al. 2004).

Understanding the dynamics and population effects of chytridiomycosis is difficult because the influence of the disease is temperature dependent (Aplin and Kirkpatrick 2000, Berger et al. 2004, McDonald et al. 2005, Piotrowski et al. 2004, Retallick 2002, Rödder et al. 2008, Savage et al. 2011). *Bd* grows optimally between 17°C and 25°C and dies above 30°C (Piotrowski et al. 2004). Prevalence of *Bd* is usually higher in cooler seasons and lower in warmer ones (Brem and Lips 2008, Chapter 5, Retallick 2002, Whitfield et al. 2012, Woodhams and Alford 2005). Similarly, prevalence of infection often differs among elevations: higher elevations are cooler and prevalence at these elevations is often higher, presumably because cooler temperatures favour the pathogen (Brem and Lips 2008, Pullen et al. 2010, Woodhams and Alford 2005). Connection from upland to lowland areas via the flow of cool water, potentially containing *Bd* zoospores, can also affect the prevalence of *Bd* infection (Chapter 4 and 5). The effects of season, elevation, and connectivity can interact in determining the prevalence of the pathogen (Chapter 4 and 5).

Although chytridiomycosis can cause population extinctions, not every population infected with *Bd* goes extinct or even declines. For example, chytridiomycosis had no apparent negative impact on adult survival rates in a population of tree frogs *(Litoria wilcoxii*; Kriger and Hero 2006). Similarly, not every individual infected with *Bd* dies; survival rates of infected sharp-snouted day frogs (*Taudactylus acutirostris)* did not differ significantly from those of uninfected individuals (Retallick et al. 2004). On the other hand, in some amphibian populations, survival rates of infected individuals are lower than those of uninfected individuals (Longo and Burrowes 2010, Murray et al. 2009, Pilliod et al. 2010). At the population level, some susceptible species can persist with the disease (Briggs et al. 2005, 2010, Tobler et al. 2012). Some species have reappeared following apparent local extirpations (McDonald et al. 2005, Retallick et al. 2004,

Rodriguez-Contreras et al. 2008). Population recovery suggests that the host-pathogen relationship has changed, either temporarily or more permanently, to favour the host. This could be caused by changes in factors such as individual and species susceptibility, environmental temperatures, sources of infection, and disease transmission rates.

I examined the influence of *Bd* infection on the common mistfrog (*Litoria rheocola*). This species declined and disappeared in the early 1990s during outbreaks of chytridiomycosis at all surveyed sites above 400 m elevation in the Australian Wet Tropics, while persisting, apparently unaffected, at sites below 400 m (McDonald and Alford 1999, Richards et al. 1993). *Litoria rheocola* has since reappeared at some sites above 400 m (McDonald and Alford 1999, McDonald et al. 2005). Using capture-mark-recapture techniques, I quantified population parameters, including survival and recapture rates, of frogs in high elevation populations and in low elevation populations that were either connected or unconnected to higher elevations by stream flow. I carried out my study over a full annual cycle, from one winter through the next. I used multistate modeling to assess my mark-recapture data and to examine rates of transition of individuals between uninfected and infected states to determine whether chytridiomycosis was an important cause of mortality of individuals. My study is the first to quantify the effects of elevation and site-connectedness on amphibians and *Bd* over a full annual cycle at replicate sites.

Materials and Methods

Study species

The common mistfrog, *Litoria rheocola*, is a small (average adult male body size 2 g, 32 mm snout-urostyle length; S.J.Sapsford, personal observation), endangered, hylid frog (IUCN 2012). It occurs near rocky, fast-flowing, rainforest streams in northern Queensland, Australia (Dennis 2012, Hoskin and Hero 2008). At night, and on rainy days, males typically perch on

rocks, logs, and stream-side vegetation near riffles (Dennis 2012, Hodgkison and Hero 2002, Hoskin and Hero 2008), and on dry days they typically shelter between moist rocks in the stream bed (E.A. Roznik, unpublished data). Females were elusive as they tended to visit the stream only briefly to breed (S.J.Sapsford, personal observation).

Study sites

     I carried out my surveys at three different site types: high elevation, contiguous low elevation, and non-contiguous low elevation. High elevation sites, following the definition of McDonald and Alford (1999), were more than 400 m above sea level (ASL). Populations of *L. rheocola* at these sites disappeared in the early 1990s and subsequently reappeared. Low elevation sites were below the 400 m cut-off; populations of *L. rheocola* at these sites are not known to have suffered declines (McDonald and Alford 1999). Contiguous low elevation sites were low elevation sites that were connected to high elevation sites by stream flow. At such sites, *Bd* zoospores may drift down from higher elevations, possibly influencing the prevalence and dynamics of *Bd* (Brittain and Eikeland 1988). Contiguous low elevation sites also receive inflow of cooler water from high elevations resulting in lower water temperatures that might affect the prevalence and dynamics of *Bd* infections (Chapter 4). Finally, non-contiguous low elevation sites were low elevation sites that were not connected to high elevation sites.

     I selected six sites in the Wet Tropics Bioregion in northern Queensland, Australia: two high elevation sites (Bobbin Bobbin Falls, 17°22'43"S 145°46'21"E; 700 m ASL and Windin Creek, 17°22'04"S 145°42'52"E; 718 m ASL, and both in Wooroonooran National Park), two contiguous low elevation sites (Frenchman Creek, 17°18'29"S 145°55'16"E; 59 m ASL, in Wooroonooran National Park, and Tully Creek, 17°46'29"S 145°38'38"E; 114 m ASL, in Tully Gorge National Park), and two non-contiguous low elevation sites (Mena Creek, 17°38'59"S 145°59'13"E; 59 m ASL, and Stoney Creek, 17°55'17"S 146°4'7"E; 18 m ASL, in Hull River

National Park). All of these creeks were surrounded by tropical rainforest and all had pools and riffles; some also had waterfalls. The nature of the creek beds varied among sites: those of Frenchman Creek, Tully Creek, and Bobbin Bobbin Falls were comprised of large boulders interspersed with sections comprised of smaller rocks (1-10 cm in diameter). The beds of Windin, Mena, and Stoney Creeks were primarily comprised of small rocks. Sampling of adult frogs took place over one full annual cycle on five different sampling occasions: austral winter (June/July 2010), spring (October 2010), summer (January 2011), autumn (March/April 2011) and the following winter (June/July 2011).

