Distribution, ecology, disease and physiology of a mountain-top endemic frog in the face of climate change: a study on *Philoria loveridgei*

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Abstract

Evidence clearly shows that climate change has affected ecosystems and individual species, challenging species survival and increasing extinction rates. Assessing species vulnerability to climate change is a key topic in ecology and conservation biology. Uncertainty remains as to which species will be most vulnerable and to what extent. An integrative framework has been developed to guide research towards evaluating species vulnerability. This approach proposes that a species’ vulnerability to climate change is dependent upon the synergistic combination of intrinsic (sensitivity) and extrinsic (exposure) factors. Following this framework, I examined how (i) sensitivity to environmental change (i.e. changes in calling phenology, thermal tolerances, and disease susceptibility) and (ii) exposure to environmental change (i.e. detectability, occupancy and species distribution, and thermal environment and microhabitat buffering) influenced the vulnerability of *Philoria loveridgei*, a direct-developing anuran, in subtropical rainforests of mid-eastern Australia. This allowed me to evaluate the vulnerability of this species to climate change.

Sensitivity results indicated that calling phenology and breeding phenology of *P. loveridgei* were influenced primarily by the spring and early summer rainfall events associated with the start of the calling season. I hypothesise that these rainfall events increase soil moisture, and hence increase *P. loveridgei* nest moisture and subsequently forming a suitable environment for egg and larval survival. These early summer rainfall events would therefore cue the onset of the calling, associated with breeding. A diurnal calling pattern was identified for *P. loveridgei*, with a higher calling frequency during the early morning hours. Temperature was identified as a secondary factor influencing *P. loveridgei* calling phenology. Thermal sensitivity tests described a thermal tolerance range (CT\text{min} = 6.8°C, CT\text{max} = 30.4 °C, preferred temperature range = 7.3 - 25°C) for *P. loveridgei*. Susceptibility to the fungal pathogen disease, chytridiomycosis, was confirmed for the first time in *P. loveridgei*; however infection intensity and prevalence were low. The species distribution model predicted a restricted geographical range for *P. loveridgei*, mainly within national parks in the mountains of mid-eastern Australia. Two variables contributed the most to *P. loveridgei* distribution: precipitation in the wettest period and maximum temperature of the warmest period. Occupancy was high throughout the current *P. loveridgei* distribution range, with different environmental variables influencing detectability (temperature and rainfall) and occupancy (canopy cover). Thermal environment and sensitivity results describe this subtropical mountain-top ectotherm as a thermoconformer and thigmotherm. Future predictions forecast the loss (to varying degrees) of suitable habitat for *P. loveridgei* under both moderate and extreme climate change scenarios. Microhabitat thermal analyses
however, suggest that the fossorial burrowing behaviour of *P. loveridgei* could buffer the effects of climate warming.

By following an integrative framework, my research makes original contributions to the scarce existing knowledge of the sensitivity and exposure factors influencing the vulnerability of *P. loveridgei* to climate change. Overall, *P. loveridgei* ecological biology is influenced by rainfall and temperature making this ectotherm vulnerable to climate change. Thermally buffered environments (i.e. rainforest and microhabitat) however, are expected to play a major role in reducing *P. loveridgei* vulnerability to a warming climate. I hypothesise that changes in rainfall and cloud patterns will have a large role in *P. loveridgei* vulnerability to climate change. This research emphasises the importance of investigating both sensitivity and exposure factors when evaluating the vulnerability of mountain-top ectotherms, such as *P. loveridgei*, to climate change. I recommend monitoring an array of populations throughout the altitudinal and latitudinal distribution with particular focus on changes in rainforest canopy cover, rainfall patterns and disease prevalence. Management should focus on ensuring the conservation of rainforest habitats in mountain-top areas within established national parks and the possibility of expanding these areas. This study highlights the importance of prioritising the conservation of rainforest habitats in subtropical mountain-top areas, to aid in the conservation of leaf litter ectotherms.
Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

Mariel Familiar López
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1. Amphibian declines

Amphibians are threatened worldwide, with many species facing population declines and extinctions (Collins and Storfer, 2003; Stuart et al., 2004; Wake and Vredenburg, 2008; Collins, 2010; Wake, 2012). Amphibian population declines are not randomly distributed; they exhibit taxonomical as well as regional patterns. Bufonidae, Leptodactylidae, Hylidae and Ranidae, are the amphibian families with the greatest number of rapidly declining species (Stuart et al., 2004). In general, tropical countries have greater amphibian species richness and many species are at risk (Wake and Vredenburg, 2008), with Neotropical species relatively more affected than other regions (Stuart et al., 2004; Lips et al., 2005). Species from Australia and New Zealand are also rapidly declining (Hero and Morrison, 2004; Stuart et al., 2004). Amphibians that inhabit montane tropical regions, especially if they are associated with streams or streamside habitats, are also more likely to be severely threatened (Hero and Morrison, 2004; Hero et al., 2005; Wake and Vredenburg, 2008).

Several countries worldwide have reported amphibian population declines at high altitudes (altitudes over 400 m. a. s. l.): Costa Rica (Pounds and Crump, 1994; Lips, 1998), Panama (Lips, 1998), Ecuador (Ron et al., 2003), United States (Davidson et al., 2013), Puerto Rico (Burrowes et al., 2004), Mexico (Lips et al., 2004), Spain (Bosch et al., 2001) and Australia (Berger et al., 1998; Hero and Morrison, 2004; Hero et al., 2005; Morrison and Hero, 2012; Hero et al., 2015), among others. This pattern suggests that amphibians inhabiting montane regions have a higher risk of decline or even extinction and therefore, are in need of research attention (Davidson et al., 2013).

Amphibian population declines were originally thought to be mainly associated with anthropogenic causes, including environmental pollution, habitat destruction, invasive species and changes in land use. Many, more recent, amphibian declines however, have occurred in relatively pristine and protected areas (termed "enigmatic declines"), suggesting a more complex problem (Stuart et al., 2004). Enigmatic amphibian population declines are a multifactorial problem with complex interactions between pollution, climate change, UV radiation and infectious disease (e.g. chytridiomycosis) (Collins and Storfer, 2003; Collins, 2010; Blaustein et al., 2011; Rohr and Palmer, 2013). These factors can be considered human-induced stressors that may also interact with natural stressors (e.g. competition,
predation, resource availability, reproduction and disease), affecting amphibians at different levels (e.g. molecular, physiological, individual, population and community) (Blaustein et al., 2011; Rohr et al., 2013).

It is important to comprehensively consider factors associated with amphibian population declines as synergistic interactions can exacerbate the effects of a single factor (Young et al., 2001; Blaustein et al., 2011; Rohr and Palmer, 2013). Furthermore, causes of amphibian population decline may vary interspecifically depending on region and species, or intraspecifically in different populations of the same species or at different life stages. In a given region, species may be more affected by one particular factor than another, or some species can be more vulnerable to a particular stressor (Hero and Morrison, 2004; Blaustein et al., 2010; Blaustein et al., 2011; Hero et al., 2015). Recently, climate change and emerging infection diseases (e.g. chytridiomycosis) have played a major role in amphibian population declines (Collins and Storfer, 2003; Stuart et al., 2004; Corn, 2005). Targeted research on these factors and their interactions are needed to elucidate their role in amphibian population declines.

1.1 Amphibian declines in Australia

Many Australian amphibian species are facing declines and extinctions. The Australian amphibian fauna is comprised of 241 recognised frog species with over 20% of these threatened, most have declined and at least three species are considered extinct (Hero et al., 2006; Anstis, 2013; Hero et al., 2015). Threatened species in this region are concentrated in upland areas (41%) rather than in lowlands (8%) (Hero and Morrison, 2004). Factors threatening Australian amphibians parallel the recognised causes for the rest of the world (e.g. habitat destruction, over-exploitation, pollution, climate change, UV radiation, infectious disease, introduction of exotic species, and pesticide use), with habitat loss the major threat (Hero et al., 2015). Infectious diseases (e.g. chytridiomycosis) and climate change have also been associated with increasing extinction risk of Australian amphibian species (Hero and Morrison, 2004; Stuart et al., 2004; Hero et al., 2006). Moreover, it has been suggested that frog species that inhabit upland areas are more likely to be threatened by chytridiomycosis and global climate change (Hero and Shoo, 2003; Hero et al., 2015).

Chytridiomycosis is the infectious disease caused by the pathogen *Batrachochytrium dendrobatidis* (*Bd*), and in Australia it has been directly associated with declines of at least five amphibian species (Hero and Morrison, 2004) and has been found in species from three amphibian families, Hylidae, Microhylidae and Myobatrachidae (Skerratt et al., 2008; Murray
et al., 2010). In general, chytridiomycosis in Australian frogs is strongly associated with aquatic habits (Kriger and Hero, 2007; Skerratt et al., 2010). Studies that directly associate climate change with Australian amphibian declines are limited (Hero and Morrison, 2004; Lemckert and Penman, 2012). Research has focused on bioclimatic modelling to predict the impacts of climate change on Australian frogs, with several studies predicting a major loss of suitable habitat for montane and/or narrow ranged endemic species (Williams et al., 2003; Thomas et al., 2004; Williams and Hilbert, 2006; VanDerWal et al., 2009; Hagger et al., 2013). In addition, Laurance (2008) suggested a climate-pathogen interaction where consecutive warm years at tropical latitudes were likely to predispose upland frog populations to disease and consequently to declines.

In Australia, amphibian declines and extinction risk are correlated with several life history traits including geographical range, altitudinal range, habitat specialisation, aquatic reproductive mode and clutch size (Williams and Hero, 1998, 2001; Hero et al., 2005; Murray and Hose, 2005; Hero and Morrison, 2012; Morrison and Hero, 2012). Upland regions in Australia have high species richness, endemism and high numbers of geographically restricted species (Hero and Shoo, 2003; Williams et al., 2003). For example, more than 50 frog species inhabit the wet subtropical region (about half are endemic) (Hines et al., 1999). Extinctions of Australian endemic frogs have occurred significantly more often in species with narrow distribution ranges (Hero and Morrison, 2004; Hero et al., 2005; Murray and Hose, 2005). Species with small geographical ranges tend to have limited tolerance to changing environmental conditions, making them more vulnerable to rapid climate change (Williams et al., 2003; Hero et al., 2005; Murray and Hose, 2005). Habitat specialisation may be related to narrow geographical ranges and in turn increase species vulnerability to severe changes in weather patterns.

Declines in eastern Australian upland frogs have been strongly associated with an aquatic life stage compared to species with terrestrial larval development. Chytridiomycosis has been suggested as a plausible explanation explaining this disparity (Hero and Morrison, 2004; Hero et al., 2005; Hero and Morrison, 2012). Little is known however, of the susceptibility of Australian terrestrial species to Bd. Terrestrial breeding frog species may be at risk of decline due to changes in weather patterns (Pounds et al., 2006; Lemckert and Penman, 2012), as decreases in rainfall and changes in cloud patterns will change habitat moisture and humidity (e.g. soil moisture), which in turn affects nesting sites and egg and larval development (Lemckert and Penman, 2012). One study has shown increased mortality
due to dry conditions affecting egg masses of the direct-developing *P. loveridgei* (Seymour *et al*., 1995).

In summary, Australian frogs are at high risk of extinction as they experience complex interactions of threatening processes (habitat loss, chytridiomycosis and climate change) as well as species vulnerability (geographic range, breeding habitat and specialisation) (Hero and Morrison, 2004, 2012). Further studies, taking several of these factors into account and focusing on species that inhabit upland areas are needed.

### 1.2 Global climate change

Climate change is a global phenomenon that will affect many environmental parameters (e.g. air and sea surface temperature, solar radiation, precipitation, cloud cover, sea level and frequency of extreme weather events) and subsequently, will have a significant impact on biodiversity (Walther *et al*., 2002; Thomas *et al*., 2004; Parmesan, 2006; Bickford *et al*., 2010; IPCC, 2014; CSIRO, 2015; Urban, 2015). Climate change impacts can be diverse, with beneficial as well as detrimental effects (Williams *et al*., 2008; Blaustein *et al*., 2010). The planet is warming up, with average surface temperatures increasing by about 0.8ºC over the last century (IPCC, 2007, 2014; CSIRO, 2015). While the global climate has fluctuated in the past, the recent observed global warming is a consequence of an increase in anthropogenic greenhouse gas concentration (Carey and Alexander, 2003; IPCC, 2007, 2014; CSIRO, 2015). For the next two decades a global warming of about 0.2ºC per decade is projected for a range of emission scenarios (SRES scenarios; IPCC, 2007), with mean air temperatures predicted to increase between 0.6 and 4.0ºC by 2100 (Liverman and O'Brien, 1991; IPCC, 2007; Rogelj *et al*., 2012). Recent climate warming projections, based on RCP’s (Representative Concentration Pathways; IPCC, 2014), estimate a wider range of temperatures with mean air temperature increases between 0.4 and 4.8ºC by the end of the century (Rogelj *et al*., 2012; IPCC, 2014). Temperature increases are not globally uniform; daily average minimum temperatures have risen faster than daily average maxima, land areas have warmed at a more rapid rate than ocean regions, and the northern hemisphere has had more pronounced warming than the southern hemisphere (Easterling *et al*., 2000; IPCC, 2007, 2014). Some ecosystems and regions are expected to be more severely affected by climate change; for example, mountain regions appear to be more sensitive to warming than other land regions (Thomas *et al*., 2004; IPCC, 2007). This is because temperatures change more rapidly with altitude (1ºC per 160 m a.s.l.) than with latitude (about 1ºC per 150 km); resulting in more rapid changes in mountain communities (McCarty, 2001).
In addition to increasing air temperature, climate change will also cause changes in precipitation patterns and there will be an increase in the frequency and severity of extreme weather events (Easterling et al., 2000). It is very likely that, in some regions, hot extremes (heat waves and dry-seasons) and heavy precipitation events will become more intense, more frequent and last longer (Walsh and Ryan, 2000; IPCC, 2007, 2014). Increases in precipitation are very likely to occur at high-latitudes, while decreases are likely in most subtropical habitats (IPCC, 2007). Changes in precipitation regimes may strongly influence, for example, the persistence of tropical forests as they could become more vulnerable to fire (Cochrane, 2003).

1.2.1 Climate change in Australia

In Australia, warming trends have mirrored global-scale patterns (Hughes, 2003; CSIRO, 2012). Since 1950, each decade has been warmer than the previous one, with the mean annual air temperatures increasing 0.7°C. Warming has occurred in all seasons, however, air temperatures have increased more in spring (0.9°C). This includes a faster rise in daily average minimum temperature (>1.1°C) compared with daily average maxima. Overall, across most of Australia the frequency of extreme cold weather events has decreased while the frequency of warm weather events has increased. In the past 50 years the Australasian region has experienced several days with record hot temperatures, suggesting that severe heatwaves and warm extremes will increase. By the year 2030, average temperatures are projected to rise by 0.6 to 1.5°C, but by 2070, warming is projected to be in the range of 1.0 to 5.0°C (CSIRO, 2014, 2015). During the past century, El Niño and La Niña events have produced hotter droughts and cooler wet periods (CSIRO, 2014, 2015). Precipitation in Australia has seen a trend towards increased spring and summer monsoonal rainfall in the north, higher than normal rainfall across the centre, and decreases in late autumn and winter rainfall across the south (CSIRO, 2014, 2015).

As with the rest of the country, southeast Queensland and northern New South Wales will have unprecedented temperature rises and changes in rainfall related to climate change (Cai et al., 2005). In Queensland, greater warming has occurred inland and some cooling has happened in the southern part of the state. Air temperature projections range from 1.0 to 2.2 °C by 2050 (Suppiah et al., 2001; Whitfield et al., 2010; CSIRO, 2014). Summer rainfall will increase between 0 to 5% in Queensland and from 10 to 20% in New South Wales (Suppiah et al., 2007). The IPCC Fourth Assessment Report projects that by 2020 this region will experience a significant biodiversity loss in some ecologically rich sites, including the Queensland Wet Tropics (IPCC, 2007). For example, one study predicted a
decrease of 50% in highland rainforest environments (habitat for many endemic vertebrates) with only 1°C of warming (Hilbert et al., 2001). A comparative extinction risk study predicts climate change to dominate extinction risk in montane Queensland forests (Williams et al., 2003). Furthermore, a recent assessment of vulnerability to climate change of subtropical rainforest vertebrate species in southeast Queensland suggested that montane frogs and reptiles were the taxa most at risk (Hagger et al., 2013).

1.3 Species vulnerability to climate change

Climate change has been recognised as a global threat to biodiversity, ecosystem functions and individual species, challenging their survival and increasing extinction risks (Thomas et al., 2004; Williams et al., 2008; Bellard et al., 2012; Foden et al., 2013; Moritz and Agudo, 2013; Pacifici et al., 2015). Evaluating risk from climate change involves understanding individual species vulnerability, which includes assessing the species’ ecological and evolutionary biology (Williams et al., 2008; Pacifici et al., 2015). Vulnerability to climate change will differ depending on each species’ sensitivity and exposure to environmental change, making some species more vulnerable than others (Moritz and Agudo, 2013). Therefore, it is important to determine which species will be most vulnerable to a warming environment (Williams et al., 2003; Thomas et al., 2004; Pimm, 2008; Blaustein et al., 2010; Penman et al., 2010; Moritz and Agudo, 2013). A general integrative framework developed by Williams et al. (2008), proposes that a species’ vulnerability would depend on its sensitivity, exposure (regional and local), resilience and potential to adapt (evolutionary and ecological) (Figure 1.1). Since then, several theoretical frameworks have been developed focusing on multiple factors involved in a species’ vulnerability to climate change, agreeing on two main components: sensitivity and exposure (Kearney and Porter, 2009; Rowland et al., 2011; Huey et al., 2012; Foden et al., 2013; Moritz and Agudo, 2013). It is generally accepted that a species’ vulnerability to climate change involves the combination of intrinsic (sensitivity) and extrinsic (exposure) factors, acting synergistically (Williams et al., 2008). Species sensitivity involves factors such as physiological tolerance limits (critical thermal limits, preferred body temperatures and activity temperatures and metabolic rates), ecological traits (behaviour, habitat or trophic specialisation, life history characteristics, biotic and abiotic interactions) and genetic diversity. Exposure factors involve regional (mesoscale changes in means and extremes of temperature and precipitation) and local (microhabitat and topographic buffering) factors (Williams et al., 2008; Moritz and Agudo, 2013).
1.4 Amphibians and climate change

Climate change may have direct (the actual cause of death) or indirect (influence factors that are the direct cause of death) impacts on amphibian population declines (Carey and Alexander, 2003; Bickford et al., 2010; Blaustein et al., 2010; Rohr and Raffel, 2010; Li et al., 2013; Rohr and Palmer, 2013; Pacifici et al., 2015). Amphibians are ectothermic animals, and all aspects of their life history are strongly influenced by external environmental conditions, including weather factors such as temperature and precipitation. Amphibians have moist skin, cutaneous respiration, shell-less eggs, complex aquatic and terrestrial life cycles, and physiological characteristics that make them sensitive to aquatic and terrestrial environmental changes (Carey and Alexander, 2003; Li et al., 2013). Amphibians are especially sensitive to climate change as it can alter several aspects of amphibian biology
including behaviour, reproduction dynamics, development, breeding phenology, distribution, physiology, foraging and disease (Blaustein et al., 2001; Corn, 2005; Sodhi et al., 2008; Blaustein et al., 2012; Li et al., 2013; Rohr et al., 2013). Amphibians, however, can respond to climate change challenges by shifting their climatic niche in three non-exclusive dimensions: time (e.g. phenology), space (e.g. distribution range) and individual (e.g. physiology) (Bellard et al., 2012).