Field methods

Prior to surveys, I placed flags at 10 m intervals along a 400 m transect at each site so I could record the location of each frog captured. Sampling was conducted over five consecutive nights at each site in each season. Adult frogs were located at night using visual and auditory cues, and were captured using a clean plastic sandwich bag worn as a glove. Following capture, all frogs were swabbed for *Bd*, measured to the nearest 0.1 mm (snout-urostyle length), weighed to the nearest 0.1 g, and given individual marks with visible implant elastomer as detailed below. To prevent disease transmission among frogs, a new pair of powder-free latex gloves was used to handle each individual. Once processing was complete, frogs were released at their capture location.

Assessing disease status

Frogs were swabbed using a sterile, fine-tip, dry rayon swab (#113, Dry swabs, Medical Wire and Equipment, Corsham, Wiltshire U.K.). The ventral side of each foot, inner thigh area, both lateral sides of the stomach, medial section of the stomach, and ventral side of each hand

were swabbed three times, because the fungus is mainly found on these areas of the body

(North and Alford 2008, Skerratt et al. 2008).

After processing, all swabs were placed in separate, labelled vials and refrigerated until

the end of sampling. All swab samples were processed using real-time quantitative PCR (Boyle

et al. 2004). Samples were run in triplicate and considered positive for *Bd* if at least two of the

three PCR reactions had numbers of zoospore equivalents that were greater than zero.

Marking technique

Each individual was marked using visible implant elastomer (VIE; Northwest Marine

Technology Inc., Shaw Island, Washington, USA). Five colours of VIE were used to create a

marking scheme: pink, orange, yellow, green, and blue. VIE was injected subcutaneously into

the inner thigh of the frog (Schmidt and Schwarzkopf 2010) using a 29-gauge insulin needle

(Terumo Medical Corporation, Elkton, Maryland, USA). Individual frogs received up to three

marks in total, with marks placed in the left leg, the right leg, or both. Needles were reused and

were sterilised before each use by placing them in 70% ethanol for at least 20 seconds

(Australian Department of Environment and Heritage 2006, Johnson et al. 2003).

Modelling overview – program MARK

I considered data from male *Litoria rheocola* only (*n* = 1340) as few females were

recaptured. Females tended to visit the stream only briefly to breed. My mark-recapture data set

consisted of live captures on five different sampling occasions. My capture histories included

information on a dynamic state variable: the infection status of each frog at every capture. I

therefore used multi-state models to analyze the effect of infection status on monthly survival

and daily recapture rates. The survival estimates (termed 'apparent survival'; White and

Burnham 1999) incorporated both mortality and emigration; survival thus equated to persistence

in the study area. In addition, multi-state models can estimate the transition probabilities between states. In my study, these transitions refer to incidence and recovery probabilities. An incidence event occurs when a healthy individual becomes infected between sampling periods (transition from *Bd*-negative to *Bd*-positive) whereas a recovery event occurs when an infected individual loses its infection (i.e., a transition from *Bd*-positive to *Bd*-negative). Incidence and recovery probabilities are critical parameters for understanding the dynamics of infectious diseases.

I used program MARK (White and Burnham 1999) to analyze my data, examining the following explanatory variables: infection status (2 levels: healthy or infected with *Bd*), population identity (6 levels: Mena Creek, Stoney Creek, Frenchman Creek, Tully Creek, Bobbin Bobbin Falls, and Windin Creek), site type (3 levels: high elevation, contiguous low elevation, and non-contiguous low elevation, and season (4 levels: winter, spring, summer, autumn). I examined a set of candidate models for their fit to the data. These models tested several combinations of the parameters (survival, recapture, incidence, and recovery) that were modelled as (1) constant over time (i.e., no influence of season), (2) time-dependent (i.e., influenced by season only), (3) different among groups (i.e., populations, site type, or *Bd* infection status), and (4) dependent on the interaction between groups (i.e., population, site type, *Bd* infection) and time (season).

I derived an estimate of lack of fit for the best model in my candidate set using the median $\hat{c}$ approach (White and Burnham 1999). To quantify the amount of over-dispersion, I calculated the value of the variance inflation factor ($\hat{c}$). The model fit the data well ($\hat{c} = 1.19$), so no adjustment for over- or under-dispersed data was necessary. I used Akaike's Information Criterion adjusted for small sample size (AIC_c; Burnham and Anderson 2002) to guide model selection. The best-supported models are those that make up the top 90% of Akaike weights, and have relative deviations from the best model of less than 2 ($\Delta AIC_c < 2$; Burnham and

Anderson 2002). If the best-supported models suggested that there was a group effect, I compared the 95% confidence intervals for the means of the groups concerned, to determine whether they differed significantly, following the approach described by Cumming et al. (2007). When they did not, I examined the magnitude of non-significant differences, and their variability, to determine whether the differences appeared genuinely unimportant or if they were potentially biologically meaningful.

Analysis of transitions of infection status

In addition to the transition probabilities calculated by MARK, I also estimated transition rates directly from my data. I measured rates of four different transitions between states for individuals recaptured across seasons: 1) individuals that were infected in one season and remained infected in the next (positive to positive; P-P), 2) individuals that recovered from one season to the next (positive to negative; P-N), 3) individuals that remained uninfected between successive seasons (negative to negative; N-N), and 4) individuals that became infected between successive seasons (negative to positive; N-P). To determine whether the proportion of individuals undergoing each transition differed significantly among site types, I used a Fisher's Exact Test.

Estimates of steady state prevalence

Although my directly estimated transition (incidence and recovery) rates and directly estimated prevalences were derived from the same data set, they were not constrained to agree; biases in frog behaviour or my techniques could have caused large divergences, making my estimated transition rates poor descriptions of infection dynamics. Close agreement between prevalences estimated from transition rates and directly from the data would suggest that the transition rates I measured provide a reasonable description of the infection dynamics in this

system. I used a cross-validation procedure to test whether transition rates estimated directly from my data could produce the observed pathogen prevalence in the frog populations. I carried out this cross-validation by assuming that prevalence results from a first-order Markov process with a 2 X 2 transition matrix with elements: P-P, P-N, N-P, N-N (where N = negative for Bd infection and P = positive for Bd infection). I calculated expected prevalence for each of two factors: season and site type. To estimate prevalence for each season, I aggregated my data across all site types to avoid small sample sizes. Similarly, to estimate prevalence for each site type I aggregated my data across seasons. I compared the expected prevalences with the observed prevalences from the diagnostic qPCR results.

Results

Of the 1340 individuals I marked, 275 were recaptured at least once. I used month (30 days) as the unit of time in my MARK analysis; monthly survival rates therefore refer to the probability that a frog survives for one month. Incidence and recovery rates refer to the probability that the frog becomes infected or loses its infection conditional on its survival during the preceding month. Recapture rates are defined relative to the duration of the sampling period and my recapture rates therefore refer to the probability of recapturing a frog over the 5-day capture interval.

Modelling using program MARK

The model with the most support (65.4% of model weight; Table 6.1) suggested that monthly frog survival was influenced by an interaction between site type and season. The best model also suggested that recapture rate was influenced by site type only, that monthly incidence of infection was influenced by season only, and that monthly recovery rate (transition of *Bd*-positive to *Bd*-negative) was constant throughout the study period (Table 6.1). The two

models that incorporated chytrid infection status in the recapture parameter or survival parameter were far less parsimonious (ΔAICc = 8.66, model weight = 0.01 and ΔAICc = 10.68, model weight < 0.005, respectively; Table 6.1) suggesting that chytrid infection had little influence on survival or recapture rates in these *L. rheocola* populations. Monthly survival estimates at all site types decreased over time, with the lowest survival occurring between autumn and winter (Fig. 6.1, Appendix 2). However, the 95% confidence intervals (CIs) overlapped substantially between winter and spring and summer and autumn (Fig. 6.1). Estimates of the probability of recapture differed significantly among site types (95% CIs do not overlap in Fig. 6.2; Cumming et al. 2007); recapture rates were highest for populations at high elevations, followed by contiguous low elevation populations, and then by non-contiguous low elevation populations (Fig. 6.2, Appendix 2). The monthly rate of incidence of infection remained low (1.3%-10.5%) for the first three time intervals (between winter and autumn) and significantly increased (51.7%) in the last interval between autumn and winter (Fig. 6.3, Appendix 2). The monthly recovery rate remained constant throughout the year at 80.3%.

Table 6.1 Candidate models used to estimate monthly survival, recapture, incidence of infection and recovery rates for populations of common mistfrogs (*Litoria rheocola*) at high, contiguous low and non-contiguous elevations over four time intervals.

Model Description				AICc	ΔAICc	Model weight	Parameters	Deviance
Survival	Recapture	Incidence of Infection	Recovery					
Site type x season	Site type	Season	Constant	2098.51	0.00	0.65	20	296.80
Site type x season	Site type + season	Season	Constant	2100.73	2.22	0.22	23	292.85
Time	Site type	Season	Constant	2102.88	4.37	0.07	12	317.52
Site type x season	Site type	Site type x season	Constant	2106.13	7.62	0.02	37	269.13
Site type + season	Site type + season	Season	Constant	2106.92	8.41	0.02	17	311.36
Season	Chytrid infection x site type	Season	Constant	2107.17	8.66	0.01	15	315.70
Site type x season	Site type	Site type + season	Constant	2107.30	8.79	0.01	31	282.84
Site type x season	Site type	Site type x season	Site Type x season	2108.62	10.11	0.00	39	267.40
Chytrid infection x season	Site type	Season	Constant	2109.19	10.68	0.00	16	315.68
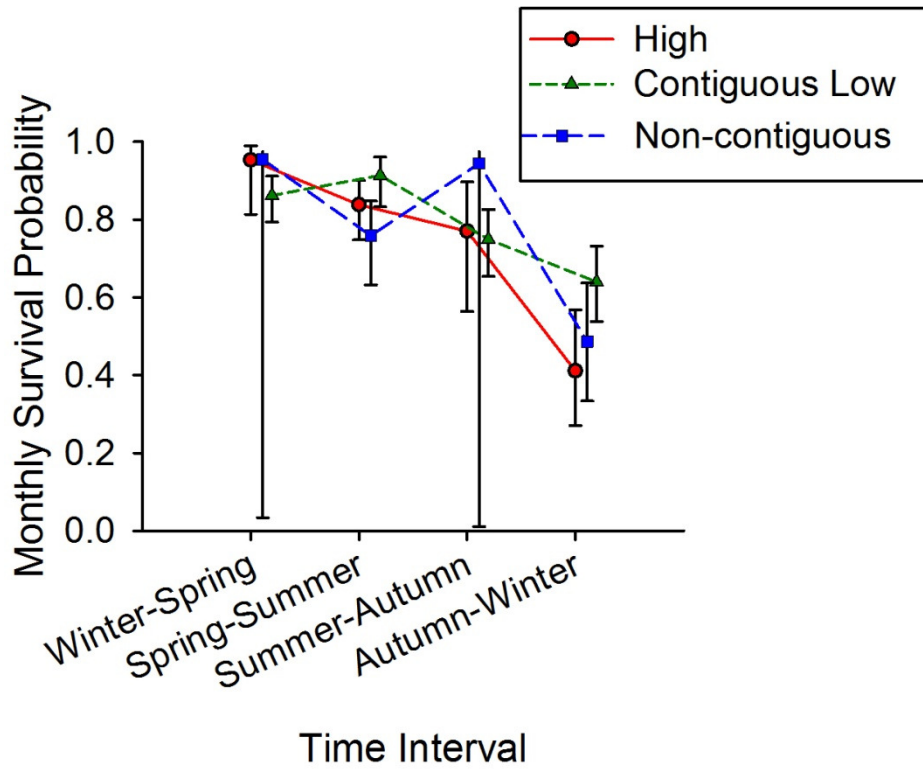
Figure 6.1 Monthly survival estimates (with 95% confidence intervals) for common mistfrog (*Litoria rheocola*) populations in northern Queensland, Australia across four time intervals.
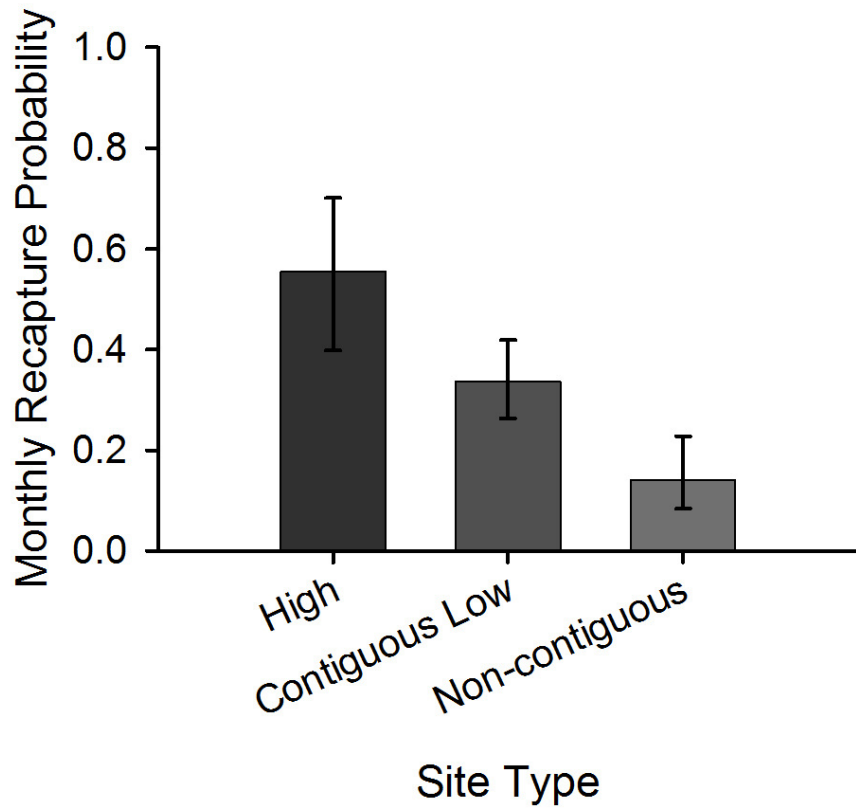
Figure 6.2 Monthly recapture estimates (with 95% confidence intervals) for common mistfrog (*Litoria rheocola*) populations in northern Queensland, Australia across four time intervals.
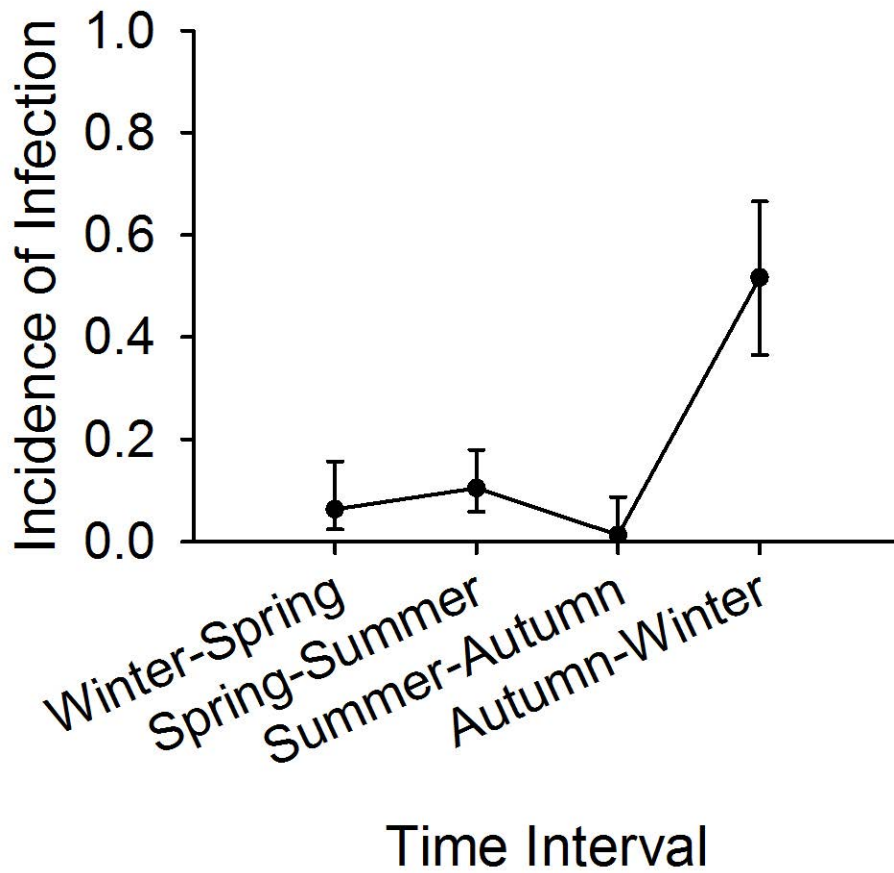
Figure 6.3 Monthly incidence of *Bd* infection in common mistfrog (*Litoria rheocola*) populations in northern Queensland, Australia across four time intervals.