### 1.4.1 Phenology and climate change

Shifts in phenology events associated with global warming have been reported for several organisms including earlier timing of spring events, breeding, leaf-unfolding, flowering, bird migration and egg-laying (Parmesan and Yohe, 2003; Root et al., 2003; IPCC, 2007; Parmesan, 2007). Amphibian species phenology changes are related to timing of breeding, period of hibernation and the ability to find food (Blaustein et al., 2001). Climate change may have diverse effects on amphibian breeding phenology with the broad general trend towards species breeding earlier (Beebee, 1995; Reading, 1998; Blaustein et al., 2001; Gibbs and Breisch, 2001; Reading, 2003; Tryjanowski et al., 2003; Todd et al., 2011). A meta-analysis study reported amphibians as the taxonomic group displaying the strongest shifts towards breeding earlier associated with global warming (Parmesan, 2007). Changes in timing of breeding of amphibians could affect fitness and/or population dynamics (Yang and Rudolf, 2010). For example, hibernating species could be vulnerable to winter relapses if they venture out too soon, exposing embryos and larvae to freezing/low temperatures and predators (Corn, 2003). Additionally, changes in precipitation patterns due to climate change, will affect terrestrial habitats (e.g. vegetation and soil moisture) influencing amphibian breeding phenology (Bickford et al., 2010; Blaustein et al., 2010). Reduced precipitation may affect reproductive phenology because breeding cues will be less frequent (e.g. rainfall events), and available breeding sites will become scarce. Species with direct-development may be more vulnerable and experience increased mortality due to drier climate conditions, with direct impacts on all life stages (Donnelly and Crump, 1998; Bickford et al., 2010; Lemckert and Penman, 2012). For example, eggs and tadpoles of these terrestrial species may become more vulnerable to mortality from drying (Donnelly and Crump, 1998; Lemckert and Penman, 2012). Drier environments may also force adult amphibians to congregate in water sources or near moist microhabitats, increasing competition and facilitating disease transmission (Bickford, 2005; Joglar et al., 2007; Bickford et al., 2010; Longo and Burrowes, 2010).
Altered breeding phenology events due to climate change may have consequences on the structure and persistence of amphibian populations, ultimately resulting in population declines (Blaustein et al., 2001; Bickford et al., 2010). Research is needed to evaluate shifts in amphibian phenology in Australian frogs; a proxy and good starting point may be short-term amphibian monitoring studies on calling phenology.

1.4.2 Distribution, abundance and climate change

Another important consequence of global warming is the alteration of species distribution and abundance (Blaustein et al., 2001; Pounds, 2001; Wake, 2012; Li et al., 2013). The general trend is for species to move towards the poles and/or to higher altitudes at rates that are consistent with recent temperature increases (Parmesan and Yohe, 2003; Root et al., 2003; Lawler et al., 2009). In the particular case of amphibian populations, present distribution ranges are a close reflection of weather patterns (rainfall and temperature) and therefore, climate change will have an impact (Williams et al., 2003; Shoo and Williams, 2005; Bickford et al., 2010). For example, in response to warming, six anuran species from the Ecuadorian Andes increased their higher altitudinal range limit (Bustamante et al., 2005). A study on European amphibians projected range contractions for all species, assuming no dispersal, with climate warming (Araújo et al., 2006). A change in geographic range can often result in narrower ranges, as the area of suitable climate decreases (Parmesan and Yohe, 2003; Root et al., 2003; Martinez-Meyer, 2005). This may be devastating for already narrowly distributed montane amphibian species, such as mountain-top frogs (e.g. Forero-Medina et al., 2011). These cool-adapted species could be forced to move further uphill to the limit of their distribution and eventually into extinction, as their thermal environment disappears (Pimm, 2008; Wake and Vredenburg, 2008; Forero-Medina et al., 2011; Hagger et al., 2013; Elsen and Tingley, 2015). A reduction in species distribution can be associated with species vulnerability and rising extinction risk (Thomas et al., 2004). This may be important for amphibian conservation, as upland habitats in Australia contain endemic and geographically restricted species that can be affected by global warming (Hero and Shoo, 2003; Hero et al., 2015).

The capacity of species to survive will be related to their ability to reach new climatically suitable areas, but this can be hampered by habitat loss and fragmentation (Thomas et al., 2004). Amphibian species will be most vulnerable if they have to shift their distributions large distances. Many amphibian species have low vagility (i.e. ability to move long distance), which will decrease with habitat loss and fragmentation (Forero-Medina et al., 2011). Amphibian species that inhabit mountain-tops are most likely to go extinct as they
lack an altitudinal range area that will allow them to move distances between mountains and have low vagility (Bickford et al., 2010; Forero-Medina et al., 2011; Laurance et al., 2011; Shoo et al., 2011). Mountain-top amphibian populations have two choices facing climate change: acclimate until adaptation occurs or die (Wake and Vredenburg, 2008). Despite this potential outcome, there are few studies that have explored the effects of climate change on amphibians in montane habitats. A general approach has relied on bioclimatic (niche) models to predicting future impact of climate change on amphibian species, such as shifts or range contractions. Predicting present and future amphibian species distributions will become important in their conservation (Williams et al., 2003; Thomas et al., 2004; Lawler et al., 2009; VanDerWal et al., 2009; Lawler et al., 2010).

1.4.3 Physiology and climate change

Tropical ectotherms, particularly amphibians, are especially threatened by climate change (Carey and Alexander, 2003; Corn, 2005); however the extent of how sensitive they will be to these changes will depend on the species’ specific physiological traits, which for most species are unknown (Huey et al., 2012; Moritz et al., 2012; Moritz and Agudo, 2013). The study of species physiological limits is emerging as a key predictor in modelling their sensitivity to rapid changes in the environment (Chown et al., 2010; Huey et al., 2012). Thermal tolerances can be related to the presence and absence of a species and hence their distribution in a particular area or habitat, as they relate to the thermal conditions under which that species can survive (Donnelly and Crump, 1998; Kearney and Porter, 2009; Huey et al., 2012).

Amphibians are a diverse group of ectotherms that live under a wide range of climate conditions and habitats, with each species having different physiological tolerances (Donnelly and Crump, 1998; Navas et al., 2008; Sunday et al., 2011). Comparative studies have suggested that tropical ectotherms have smaller thermal tolerances than their temperate equivalents (Navas, 1997; Sunday et al., 2011; Huey et al., 2012). Furthermore, frogs that inhabit tropical mountains experience small temperature variations, suggesting that they may have limited acclimation ability in rapidly changing thermal regimes (Wake and Vredenburg, 2008). It has also been suggested that species that have evolved under reduced natural temperature variation will have narrow thermal tolerances (Addo-Bediako et al., 2000; Deutsch et al., 2008; Huey et al., 2009; Huey et al., 2012; Seebacher et al., 2015). Additionally, tropical ectotherms may live closer to their thermal optimum temperature and hence will be less adapted to changes in environmental temperatures (Huey et al., 2009; Kearney and Porter, 2009; Forero-Medina et al., 2011; Huey et al., 2012). For example, a
study in European beetles found that species most vulnerable to increases in temperature are those that have low thermal tolerances and low acclimation ability (Calosi et al., 2008). Moreover, some studies suggest that tropical ectothermic animals may be more threatened by global warming than temperate organisms (Deutsch et al., 2008; Huey et al., 2009). Having a good understanding of a species’ vulnerability to climate warming will come from a robust understanding of its sensitivity and response to temperature, especially body temperature in ectothermic animals (Williams et al., 2008; Huey et al., 2012).

Tropical species that are thermally specialised may also show a tendency towards being altitudinal specialised (e.g. Philoria spp.) (Laurance et al., 2011). This may be reflected in the diversity of restricted endemic species that inhabit cool mountain areas of the tropics (Fjeldsaå and Lovett, 1997; Rahbek, 1997; Ricketts et al., 2005). These montane species may also be at high risk of extinction due to global warming (Williams et al., 2003; Thomas et al., 2004; Ricketts et al., 2005; Elsen and Tingley, 2015). Investigating thermal tolerances of tropical montane ectotherms is important for elucidating possible impacts of climate change.

Variation in environmental temperature, due to climate change, may alter ectotherm body temperature and in turn affect their physiological performance. The physiological impacts of warming will depend on the species field body temperature in relation to its optimal temperature. This may affect each species differently depending on whether the body temperature is lower, equal to or higher than the optimal temperature for that particular species (Huey et al., 2012). These effects of warming may be analysed using performance curves (Figure 1.2a): if the body temperature ($T_b$) of the species is lower than the optimal temperature ($T_o$) then an rise in $T_b$ due to warming will actually enhance fitness (Figure 1.2b-A). If prior to warming the $T_b$ is similar to the $T_o$ then an increase in $T_b$ will have little impact (Figure 1.2b-B). If the $T_b$ increases higher than the $T_o$ then warming may have significant effects because performance declines rapidly when $T_b$ is higher than $T_o$ (Figure 1.2b-C) (Huey et al., 2012). Performance curves are useful for understanding how physiological variation may influence species responses to climate change (Buckley, 2008).
The magnitude with which climate change will potentially impact a species physiology will also depend on their capacity to buffer the effects by migration, shifts in microhabitat choice, or acclimatisation, and the evolutionary response of physiological tolerances (Williams et al., 2008; Kearney and Porter, 2009; Scheffers et al., 2014). For example, a study by Shoo et al. (2010) found that boulder microhabitats in Australia could buffer extreme temperatures by as much as 10°C. Hence, research on ectotherm responses to climate change should not only consider physiological sensitivity to temperature but the capacity to buffer the impacts through behaviour. This may be achieved by undertaking a multidisciplinary approach involving research on thermoregulation and species distribution (Kearney and Porter, 2009).

1.4.4 Disease and climate change

An important recognised factor in the amphibian crisis is the spread of contagious diseases (e.g. chytridiomycosis), as they can increase the risk of decline in amphibians (Stuart et al., 2004). There are several amphibian pathogens including bacteria, viruses, protozoans, trematode parasites, oomycetes and fungi (Kiesecker, 2011). Associations between pathogens, environmental change (e.g. climate change) and amphibian declines have been reported in the literature (Rohr et al., 2008; Li et al., 2013; Rohr and Palmer, 2013; Rohr et al., 2013). Global climate change may have direct or indirect impacts on amphibian declines, as it has the potential to alter host-pathogen dynamics (Carey and Alexander, 2003; Blaustein et al., 2010; Rohr and Palmer, 2013; Rohr et al., 2013). For example, a study showed a relationship between changes in ultraviolet-B radiation, due to climate change, and an increased vulnerability to the amphibian pathogen *Saprolegnia ferax*.
(Kiesecker et al., 2001). Another study linked the effects of global warming to female body condition as a possible explanation of amphibian declines (Reading, 2007). Several researchers have also reported a link between the amphibian pathogen *Batrachochytrium dendrobatidis* (*Bd*) and global warming leading to amphibian population declines and extinctions (Pounds, 2001; Burrowes et al., 2004; Pounds et al., 2006; Bosch et al., 2007; Longo et al., 2010). Pounds et al. (2006) proposed a climate-linked epidemic hypothesis which states that temperatures in upland areas are shifting towards the *Bd* optimum thus encouraging disease outbreaks. Consistent with this climate-linked hypothesis, a study by Bosch et al. (2007) found that rising temperatures in montane areas in central Spain were associated with increases in chytridiomycosis. Another study, on direct-developing frogs, described *ex situ* and *in situ* evidence of the climate linked epidemic hypothesis (Longo et al., 2010). In Australia, Laurance (2008) proposed a climate-pathogen interaction that predisposed upland frogs to disease. Even though the climate-linked epidemic hypothesis has been questioned by some researchers (for review see Blaustein et al., 2010), it is widely accepted that climate change may be a key stressor associated with disease dynamics and consequently, threatened species survival (Pounds et al., 1999; Carey and Alexander, 2003; Pounds et al., 2006; Bosch et al., 2007; Rohr et al., 2008; Rohr and Raffel, 2010). Irrespective of how controversial the hypothesis is, it is still important to understand how climate change will affect host-pathogen relationships, particularly in high altitude amphibian species (Muths et al., 2008; Muths and Hero, 2010; Li et al., 2013; Rohr et al., 2013). Recently it has been suggested that to better understand host-pathogen dynamics, climate-disease models should integrate host and pathogen physiology and disease ecology (Rohr et al., 2013). Additionally, climate modelling research should include the use of climatic variances and extremes, given that these changes are a characteristic of the forecasted climate change (Rohr and Raffel, 2010; Rohr et al., 2013). Continuing research on *Bd* will be important in resolving global amphibian declines (Hero and Morrison, 2004). Furthermore, research on this topic will provide knowledge on the environmental limitations of *Bd* in wild amphibian populations and hence aid conservation of amphibian species (Muths and Hero, 2010; Rohr et al., 2011). Increases in temperature and fluctuations in precipitation due to climate change may be a stressor that could be associated with disease outbreaks further threatening high altitude species and populations (Pounds et al., 2006; Kiesecker, 2011; Rohr et al., 2013).

In summary, climate change will have different direct and indirect effects on amphibian species, at an individual, population and community level. Research should therefore take a multidisciplinary approach to understand these effects and the links between them (Blaustein et al., 2010).
1.5 Study species general characteristics

There are 27 Australian terrestrial (direct-developing) species of frog from two families and four genera (Microhylidae: *Cophixalus* and *Austrochaperina*; and Myobatrachidae: *Assa* and *Philoria*) (Anstis, 2013). *Philoria* species are endemic, montane, terrestrial frogs with direct-developing eggs that inhabit high altitude forest (Anstis, 2013). Presently, there are six recognised species in the genus: *Philoria loveridgei*, *P. kundagungan*, *P. sphagnicola*, *P. pughi*, *P. richmondensis* and *P. frosti* (Knowles *et al.*, 2004). All species, except *P. frosti*, have small geographical ranges along the top of the eastern escarpment of the Great Dividing Range (southeast Queensland and eastern New South Wales), where there is subtropical rainforest with cool temperatures (Knowles *et al.*, 2004). Specifically, they inhabit boggy headwaters or soaks on the forest floor and seepage zones in mid to high altitude (above 600 m a.s.l.) subtropical rainforest (Hines *et al.*, 1999). They are among the rarest vertebrates in eastern Australia and all species are habitat specialists with distributions restricted to high mountains (Hines *et al.*, 1999; Knowles *et al.*, 2004).

To date there are no reported declines for *Philoria* frogs (Hines *et al.*, 1999; Hero *et al.*, 2005), even though they have range sizes similar to the majority of declining amphibian species found in a study of the endemic Australian frog fauna (Murray and Hose, 2005). There is also little information about their distribution, ecology, non-breeding habitat requirements, population size, structure or dynamics, as little work has been done on this group of frogs (Hines *et al.*, 1999). To improve conservation of these species, research is needed in the major gaps identified: species distribution, abundance, physiology, disease, phenology and ecology. Additionally, research should be done in assessing climate change impacts in this highly vulnerable group of frogs.

Although *Philoria* frogs are diurnal they are difficult to find as they exhibit fossorial habits and produce soft mating calls. They are considered mesic forest frogs that are non-stream breeding (Hines *et al.*, 1999), but an association between breeding and ground water seepage (related to soil moisture) has been observed for the genus. Breeding occurs in well-covered sites such as small burrows or chambers excavated in the ground and under leaf litter or rocks in seepage areas at the heads of streams. All *Philoria* species lay their eggs inside this small nest, where the tadpoles remain throughout their entire development until they emerge as metamorphs (Seymour *et al.*, 1995; Knowles *et al.*, 2004).
In subtropical Australia, mesic forest frogs are predominantly dependent upon rainforest and wet sclerophyll forest communities. This vegetation is typically found in the foothills and ranges of the Great Divide. Historically, as in other areas of Australia, large areas of rainforest have been lost due to timber harvesting, hydrological changes, cattle grazing, altered fire regimes, feral animals and weed invasion. The effects of these disturbances on Philoria species are unknown but from the little knowledge of the groups’ ecology they are likely to be highly detrimental (Hines et al., 1999).

1.5.1 Philoria loveridgei (Loveridge’s mountain frog)

This species is a small frog with an adult snout-vent length (SVL) size of 32 mm for females and 30 mm for males (Figure 1.3a-b). Body shape is moderately robust and pear-shaped with a head that is shorter than wide and short limbs. The snout is rounded in dorsal and profile view. Tympanum is very small and almost indistinct. Dorsal colour may vary from a light brown to reddish-brown and grey from light grey to very dark grey. Individuals often present a wide dark grey patch over the pelvic region and a narrower irregular band across the shoulders or a much wider patch over most of the dorsum. Additionally, a visible well developed dark brown or black stripe extending from the snout, through the eye, to the base of the arm, is present. Eyes have a horizontal pupil with iris colour bronze-gold above the pupil and brown below. The ventral surface is usually of a uniform pale colour, but some individuals have a light mottling on the throat and under surfaces of hind limbs. Fingers and toes are unwebbed, with male nuptial pads weakly developed (first finger) and female spatula present in first and second finger (Knowles et al., 2004; Anstis, 2013).

This species seems to have a short breeding season in spring and summer months (Knowles et al., 2004; Anstis, 2013). Males call from or near small chambers they construct in the moist forest floor. Females lay egg masses in these nests that are flask-shaped burrows in soft, moist soil under logs, stones or forest littler (Knowles et al., 2004). The eggs of P. loveridgei are deposited in non-foamy jelly like nests, with relatively low clutch sizes (35 eggs) (Seymour et al., 1995). The embryos of this species hatch at Gosner stage 18-20. The embryos hatch within the egg mass and the larvae complete metamorphosis within the jelly, without the need to feed and emerge as juveniles (Moore, 1961; Seymour et al., 1995).

The conservation status of P. loveridgei is, listed as “Vulnerable” under the New South Wales Threatened Species Conservation Act, 1995, and as “Rare” in the Queensland Nature Conservation Act, 1994 (Hines et al., 1999; Knowles et al., 2004).
1.6 Aims

The general aim of this thesis was to provide a better understanding of the distribution, ecology, disease and thermal physiology of a mountain-top endemic frog (*Philoria loveridgei*), and assess its vulnerability to potential impacts of climate change (Figure 1.4).