Transition probabilities and mean prevalences of infection

Similar to transition probabilities estimated in MARK, the proportions of all four possible transitions of infection status (P-P, P-N, N-P, and N-N) estimated directly from my data did not differ significantly among site types (Table 6.2; Fisher's Exact Test, $p = 0.261$). Although not statistically significant, the transition rate from the uninfected to the infected state at high elevation sites (19.6%, Table 6.2) was almost twice that at contiguous low (12.0%, Table 6.2) and non-contiguous low sites (9.1%, Table 6.2). The rate of recovery (transition from *Bd*-positive to *Bd*-negative) was more similar across site types; on average, 78.3% to 80% of infected individuals lost their infections between seasons. Transition rates differed significantly among seasons (Table 6.2; Fisher's Exact Test, $p < 0.0001$). The rate of appearance of new infections was much higher for the autumn-winter interval (44%, Table 6.2) than any other period of the year. The proportion of recoveries was high for all intervals (74.1 to 89.5%, Table 6.2) except the autumn-winter interval (0.0%), however, that estimate was based on a small sample size ($n = 1$). The highest rate of recovery occurred over the spring-summer interval, when temperatures increased sharply.

Estimates of steady state prevalence

The estimates of steady-state prevalence of *Bd* for each site type closely matched the observed prevalences for high and contiguous low elevation populations (Table 6.3, correlation of all predicted versus observed prevalences, r = 0.837, p = 0.009). This suggests that in most cases the transition rates I estimated from my recaptures closely reflected real rates of transition in the populations I surveyed, and that my estimates are not highly biased, for example by effects of infection on recapture probability. The non-contiguous low elevation populations had the biggest mismatch between the predicted (10.2%, Table 6.2) and observed prevalence (27.4%), suggesting that my observed transitions for sites of this type underestimate rates of

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incidence, overestimate rates of recovery, or both. My estimates of transition probabilities may

have been less accurate for this site type because the proportion of individuals recaptured was

lower than at either of the other site types (Fig. 6.2).

Table 6.2 Observed numbers and rates of transitions between infection states observed in individual frogs. Only transitions observed between successive seasons are included.

Category	Number of transitions observed				Proportion of initially negative that		Proportion of initially positive that	
	Negative to negative	Negative to positive	Positive to negative	Positive to positive	Remained negative	Became positive	Remained positive	Became negative
By seasons across all site types								
Summer-Autumn	35	1	1	3	0.972	0.028	0.250	0.750
Winter-Spring	57	4	7	20	0.934	0.066	0.259	0.741
Autumn-Winter	14	11	1	0	0.560	0.440	1.000	0.000
Spring-Summer	64	11	2	17	0.853	0.147	0.105	0.895
By site type across all seasons								
High	45	11	5	18	0.804	0.196	0.217	0.783
Contiguous low	95	13	5	18	0.880	0.120	0.217	0.783
Non-contiguous low	30	3	1	4	0.909	0.091	0.200	0.800

Table 6.3 Observed prevalence of *Bd* in each season across all site types and in each site type across seasons, and steady-state prevalence predicted from observed rates of transition (Table 6.2).

Category	Prevalence of infection	
	Measured	Predicted from transition matrix
By seasons across all site types		
Winter 1	0.345	0.473
Spring	0.230	0.157
Summer	0.094	0.140
Autumn	0.053	0.059
Winter 2	0.567	0.473
By site type across all seasons		
High	0.273	0.200
Contiguous low	0.160	0.133
Non-contiguous low	0.274	0.102

Discussion

Survival

My mark-recapture models indicate that frog survival depended on season in a manner that differed among site types, but was not influenced by individual infection status. Monthly rates of survival were roughly 80% or greater at all site types across three of the four seasonal intervals I examined. The drop in survival in the last time period may be an artefact of the low

recapture rates for that time period resulting in estimates with low precision (Faustino et al. 2004). Low recapture rates could have been caused by the unusually cold conditions (mean temperature for June 2011 = 13.8°C, long-term mean temperature for June = 15.8°C; Bureau of Meteorology 2012) during that period resulting in low levels of frog activity.

This study found no effect of *Batrachochytrium dendrobatidis* infection on the survival rate of *Litoria rheocola*. This result was surprising because *Bd* is known to cause mortality in *L. rheocola* (Woodhams and Alford 2005) and because I observed severely infected and dying frogs during the study. My results are similar to those of Kriger and Hero (2006), who found no effect of *Bd* infection on the survival of *Litoria wilcoxii*. In contrast, several other studies on a variety of frog species have reported differences in survival rates between infected and uninfected individuals (Longo and Burrowes 2010, Murray et al. 2009, Pilliod et al. 2010).

The lack of a significant influence of *Bd* on survival of *L. rheocola* is interesting given that populations of *L. rheocola* suffered drastic *Bd*-associated declines in the 1990s, when populations were extirpated from high elevation sites (McDonald and Alford 1999, Richards et al. 1993). Since then, some high elevation populations of *L. rheocola* have reappeared, although *Bd* is now endemic in these areas and still kills some individual frogs (S. J. Sapsford personal observation, Woodhams and Alford 2005). It therefore appears that mortality in my populations is compensatory, since these individual-level effects were not reflected in overall estimates of population survival rates. Other mark-recapture studies have found similar results. In Switzerland, *Bd*-infected populations of the endangered midwife toad (*Alytes obstetricians)* remained stable and did not have lower population growth rates than uninfected populations (Tobler et al. 2012). Similarly, mountain yellow-legged frogs (*Rana sierrae*) can persist at low population densities with endemic *Bd* (Briggs et al. 2010). While the reasons behind variation among species and populations in their ability to coexist with *Bd* remain unknown, long-term coexistence of frogs with the pathogen requires compensatory Bd-induced mortality so that

chytridiomycosis does not change the overall population growth rate. If *Bd*-induced mortality

was not compensatory (i.e., if it was additive to existing sources of mortality), infected

populations would experience ongoing declines. Lowland populations of *L. rheocola* have

persisted with endemic *Bd* infections for at least 20 years (Australian Department of

Environment and Heritage 2006), suggesting that in these populations, *Bd*-induced mortality

must be balanced by a decrease in mortality from other sources. My result is consistent with the

observation that *L. rheocola* and many other species have now coexisted for long periods of

time with *Bd*, despite the fact that chytridiomycosis produces some individual mortality (Briggs et

al. 2005, 2010, Puschendorf et al. 2011, Woodhams and Alford 2005).

Recapture

I found that recapture rates differed among site types, but were not significantly

influenced by season or infection status. My result is similar to a mark-recapture study by

Murray et al. (2009) who found no evidence that recapture rates depended on infection status in

Pearson's treefrogs (*Litoria pearsoniana*). Given that infection status does not affect recapture

rate, it seems plausible that infection status does not affect the initial capture rates, and that my

estimates of *Bd* prevalence should therefore be unbiased (Faustino et al. 2004).

I recaptured relatively few individuals at the non-contiguous low elevation sites (63

recaptured out of 498 marked). Many more new individuals were marked during each sampling

period at both contiguous ($n = 628$) and non-contiguous low elevation sites ($n = 498$) than at

high elevation sites ($n = 213$). A possibility is that population size influenced recapture

probabilities: larger populations may make recaptures less likely as rates of initial captures

increase. However, other variables could have influenced recapture rates including invasive

species (cane toads are present at some of the sites), site characteristics, and weather.

Infection transitions and estimates of prevalence

Site type did not significantly influence the rates of incidence of infection (Table 6.1), although my parameter estimates showed a more than twofold difference between high sites and low non-contiguous sites (Table 6.2). However, I observed very few transitions between states in low non-contiguous sites (Table 6.2), so my estimates for those sites have very low precision. As confirmed by both methods (MARK and my calculations directly from data), rates of incidence of infection differed strongly among seasons (Fig. 6.3, Table 6.2). The incidence of new infections was highest between autumn and winter and lowest between summer and autumn (Fig. 6.3, Table 6.2). It is possible that there is a temperature-related mechanism behind the differences in incidence of infection among seasons. During the summer months, temperatures were frequently above 25°C (Chapter 5), which substantially slows *Bd* growth, and were sometimes above 30°C (1.2% of recorded temperatures; Chapter 5), which is within the range that kills *Bd* (Longcore et al. 1999, Piotrowski et al. 2004). In comparison, during winter months, temperatures were frequently between 17°C and 23°C (Chapter 5), which is within the optimal growth range of *Bd* (Piotrowski et al. 2004). Recovery rates (transition from *Bd*-positive to *Bd*-negative) were more constant across seasons suggesting that transitions from infected to uninfected status may be relatively independent of environmental conditions, perhaps affected more strongly by the frog immune system (Woodhams et al. 2010).