1.6.1 Specific research project aims:

- **Chapter 2** investigates the relationships between temperature, rainfall and calling phenology (time and duration) of *P. loveridgei* in high altitude subtropical rainforests of southeast Queensland.

- **Chapter 3** examines the distribution (present and future), detectability and occupancy of *P. loveridgei* in subtropical rainforests of southeast Queensland and northern New South Wales.

- **Chapter 4** investigates the critical thermal tolerance of *P. loveridgei* in subtropical rainforests of southeast Queensland and northern New South Wales.

- **Chapter 5** assesses the infection status of *P. loveridgei* with the pathogen *Batrachochytrium dendrobatidis* in subtropical rainforests of southeast Queensland and northern New South Wales.

- **Chapter 6** provides an assessment of the likely impacts of climate change on *P. loveridgei*.
Figure 1.4 Conceptual framework followed to assess *Philoria loveridgei* vulnerability to climate change (adapted from Williams et al., 2008). Colours distinguish exposure (orange) and sensitivity (yellow) components examined in this study.

1.7 Content and thesis structure

This thesis is structured as a series of manuscripts following Griffith University “Thesis as a series of publish and unpublished papers” (http://www.griffith.edu.au/higher-degrees-research/current-research-students/thesis/preparation/thesis-as-a-series-of-published-unpublished-papers) including a thesis general introduction and discussion chapters. This thesis is organised into six chapters: general introduction (Chapter 1), four results chapters (Chapters 2-5) and general discussion (Chapter 6). Each result chapter is structured in a manuscript form specifically formatted to meet the requirements of the academic journal to which it will be submitted. As a result of this structure there is some repetition (e.g. study site, study species, references and figures) among chapters throughout the thesis.
1.7.3 Publications

Publications that will be submitted derived from this research are as follow:


1.7.2 Industry monographs


1.7.3 Conference abstracts


(2) Familiar López, M., Lollback, G. and Hero, J-M., (2014) Distribution, ecology, disease and physiology of mountain-top endemic frogs in the face of climate change: A
study on *Philoria* spp. Tropical Research Network Conference (oral presentation) Cairns, Australia.


(10) **Familiar López, M.** and Hero, J-M. (2012) Distribution, ecology, disease and physiology of mountain-top endemic frogs in the face of climate change: A study on *Philoria* sp. Student Research Symposium (oral presentation), Environmental Futures Centre, Griffith University, Brisbane, Australia. **Best Presentation Award.**

1.8 References


CSIRO (2015) *Climate change: science and solutions for Australia*. CSIRO Publisher, Australia.


CHAPTER 2 –
Calling phenology of the mountain-top frog

*Philoria loveridgei*

The introduction chapter provides a synthesis of the global amphibian population crisis and the role different factors (e.g. climate change) have had. The review included specific sections on Australian amphibian population declines and the causative factors identified to date.

The current results chapter describes the association between abiotic factors and the calling phenology of *P. loveridgei* within two national parks in mid-eastern Australia. Calling phenology is a first sensitivity factor assessed in this thesis to evaluate the vulnerability of *P. loveridgei* to climate change.

This chapter will be submitted as a research paper to the journal Austral Ecology and has been formatted to fit the journal style. The citation will be as follows:


Co-authors of this manuscript are my thesis supervisors, Dr Gregory Lollback, Dr David Newell and Prof. Jean-Marc Hero. My (Mariel Familiar López) contribution to the manuscript included the initial concept and survey design, field sample collection, data analyses and manuscript preparation.
Mariel Familiar López (corresponding author)

Gregory Lollback (Associate Supervisor)

David Newell (External Supervisor)

Jean- Marc Hero (Principal Supervisor)
2.1 Abstract

Worldwide, amphibian populations have declined, highlighting the need for effective and long-term monitoring programs to distinguish between natural population fluctuations and declines. Amphibian phenology and population dynamics are strongly influenced by environmental cues. Elucidating the abiotic and biotic factors associated with breeding/calling behaviour in anurans is important for understanding breeding phenology and designing appropriate monitoring strategies. An effective and widely used approach is to undertake anuran calling surveys as a surrogate for breeding activity. Herein we examined the calling phenology (seasonal and daily patterns) of Philoria loveridgei, a cryptic rainforest anuran, and described the environmental parameters influencing this behaviour. Four automated recording devices were established in subtropical rainforest in southeast Queensland, Australia. Recorders were set to record the acoustic environment every 10 min/hour during the activity season of P. loveridgei. These recordings were conducted over three years (2011-2014). Automated recognition software was used to scan and detect P. loveridgei vocalisations within the recordings. Calling activity of P. loveridgei was detected across all hours of the day, however, results indicated a diel variation. Results of this study identified a diurnal calling pattern for P. loveridgei, with a higher calling frequency during the early morning hours. The core calling period (months with the highest frequency of calling activity) for P. loveridgei is narrow, within the months of November and December. The environmental variables influencing P. loveridgei calling behaviour were rainfall, season and temperature. Results suggest spring rainfall events cue the onset of the calling activity, associated with breeding behaviour. Our findings confirm the phenology of P. loveridgei populations and demonstrate the utility of automated calling surveys to elucidate phenology patterns. Improved knowledge of anuran calling behaviour and the abiotic factors that influence it permits the planning of better species-specific monitoring strategies, including for threatened species.

Keywords: Australia, amphibian, automated surveys, rainfall, rainforest.
2.2 Introduction

Amphibians are the most threatened vertebrate group worldwide, with several species facing population declines and extinctions (Stuart et al., 2004; Wake and Vredenburg, 2008). Australian amphibians are no exception with over 20% species threatened (Hero et al., 2006; Hero et al., 2015). Amphibian phenology is strongly influenced by environmental cues, which can be affected by a changing environment (Corn, 2005; Parmesan, 2007; Todd et al., 2011). Amphibian phenological responses to climate change have included shifts in the timing of breeding events, hibernation periods of and geographic distribution (Gibbs and Breisch, 2001; Corn, 2003; Todd et al., 2011; Li et al., 2013). In light of the amphibian crisis, continuous monitoring of amphibian population is required to elucidate population fluctuations from declines (Kirlin et al., 2006). Establishing effective, efficient and long-term monitoring strategies is the fundamental approach (Weir and Mossman, 2005; Newell et al., 2013; Quick et al., 2015; Willacy et al., 2015). Monitoring of amphibian populations has subsequently increased to facilitate effective conservation and management strategies (Mossman et al., 1998; Pellet and Schmidt, 2005; Weir and Mossman, 2005).

Acoustic surveys have been widely used to monitor anuran breeding dynamics (Bridges and Dorcas, 2000; Kirlin et al., 2006; Steelman and Dorcas, 2010). Male anurans produce acoustic signals to advertise and attract mates at potential breeding sites. These advertisement calls are species-specific, confirming the presence of individual species without visual encounters (Lemckert and Mahony, 2008). Acoustic surveys of anuran species can therefore be used to monitor calling behaviour and breeding phenology, particularly for cryptic anuran species (Pough et al., 2004; Weir and Mossman, 2005; Saenz et al., 2006). Calling behaviour in anurans is dynamic: it changes with latitude (Lemckert and Mahony, 2008) and altitude (Morrison and Hero, 2003; Hartel et al., 2007), and is influenced by several abiotic factors including rainfall (Jensen et al., 2003; Kirlin et al., 2006; Saenz et al., 2006), wind (Robertson, 1986; Henzi et al., 1995), humidity (Bellis, 1962), barometric pressure (Steelman and Dorcas, 2010) and temperature (Blaustein et al., 2001; Oseen and Wassersug, 2002; Saenz et al., 2006; Steelman and Dorcas, 2010; Willacy et al., 2015).

Although temperature and rainfall are the most common variables influencing breeding behaviour, factors associated with calling behaviour are species-specific (Oseen and Wassersug, 2002; Pellet and Schmidt, 2005; Kirlin et al., 2006). To increase the detectability probability of a species during calling surveys for monitoring purposes, it is important to consider species-specific environmental factors (Pellet and Schmidt, 2005; Kirlin et al., 2006; Steelman and Dorcas, 2010). Climate conditions influence calling behaviour and
consequently restrict calling to specific periods of time, termed core calling periods. Recognising these core periods is important for planning surveys, particularly for rare or cryptic species (Pellet and Schmidt, 2005; Lemckert and Mahony, 2008). For most amphibians, however, information on their calling behaviour is poor (Lemckert and Mahony, 2008; Willacy et al., 2015). Determining calling phenology and the associated climate factors are important for effectively monitoring frog populations (Pellet and Schmidt, 2005; Kirlin et al., 2006; Koch and Hero, 2007; Lemckert and Mahony, 2008; Steelman and Dorcas, 2010).

Calling surveys allow the investigation of multiple aspects of amphibians behaviour, including monitoring distribution and abundance of anurans, determining detection and occupancy probabilities and assessing populations dynamics (Pellet and Schmidt, 2005; Weir and Mossman, 2005). Data recorded from calling surveys can be used to determine the best time to conduct anuran surveys (higher detection probability), as they gather baseline data on breeding activity for specific species (Pellet and Schmidt, 2005). Automated acoustic surveys are commonly used to monitor anuran calling activity, with several advantages including (i) data collection possible for extended periods of time, (ii) simultaneously data collection from different sites covering large areas, (iii) minimal disturbance to the species, and (iv) relative low cost (Bridges and Dorcas, 2000; Oseen and Wassersug, 2002; Todd et al., 2003; Weir and Mossman, 2005; Saenz et al., 2006; Steelman and Dorcas, 2010; Willacy et al., 2015). Automated acoustic surveys are also suitable for identifying diel patterns that can be used to plan future surveys (Oseen and Wassersug, 2002; Todd et al., 2003; Weir and Mossman, 2005; Saenz et al., 2006; Steelman and Dorcas, 2010; Croker and Kottege, 2012).

This study examines the calling phenology (seasonal and daily patterns) of Philoria loveridgei and the environmental parameters associated with this behaviour. The specific aims were to (i) determine the environmental parameters (i.e. temperature and rainfall) related to P. loveridgei calling activity, (ii) define calling activity patterns that would increase the detection probability of the species allowing for the optimisation of future surveys, and (iii) collecting P. loveridgei calling phenology baseline data for future assessments of population dynamics under climate change.

2.3 Methods

2.3.1 Study area and species

This study was conducted in two national parks (Springbrook and Lamington National Park) located in southeast Queensland, comprising part of the Tweed Caldera Rim in mid-
eastern Australia (Figure 2.1). This area encompasses Gondwana Rainforests, listed as World Heritage forest in Australia. Acoustic sampling of the environment was undertaken at four sites, two in each national park (Figure 2.1 and Table 2.1). Sites were selected based on the high occupancy of *P. loveridgei* (see Chapter 3). Acoustic sampling took place over three years, including three *P. loveridgei* breeding seasons, from October 2011 to March 2014.

*Figure 2.1* Study area indicating the location of the four sites where Wildlife Acoustics SM2 recorders were deployed (red dots) in southeast Queensland, Australia.

*Philoria loveridgei* is a mountain-top frog endemic to the Tweed Caldera Rim in mid-eastern Australia (Knowles *et al.*, 2004). This species is a rare vertebrate in eastern Australia, as it is a habitat specialist with a distribution restricted to high mountains (above 600 m a.s.l.) in subtropical rainforests (Hines *et al.*, 1999; Knowles *et al.*, 2004). *Philoria*
*loveridgei* is a terrestrial anuran that has direct-developing tadpoles laid as eggs inside burrows constructed under leaf litter or rocks on the rainforest floor. Male frogs call from inside or near burrows producing a soft, short and guttural “bo-r-k” sound (Knowles *et al.*, 2004; Anstis, 2013). It is a small frog that can reach an adult snout-vent length (SVL) of 32 mm for females and 30 mm SVL for males (Seymour *et al.*, 1995; Knowles *et al.*, 2004; Anstis, 2013). A recent assessment of subtropical rainforest vertebrate species of eastern Australia noted this frog species as being highly vulnerable to climate change (Hagger *et al.*, 2013).

### 2.3.2 Data collection

Automated recording systems were used to determine the calling activity of *P. loveridgei* frogs in southeast Queensland. Four Song Meter (SM2, Wildlife Acoustics) devices were established in the rainforest at four sites to concurrently sample the acoustical environment (Figure 2.1). SM2 recorders were deployed near known *P. loveridgei* populations, based on historical records (WildNet data base, Environmental Protection Agency, 2004). Recording systems were internally setup with four 32 GB digital sound cards and powered with four D alkaline batteries. All SM2 recorders were programmed to record the first 10 minutes of every hour for the 24 hour day period, at a stereo sample rate of 44100Hz. SM2 were contained within metal mesh cases for microphone protection and mounted on a metal stake approximately 1 m above the ground. All data recorded were periodically collected (≈ 50 days) and transferred to portable external hard drives for storage and analyses. Rainforest air temperature was measured, at each site, by the inbuilt thermocouple sensor (± 2°C accuracy) in the SM2. Rainfall data were obtained from the nearest Australian Government Bureau of Meteorology meteorological station.
Table 2.1 Location of SM2 recorder deployment sites and breeding seasons surveyed of Philoria loveridgei calling phenology.

<table>
<thead>
<tr>
<th>National Park</th>
<th>Site</th>
<th>Altitude (m)</th>
<th>Season</th>
<th>Breeding seasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Springbrook</td>
<td>Best of All Lookout</td>
<td>986</td>
<td>1</td>
<td>October – January 2011/2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>October – January 2012/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>October – January 2013/2014</td>
</tr>
<tr>
<td></td>
<td>Bilborough</td>
<td>848</td>
<td>1</td>
<td>October – January 2011/2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>October – January 2012/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>October – January 2013/2014</td>
</tr>
<tr>
<td>Lamington</td>
<td>Binna Burra 1</td>
<td>1020</td>
<td>1</td>
<td>October – January 2011/2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>October – January 2012/2013</td>
</tr>
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<td></td>
<td></td>
<td>3</td>
<td>October – January 2013/2014</td>
</tr>
<tr>
<td></td>
<td>Binna Burra 2</td>
<td>625</td>
<td>1</td>
<td>October – January 2011/2012</td>
</tr>
</tbody>
</table>

2.3.3 Data analysis

All field recordings were processed with the automated sound recognition software Song Scope (Wildlife Acoustics). Song Scope implements a pattern recognition algorithm to efficiently detect a signal (i.e. frog vocalisation) in long field recordings. Firstly, we temporally and spectrally identified 46 individual vocalisations (annotations) from 12 recording files and labelled them to individual level using the spectrogram visualisation tool of the software. Annotations were derived from audio files from recordings from all four study sites representing the range of individual and geographic variation. Secondly, we built a recogniser or reference vocalisation derived from the annotations previously selected. The recogniser contains all the specific characteristics of the target vocalisation (training data) and is used to detect similar vocalisations in field recordings. To create a viable recogniser, multiple parameters were manually adjusted including: sample rate, frequency minimum, frequency range, dynamic range, fast fourier transform (FFT), maximum syllable, maximum syllable gap and maximum song. Once the recogniser was built field recordings were batch scanned using the software Song Scope.

To test the accuracy of the software in detecting P. loveridgei vocalisations we followed the methods described by Willacy et al. (2015) and Waddle et al. (2009). After estimating the false positives and negatives rates, we further filtered the output result data
setting a minimum value of quality of 50 and score of 60. Additionally, recording data were manually checked for false positives at the earliest and latest dates around the core calling season and were removed from analyses if found.

The presence or absence of calling activity was determined for each recording and used as a dichotomous dependent variable (1, 0) to define daily patterns at an hourly level over time. Survey effort (number of total recordings) was used to standardise the calling activity (presence of a call at hourly and daily levels), for all sites and seasons. Chi- squared tests were used to determine if calling activity was uniformly distributed over time (24 h), months, seasons (season 1: 2011-2012, season 2: 2012-2013, season 3: 2013-2014) and sites (Table 2.1). The daily likelihood of *P. loveridgei* calling was analysed using logistic regression models. To avoid pseudo-replication, calling data was transformed from an hourly level to a daily measure. Logistic regression models included the predictor variables months, season (see above), site (Table 2.1), daily air temperature (°C) and daily rainfall (mm). Models were chosen *a priori*. The best fitting model was identified by the lowest Akaike Information Criterion (AIC) value and a large AIC weight. Analyses were conducted using SPSS software version 22 (I.B.M. Corp, 2013).

### 2.4 Results

The SM2 devices recorded a total of 40871 recordings from October 2011 to February 2014. This is the equivalent of approximately 6812 h of acoustic field sampling (calling surveys). From all the acoustic data recorded, only 5075 recordings contained *P. loveridgei* vocalisations. The positive rate of the presence of *P. loveridgei* vocalisation was 84% and the false negative rate was 12%. Sounds misidentified by the software as *P. loveridgei* vocalisation mostly included birdcalls, rainfall and background noise.

*Philoria loveridgei* calling were detected at all study sites and seasons, starting from September and lasting until January (Table 2.2). At every site and for every season, we did not detect *P. loveridgei* vocalisations after January, indicating the end of the calling activity for this species. There was some site variation in core calling months, mostly due to unintended sampling bias (i.e. logistical constrains and equipment failure). When data were pooled however, core calling activity was greater than expected for the months of November and December in season 1 ($\chi^2_{(4)} = 1856.9, p < 0.001$) and season 2 ($\chi^2_{(11)} = 2310.6, p < 0.001$), and October in season 3 ($\chi^2_{(7)} = 606.2, p < 0.001$) (Figure 2.2).
Table 2.2 Summary of calling activity within breeding seasons of *Philoria loveridgei* in each site.

<table>
<thead>
<tr>
<th>National Park</th>
<th>Site</th>
<th>Season</th>
<th>Calling activity (start and end dates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Springbrook</td>
<td>Best of All</td>
<td>1</td>
<td>20 October 2011 to 1(^{st}) January 2012</td>
</tr>
<tr>
<td></td>
<td>Lookout</td>
<td>2</td>
<td>19 October 2012 to 20 January 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>25 September to 19 December 2013</td>
</tr>
<tr>
<td></td>
<td>Bilborough</td>
<td>1</td>
<td>20 October 2011 to 23 January 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>12 October 2012 to 18 January 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>25 September 2013 to 31(^{st}) January 2014</td>
</tr>
<tr>
<td>Lamington</td>
<td>Binna Burra 1</td>
<td>1</td>
<td>31(^{st}) October 2011 to 4 January 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>8 September to 25 January 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>4 October to 17 November 2014</td>
</tr>
<tr>
<td></td>
<td>Binna Burra 2</td>
<td>1</td>
<td>1(^{st}) November to 6 January 2012</td>
</tr>
</tbody>
</table>

Figure 2.2 Summary of the proportion of daily recordings with *Philoria loveridgei* calling in each month and season (all sites combined). No calling was detected between February and August.

*Philoria loveridgei* calling activity was detected across all hours of the day at all sites and seasons (Figure 2.3). Diel variation, however, was found in the hourly calling activity.
pattern with the frequency of calling not uniformly distributed throughout the day ($\chi^2_{(23)} = 1909.19, p < 0.001$). There was one distinct peak in the morning (5:00 – 10:00), and a distinct decrease in calling activity in the evening (18:00) (Figure 2.3). The calling pattern varied among seasons ($\chi^2_{(2)} = 460.95, p < 0.001$), sites ($\chi^2_{(3)} = 559.02, p < 0.001$) and months ($\chi^2_{(11)} = 4278.35, p < 0.001$), with a general diurnal calling pattern. Hourly calling activity of *P. loveridgei* was recorded at a wide range of temperatures (4.4 - 27.9°C, mean 14.58 ± 3.0°C). The highest frequency of calling activity occurred between 14 - 16°C, while the lowest frequencies occurred with temperatures > 25°C and < 6°C.

![Figure 2.3](image)

**Figure 2.3** Summary of *Philoria loveridgei* calling pattern over a 24 h period (all sites and seasons combined).

We analysed 15 *a priori* models to evaluate the factors associated with calling activity in *P. loveridgei*, and we present the top five models herein (Table 2.1). The best model included season and daily rainfall as variables influencing calling in *P. loveridgei*, with a negative relationship between rainfall and calling activity. The best model was statistically significant ($\chi^2_{(15)} = 779.96, p < 0.001$), had a good fit to the data (Pearson $\chi^2 = 288.58$, df = 1357, $p = 1.0$, deviance $\chi^2 = 261.14$, df = 1357, $p = 1.0$) and explained 54% of the variance (Nagelkerke $R^2$). The next best model also included temperature as a predictive variable influencing calling behaviour, however the performance of this model was low. Models which
contained temperature, site and month performed relatively poorly. Daily calling for each season (all sites combined) was 52% for season 1, 43% for season 2 and 51% for season 3.

**Table 2.3** Summary of the five best logistic regression models describing calling activity of *Philoria loveridgei*, ranked by AIC.

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
<th>K</th>
<th>-2Log likelihood</th>
<th>ΔAIC</th>
<th>AIC wgt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season, rainfall</td>
<td>1090.09</td>
<td>3</td>
<td>948.09</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Season, temperature, rainfall</td>
<td>1095.76</td>
<td>4</td>
<td>947.76</td>
<td>5.68</td>
<td>0.059</td>
</tr>
<tr>
<td>Site, temperature</td>
<td>1096.23</td>
<td>3</td>
<td>944.23</td>
<td>6.14</td>
<td>0.046</td>
</tr>
<tr>
<td>Site, season, temperature</td>
<td>1099.84</td>
<td>4</td>
<td>941.84</td>
<td>9.76</td>
<td>0.008</td>
</tr>
<tr>
<td>Month, rainfall</td>
<td>1104.54</td>
<td>3</td>
<td>938.54</td>
<td>29.41</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Daily rainfall was one of the variables predicted to influence calling activity of *P. loveridgei* and we examined this relationship for each site and season. Herein, we show this relationship for the two sites at Springbrook National Park in the first two seasons (Figures 2.4 and 2.5).

**Figure 2.4** Relationship between calling activity (proportion of recordings with calls) and daily cumulative rainfall at Best of All Lookout (Season 1).
Figure 2.5 Relationship between calling activity (proportion of recordings with calls) and daily cumulative rainfall at Bilborough (Season 2).

2.5 Discussion

Results of this study identified a narrow core calling period (months with the highest frequency of calling activity) for *P. loveridgei* in the months of November and December. Calling activity, however, started as early as September and lasted until late January in some sites. Outside of the core calling period, calling activity may occur at very low frequencies and is unpredictable. Although calling activity does not necessarily define the breeding period of a species, concentrated calling for two months is likely to be strongly associated with breeding (Lemckert and Mahony, 2008). Hence, calling behaviour can be used as a surrogate for breeding activity (Saenz *et al.*, 2006). Breeding activity of *P. loveridgei* has been described as occurring in spring and summer months (Seymour *et al.*, 1995; Knowles *et al.*, 2004; Anstis, 2013). Our results refine the *P. loveridgei* breeding season to November and December. Additionally, increased evidence of recruitment was observed during these months (MFL personal observation). A recent study, described September to November as the peak calling and breeding months of *Philoria richmondensis* (Willacy *et al.*, 2015). Differences between core calling months for these sister species may reflect their latitudinal distribution or habitat specialisation inside their highly narrow and fragmented distributional range (Lemckert and Mahony, 2008; Willacy *et al.*, 2015).
Alternatively, this result could arise as a response to spatial and temporal differences in rainfall patterns. The onset of calling activity follows the start of the wet season, commencing after high rainfall events in spring. The frequency of daily calling increases with the increase of rainfall, however, it decreases and finishes before the wet season ends.

*Philoria loveridgei* calling behaviour was not randomly distributed throughout the 24h diel period. Calling activity was constant during the day, with a distinct peak of activity in the early morning (5:00 – 10:00) and a distinct decrease in the evening (18:00). This result is similar to the diurnal pattern described for *P. richmondensis*, with daily calling activity associated with a morning and evening period (Willacy *et al.*, 2015). Our results confirm the diurnal calling behaviour of this anuran genus. A study on three anurans from North Carolina (*Pseudacris crucifer*, *P. feriarum* and *Rana sphenocephala*) also described daily temporal variation in calling activity, with distinct peaks for each species (Steelman and Dorcas, 2010). Other studies have reported daily temporal variation in calling activity patterns, describing different nocturnal activity peaks depending on the anuran species (Bridges and Dorcas, 2000; Oseen and Wassersug, 2002; Todd *et al.*, 2003). Overall, our results indicate that calling activity patterns, although similar in related specie, are species-specific. This specificity highlights the need to understand the calling behaviour of individual species before conducting acoustic surveys.

The best model indicated that the environmental factors influencing calling behaviour of *P. loveridgei* were rainfall (negative association) and season. Seasonal variation (variation between the different sample seasons) in calling activity of *P. loveridgei* indicates that calling behaviour is significantly associated with environmental conditions, which changed between years during this study. This was evident as the start and end of the calling activity period changed between seasons and sites. These results agree with previous studies describing a stronger influence of climate factors on the calling activity of species with short calling periods (Oseen and Wassersug, 2002). Even though there were differences in calling activity between seasons, rainfall was the main factor influencing calling behaviour in *P. loveridgei*. Daily rainfall also influenced detection probabilities of *P. loveridgei*, further indicating the importance of this variable (see Chapter 3). Rainfall was negatively associated with calling activity of *P. loveridgei*, which contrasts with previous studies where rainfall is positively related to anuran calling activity (Kirlin *et al.*, 2006; Saenz *et al.*, 2006). One possible explanation is that rainfall noise decreased the ability of the automated recognition software to detect *P. loveridgei* vocalisations, due to the similar frequency range between them. We are confident, however, that the post filtering and manual testing of the output data would
have minimised this outcome. Alternatively, as heavy rainfall periods could act as acoustic interference (Oseen and Wassersug, 2002) P. loveridgei males could decrease their calling during heavy rainfall events (24 h rainfall) and resume calling immediately after the rainfall in order to maximise their acoustical signal. This negative relationship suggests that rainfall is a cue for the onset of calling in P. loveridgei, but becomes less important after calling begins. A similar negative relationship between rainfall and calling activity was described for P. richmondensis (Willacy et al., 2015). We hypothesise that early spring rainfall events would increase soil moisture, which would increase Philoria spp. nest moisture and subsequently increase eggs and larval survival. These early spring rainfall events would therefore be a cue for breeding behaviour. For example, a study on calling activity of Rana sphenoecephala found that calling activity was cued by rainfall (Saenz et al., 2006). At the daily level, we suggest that the calling activity of P. loveridgei coincides with the start of the wet season, highlighting the influence of this cue for the onset of calling activity and possibly breeding behaviour.

The second best model included temperature as a variable influencing calling activity of P. loveridgei. This result agrees with previous research of anuran calling behaviour (Navas, 1996; Oseen and Wassersug, 2002; Pellet and Schmidt, 2005; Kirlin et al., 2006; Saenz et al., 2006; Steelman and Dorcas, 2010; Willacy et al., 2015). The highest frequency of calling activity in P. loveridgei occurred between 14 - 16°C. This result concurs with the temperatures of 15 - 16°C influencing calling activity in the sister species P. richmondensis (Willacy et al., 2015). Two other studies on detection probabilities of calling activity of anurans, from Switzerland and the United States, reported a strong association of calling activity with temperature (Pellet and Schmidt, 2005; Kirlin et al., 2006). Our detection probability results of P. loveridgei also support temperature as a variable influencing calling activity, with temperatures below 21°C having higher detection probabilities (see Chapter 3). Additionally, P. loveridgei body temperature preferences have a mean of 15.4°C (see Chapter 4). In summary, the results of this study indicate that the environmental variables influencing P. loveridgei calling behaviour, and possibly breeding behaviour, are season, rainfall and temperature.

Previous studies have used automated recording systems to effectively study anuran calling behaviour and the environmental factors influencing calling (Oseen and Wassersug, 2002; Todd et al., 2003; Saenz et al., 2006; Steelman and Dorcas, 2010; Willacy et al., 2015). The results of this study demonstrate the utility of automated recordings systems to survey and monitor the calling behaviour for cryptic species. Automated calling surveys are
advantageous because they allow data to be recorded simultaneously from different sites, covering large areas and with minimal disturbance to anurans (Bridges and Dorcas, 2000; Todd et al., 2003; Steelman and Dorcas, 2010; Willacy et al., 2015). Recording devices, however, are generally expensive and potentially limits the number of survey sites (Willacy et al., 2015). While useful it is important to bear in mind that this approach produces large amounts of recording data that can be time consuming to analyse. Automated sound recognition software (e.g. Song Scope) are a quick and practical approach to analyse large sound data sets thereby reducing processing times. These automated systems, can have relatively large uncertainty and error rates, particularly false positives (Waddle et al., 2009) that must be considered when using this approach. To overcome these issues, time must be spent fine tuning the automated scanning software and filtering the resulting data (Todd et al., 2003). In general, automated acoustic surveys and automated sound analyses are a valuable monitoring strategy that can be used for several conservation outcomes, particularly to improve the effectiveness of long-term monitoring programs (Bridges and Dorcas, 2000).

To effectively and efficiently undertake calling surveys, it is important to consider species-specific core calling periods in conjunction with the environmental conditions influencing this activity (Bridges and Dorcas, 2000; Lemckert and Mahony, 2008; Steelman and Dorcas, 2010). The results of this research are useful for planning specific times to undertake *P. loveridgei* surveys. As rainfall, season and temperature influence calling behaviour we can fine-tune monitoring strategies to increase detectability of *P. loveridgei*. Our results suggest that the best time to conduct *P. loveridgei* calling surveys, with increased likelihood of detection, is during the morning between 6:00 and 10:00, preferably in the months of November and December, after the wet season has started and at air temperatures between 14 - 16°C. This recommendation is not limited to calling surveys; it can also be used for *P. loveridgei* encounter surveys.

In summary, data from automated calling surveys can be used to develop species-specific survey protocols to monitor anuran populations over extended periods of time. This can aid in elucidating population trends from phenological changes caused by climate change. The information gathered in this study provides suitable baseline data to be used in future population dynamic studies of *P. loveridgei*, allowing for comparisons and trends to be identified, particularly in relation to climate change.
2.6 Acknowledgments

This research was funded by Tricia Waters (OEH) and the Advisory Committees of the Gondwana Rainforests of Australia World Heritage Area (DECCW-720-2010). MFL was also partially funded by School of Environment, Griffith University. This study was conducted under a Scientific Purpose permit issued by the Department of Environment and Resource Management (WITK10308811), and was approved by Griffith University Animal Ethics Committee (ENV/21/12AEC). We thank Dr Clare Morrison for her valuable mentorship and comments on earlier drafts of this manuscript. We thank Harry Hines for assistance with site selection, Michael Mahony for partial funding, and Rosie Willacy for valuable assistance with data analyses. A special thanks to all field volunteers.

2.7 References


CHAPTER 3 –
Climate and occupancy modelling for predicting
*Philoria loveridgei* distribution and abundance

The previous results chapter examined calling phenology of *P. loveridgei* within two national parks of mid-eastern Australia. Calling phenology is one of the sensitivity factors assessed in this thesis to evaluate the vulnerability of *P. loveridgei* to climate change. Chapter 2 concluded that calling phenology of *P. loveridgei* is primarily associated with rainfall and temperature, varying between seasons.

The current results chapter examines exposure factors by modelling *P. loveridgei* current distribution, estimating occupancy and detectability probabilities and forecasting future changes to the species distribution under moderate and extreme climate change scenarios.

This chapter will be submitted as a research paper to the journal of Diversity and Distribution and has been formatted to fit the journal style. The citation will be as follows:

**Familiar López, M., Lollback, G., Newell, D. and Hero, J-M. (in preparation)** Will climate change jeopardise the survival of a subtropical mountain-top frog (*Philoria loveridgei*)? *Diversity and Distribution*

Co-authors of this manuscript are my thesis supervisors, Dr Gregory Lollback, Dr David Newell and Prof. Jean-Marc Hero. My (Mariel Familiar López) contribution to the manuscript included the initial concept and survey design, field sample collection, modelling analyses and manuscript preparation.
Mariel Familiar López (corresponding author)

Gregory Lollback (Associate Supervisor)

David Newell (External Supervisor)

Jean- Marc Hero (Principal Supervisor)
3.1 Abstract

Aim

The primary aim of this study was to model the current distribution of *Philoria loveridgei* and predict its future distribution under moderate and extreme climate change scenarios. Our secondary aim was to use occupancy modelling and additional surveys inside the predicted distribution area to evaluate the species distribution model (SDM).

Location

Subtropical mid-eastern Australia.

Methods

Using 87 spatially unique presence records of *P. loveridgei* we built a SDM using the maximum entropy algorithm (Maxent). We then established and surveyed 42 transects inside the predicted distribution area to estimate the detectability probability and the proportion of area occupied by *P. loveridgei*. Finally, we modelled the future species distribution under moderate and extreme climate change scenarios using representative concentration pathways predicted for 2050 and 2070.

Results

The current distribution of *P. loveridgei* was modelled throughout a narrow geographic range (650.71 km²) in the mountains of mid-eastern Australia. Average occupancy was 0.773, which was generally higher than what Maxent predicted. Variables influencing detectability and occupancy estimates included number of repeated surveys, temperature, rainfall and canopy cover. Maxent predictions suggested suitable climate conditions for *P. loveridgei* will be lost to varying degrees under both climate change scenarios, with the total loss of suitable areas under the extreme scenario by 2070.

Main conclusions

Populations of *P. loveridgei* are presently distributed throughout most of the areas predicted as climatically suitable by Maxent, confirmed by the occupancy designed field surveys. This outcome supports the use of field surveys based on occupancy design as a practical tool to evaluate SDM. Climate change is predicted to jeopardise the survival of *P. loveridgei* (a mountain-top frog) however, microclimate buffering could play a major role in their ability to survive.

Keywords: Amphibian declines, Australia, detection probability, habitat suitability, Maxent, PRESENCE, occupancy, survey effort.
3.2 Introduction

It is now evident that climate change will have a significant impact on biodiversity and ecosystems, challenging species survival (Williams et al., 2003; Thomas et al., 2004; Pimm, 2008; Williams et al., 2008; Blaustein et al., 2010; Penman et al., 2010; Moritz & Agudo, 2013; Pacifi et al., 2015). As a group, ectotherms and amphibians in particular are highly vulnerable to the effects of climate change (Carey & Alexander, 2003; Corn, 2005; Deutsch et al., 2008; Hagger et al., 2013; Li et al., 2013; Seebacher et al., 2015). Climate change will influence individual species differently and can alter some species distributional ranges (e.g. Wilson et al., 2007; Penman et al., 2010). The general trend is that species will move towards the poles and/or to higher altitudes mirroring the increase in temperature (Parmesan & Yohe, 2003; Root et al., 2003; Wilson et al., 2005; Wilson et al., 2007; Lawler et al., 2009). Shifts in distribution may cause geographical range contractions resulting in a significant reduction of areas occupied by a species, leading to species extinction (Thomas et al., 2006; Pimm, 2008). Of particular concern are mountain-top species which have been reported to respond more rapidly to environmental change (Wilson et al., 2007; Laurance et al., 2011). These cool-adapted species could be forced to move further uphill to the limit of their distribution and eventually into extinction, as their thermal environment disappears (Pimm, 2008; Wake & Vredenburg, 2008; Forero-Medina et al., 2011; Hagger et al., 2013) (Figure 3.1).

Figure 3.1 Conceptual diagram predicting the movement of amphibians up mountains in response to global warming. Symbols are courtesy of the Integration and Application Network, University of Maryland Centre for Environmental Science (ian.umces.edu/symbols/). Forecasting responses to future climate scenarios is critical for
long term species management and conservation, and for prioritisation of resources (e.g. Fouquet et al., 2010). In this context, species distribution modelling (SDM) has been widely and successfully used to predict species geographical ranges and aid conservation and management strategies (e.g. Baldwin, 2009; Elith et al., 2010; Peterson et al., 2011). There are several modelling frameworks available for predicting species distributions, however, the maximum entropy (Maxent) approach has proven better than other models that rely on presence only data (Elith et al., 2006; Phillips et al., 2006; Elith et al., 2011). In general, SDM are built by associating occurrence data (presence/absence or presence only) of a species with abiotic and/or habitat variables to develop models of the ecological requirements of the species and produce distribution prediction maps. These models identify a species realised niche describing the conditions under which a species has been found to occur, and allowing future projections (e.g. Martinez-Meyer, 2005; Elith et al., 2006). Given the widespread use of SDM, evaluating their accuracy is important with many using some of the original dataset to test the predictability of the model. A common evaluating approach is to partition the occurrence data set used to build the models. This method thereby reduces the data used to build the models and can lead to reduced accuracy (Stockwell & Peterson, 2002). Another approach is to collect supplementary and independent occurrence data based on additional surveys to evaluate the accuracy of the SDM (e.g. Elith et al., 2006). Fewer studies, however, have used occupancy models to design surveys and collect the additional occurrence data required to validate the SDM (Newbold et al., 2010; Gormley et al., 2011; Peterman et al., 2013).

Occupancy models use a likelihood based approach to estimate the proportion of sites occupied by a species when its detection probability is <1. Repeat surveys allow researchers to model changing probabilities of detection and occupancy of a species using covariates (e.g. environmental variables) (MacKenzie et al., 2002; MacKenzie, 2006). Occupancy modelling is a flexible approach that has been applied to a variety of research studies and different taxa with optimal results (see Bailey et al., 2013). Accounting for imperfect detection of a species is important when surveying species that are difficult to detect (e.g. cryptic species), allowing for a more precise estimation of occupancy and hence, its distribution (Bailey et al., 2004).