In conclusion, I demonstrated that a frog species that has been highly susceptible to *Bd* during past epidemic outbreaks can persist with *Bd* in the wild. I found individuals dying of chytridiomycosis, but overall mortality rates did not differ significantly between infected and uninfected frogs, suggesting that mortality is compensatory in these endemically infected populations. The exact mechanisms underlying the ability of *L. rheocola* and other frog species to persist and co-exist with *Bd* remain unknown. Future studies of how these frog species

maintain viable populations in the face of *Bd* infection will be critical to understanding the effects

of this pathogen on amphibian populations.

Chapter 7 : General Discussion

Introduction

Infectious diseases have caused substantial declines in several wildlife species (Dobson and Foufopoulos 2001). For example, chronic wasting disease (CWD), a fatal neurodegenerative disease, has become a long-term concern for management of North American deer populations; where the disease has become endemic, it has caused decreased survival and declines in deer abundance (Miller and Wild 2004, Robinson et al. 2012). Disease emergence usually occurs when there is a change in the ecology of the host, pathogen, or both (Daszak et al. 2000). Wildlife diseases that can infect multiple host species are more likely to cause declines; some pathogens have crossed both species and geographical boundaries to infect many wildlife populations resulting in epidemics (Dobson and Foufopoulos 2001). Diseases that cross species and geographical boundaries can potentially be transmitted among wildlife populations, domestic animal populations, and human populations (Cleaveland et al. 2001, Daszak et al. 2000, Gortazar et al. 2007). For example, the H5N1 avian flu can be transmitted from wildlife to human populations, causing devastating effects to both wildlife and human health (Yen and Peiris 2012). Another example is canine distemper; high prevalence of the canine distemper in domestic dogs has caused high incidence of canine distemper in wild dogs, causing major declines in endangered wild dog populations (Cirone et al. 2004, Daszak et al. 2000, McCarthy et al. 2007).

A pathogen that can only infect a single host cannot survive when host density is below a critical threshold (McCallum and Dobson 1995, Pedersen et al. 2007), and so dies out quickly. Pathogens with multiple hosts can persist in susceptible host populations even when host density is low (Daszak et al. 1999, McCallum and Dobson 1995, Pedersen et al. 2007).

Reservoir hosts, which are typically abundant, widespread species, are more resistant to the pathogen than more susceptible host species, and can maintain virulent pathogens endemically (Blaustein et al. 2005, Brunner et al. 2004, de Castro and Bolker 2005, McCallum and Dobson 1995). Maintenance of a virulent pathogen by a less susceptible host can drive highly susceptible hosts to extinction. Despite the potential importance of reservoir hosts in disease persistence, little is understood about the interactions between susceptible hosts, reservoirs, and pathogens for most host-pathogen systems. Understanding transmission of pathogens among multiple hosts may provide ways of mitigating host decline by reducing transmission rates.

Chytridiomycosis

Chytridiomycosis is an amphibian disease caused by the pathogenic fungus, *Batrachochytrium dendrobatidis* (*Bd*). It has caused mass mortalities, declines and extinctions of amphibian populations and species worldwide (Berger et al. 1998, Bosch et al. 2001, Bradley et al. 2002, Lips et al. 2006, Longcore et al. 1999, Rachowicz et al. 2006). In many cases, the disease has extirpated populations, causing the loss of more than 50% of the species of amphibians at some sites (Lips 1999, Lips et al. 2006). In most cases, amphibian species that declined during epidemics of chytridiomycosis co-occurred with species that did not decline (Lips et al. 2006, Retallick et al. 2004). These non-declining species can be infected by *Bd,* but individuals typically survive and can persist with infections, and, thus, act as reservoirs for the disease. Tadpoles, even those of species that are severely affected by chytridiomycosis in their terrestrial stages, can often persist with infections and are thus likely to be an important potential reservoir host for *Bd* (Brunner et al. 2004).

Tadpoles can act as intraspecific reservoirs for *Bd* because *Bd* infects the mouthparts of tadpoles, usually without fatal effects (Blaustein et al. 2005, Lamirande and Nichols 2002). As

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they often occur in high abundance in stream environments and are closely associated with many adult frog populations, they can readily transmit *Bd* to adults and other amphibian species (Blaustein et al. 2005, Lamirande and Nichols 2002). Tadpoles can transmit infections to adult and recently metamorphosed amphibians, and some species may also maintain their own infections through metamorphosis (Brunner et al. 2004, Marantelli et al. 2004). The diversity of tadpole morphology, physiology, behaviour, and developmental strategies is likely to cause variations in the host-pathogen relationship as well as variations in the success of disease transmission between tadpoles and adult frogs. Thus, for different amphibian populations, it is not only important to understand disease dynamics in the susceptible host but also in sympatric species and life stages that may be responsible for disease transmission. For example, tadpole infection could affect the health and disease dynamics of *Bd* in adult populations by causing disease persistence.

Aims and approach

My study sites were located at three different site types. In the Wet Tropics Bioregion, Australia, high elevations are considered regions that are higher than 400 m above sea level (ASL; McDonald and Alford 1999). High and low elevation areas are often connected by streams, making them contiguous, such that water can flow from high to low elevation sites. I refer to low elevation sites connected to high elevations as contiguous low elevation sites. In such sites, *Bd* zoospores may be carried from high to low elevation, influencing prevalence of *Bd* in the populations at lower elevations through drift (Brittain and Eikeland 1988). Cooler water will flow from high elevations to low elevations, potentially reducing maximum water temperatures. Not all low elevation sites, however, are connected to high elevation sites. Some low elevation streams have no adjacent regions higher than 400 m. I refer to these areas as non-contiguous low elevation sites.

The general aim of my study was to examine the effects of *Bd* on the endangered, stream-associated, common mistfrog (*Litoria rheocola*), particularly the diseases effects on population parameters such as survival and recapture rates, and how these change among seasons and differed among site types. To achieve this, I examined and marked individuals of *L. rheocola* in northern Queensland, Australia. I surveyed six populations at three different site types: high elevation, contiguous low elevation, and non-contiguous low elevation and in different seasons: winter, spring, summer, and autumn. My specific aims within each chapter were to:

Chapter 2: determine if visible implant elastomer (VIE) marking affected the movement and survival of *L. rheocola,*

Chapter 3: determine whether VIE is an effective and viable long-term marking technique,

Chapter 4: examine and describe how *Bd* infection differed among populations of tadpoles at different site types and among seasons, and determine whether factors such as season, size of tadpoles, and drift (flow of infectious propagules downstream) drove prevalence of *Bd* in tadpoles,

Chapter 5: examine and describe how *Bd* infection differed among populations of adult frogs at different site types and among seasons

Chapter 6: examine and describe the population dynamics (survival and recapture rates) of adult frogs at different site types and among seasons

Development of techniques

To examine and describe infection and population dynamics of *Bd* in adult and tadpole populations I first had to determine the efficacy of a new marking technique. Visible implant

elastomer (VIE) has been used extensively in mark-recapture studies of fishes (Astorga et al. 2005, Bolland et al. 2009, Brennan et al. 2007, Bushon et al. 2007, Catalano et al. 2001, Claverie and Smith 2007, Curtis 2006, Goldsmith et al. 2003), but has been used only recently to mark amphibians. Lab studies have demonstrated that VIE can be used to mark amphibians (Bailey 2004, Heard et al. 2008, Heemeyer et al. 2007, Nauwelaerts et al. 2000, Schmidt and Schwarzkopf 2010). Marks remain visible and readable for the duration of short-term field studies (Campbell et al. 2009, Hoffman et al. 2008). However, before using any marking technique, it is important to know its affects on behaviour and survival of the species being marked (Donnelly et al. 1994, Ferner 2010). I found, in a 3-week study, that VIE marking in *L. rheocola* did not significantly reduce frog survival or movement (Chapter 2). In addition, after a one year field study, all VIE marks were retained and remained visible and easily identifiable (Chapter 3). Therefore, I could conduct an unbiased mark-recapture study of *L. rheocola* using this technique.

Infection dynamics of *Bd* in tadpoles

My results demonstrated that infection prevalence was significantly affected by season: all site types had higher prevalence of *Bd* in winter than in summer. Infection prevalence was also affected by site type. *Litoria rheocola* tadpoles at all low elevation sites had lower maximum prevalences than those at high elevation sites. There was a significant interaction between the effects of season and site type on the prevalence of *Bd* in tadpoles. Seasonal changes were more prominent at high elevation sites than at low elevation sites, and the exact patterns of seasonal change differed among sites of all three types. The difference in patterns of prevalence of *Bd* between contiguous and non-contiguous low elevation sites was probably caused by the water flow connection between contiguous low elevation sites and high elevation site. It is possible the downstream transport of infectious propagules caused high infection in

contiguous low elevation tadpole populations; this effect may also have been caused in part or in whole by the more moderate seasonal temperature changes caused by the influx of water from high elevation sites into contiguous low elevation sites.

I also found that larger tadpoles had higher prevalences of *Bd* infection than smaller tadpoles. Since larger tadpoles were more abundant in winter than summer, and tadpoles remained in the environment for a year, the annual cycle of *Bd* prevalence could have been driven largely by tadpole demography.

Infection dynamics of *Bd* in adult frogs

In adult *L. rheocola* populations, the prevalence of *Bd* fluctuated seasonally; it was highest in winter across all elevations. Site type also had a significant effect on prevalence of *Bd*. No infections were detected in summer in both the contiguous and non-contiguous low elevation sites; however, infections reappeared in autumn. In comparison, infection persisted throughout the year in populations at high elevation sites. My results indicated that reservoir hosts played a role in the persistence of *Bd* in both contiguous and non-contiguous low elevations. In summer, tadpoles in contiguous low elevation sites had a high prevalence of *Bd* (58%) suggesting that they may be acting as a reservoir in these populations. However, tadpoles in one of the non-contiguous low elevation sites were not infected with *Bd* in summer. Thus, in non-contiguous low elevation sites, tadpoles may not be an important reservoir for this disease.

Population dynamics of adult frogs

Adult survival was influenced by season and site type with probability of survival remaining high (~80%) from winter to autumn but declining in the following winter. Recapture

rates were influenced by site type only with the highest recapture probability at high elevation sites. Model estimates indicated that neither survival nor recapture rates were influenced by chytrid infection at any site type. The lack of influence of chytrid infection suggests that occasional instances of mortality caused by *Bd* at the individual level do not translate into low survival rates at the population level. The rate of incidence of infection was influenced by season and remained low between winter and autumn but peaked between autumn and winter. The rate of recovery from infection (tranisition from *Bd*-positive to *Bd*-negative) remained almost constant throughout the study, with approximately 80.3% of infected individuals clearing their infections each month. It appears that in the populations I studied, disease-induced mortality is compensatory rather than additive allowing *Litoria rheocola* populations, previously at risk of decline, to persist with the disease.

In summary, this thesis presents the first comprehensive overview of the infection dynamics of *Bd* in a stream-associated amphibian community. Tadpoles could be acting as reservoirs for *Bd* in these stream environments, causing disease persistence in adult populations that might otherwise be uninfected. I have provided evidence that the infection dynamics of *Bd* differ among high elevation, contiguous low elevation, and non-contiguous low elevation sites in both adults and tadpoles. Demographic parameters, such as survival and recapture rates, also differed among site types. My thesis also provides evidence that a species that was locally extinct due to *Bd* at high elevations is recovering at high elevation sites, and may now be persisting and coexisting with this fungal pathogen.

Implications and future directions

My results have several important implications for the study of amphibians, as well as for the conservation of my study species. Visible implant elastomer (VIE) marking appears to have no effects on survival or movement of *Litoria rheocola.* This is a key assumption of mark-

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recapture studies (Pollock et al. 1990, Ferner 2010). VIE is therefore a safe and effective

alternative to more invasive and potentially harmful techniques such as toe-clipping. Since VIE

marks are long-lasting and remain visible for long periods, they allow for long-term study of

animals. This has important implications for future research as approximately one third of all

amphibian species are considered threatened and a further 22% are categorized as "data

deficient" (IUCN 2012). Effective and safe marking techniques allow mark-recapture studies to

be conducted which provides measurement of basic biological and ecological attributes of

species that may otherwise be unknown.

Increased larval tolerance and/or resistance could affect pathogen virulence (Woolhouse

et al. 2001), environmental zoospore density, and transmission of infection to other hosts.