In this context both frameworks, species distribution and occupancy modelling, are widely used to predict species occurrence and identify the important environmental characteristics associated with it, however, they are not often used together (Newbold et al., 2010; Gormley et al., 2011; Peterman et al., 2013). The primary aim of this study was to
model the current distribution of *Philoria loveridgei* mountain-top frog, and predict its future distribution under moderate and extreme climate change scenarios. Our secondary aim was to use occupancy modelling and additional surveys inside the predicted distribution area to evaluate the species distribution model (SDM).

### 3.3 Methods

#### 3.3.1 Study species and study area

*Philoria loveridgei* is a habitat specialist frog restricted to subtropical rainforest and is a rare vertebrate in mid-eastern Australia (Hines *et al.*, 1999; Knowles *et al.*, 2004). This mountain-top (above 600 m a.s.l.) frog is endemic to the Tweed Caldera Rim in southeast Queensland and northern New South Wales, Australia (Knowles *et al.*, 2004). This highly cryptic frog specie is diurnal, terrestrial and has direct-developing tadpoles laid as eggs inside burrows constructed under leaf litter or rocks on the rainforest floor. Male frogs call from inside or near burrows producing a soft species-specific vocalisation which is short and has a guttural “bo-r-k” sound (Knowles *et al.*, 2004; Anstis, 2013). A recent assessment of rainforest vertebrate species of Australia identified this frog species as highly vulnerable to climate change mainly because of its restricted distribution (Hagger *et al.*, 2013; Hero *et al.*, 2015).

The field component of this study was conducted in five national parks within the Tweed Caldera Rim including Springbrook, Lamington (Green Mountains and Binna Burra sections), Nightcap, Mount Warning and Border Ranges National Parks (Figure 3.2 and Table 3.1). This area is a core region of subtropical rainforest encompassing World Heritage listed Gondwana Rainforests Reserves (McDonald, 2010). Field sampling took place between November 2012 and January 2013.

#### 3.3.2 Species distribution modelling (SDM)

A SDM framework was used to estimate the optimal probability distribution of *P. loveridgei* and predict its future distribution under different climate change scenarios. The SDMs were built using the maximum entropy algorithm (Maxent; Phillips *et al.*, 2006). This a machine learning method that finds the predicted distribution based on the maximum entropy principle (Phillips *et al.*, 2006). To generate species probability models, Maxent requires presence only records and incorporates interaction effects of environmental and/or habitat variables. Additionally, this method estimates the contribution of each explanatory variable to the SDM. Maxent calculates the area under the curve (AUC) of each model allowing for model success to be tested (Elith *et al.*, 2006; Phillips *et al.*, 2006). Maxent is widely used
and has proven to be a reliable and robust approach for estimating species distribution (Elith et al., 2006).

We verified and used 87 spatially unique presence records of *P. loveridgei* from the WildNET Database 2011 (Environmental Protection Agency, 2004, WildNet) and Harry Hines (verification of the data, Queensland Department of Environment and Resource Management). We selected nine climate layers obtained from Worldclim data (30 arc seconds) including five temperature variables (bio1: annual mean temperature, bio4: temperature seasonality, bio5: maximum temperature of warmest period, bio8: mean temperature of wettest quarter, and bio10: mean temperature of warmest quarter) and four precipitation variables (bio13: precipitation of wettest period, bio15: precipitation seasonality, bio16: precipitation of the wettest quarter and bio18: precipitation of warmest quarter). The same predictor variables were used to model the future species distribution for 2050 and 2070 under two climate change scenarios. We used representative concentration pathways scenarios (RCP) derived from Hadley Centre Global Environmental Model version 2 (HadGEM2-ES) and based on an extreme (RCP8.5) and moderate (RCP6.0) scenario of global warming (Hijmans et al., 2005; IPCC, 2014).

Maxent was set to run 15 replicates (subsample type), with 5000 iterations and with 75% of presence records as training data leaving 25% for a first evaluation of model performance. All other settings were maintained as default. Total modelled area was estimated in ArcGIS (ESRI, 2015) classifying the logistic output maps into three categories based on habitat suitability; high suitability >0.70, moderate suitability between 0.50 – 0.69 and low suitability <0.49. The minimum probability of suitable habitat modelled was set using the minimum training presence logistic threshold.

### 3.3.3 Occupancy survey design

To evaluate the SDM of *P. loveridgei* we performed additional independent surveys based on occupancy to ground-truth the model and evaluate its predictive accuracy. Since we aimed to find areas where *P. loveridgei* was present, selection of sites for transect establishment was based on the SDM selecting areas with >50% predicted environmental suitability. Forty-two transects were established within five national parks to cover the altitudinal and geographical range predicted for the species (Figure 3.2 and Table 3.1). For each national park, transects were selected systematically by first mapping all the potential frog sites in the area using ArcGIS (ESRI, 2015) then selecting sites located approximately 200 m apart to ensure independence. Potential sites for this frog species are usually located
in boggy headwaters in rainforest gullies (M. Familiar López pers. obs.) and standardised transects were placed down the middle of these. Gullies were typically 15 m wide with rock, mud or vegetation ground substrate.

Individual *P. loveridgei* frogs were detected acoustically and counted during low-pause walks (~30 min) along 100 m transects. All transects surveyed were 100 m length except for Mount Warning National Park, where 50 m transects were established due to rugged steep terrain. Each transect was surveyed on five occasions (MacKenzie *et al.*, 2002; MacKenzie & Royle, 2005) in one season. To maximise detectability we conducted diurnal surveys in late spring and early summer following periods of rainfall, when *P. loveridgei* calling activity is known to peak (see Chapter 2). All transects within each site (national park) were sampled on the same day. Surveys took place in a variety of environmental conditions with survey-specific environmental data being recorded including: temperature, humidity, rainfall, cloud cover and the number of frogs heard. For each site, additional rainfall data were obtained from the nearest Australian Government Bureau of Meteorology meteorological stations. At the end of all occupancy surveys we estimated for each site the altitude, presence/absence of *Helmholtzia* plants, aspect and canopy cover.

Relative frog abundance for each transect was quantified as the maximum number of frogs heard among the five survey occasions. The maximum number of frogs was doubled for Mount Warning National Park transects to correct for the 50 m transects established at that site. The relative population abundance for each location (national park) was calculated as the mean of the relative abundances of each transect.

### 3.3.4 Occupancy modelling

Occupancy modelling followed the methods described by MacKenzie *et al.* (2002) using PRESENCE software (Hines, 2010). This method is a statistical approach for site occupancy data that describes maximum likelihood estimations. The model develops estimates that describe the probability of an area being occupied (PAO) by a species, if detection is <1. In field surveys, the detection of a species at a site cannot be guaranteed even when it is present. The naïve estimate of PAO is calculated as the number of sites where the species was detected divided by the total number of sites surveyed. This estimate will underestimate the true PAO. Therefore, MacKenzie’s method follows repeated surveying of the study sites where the probability of detecting the species of interest can be estimated and an unbiased PAO can be obtained (MacKenzie *et al.*, 2002). This method allows for
occupancy and detection probabilities to be estimated as a function of site-specific and sampling-occasion covariates, respectively.

To estimate *P. loveridgei* occupancy, we first modelled the sampling-occasion covariates that influence detection probability by maintaining the site occupancy constant in PRESENCE. Secondly, we modelled the frog species occupancy incorporating the site-specific covariates to the best detection probability model. Each model is ranked in PRESENCE according to the estimated Akaike Information Criterion (AIC) which we corrected for a small sample size (AICc) (Johnson and Omland 2004), using the 42 sites sampled. The best fitting model was identified by a low AICc value and a large AICc weight. The covariates used for detectability estimates (*p*) were the number of repeated surveys (total of 5 visits), temperature (°C), humidity (%), cloud cover (%), rainfall (mm) and number of frogs heard per survey. The covariates used for occupancy estimates (*psi*) included altitude (m a.s.l.), canopy cover (%), presence/absence of *Helmholtzia* plants and aspect (circular degrees transformed). All variables were used to estimate detectability and occupancy after testing for collinearity between continuous variables showed correlation coefficient values *r* < 0.7 (Garden *et al*., 2007). Linear regression analyses were used to test for relationships between occupancy probabilities, canopy cover and Maxent predicted habitat suitability probabilities and canopy cover (R Development Core Team, 2015).

### 3.4 Results

#### 3.4.1 Maxent SDM

The spatial distribution model of *P. loveridgei* predicted a narrow geographical range within the Tweed Caldera Rim region, covering a total area of 650.71 km². The Maxent model was robust and had a good fit indicated by the high AUC statistic (AUC = 0.992, SD = 0.002). Areas with more suitable conditions and higher probability of *P. loveridgei* occurrence are distributed in small fragmented sections (42.39 km²) high in the mountains (mean altitude 904 m a.s.l.), mainly in Lamington National Park. Areas of moderate suitability were predicted in larger sections (170.69 km²) all within existing national parks (mean altitude 723 m a.s.l.). All the areas with high and moderate suitable conditions were predicted within established national parks. Additional areas of lower suitability and low probability of occurrence for *P. loveridgei* were predicted mainly in larger areas (437.63 km²) at lower altitudes (mean altitude 568 m a.s.l.) (Figure 3.2). The Maxent analyses of variable contribution identified precipitation of wettest period and maximum temperature of warmest period as contributing the most to SDM: with relative contribution of 80% between them. Precipitation of the wettest period (47.2%) was the variable with the highest explanatory
power in the model, positively related to current distribution of *P. loveridgei*. Habitat suitability of *P. loveridgei* was reduced by an increase in the maximum temperature of the warmest period (32.6%). This relationship suggests that increasing precipitation in the wettest period and cool maximum temperatures are the primary correlates for predicting the distribution of *P. loveridgei*.

Figure 3.2 Spatial distribution model for *Philoria loveridgei* classified into three habitat suitability probabilities, high (> 0.70) dark green, moderate (0.50 – 0.69) light green, and low (> 0.49) light blue. Red dots indicate sites surveyed for the occupancy model.

3.4.2 Occupancy modelling

We detected 364 *P. loveridgei* vocalisations on 73 of 205 survey occasions with an overall mean of 2/5 survey visits. Frogs were detected on 31 of the 42 transects in all the national parks surveyed. The naïve PAO was 0.738 and the mean model estimated
occupancy was 0.773 (SE = 0.07). These results describe a high occupancy within the SDM predicted for *P. loveridgei*. There was, however, no significant relationship between Maxent model probability values and the modelled occupancy probabilities ($R^2 = 0.01$, SE = 0.01, $p = 0.5$). Occupancy probabilities were high in areas predicted by Maxent with moderate habitat suitability. Areas of high predicted suitability had lower occupancy estimates (Figure 3.3). This suggests that the best Maxent model underestimates the occupancy probability of *P. loveridgei* in areas predicted to have high habitat suitability.

![Figure 3.3](image)

**Figure 3.3** Relationship between Maxent suitable habitat probabilities and occupancy model probabilities. The Maxent model underestimates the occupancy of *Philoria loveridgei* in areas predicted with moderate suitability (0.50 – 0.69).

Occupied areas were distributed over a range of altitudes. The lowest occupied transect (683 m a.s.l.) was located within Nightcap National Park and the highest (1083 m a.s.l.) in Border Ranges National Park (Table 3.1). Climate conditions varied during the five
survey visits with temperatures at time of survey ranging from 10.2 - 27.5°C, humidity from 57.8% - 100% and cloud cover from 0 - 100%. Surveys were conducted during different times of day ranging from 7:35 to 17:30. Rainforest canopy cover on transects ranged from 72 - 93% and aspect from 5 - 342°. The relative abundance of calling *P. loveridgei* males on a single survey ranged from 1 - 18 frogs with an average of 5 frogs/ 100 m over all transects. Relative population abundance varied between national parks with Lamington National Park supporting the largest frog population (Table 3.1).

**Table 3.1** Summary of the 42 transects established for occupancy surveys of *Philoria loveridgei*. Relative population is the maximum average number of frog per transect within each national park.

<table>
<thead>
<tr>
<th>National Park</th>
<th>Altitude range (m asl)</th>
<th>Transects occupied/ total surveyed</th>
<th>Relative population (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Border Ranges</td>
<td>769 - 1083</td>
<td>4/5</td>
<td>7 (5.6)</td>
</tr>
<tr>
<td>Mount Warning</td>
<td>564 - 848</td>
<td>2/5</td>
<td>9 (7.0)</td>
</tr>
<tr>
<td>Springbrook</td>
<td>815 - 986</td>
<td>4/4</td>
<td>9 (2.9)</td>
</tr>
<tr>
<td>Lamington (Binna Burra)</td>
<td>826 - 1021</td>
<td>11/13</td>
<td>7 (6.8)</td>
</tr>
<tr>
<td>Lamington (Green Mountains)</td>
<td>909 - 1062</td>
<td>7/9</td>
<td>5 (4.6)</td>
</tr>
<tr>
<td>Nightcap</td>
<td>683 - 793</td>
<td>3/6</td>
<td>4 (4.6)</td>
</tr>
</tbody>
</table>

Using PRESENCE we built and analysed 14 possible models estimating detectability and occupancy probabilities of *P. loveridgei* (Table 3.2 and Table 3.3). The best detection model indicated that temperature and the number of repeated surveys were the sampling-occasion covariates influencing detection probability of *P. loveridgei*. Detectability decreased with increasing temperatures and was higher during the first two survey visits (mean \( p = 67\% \)) and was lowest in the last survey (mean \( p = 7\% \)) (Figure 3.4). Only temperature and the number of repeated surveys were used to assess site occupancy for *P. loveridgei* as this model was 2.8 times more likely than the next best model (Table 3.2). Canopy cover was the site-specific covariate that best predicted the occupancy of *P. loveridgei* (Table 3.3). The relationship between occupancy probabilities and canopy cover was significant showing higher occupancy probabilities with increasing canopy cover \( R^2 = 0.999, \ SE = 0.0003, \ p < 0.001 \). Models that included the number of frogs heard per survey round, altitude and the presence of *Helmholtzia* plants were excluded from the results, because the models including these variables did not converge.
Table 3.2 Model selection results of detection of *Philoria loveridgei* ranked by AICc.

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
<th>K</th>
<th>ΔAICc</th>
<th>AICc wgt</th>
</tr>
</thead>
<tbody>
<tr>
<td>psi(.), p(survey, temperature)</td>
<td>220.10</td>
<td>7</td>
<td>0</td>
<td>0.66</td>
</tr>
<tr>
<td>psi(.), p(survey, temperature, rain)</td>
<td>221.50</td>
<td>8</td>
<td>1.39</td>
<td>0.33</td>
</tr>
<tr>
<td>psi(.), p(survey, rain)</td>
<td>229.21</td>
<td>7</td>
<td>9.11</td>
<td>0.01</td>
</tr>
<tr>
<td>psi(.), p(survey)</td>
<td>234.27</td>
<td>6</td>
<td>14.17</td>
<td>0.001</td>
</tr>
<tr>
<td>psi(.), p(temperature, rain)</td>
<td>246.84</td>
<td>3</td>
<td>26.74</td>
<td>0.00</td>
</tr>
<tr>
<td>psi(.), p(rain)</td>
<td>253.13</td>
<td>2</td>
<td>33.02</td>
<td>0.00</td>
</tr>
<tr>
<td>psi(.), p(temperature)</td>
<td>257.39</td>
<td>2</td>
<td>37.28</td>
<td>0.00</td>
</tr>
<tr>
<td>psi(.), p(.)</td>
<td>258.98</td>
<td>2</td>
<td>38.87</td>
<td>0.00</td>
</tr>
<tr>
<td>psi(.), p(humidity)</td>
<td>259.20</td>
<td>2</td>
<td>39.10</td>
<td>0.00</td>
</tr>
<tr>
<td>psi(.), p(cloud cover)</td>
<td>259.69</td>
<td>2</td>
<td>39.58</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 3.3 Model selection results of occupancy of *Philoria loveridgei* ranked by AICc.

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
<th>K</th>
<th>ΔAICc</th>
<th>AICc wgt</th>
</tr>
</thead>
<tbody>
<tr>
<td>psi(canopy cover), p(survey, temperature)</td>
<td>219.68</td>
<td>7</td>
<td>0</td>
<td>0.37</td>
</tr>
<tr>
<td>psi(.), p(survey, temperature)</td>
<td>220.10</td>
<td>7</td>
<td>0.42</td>
<td>0.30</td>
</tr>
<tr>
<td>psi(canopy cover), p(survey, temperature, rain)</td>
<td>221.06</td>
<td>8</td>
<td>1.38</td>
<td>0.18</td>
</tr>
<tr>
<td>psi(.), p(survey, temperature, rain)</td>
<td>221.49</td>
<td>8</td>
<td>1.81</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Figure 3.4 Relationship between temperature at time of survey and estimates of detectability for *Philoria loveridgei*.

3.4.3 Future projections of SDM

In general, the climate change models forecast a substantial decrease in area and spatial distribution of the climate envelope for *P. loveridgei*, resulting in an increasingly narrow and fragmented geographical distribution (Figure 3.5a-d). Results for 2050 and 2070 under RCP6.0 and RCP8.5 climate scenarios forecast a complete loss of highly suitable areas. Maxent forecast a loss of 89% of climatically suitable areas for *P. loveridgei* by 2050 under a moderate climate change scenario (Figure 3.5a). Under the extreme climate change scenario the model forecast a 92% decrease in area with suitable conditions (Figure 3.5b). Most of the southeast sites will decrease, narrowing the species distribution. Under the RCP6.0 scenario, the model forecast the loss of all moderately suitable areas by 2070 (Figure 3.5c). This indicates that climate suitability will decrease to small fragments of the current distribution located mainly in the mountain-tops in Lamington National Park. Results of the extreme climate change scenario indicate severe loss of all suitable areas, with no area forecasted as climatically suitable for *P. loveridgei* (Figure 3.5d).
Figure 3.5 Future predicted distribution models for *Philoria loveridgei* classified into three habitat suitability probabilities, high (> 0.70) dark green, moderate (0.50 – 0.69) light green, and low (> 0.49) light blue. **a)** Under the RCP6.0 scenario by 2050, **b)** under the RCP8.5 scenario by 2050, **c)** under the RCP6.0 scenario by 2070 and **d)** under the RCP8.5 scenario by 2070.