Prevalence of *Bd* in tadpoles was elevated throughout the year even when prevalence of *Bd* in

adults was low. Therefore, as suggested by others (Blaustein et al. 2005, Brunner et al. 2004),

tadpoles can act as an effective intraspecific reservoir and be responsible for the persistence of

infection in adult populations. Tadpoles can maintain *Bd* in the environment when adult host

densities are low and when environmental conditions limit or eliminate infections in adults, such

as during warm summers. It is possible that adult *L. rheocola* populations at both contiguous low

and non-contiguous low elevation sites that can lose their infections in summer might remain

uninfected if reservoir hosts were not present. As this study is the first to present these results it

is pertinent to conduct similar studies in other amphibian populations. Knowing the infection

dynamics of *Bd* in multiple sympatric hosts will increase understanding of *Bd* and how it persists

in and is transmitted within and among species and populations. This information will ultimately

be important in managing populations affected by this pathogen.

I found that topography plays an important role in the infection dynamics of *Bd* in these

stream communities. Low elevation sites connected to a high elevation were influenced by

some combination of the flow of cooler water downstream and the downstream transport of

infectious zoospores. Tadpoles at low elevation sites, influenced by water flow from high elevation sites (contiguous low), had higher rates of infection than tadpoles at non-contiguous low elevation sites. Differences in infection dynamics have important implications for the roles of reservoir hosts in amphibian communities: tadpoles at contiguous low elevations are potentially more effective reservoirs than tadpoles at non-contiguous elevations. However, since this study was the first to demonstrate this, future study is needed to verify the effects of water flow from high to low elevations in other amphibian populations.

Lastly, given that *L. rheocola* was extirpated from all known sites at high elevations when chytridiomycosis first emerged, and has been slowly recovering its original range since sometime in the 1990s, it is possible that it is developing resistance or tolerance to this pathogen (however, it is unknown how these species have recovered). This could have implications for the future of conservation of amphibian populations worldwide but further research is required to determine how and why this may be happening. Furthermore, if differences in host defence can be determined, this knowledge may help protect species that are still highly susceptible to the amphibian chytrid fungus.

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Appendix 1: Data on the prevalence of *Bd* infection for *Litoria rheocola*, *Litoria nannotis*, *Litoria serrata*, and *Nyctimystes dayi* tadpoles and when they occurred in the six populations surveyed

Table 1: The number of *Litoria rheocola* (the common mistfrog) that occurred across six sites and seasons and the number that were infected with *Batrachochytrium dendrobatidis* in northern Queensland, Australia

Season	Bobbin		Windin		Frenchman		Tully		Mena		Stoney	
	# Caught	# Infected	# Caught	# Infected	# Caught	# Infected	# Caught	# Infected	# Caught	# Infected	# Caught	# Infected
Winter	17	15	21	19	31	17	18	15	40	4	47	1
Spring	11	6	15	12	36	21	4	4	40	26	39	19
Summer	13	1	17	4	22	12	14	8	40	1	16	9
Autumn	-	-	24	11	28	11	7	1	9	0	34	6
Winter 2	15	14	33	26	39	26	12	11	39	14	40	39

Table 2: The number of *Litoria nannotis* (the waterfall frog) that occurred across six sites and seasons and the number that were infected with *Batrachochytrium dendrobatidis* in northern Queensland, Australia

Season	Bobbin		Windin		Frenchman		Tully		Mena		Stoney	
	# Caught	# Infected	# Caught	# Infected	# Caught	# Infected	# Caught	# Infected	# Caught	# Infected	# Caught	# Infected
Winter	12	10	9	9	-	-	37	33	-	-	-	-
Spring	8	3	4	1	-	-	34	28	-	-	-	-
Summer	4	0	5	1	1	1	42	29	-	-	-	-
Autumn	-	-	14	10	-	-	53	44	-	-	-	-
Winter 2	23	21	7	7	-	-	47	45	-	-	-	-

Table 3: The number of *Litoria serrata* (the green-eyed treefrog) that occurred across six sites and seasons and the number that were infected with *Batrachochytrium dendrobatidis* in northern Queensland, Australia

Season	Bobbin		Windin		Frenchman		Tully		Mena		Stoney	
	# Caught	# Infected	# Caught	# Infected	# Caught	# Infected	# Caught	# Infected	# Caught	# Infected	# Caught	# Infected
Winter	-	-	-	-	-	-	-	-	-	-	3	0
Spring	20	2	26	2	4	0	1	0	-	-	1	0
Summer	23	0	9	0	2	0	5	2	-	-	-	-
Autumn	-	-	2	0	-	-	-	-	-	-	-	-
Winter 2	2	1	-	-	-	-	-	-	1	0	-	-

Table 4: The number of *Nyctimystes dayi* (the Australian lace-lid frog) that occurred across six sites and seasons and the number that were infected with *Batrachochytrium dendrobatidis* in northern Queensland, Australia

Season	Frenchman		Tully	
	# Caught	# Infected	# Caught	# Infected
Winter	-	-	1	1
Spring	-	-	-	-
Summer	-	-	-	-
Autumn	2	0	-	-
Winter 2	1	1	1	1

Appendix 2: Supporting Information for multistate model analysis in MARK

Table 1: Mean survival estimates and 95% confidence intervals for *Litoria rheocola* populations at each site type and season calculated from multi-state models in MARK

Site type	Season	Mean survival (%)	95% CIs
High	Winter-spring	95.3	81.3 – 99.0
	Spring-summer	83.8	74.8 – 90.0
	Summer-autumn	77.0	56.4 – 89.7
	Autumn-winter	41.1	27.0 – 56.8
Contiguous low	Winter-spring	84.9	78.1 – 89.9
	Spring-summer	90.1	82.0 – 95.7
	Summer-autumn	73.6	64.2 – 81.3
	Autumn-winter	62.8	52.6 – 71.9
Non-contiguous low	Winter-spring	97.9	5.91 – 1.00
	Spring-summer	78.4	65.7 – 87.2
	Summer-autumn	96.9	3.61 – 1.00
	Autumn-winter	51.1	35.9 – 66.2

Table 2: Mean recapture estimates and 95% confidence intervals for *Litoria rheocola* populations at each site type and season calculated from multi-state models in MARK

Site type	Mean recapture (%)	95% CIs
High	55.4	39.8 – 70.1
Contiguous low	33.7	26.3 – 41.9
Non-contiguous low	14.1	8.40 – 22.8

Table 3: Mean incidence of infection estimates and 95% confidence intervals for *Litoria rheocola* populations at each site type and season calculated from multi-state models in MARK

Season	Mean incidence of infection (%)	95% CIs
Winter – spring	6.33	2.39 – 15.7
Spring – summer	10.5	5.89 – 18.0
Summer – autumn	1.31	0.00 – 8.72
Autumn - winter	51.7	36.5 – 66.5