3.5 Discussion

*Philoria loveridgei* frogs are restricted to a narrow geographical distribution in the forested mountain-tops that form the Tweed Caldera Rim, mid-eastern Australia. Occupancy designed field surveys found a high proportion of area occupied by *P. loveridgei* frogs within the SDM, confirming they are distributed throughout most of the areas predicted as
climatically suitable. This outcome supports the importance of field surveys based on occupancy design (MacKenzie et al., 2002) as an effective tool to accurately evaluate SDM. Other studies have used field surveys based on occupancy methods to evaluate the accuracy of SDM with a positive relationship between the two models (Newbold et al., 2010; Gormley et al., 2011; Peterman et al., 2013). Contrary, we found no positive relationship between occupancy probabilities and Maxent probabilities. This results could be attributed to the under estimation of occupancy of P. loveridgei in the predicted moderate habitat suitability. By using easily accessible and free climate layers (Worldclim data; Hijmans et al., 2005) in combination with a user-friendly and free distribution modelling algorithm (Maxent; Phillips & Dudik, 2008) we successfully built a distribution model that accurately delimited the geographical outer limits likely to contain P. loveridgei frogs. A limitation of climate only SDM are their coarse spatial resolution, which may not be appropriate for matching the landscape extent of the study species or the process being modelled (Peterman et al., 2013). Our study indicates, however, that for cryptic and specialist species with few known localities they are an effective base line approach to identify new survey areas with high probability of occupancy. Results from other studies have shown that species with smaller ranges are modelled more accurately, maybe due to their more specific climate and habitat requirements (Newbold et al., 2010). This suggests that coarse resolution climate based SDM can be useful tools for predicting species distribution limits of restricted distributed species. Additionally, the ability of the climate only model to accurately predict current areas of P. loveridgei presence suggest that climate factors are important components of the distribution of this species at a larger scale.

Precipitation in the wettest period and maximum temperature of the warmest period best explained current suitable areas for the occurrence of P. loveridgei. These results corroborate previous observations that P. loveridgei has a limited distribution in cool rainforest habitats at high altitudes (above 600 m a.s.l.) and is strongly associated with high rainfall (Hines et al., 1999; Knowles et al., 2004). Similar results have also been reported for the predicted distributions of other amphibian species (e.g. Ficetola et al., 2007; Urbina-Cardona & Loyola, 2008; Fouquet et al., 2010), where temperature and precipitation factors were identified as the most important variables contributing to SDM. Being ectotherms, amphibians are highly influenced by climate factors (Carey & Alexander, 2003; Li et al., 2013), making climate based SDM a useful and reliable tool to model their distribution limits. Our results suggest that our best Maxent model underestimates the occurrence probability of P. loveridgei. Areas predicted by the Maxent model as having moderate habitat suitability had high occupancy probability values similar to areas predicted to have high habitat suitability. Occupancy surveys indicated no difference in proportion of area being occupied
between moderate and high suitable areas modelled by Maxent. This result may be explained by the fact that the SDM identified the fundamental niche of *P. loveridgei* at a landscape scale but not the realised niche (e.g. microhabitat) (see Chapter 4) (Martinez-Meyer, 2005). SDM built with large scale climate variables (e.g. temperature and precipitation) are good predictors of species fundamental niche, however they lack fine scale resolution to determine habitat components influencing the occurrence and distribution of a species (Martinez-Meyer, 2005; Peterman et al., 2013). Fine scale models that include habitat variables associated with microhabitat use can be important to further define the boundaries of SDM (Kearney & Porter, 2004). In this context, occupancy models are a practical approach to estimate fine scale abiotic and biotic factors associated with an area occupied by a species (MacKenzie, 2006).

Occupancy models estimated two fine scale variables associated with detection probability (temperature and the number of repeated surveys) and one with occupancy (canopy cover). These results are similar to other studies on forest amphibian species which reported that these variables influenced detection and occupancy (MacKenzie et al., 2002; Mazerolle et al., 2005; Pellet & Schmidt, 2005; Weir & Mossman, 2005; Kirlin et al., 2006; Kéry et al., 2013; Peterman et al., 2013). Theoretical results on the number of visits required to accurately detect if a species is in a site, indicate a minimum of three surveys is needed when detection probability is > 0.6 and occupancy is > 0.7 (MacKenzie et al., 2002; MacKenzie & Royle, 2005). Detection probabilities of *P. loveridgei* were high (mean \( p = 0.67 \)) in the first two survey visits, corroborating that a lower minimum number of visits is necessary to be certain of the presence (i.e. detection) of this species in a site, under adequate environmental conditions. A study on anurans from Switzerland, also suggest when detection probabilities are high (\( p = 0.743 \)) a minimum of three visits at an average temperature of 13°C is required to be certain of the presence of *Hyla arborea* (Pellet & Schmidt, 2005). We recommend a minimum of three surveys per site, under the right conditions (see below), would be necessary to accurately confirm the presence of *P. loveridgei* in an area. The first surveys of this study took place on late November and early December, and were identified as the best months to undertake surveys with a high probability (> 60%) of detecting this species (see Chapter 2). Additionally, surveys for *P. loveridgei* species should be carried out when air temperatures are below 21°C, to have > 50% likelihood of detecting this frog (see Chapter 2 and 4). Pellet and Schmidt (2005) described that detection probabilities varied strongly with temperature, highlighting the importance of considering this factor when surveying. The best competing detectability model included rainfall as a predictor covariate influencing detection probabilities. We hypothesis that detection of this species increases after the start of the wet season, because it is
associated with the start of the breeding season (see Chapter 2), and decreases after breeding events occur. Early spring rainfall events would increase soil moisture, which would increase *P. loveridgei* nest moisture increase the eggs and larval survival (see Chapter 2). This explains why rainfall could be an important secondary covariate associated with detection probabilities.

The probability of an area being occupied by *P. loveridgei* increased with canopy cover, confirming this species association with closed rainforest. A similar finding was reported by Peterman *et al.* (2013) where an increase in canopy cover was associated with higher probabilities of ponds being occupied by *Ambystoma jeffersonianum* a forest salamander species. Altitude and the presence of *Helmholtzia* plants did not converge in any occupancy model, however, they could be useful for survey planning. All transects where *Helmholtzia* plants were present were occupied by *P. loveridgei* frogs, but the converse did not hold (i.e. *P. loveridgei* were also found on transect without *Helmholtzia*). We suggest this plant is a surrogate for identifying sites with high soil moisture, although this was a variable we did not measure. This plant is big and easy to identify, hence it could be a habitat characteristic to assist with identification of possible survey areas for *P. loveridgei*, within closed subtropical rainforests. The SDM predicted areas of high and moderate habitat suitability at altitudes above 700 m a.s.l., suggesting a mountain-top restricted distribution range. Altitude, however, was a poor predictor of occupancy. This result may be explained by the fact that occupancy surveys where only undertaken at altitudes above 600 m a.s.l.

The relative abundance results indicated that although they have a narrow distribution within subtropical rainforest, *P. loveridgei* populations seem to be locally abundant through their distribution and can reach densities of 18/100m. Within the five national parks, Lamington National Park harbours the largest population of *P. loveridgei*. This national park was also predicted to contain large areas of high and moderate habitat suitability, corroborating the high occupancy found. A previous study demonstrated that range-restricted specialist species (small distribution ranges and narrow environmental niches) tend to have high uniform local abundances within their area of distribution (Williams *et al.*, 2009). Our relative population abundance results corroborate this study.

Species with small geographical ranges tend to have limited tolerance to changing environmental conditions, making them more vulnerable to rapid climate change (Williams *et al.*, 2008; Laurance *et al.*, 2011). Additionally, this species may be habitat specialists increasing their vulnerability when faced with significant changes in weather patterns.
(Williams et al., 2003; Hero et al., 2005; Murray & Hose, 2005). Our Maxent predictions suggest suitable habitat areas for the occurrence of *P. loveridgei* will be severely affected by a warming climate, with no suitable areas remaining by 2070 under an extreme scenario. The projected habitat suitability distributions suggest a loss of suitable areas from 89 - 100% by 2070. Even under a moderate scenario (RCP6.0) the forecast estimates a significant reduction of suitable habitat areas and severe fragmentation of the remaining sites. A study modelling the distribution of rainforest plants along altitudinal gradients in eastern Australia suggested a continuing contraction of climate suitable envelopes for cool highland rainforest by 2050 (Mellick et al., 2013). *Nothofagus* species, which typify the canopy of montane cool subtropical rainforests, are contracting and will be increasing vulnerable under climate warming (Mellick et al., 2013). This finding adds to our forecast of potential loss of suitable habitat areas for *P. loveridgei* which will increase their vulnerability to climate change. *Philoria loveridgei* current mountain-top distribution may have been delimited by rainforest contractions following previous climate warming during past glacial cycles, confining this species to cooler and higher altitude rainforest (Knowles et al., 2004; Hagger et al., 2013). Regionally endemic mountain-top cool adapted species may be particularly vulnerable to climate change because, in response to a warming climate, they will be unable to move to higher altitudes (Hagger et al., 2013). If these species have low vagility, they will also be unable to move to cooler latitudes. In the past, perhaps, these cool specialised frogs may have narrowed their range by moving to higher altitudes during historic periods of dramatic climate change (e.g. Pleistocene). Following these findings, we would expect the survival of *P. loveridgei* to be jeopardised by climate change, exacerbating a previous report (Hagger et al., 2013). This assessment, however, is based on large scale climate SDM that measure a species exposure to climate change. Fine scale data measuring sensitivity would be needed to fully understand the full impact of a warming environment on *P. loveridgei*. For example, thermally buffered environments (rainforest and microhabitats) can play an important role for mountain-top endemics that are unable to shift geographical ranges (Scheffers et al., 2013) (see Chapter 4).

*Philoria loveridgei* is a highly cryptic frog rarely encountered even in known sites, making it hard to detect and survey. Our dual modelling approach was effective for identifying areas with suitable climate conditions to surveys with occupancy probabilities. This method could reduce the cost of surveys by allowing the prioritisation of surveys and more strategic planning of survey planning. Few studies have used this dual approach to evaluate SDM (Newbold et al., 2010; Gormley et al., 2011) and only one has used it to guide survey efforts (Peterman et al., 2013). However, they have all concluded that even a small amount of field surveys are good to test SDM accuracy. We highlight the use of this dual
method to reduce the cost of survey efforts by determining priorities for strategic survey and monitoring programs for other species too. Additionally, this method can be used to determine the occupancy status of an area and to identify habitat features associated with the presence of a species for management and conservation purposes.

In conclusion, results from this study indicate that *P. loveridgei* is vulnerable to climate change, indicated by the predictive models. Occupancy and detectability is strongly influenced by abiotic factors (temperature, rainfall and canopy cover), that can be significantly altered by a warming climate. This study is the first to provide data on the current distribution, relative abundance, occupancy and detectability of *P. loveridgei* and provides base line information for future population dynamic studies of this species. These include data comparisons and assessment of changes in distribution, relative abundance, occupancy and detectability. Further work on *P. loveridgei* can build to determine whether populations are stable or decreasing, as populations could decline and possible become extinct due to drastic climate change or other factors including disease outbreaks (see Chapter 5).

### 3.6 Acknowledgments

This research was funded by Tricia Waters (OEH) and the Advisory Committees of the Gondwana Rainforests of Australia World Heritage Area (DECCW-720-2010). MFL was also partially funded by School of Environment, Griffith University. This study was conducted under a Scientific Purpose permit issued by the Department of Environment and Resource Management (WITK10308811), and was approved by Griffith University Animal Ethics Committee (ENV/21/12AEC). We thank Dr Clare Morrison for her valuable mentorship and comments on earlier drafts of this manuscript. We thank Harry Hines, and Michael Mahony for assisting with the verification of species presence records. A special thanks to all field volunteers.

### 3.7 References


CHAPTER 4 –

Thermal environment and thermal physiology tolerances of *Philoria loveridgei* in the face of climate change

The previous results chapter examined the potential of *P. loveridgei* to survive climate change by modelling its distribution (present and future), occupancy and detectability probabilities. Distribution and occupancy modelling are the first of the exposure factors assessed in this thesis to evaluate the vulnerability of *P. loveridgei* to climate change. Chapter 3 described the restricted geographical range of *P. loveridgei*, mainly within national parks in the mountains of mid-eastern Australia. Future predictions forecasted the loss (to varying degrees) of suitable habitat for *P. loveridgei* under moderate and extreme climate change scenarios.

The current results chapter examines a second sensitivity factor by investigating the thermal physiology of *P. loveridgei* within six national parks covering the altitudinal and geographical range of the species. The local rainforest environmental change and microhabitat buffering is also explored as additional exposure factors.

This chapter will be submitted as research paper to the Journal of Thermal Biology and has been formatted to fit the journal style. The citation will be as follows:


Co-authors of the manuscript include an external adjunct advisor, Prof. Andrew Krockenberger (whose area of expertise includes thermal physiology of tropical organisms) and my thesis supervisors: Dr Gregory Lollback and Prof. Jean-Marc Hero. My (Mariel Familiar López) contribution to the manuscript included the initial concept and survey design, field sample collection, data analyses and manuscript preparation. The thermal techniques and equipment used in this study were developed by Andrew Krockenberger.
Mariel Familiar López (corresponding author)

Andrew Krockenberger (Co-author)

Gregory Lollback (Associate Supervisor)

Jean- Marc Hero (Principal Supervisor)
4.1 Abstract

Biodiversity is threatened by climate change. The extent to which individual species will be sensitive to environmental changes will, however, depend on a combination of exposure, behaviour and physiology factors that are unknown for most species. The study of species’ physiological limits is key to modelling their sensitivity to rapid environmental change. One approach to assess thermal tolerances is to evaluate the critical thermal limits ($CT_{min}$ and $CT_{max}$) and preferred temperatures ($T_{pref}$). Herein, we measured the critical thermal limits and preferred temperatures of six populations of *Philoria loveridgei* throughout its range. We also evaluated the vulnerability to climate change of *P. loveridgei* by assessing its warming tolerance. Frogs were heated or cooled at a rate of 0.5°C per minute and their behaviour monitored to determine critical thermal limits. A thermal gradient with hot (35°C) and cold (10°C) extremes was used to estimate preferred temperatures ($T_{pref}$). *Philoria loveridgei* displayed a thermal tolerance range ($CT_{min} = 6.8°C; CT_{max} = 30.4°C$) and a $T_{pref}$ of 15.4°C. There was no significant relationship between critical limits or preferred temperatures with altitude. Frog body and burrow temperatures at time of capture were related indicating that *P. loveridgei* is a thermocorformer and thigmotherm. The thermal properties of the microhabitats were relatively stable compared with fluctuating rainforest air temperature, suggesting that microhabitats effectively buffered extreme temperatures and could provide a refuge from climate change. Our data highlights the importance of considering microhabitat refuges when investigating the vulnerability of terrestrial ectotherms to climate change. Thermoconformers and thigmotherms, like *P. loveridgei*, could physiologically cope with increases in environmental temperatures, if buffered from temperature fluctuations by their microhabitat (leaf litter and burrows), and/or macrohabitat (the rainforest itself).

**Keywords:** Australia, thermoconformer, thigmotherm, subtropical rainforest, warming tolerance.
4.2 Introduction

Climate change is a global phenomenon affecting multiple environmental parameters (e.g. air temperature, precipitation, cloud cover and the frequency of extreme weather events) that will challenge the survival of many species worldwide (IPCC, 2014; Pounds et al., 2006; Urban, 2015). It has been recognised that tropical and subtropical regions have higher species extinction risk due to climate change, including within Australia (Urban, 2015; Williams et al., 2003). Mountain-tops are particularly important because they harbour thermally specialised species (e.g. cool-adapted) that would be highly susceptible to climate warming (Laurance et al., 2011). There are differences between taxonomic groups in their vulnerability to climate change, with ectotherms (e.g. amphibians) reportedly more at risk (Deutsch et al., 2008; Duarte et al., 2012; Hagger et al., 2013; Rohr and Palmer, 2013). The increase in extreme weather events predicted with climate warming could have devastating consequences for ectotherms as they may cause significant physiological responses (e.g. exceed physiological limits) exposing them to physiological stress, ultimately leading species and/or population to extinctions (Sinervo et al., 2010). An accepted, general prediction is that cool-adapted species are more vulnerable to extinction due to climate change (e.g. Laurance et al., 2011). Consequently, evaluating the vulnerability of these organisms to climate change is critical, particularly for ectothermic taxa such as amphibians (Hagger et al., 2013; Williams et al., 2008).

The extent of how sensitive a species will be to environmental changes will depend on their exposure, plasticity and physiological traits, which for most species are unknown (Huey et al., 2012; Moritz et al., 2012; Williams et al., 2008). Evaluating species' physiological limits and thermal tolerances has been widely used to model their sensitivity to rapid environmental changes (Chown et al., 2010; Huey et al., 2012; Rezende et al., 2011). Critical thermal limits (CT minimum and CT maximum) and preferred temperatures are often used to define thermal tolerances (Lutterschmidt and Hutchison, 1997; Rezende et al., 2011; Terblanche et al., 2011). These limits are defined as the temperatures at which locomotion activity becomes disorganised and individuals lose their ability to escape hostile conditions or respond to threatening situations (Hutchison, 1961). Thermal limits tend to reflect a species' thermal ecology, such that tropical species exposed to low seasonal variation are expected to have narrower thermal ranges when compared to temperate species (Deutsch et al., 2008). Moreover, understanding the thermal limits of ectotherms is essential for forecasting their response to climate change (Shoo et al., 2010; Sinervo et al., 2010; Terblanche et al., 2011). In addition, estimating a species' tolerance to warming within their habitat adds to the vulnerability assessment, because it estimates the amount of
environmental warming a species can tolerate before reaching detrimental, or even fatal, physiological limits. Species with large warming tolerance (WT) could potentially tolerate substantial warming before reaching their physiological limit, hence they would be less vulnerable to environmental warming (Deutsch et al., 2008; Duarte et al., 2012; Scheffers et al., 2013; Simon et al., 2015).

Amphibians are a diverse group of ectothermic vertebrates that live under a wide range of climate conditions and habitats, with each species expected to have different physiological tolerances (Donnelly and Crump, 1998). Temperature is an environmental factor that affects the survival of amphibians, because environmental temperatures directly influence their physiological and behavioural performance (Zheng and Liu, 2010), driving reproduction and survivorship (Rohr and Palmer, 2013) (see Chapter 2). In general, an amphibian’s body temperature is directly related to the thermal environment in which they live and thus they are termed thermoconformers (Alford and Lutterschmidt, 2012; Navas and Araujo, 2000; Pough et al., 2004). Amphibians at high altitudes may be partial thermoconformers, as they passively tolerate thermal fluctuations in air temperatures by using thermally buffered substrates (Navas, 1996, 1997).

The magnitude to which climate change will potentially impact a species’ physiology will also depend on its capacity to buffer its effects by migration, shifts in microhabitat choice, acclimatisation and the evolutionary response of physiological tolerances (Williams et al., 2008; Kearney et al., 2009). Therefore, evaluating an ectotherm’s environment will also help assess its vulnerability to climate change. This approach should include assessing species-specific microhabitats, as these microhabitats could buffer the thermal environment experienced by an individual and act as a refuge from climate change (Scheffers et al., 2013; Scheffers et al., 2014; Shoo et al., 2010).

In this study we examined the critical thermal limits (CT$_{\text{max}}$ and CT$_{\text{min}}$), preferred temperatures (T$_{\text{pref}}$) and the thermal environment (microhabitats and ambient temperatures) of the mountain-top frog *Philoria loveridgei*, in six populations across different altitudes throughout its distribution range (geographical and altitudinal). We also evaluated the vulnerability of *P. loveridgei* to climate change by assessing its warming tolerance (WT).
4.3 Methods

4.3.1 Study species

*Philoria loveridgei* is a mountain-top frog endemic to the Tweed Caldera Rim in mid-eastern Australia (Knowles et al., 2004). This species is a habitat specialist with a distribution restricted to high mountains (above 600 m a.s.l.) in the Gondwana Rainforests making it a rare vertebrate in eastern Australia (Hines et al., 1999; Knowles et al., 2004). *Philoria loveridgei* is diurnal (see Chapter 2), terrestrial and has direct-developing tadpoles laid as eggs inside burrows constructed under leaf litter or rocks on the rainforest floor. Adult frogs inhabit shallow burrows under humid rainforest floor at the headwaters of streams. *Philoria loveridgei* is a small frog that can reach an adult snout-vent length (SVL) of 32 mm for females and SVL of 30 mm for males (Anstis, 2013; Knowles et al., 2004; Seymour et al., 1995). It has a short breeding season that ranges from October to January (Anstis, 2013; Knowles et al., 2004) (see Chapter 2). A recent assessment of the subtropical rainforest vertebrate species of eastern Australia suggests this frog species could be highly vulnerable to climate change because its restricted distribution (Hagger et al., 2013). To date, only one study has evaluated the thermal physiology of *P. loveridgei*, with only two individuals tested (Brattstrom, 1970).

4.3.2 Study area, field surveys and sampling

This study was conducted in national parks within the Tweed Caldera region (mid-eastern Australia), located in Gondwana Rainforests of Australia World Heritage Area (Figure 4.1). To cover the altitudinal and distributional range of *P. loveridgei* sampling was undertaken at six sites, including three sites in southeast Queensland (Springbrook National Park) and three sites in northern New South Wales (Nightcap and Border Ranges National Parks) (Figure 4.1 and Table 4.1), ranging from 683 to 1083 m in altitude. Data was collected between October 2013 and January 2014.

Intensive frog surveys were conducted to capture a minimum of four frogs at each of the six sites. Surveys consisted of listening for male vocalisations and triangulating to find the source of the call. *Philoria loveridgei* frogs are highly cryptic terrestrial anurans due to their fossorial behaviour, making them hard to find. Frogs were found by carefully excavating under forest leaf litter or rocks searching for small burrows. Immediately after a frog was found its body temperature ($T_b$) was measured using a non-contact infrared thermometer (Digitech, Compact Infrared Thermometer QM-7221) applied to the middle of the dorsum. Burrow temperature was recorded by inserting a digital thermometer inside each burrow immediately after a frog was found (herein burrow temperature) (Wide Range Barrel
thermometer 31/149/0). All burrows were individually marked to prevent resampling the same individual and to allow return of individual frogs to their site of capture. To determine the environmental thermal profile of each site, one iButton® data logger was set 1 m above the ground to constantly monitor rainforest air temperatures. At each site, three iButtons® (Thermochron DS1921G) were set inside frog burrows to continuously monitor burrow temperatures (herein microhabitat temperature). In no instance were iButtons® exposed to incident solar radiation.

Figure 4.1 Study area indicating the location of the six *P. loveridgei* sites (red diamonds) surveyed in the national parks (green areas) throughout the species range in southeast Queensland (light green) and northeast New South Wales (dark green), Australia.

Frog sampling was biased towards male frogs as they remain in their burrows when calling to attract females. The morphological characteristics of all individuals, including weight and SVL, were measured before any testing. All animals were individually handled inside disposable plastic freezer bags to minimise water loss and disease transfer.
4.3.3 Thermal Physiology

Thermal physiology measurements were conducted in the field at each site to examine critical thermal maximum (CT\text{max}), critical thermal minimum (CT\text{min}) and preferred body temperatures (T_{\text{pref}}) of *P. loveridgei* individuals. A minimum of four frogs and a maximum of 12 frogs were tested at each site (Figure 4.1 and Table 4.1). All measurements were conducted in the field in close proximity to the site of capture to minimise stress and duration of captivity.

The loss of righting response (LRR) was used as the criterion to determine critical thermal limits (CT\text{max} and CT\text{min}) following a dynamic method (Brattstrom, 1968; Cowles and Bogert, 1944; Lutterschmidt and Hutchison, 1997). Each frog was tested only once, for either a CT\text{max} or CT\text{min} measurement and with the exception of one female, only male frogs were tested for critical thermal limits. All other captured females were gravid (N = 6).

Individual frogs were placed inside a clear cylindrical plastic chamber, floated on a water bath that was gradually heated or cooled at a rate of 0.5°C per minute and LRR tested (Figure 4.2a). Initial body temperature was measured using a non-contact infrared thermometer, at the middle of the frog’s dorsum. The LRR consisted of gently rotating the chamber to place the frog on its dorsum and waiting for it to reposition or right itself. The LRR test was repeated once every 0.5°C increase or decrease in temperature. We defined the critical limit as the first temperature (measured as air temperature inside the chamber) at which the frog failed to right itself within one minute of being placed on its dorsum. Immediately after frogs were tested for CT\text{max} individuals were placed inside clean plastic bags and floated on cool water to lower their body temperature until they reached their initial body temperature. Recovery from CT\text{min} involved immediately placing each frog inside a clean plastic bag and holding it between the researcher’s hands to increase body temperature until the initial body temperature was reached. Frogs were then kept at ambient temperature until being released at their place of capture.

Preferred temperatures (T_{\text{pref}}) were measured using a 1.5 m long, two-lane thermal gradient (Figure 4.2b). The thermal gradient was set up using Peltier heating/cooling plates at each end of an aluminium base to establish extreme temperatures of approximately 35°C at the hot end and 10°C at the cold. Twelve thermocouples were set up every 10 cm along the gradient and connected to a DataTaker® (dual channel data taker DT85) to continuously (every minute) record temperatures along the gradient. The gradient surface was covered with thin moist foam to provide a more natural substrate This gradient system allowed
simultaneous testing of two frogs. Gradient lanes were separated with an opaque plastic wall that impeded visibility between frogs. Initial body temperature was measured using a non-contact infrared thermometer that was pointed at the middle of the frog’s dorsum. At the start of the test frogs were released into the centre of the gradient in a low light environment and left undisturbed over a two hour period. Their position was recorded every minute using a time lapse camera controlled by a computer interface. The preferred temperature was determined by the median of the distribution of temperatures selected by each frog across the experiment period. Firstly, the position of each frog in the gradient was analysed using the image software ImageJ (Rasband, 1997-2015), by recording the number of pixels from the cold end of the gradient to the middle of the frog’s dorsum in each photo. The position of each thermocouple on the gradient was also converted to a pixel number using the same method. Secondly, each frog’s position was transformed into a selected substrate temperature using a 5th order polynomial calibration curve on the temperatures recorded by the thermocouples for each minute (Microsoft Excel). Lastly, we calculated the $T_{\text{pref}}$ of each frog as the median and interquartile range of the 120 measurements recorded across the two hour experiment and used this value for the analyses (R Development Core Team, 2015). A correction factor was applied to $T_{\text{pref}}$ before any analysis was done, to adjust the measured temperature to the frogs $T_b$. We calculated this factor as the average of subtracting the end $T_b$ of frogs (measured with the non-contact infrared thermometer) from the corresponding substrate temperature (measured by the thermocouples) recorded at the last position of the frog.

For all physiology measurements, no acclimatisation was done as the tests were conducted in the field shortly (no more than 2 h) after animals were captured. Each frog’s wellbeing was constantly monitored for the duration of the experiments and no mortality occurred. The thermal gradient and critical temperature chambers were cleaned and disinfected thoroughly between frogs, using 1% bleach solution and rinsed thoroughly with water after bleaching (Phillott et al., 2010). Foam substrate from the thermal gradient was replaced between frogs. At the end of both tests each frog was returned to its original site of capture.
Figure 4.2 Thermal physiology  

a) critical thermal limit chamber (transparent cylinder with yellow lid) floated on water bath.  

b) two lane thermal gradient covered with metallic mesh (frogs in centre of both lanes).

4.3.4 Data analysis

All statistical analyses were conducted using R software (R Development Core Team, 2015). Pearson correlations and linear regressions analysis were used to assess the relationships between CT\text{max}, CT\text{min} and T\text{pref} and explanatory variables including: altitude, SVL, body weight, T\text{b} (at time of capture), burrow temperature (at time of capture) and air temperature. A correlation and paired t-tests were used to analyse the relationship among daily mean rainforest air temperatures and daily mean microhabitat temperatures. We also analysed the average air temperatures of the previous 90 days needed to equal the mean microhabitat temperatures. This was done by performing a sequence of mean air temperatures over 90 days subtracted from microhabitat temperatures and running a linear regression.

To evaluate \textit{P. loveridgei} tolerance to a warming environment we calculated three different measurements: 1) under extreme temperature (WT), 2) under mean temperatures (WT\text{n}) and 3) within microhabitat under extreme temperature (WTh). Warming tolerance (WT) was estimated by subtracting the maximum air temperature (T\text{max}) from the mean CT\text{max} for each site (i.e. WT = CT\text{max} \text{ - } T\text{max}). Tolerance under mean temperatures (WT\text{n}) was estimated by subtracting the mean maximum air temperature from CT\text{max} for each site (i.e. WT\text{n} = CT\text{max}
– mean $T_{\text{max}}$). Microhabitat specific warming tolerance ($WT_h$) was calculated by subtracting the mean maximum microhabitat temperature from the mean $CT_{\text{max}}$ for each site ($WT_h = \text{mean } CT_{\text{max}} - \text{mean microhabitat } T_{\text{max}}$) (Deutsch et al., 2008; Scheffers et al., 2013).

### 4.4 Results

Critical thermal temperatures were measured for 47 individual frogs (46 males, 1 female). *Philoria loveridgei* displayed a narrow thermal tolerance range (Figure 4.3 and Table 4.1). The average $CT_{\text{min}}$ was $6.8 \pm 1.5^\circ\text{C}$ (mean ± SD) ($n = 23$, range 5 - 11°C), and the average $CT_{\text{max}}$ was $30.4 \pm 1.1^\circ\text{C}$ ($n = 24$, range 28 - 32.5°C). There was no significant correlation between altitude and either $CT_{\text{min}}$ ($r = 0.11$, $n = 21$, $P = 0.62$) or $CT_{\text{max}}$ ($r = 0.14$, $n = 22$, $P = 0.50$) of *P. loveridgei*, suggesting critical temperatures do not differ greatly along altitudinal or latitudinal gradients for this species (Figure 4.3).

Preferred temperature was measured for 48 *P. loveridgei* frogs (41 males, 7 females). Twelve frogs became inactive during $T_{\text{pref}}$ measurements by positioning themselves at one extreme of the gradient experiencing extreme hot (over 35°C) or cold (under 10°C) temperatures for extended time periods (over 40 min). To avoid using measurements from inactive frogs, we excluded these individuals (10 males, 2 females) from subsequent analyses. The remaining 36 frogs exhibited a wide range of $T_{\text{pref}}$ with an average of $15.4 \pm 6.0^\circ\text{C}$ (range 7.3 - 25.0°C) (Figure 4.4 and Table 4.1). No significant association was found between altitude and $T_{\text{pref}}$ ($r = 0.03$, $n = 36$, $P = 0.31$). There were no correlations between $T_{\text{pref}}$ and critical thermal limits.

Male frogs had mass of $2.5 \pm 0.2$ g ($n = 46$) and SVL of $19.0 \pm 4.0$ mm. Female frogs weighed $2.8 \pm 0.6$ g ($n = 8$) with SVL of $29.0 \pm 2.0$ mm. There were no correlations between body mass or SVL and $T_{\text{pref}}$ or critical thermal limits. Mean $T_b$ at time of capture was $16.3 \pm 2.1^\circ\text{C}$ (range 12.6 - 22.2°C). Average burrow temperature at time of capture was $16.8 \pm 1.4^\circ\text{C}$ (range 13.6 - 20.1°C). Burrow temperatures (at time of capture) and $T_b$ were significantly correlated ($r = 0.560$, $n = 54$, $P < 0.001$) and the difference between means was significant ($t = 2.17$, $n = 54$, $P = 0.034$), however, the effect size was less than 1°C. These results suggest that *P. loveridgei* are thermoconformers and thigmotherms. There was no significant correlation between altitude and $T_b$ ($r = -0.019$, $n = 54$, $P = 0.893$), or altitude and burrow temperature ($r = 0.26$, $n = 52$, $P = 0.06$).

The rainforest air temperatures were relatively stable throughout the study period, which included the Australian summer months where higher temperatures are typically
Table 4.1 Geographic variation in mean critical thermal temperatures ($CT_{\text{max}}$ and $CT_{\text{min}}$), median preferred temperatures ($T_{\text{pref}}$) and mean body temperature ($T_b$) (at time of capture) of *Philoria loveridgei*. The numbers of individual frogs are shown in brackets ($n$).

<table>
<thead>
<tr>
<th>Site</th>
<th>National Park</th>
<th>Altitude (m)</th>
<th>$T_{\text{pref}}$ °C ($n$)</th>
<th>$CT_{\text{max}}$ °C ($n$)</th>
<th>$CT_{\text{min}}$ °C ($n$)</th>
<th>$T_b$ °C ($n$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bar Mt 1</td>
<td>Border Ranges</td>
<td>1083</td>
<td>16.72 ± 4.8 (11)</td>
<td>30.9 ±0.65 (5)</td>
<td>6.9 ±1.1 (5)</td>
<td>16.6 ±0.82 (12)</td>
</tr>
<tr>
<td>Bar Mt 2</td>
<td>Border Ranges</td>
<td>977</td>
<td>13.39 ± 3.1 (7)</td>
<td>31.2 ± 0.57 (5)</td>
<td>7.1 ±1.4 (5)</td>
<td>17.9 ±2.7 (13)</td>
</tr>
<tr>
<td>Best of All Lookout</td>
<td>Springbrook</td>
<td>986</td>
<td>15.71 ± 7.7 (9)</td>
<td>29.6 ±1.0 (5)</td>
<td>6.8 ±2.7 (5)</td>
<td>15.0 ±1.5 (12)</td>
</tr>
<tr>
<td>Bilborough 2</td>
<td>Springbrook</td>
<td>848</td>
<td>17.59 ± 9.5 (2)</td>
<td>30.75 ±0.76 (3)</td>
<td>7.5 ±1.0 (3)</td>
<td>15.4 ±0.88 (6)</td>
</tr>
<tr>
<td>Bilborough 1</td>
<td>Springbrook</td>
<td>815</td>
<td>18.65 ± 8.9 (3)</td>
<td>30.0 ±0.35 (2)</td>
<td>5.5 ±0.71 (2)</td>
<td>13.8 ±0.82 (4)</td>
</tr>
<tr>
<td>Mt Nardi</td>
<td>Nightcap</td>
<td>683</td>
<td>11.14 ± 5.2 (4)</td>
<td>31.25 ±0.19 (4)</td>
<td>6.5 ±0.5 (3)</td>
<td>17.3 ±1.6 (7)</td>
</tr>
</tbody>
</table>
expected (Figure 4.5 a), c) and e). During the study period, mean maxima and minima at Best of All Lookout were 19.4°C and 15°C, at Bilborough 19.5°C and 14.8°C and at Mt Nardi 19.9°C and 16.7°C, respectively. Air temperature was significantly correlated with microhabitat temperature at these three sites (Best of All Lookout $r = 0.9$, $n = 208$, $P < 0.001$; Bilborough $r = 0.9$, $n = 208$, $P < 0.001$ and Mt Nardi $r = 0.8$, $n = 155$, $P < 0.001$) (Figure 4.5 b), d) and f). There was a time lag of 84 days’ to equal the average temperature within the microhabitat and the averaged air temperatures (average between the three sites). Warming tolerance was only estimated for these three sites where temperature data was available. We were unable to compare the temperature data of the other sites as several iButtons® failed. Warming tolerance was negative at two of the study sites, indicating that maximum air temperatures are higher than the $CT_{max}$ of $P. loveridgei$. This suggests that maximum air temperatures reached $CT_{max}$ at least once during the study period. Interestingly, $WT_n$ was positive showing that mean air temperatures has several degrees were several degrees cooler than lethal temperatures for $P. loveridgei$. Additionally, the $WT_n$ estimated temperatures were lower than $WT_n$ (Table 4.2).

Table 4.2 Warming tolerance ($WT$), mean warming tolerance ($WT_n$) and microhabitat warming tolerance ($WT_h$) of $Philoria loveridgei$ at three study sites. $WT_{h1}$ and $WT_{h2}$ represent measurements from two different burrows. All temperatures were measured for the duration of the study period.

<table>
<thead>
<tr>
<th>Site</th>
<th>$WT$ (°C)</th>
<th>$WT_n$ (°C)</th>
<th>$WT_{h1}$ (°C)</th>
<th>$WT_{h2}$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best of All Lookout</td>
<td>-3.5</td>
<td>10.2</td>
<td>12.2</td>
<td>11.3</td>
</tr>
<tr>
<td>Bilborough 2</td>
<td>-2.1</td>
<td>11.8</td>
<td>14.1</td>
<td>14.6</td>
</tr>
<tr>
<td>Mt Nardi</td>
<td>6.4</td>
<td>11.2</td>
<td>12.6</td>
<td>13.7</td>
</tr>
</tbody>
</table>
**Figure 4.3** Critical thermal limits $CT_{\text{max}}$ (solid triangle) and $CT_{\text{min}}$ (solid circle), body temperatures (solid diamonds) and burrow temperatures (unfilled diamonds) of *Philoria loveridgei* from the six study sites ($n = 47$).

**Figure 4.4** Range of preferred temperatures ($T_{\text{pref}}$) of *Philoria loveridgei* at each of the six study sites ($n = 36$).
**Figure 4.5** Daily maximum air temperatures (black dash line) and daily minimum air temperatures (grey dash and dot line) a) Best of All Lookout, c) Mt Nardi and d) Bilborough. Mean air temperatures (black dash line) and two mean microhabitat temperatures (solid lines) b) Best of All Lookout, d) Mt Nardi and f) Bilborough. All y-axis start at 10°C.
4.5 Discussion

The results of this study showed that *P. loveridgei*, a mountain-top frog, had a narrow tolerance range (CT\text{max} - CT\text{min}) for a subtropical thermoconformer living in a highly buffered thermal environment. Our data indicated that can *P. loveridgei* tolerate cold temperatures with a mean CT\text{min} of 6.8°C (± 1.5°C), consistent with its cryophilic (cold tolerant) classification (Brattstrom, 1970). In contrast, the estimated CT\text{max} indicated a mean of 30.4°C (±1.1°C), a relatively low value when compared with other amphibians (Brattstrom, 1968, 1970; Hutchison, 1961; Sunday et al., 2011; Zheng and Liu, 2010), and with other terrestrial direct-developing amphibian species (Scheffers et al., 2013; Sunday et al., 2011). This low value may be due to the small body size of this species (Heatwole et al., 1968; Seibel, 1970; Zheng and Liu, 2010) or to the exposure to relatively lower year round maximum temperatures (Duarte et al., 2012). In contrast, the CT\text{max} of *P. loveridgei* is higher when compared with two species of the same genus *P. frosti* (28.8°C) and *P. sphagnicola* (25.8°C) (Brattstrom, 1970). This could be explained by the latitudinal pattern of distribution of this genus, as *P. loveridgei* is the most northern of these three species. Several studies have reported higher CT\text{max} in amphibian species that inhabit tropical or subtropical areas compared to more temperate species (Brattstrom, 1970; Duarte et al., 2012; Sunday et al., 2011). The CT\text{max} estimated in this study is similar in magnitude (30.5°C) to that reported more than 40 years ago by Brattstrom (1970), who measured only two *P. loveridgei* individuals acclimated at 10°C, reinforcing the previous knowledge that CT\text{max} is a conservative trait (Addo-Bediako et al., 2000). Variations in critical limits and altitude have been reported, with CT\text{max} varying less with altitude than CT\text{min} (Sunday et al., 2011). Our data showed no significant difference in either critical limit in populations from different altitudes. These results are in agreement with the findings of Zheng and Liu (2010) who reported no change in laboratory measurements of CT\text{max} of acclimated *Paa spinosa* frogs from six populations in China. Our results contrast, however, with field CT\text{min} measurements of cane toads in Australia, where lower CT\text{min} were observed at higher altitude sites compared to lower sites (McCann et al., 2014). This difference, however, disappeared after laboratory acclimation of toads.

*Philoria loveridgei* is considered an endemic montane relict species (Brattstrom, 1970), from subtropical rainforests in mid-eastern Australia. Rainforest species that have evolved under reduced natural temperature variation, such as *P. loveridgei*, are expected to have reduced thermal tolerances because they may have become thermally specialised due to the reduced temperature variations they experience (Addo-Bediako et al., 2000; Deutsch et al., 2008; Huey et al., 2009; Laurance et al., 2011). Our results showed a narrow (23.6 °C)
thermal tolerance range reflecting the narrow thermal range of ambient temperatures experienced in the rainforest (mean 12 - 24°C), and the narrow thermal range measured within microhabitat temperatures (mean 15 – 19°C). Narrow thermal tolerances have been reported for other tropical and subtropical amphibian species including *Dendrobates auratus*, *Craugastor fleischmanni*, *Eleutherodactylus coqui* and *Pachymedusa dacnicolor* (see Appendix S1 in Sunday et al., 2011). Comparisons with these data must be interpreted with caution as different end points (LRR, onset of muscular spasm or lethal tolerance limit) were used to determine critical thermal limits (Lutterschmidt and Hutchison, 1997; Rezende et al., 2011). Our results support the expected trend that terrestrial amphibians living in subtropical rainforest have narrower thermal tolerance ranges (Addo-Bediako et al., 2000; Huey et al., 2012; Sunday et al., 2011). It has been posited that frogs inhabiting tropical and subtropical mountains and experience small temperature variations, may have limited acclimation ability in rapidly changing thermal environments thereby increasing their vulnerability to global warming (Wake and Vredenburg, 2008). In *P. loveridgei* however, microhabitat buffering could enhance its ability to acclimate enabling tolerance to slow seasonal changes.

The $T_{\text{pref}}$ results indicated a wide array of temperatures (7.3 - 25.0°C), suggesting that *P. loveridgei* frogs do not have a restricted preferred body temperature conforming to ambient temperatures, including high temperatures. This result implies that *P. loveridgei* frogs are thermoconformers. Our $T_{\text{pref}}$ results did not vary significantly with altitude, contrasting the previous report by Zheng and Liu (2010) which found an altitudinal association with $T_{\text{pref}}$ for the mountain spiny-frog (*P. spinosa*). These contrasting results could be due to differences in experimental design. In our study we measured $T_{\text{pref}}$ in the field over a 2 h testing period, compared to the laboratory-controlled environment and 10 h testing period used by Zheng and Liu (2010).

*Philoria loveridgei* $T_b$ were significantly correlated to burrow temperatures at time of capture, further confirming that this species is a thermoconformer. These findings are consistent with previous studies where frog’s body temperatures were closely related to substrate temperatures (Navas, 1996; Navas et al., 2013). Although the expected trend is a decrease in amphibian $T_b$ with altitude (Navas et al., 2013), our results show no significant correlation between $T_b$ and altitude. This may reflect the relatively narrow altitudinal range encompassed in the distribution of *P. loveridgei* (600 – 1300 m).
Figure 4.6 Theoretical thermal model for *Philoria loveridgei* based on the data measured in this study. Dashed line represents a theoretical performance curve (Huey et al., 2012), solid boxes represent observed thermal ranges.

As expected, rainforest air temperatures were correlated with microhabitat temperatures. *Philoria loveridgei* inhabits closed canopy rainforests, which are a thermally stable environment, providing a first buffer to increasing temperatures (see Chapter 3). Microhabitat temperatures, in general, had a narrower range of values than rainforest air temperature, suggesting that microhabitats provide a more stable thermal environment. Stable thermal microhabitats indicate they present fewer peak temperatures, buffering frogs from extreme air temperatures (e.g. very hot or very cold days). Microhabitat buffering becomes more evident as there is a long lag period (prior 84 days’) for microhabitat temperatures to equal rainforest air temperatures. With the predicted increase in severity and frequency of extreme weather events such as heat waves (IPCC, 2014), microhabitats (e.g. burrows) that can act as thermal buffers will be increasingly important as they will enable ectotherms to tolerate future warming. For example, Shoo et al. (2010) found that boulder microhabitats in Australia could buffer extreme temperatures by as much as 10°C. Our data showed that microhabitats (burrows) will effectively buffer extreme temperatures keeping thermal conditions below *P. loveridgei* physiological limits. This is important because changes in environmental conditions due to climate warming may shift maximum temperatures to exceed *P. loveridgei* physiological tolerance limits. Although our WT results
indicate that rainforest maximum temperatures sometimes reach and occasionally exceed $P. \textit{loveridgei}$ upper physiological limit (CT$_{\text{max}}$). Microhabitat buffering will decrease the effects of these extreme events. This was evident from our WT$_m$ results, which indicated that $P. \textit{loveridgei}$ microhabitat buffers rainforest air temperature by ≈1 - 3°C. This agrees with other similar amphibian studies where microhabitat temperatures are reported to mitigate raising maximum environmental temperatures (Scheffers et al., 2013; Scheffers et al., 2014). Our results suggest that $P. \textit{loveridgei}$ could tolerate environmental warming by maintaining its burrowing behaviour, and that extreme events will be effectively buffered during predicted low and moderate global warming scenarios. If extreme predicted global warming scenarios eventuate, microhabitat buffering will be less effective and fail to provide adequate refuge.

Results from this study propose that increasing temperatures due to climate changes are unlikely to drive adult $P. \textit{loveridgei}$ physiological limits outside their thermal tolerance range in the short to medium term. We did not however, account for the potential impacts of temperature in other life stages (eggs and metamorphs). A recent study found that eggs from direct-developing frogs laid in exposed leaves or in bird’s nest ferns, were more vulnerable to increased temperatures than aquatic breeder species (Scheffers et al., 2013). Our findings suggest this would not be the case for $P. \textit{loveridgei}$ as this species lays its eggs inside the thermally buffered microhabitats (burrows), providing protection against warming temperatures. Climate warming will not only increase air temperature, but will also affect terrestrial environment moisture content (Pounds et al., 2006) increasing the vulnerability of terrestrial leaf litter ectotherms (Duarte et al., 2012). Our study did not account for the potential impacts of changes in evaporative water loss or rainfall patterns. A modelling study showed the average population extirpation risk of montane biodiversity increased 10-fold when changes in rainfall were included to the models (McCain and Colwell, 2011). It is possible that $P. \textit{loveridgei}$ could experience decreased microhabitat moisture (soil and leaf litter moisture) and increased cutaneous evaporative water loss. Changes in rainfall patterns could decrease soil moisture which would decrease nest moisture decreasing eggs and larval survival (see Cahpter 2). This could affect all stages of development of this species, particularly metamorphs due to their high surface area to volume ratio and terrestrial-laid eggs which cannot relocate to more favourable microhabitats as conditions change. Any decrease in egg and larval survival could reduce juvenile recruitment rates affecting population size and leading to declines (Duarte et al., 2012).

In summary, our results suggest that subtropical thermoconformers like $P. \textit{loveridgei}$ could physiologically cope with increases in environmental temperatures, as they are
buffered from air temperature fluctuations within their microhabitat (leaf litter and burrows). Our data highlights the importance of considering microhabitat refuges when investigating the warming vulnerability of terrestrial ectotherms. This study provides valuable insight into the thermal biology of ectotherms restricted to mountain-tops, and our understanding of their vulnerability to climate change. Future research should focus on modelling microhabitat usage of this ectotherm beyond burrows, by gathering more detailed geo-climatic (e.g. soil moisture) and physiological data (e.g. operative temperatures and water loss) (Kearney and Porter, 2009). This could be done using a combination of agar models, soil moisture sensors and mechanistic niche modelling, among others (Kearney et al., 2009; Kearney et al., 2013; Navas and Araujo, 2000; Sinervo et al., 2010). Our results provide a better understanding of the vulnerability of *P. loveridgei* to climate change and extinction risk, which will aid conservation planning and management of this endemic terrestrial amphibian.

### 4.6 Acknowledgments

This research was supported by Griffith University and James Cook University Collaborative Grants Scheme. Funding from Tricia Waters (OEH) and the Advisory Committees of the Gondwana Rainforests of Australia World Heritage Area to MFL, GI and JMH are acknowledged. MFL was also partially funded by School of Environment, Griffith University. This study was conducted under a Scientific Purpose permit issued by the Department of Environment and Resource Management (WITK10308811), and was approved by Griffith University Animal Ethics Committee (ENV/21/12AEC). We thank Dr Clare Morrison for her valuable mentorship and comments on earlier drafts of this manuscript. A special thanks to all field volunteers.

### 4.7 References


CHAPTER 5 –

*Philoria loveridgei* susceptibility to chytridiomycosis

The previous results chapter examined the thermal physiology of *P. loveridgei* within six national parks covering the altitudinal and geographical distributional range of the species. Thermal physiology is a second sensitivity factor assessed in this thesis to evaluate the vulnerability of *P. loveridgei* to climate change. The conclusion drawn in chapter 4 was that *P. loveridgei* is a thermoconformer and thigmotherm with a narrow thermal tolerance range. It also describes the importance of habitat (i.e. rainforest canopy cover) and microhabitat (i.e. burrows) thermal buffering.

The current results chapter examines a third sensitivity factor by investigating the susceptibility of *P. loveridgei* to the fungal disease chytridiomycosis. To the best of our knowledge no work has been published on the susceptibility of this direct-developing species to chytridiomycosis and the prevalence of the fungus in *P. loveridgei* wild populations.

This chapter will be submitted to the journal EcoHealth and has been formatted to fit the journal style. The citation will be as follows:


Co-authors of this manuscript are an external adjunct advisor, Assoc. Prof. Vance T. Vredenburg (whose area of expertise includes amphibian declines and chytridiomycosis) and my thesis supervisors Dr Gregory Lollback and Prof. Jean-Marc Hero. My (Mariel Familiar López) contribution to the manuscript included the initial concept and survey design, field sample collection, statistical analyses and preparation of the manuscript. Laboratory sample analyses involving PCR were conducted by Vance T. Vredenburg.
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Jean- Marc Hero (Principal Supervisor)
5.1 Abstract

Amphibians are declining worldwide, with many species facing significant declines and even extinction. Chytridiomycosis is an infectious disease caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*) that has been identified as a major cause of amphibian declines globally and specifically linked to species extinctions in Australia. This fungal disease is widespread among Australian frogs, especially in aquatic breeding species, but is rarely detected in terrestrial direct-developing species. The aim of this study was to determine the susceptibility and presence of *Bd* in wild populations of a threatened direct-developing frog (*Philoria loveridgei*). We hypothesise that populations of *P. loveridgei* will present low levels of *Bd* infection and prevalence because of their terrestrial breeding behaviour. Frog skin swabs and real-time quantitative PCR analyses were used to test for *Bd* intensity and prevalence in 79 *P. loveridgei* frogs (71 males, 8 females). *Bd* was detected in seven individuals (6 males, 1 female). The pathogen was present in two of the eleven sites surveyed and was found at low infection intensity and prevalence. This study is the first to report *Bd* in wild populations of *P. loveridgei*. No population declines have been reported for this species, suggesting wild populations occur in an enzootic state with *Bd* and that host populations are not severely affected. Seasonal monitoring of populations, including dynamic information on *Bd* infection of host, should be undertaken to evaluate and manage the conservation status of this threatened species.

**Keywords:** Australia, *Batrachochytrium dendrobatidis*, prevalence, *Philoria loveridgei*, terrestrial.
5.2 Introduction

Amphibians are declining worldwide, with many species facing significant declines and extinction (Collins and Storfer 2003; Stuart et al. 2004; Wake and Vredenburg 2008; Wake 2012). Australian amphibians are no exception; of the 241 recognised species over 20% are threatened, most have declined, and at least three species are considered extinct (Hero et al. 2006; Hero et al. 2015). A significant proportion of threatened Australian species are concentrated in upland areas (41%) (Hero and Morrison 2004; Hero et al. 2006; Hero et al. 2015).

Globally, climate change and infectious diseases (e.g. fungal infections) have played a major role in population declines, and Australia is no exception (Collins and Storfer 2003; Stuart et al. 2004; Collins 2010). Principal among infectious diseases is chytridiomycosis a fungal infection caused by *Batrachochytrium dendrobatidis* (*Bd*) and positively identified as a cause of amphibian declines worldwide and in Australia (Berger et al. 1998; Daszak et al. 1999; Longcore et al. 1999; Lips et al. 2006; Skerratt et al. 2007; Wake and Vredenburg 2008; Fisher et al. 2009b). Amphibian species exhibit diverse susceptibility to *Bd* infection: some species do not exhibit any apparent symptoms while others are extremely vulnerable, frequently succumbing to cardiac arrest (Rollins-Smith 2009; Voyles et al. 2009; Kilpatrick et al. 2010; Murray et al. 2010b). This disparity is reflected in the variation in *Bd* prevalence among species and sites, as well as differences in intensity of infection among species worldwide (for review see Kilpatrick et al. 2010). Subsequently, chytridiomycosis can have different impacts on wild amphibian populations ranging from no apparent effect to devastating entire amphibian populations (Berger et al. 1998; Briggs et al. 2005; Kriger and Hero 2006b; Lips et al. 2006; Rachowicz et al. 2006; Vredenburg et al. 2010). In Australia, *Bd* has been directly associated with declines of at least five amphibian species (Hero and Morrison 2004) and has been found in species from three amphibian families (Hylidae, Microhylidae and Myobatrachidae) (Skerratt et al. 2008; Murray et al. 2010a). The pathogen is widespread throughout the coastal zone of Australia (Berger et al. 1998; Murray et al. 2011), mainly on the eastern seaboard of Australia (Murray et al. 2010a).

Disease transmission involves complex relationships between several factors that can facilitate or hinder transmission, including: host behaviour, environmental factors, host microhabitat, host immune system, and host skin microbiota, among others (Harris et al. 2006; Rowley and Alford 2007; Rollins-Smith 2009; Rollins-Smith et al. 2011; Voyles et al. 2011; Hauselberger and Alford 2012; Becker et al. 2014). Australian *Bd* studies have revealed that *Bd* is strongly associated with aquatic habitats (Kriger and Hero 2007a) and in
frogs that hibernate in aquatic microhabitats rather than terrestrial habitats (Longcore et al. 2007; Skerratt et al. 2010). This pattern of aquatic amphibian species presenting higher infection and prevalence rates has also been reported among other amphibian species worldwide and has lead researchers to hypothesise that terrestrial species may avoid Bd infection through microhabitat protection (Berger et al. 1998; Lips et al. 2003; Kriger and Hero 2007a; Brem and Lips 2008; Catenazzi et al. 2011). Terrestrial amphibians, however, can be infected with Bd, with some species experiencing population declines (Berger et al. 1998; Bell et al. 2004; Burrowes et al. 2004; Lips et al. 2006; Kriger and Hero 2007a; Brem and Lips 2008; Longo and Burrowes 2010; Catenazzi et al. 2011). Little is known of the prevalence of Bd in Australian terrestrial (direct-developing) species although they include 27 species from two families and four genera (Microhylidae: Cophixalus and Austrochaperina; and Myobatrachidae: Assa and Philoria) (Hero et al. 2005; Anstis 2013; Hero et al. 2015). To the best of our knowledge, only a few Australian direct-developing species have been reported with Bd infections. Negative Bd infection was reported on direct-developing Australian microhylids of the Wet Tropics (Hauselberger and Alford 2012). However, Bd infection was reported for Cophixalus ornatus and Assa darlingtoni (Kriger and Hero 2006a, 2007a). Population declines have not been associated with Bd among direct-developing species in Australia (Hero et al. 2005; Hero et al. 2006; Hero et al. 2015). These limited and inconclusive results warrant further investigation of Bd susceptibility in Australian terrestrial amphibians.

Philoria loveridgei is a mountain-top species endemic to the Gondwana Rainforest of the Tweed Caldera Rim in mid-eastern Australia (Knowles et al. 2004). This species is diurnal and terrestrial with direct-developing tadpoles laid as eggs in burrows under leaf litter or rocks on the forest floor. Tadpoles develop inside their burrows in a gel-like substrate deposited by the female until young metamorphic frogs emerge (Seymour et al. 1995; Knowles et al. 2004; Anstis 2013). Frog declines in montane areas of eastern Australia have been strongly associated with species presenting an aquatic life stage (Hero et al. 2005; Hero and Morrison 2012), and this has been linked to Bd (Longcore et al. 1999; Piotrowski et al. 2004; Berger et al. 2005a; Longcore et al. 2007). In this study we examine the prevalence and infection intensity of Bd in wild P. loveridgei populations. We hypothesise that P. loveridgei will present low levels of infection intensity and prevalence of Bd suggested by their terrestrial breeding behaviour.
5.3 Methods

5.3.1 Study area and sample collection

This study was undertaken in national parks in the Tweed Caldera Rim, located in mid-eastern Australia within Gondwana Rainforests of Australia World Heritage Area (Figure 5.1). Frog sampling was conducted at eleven sites which cover the altitudinal and distribution ranges of *P. loveridgei*, including seven sites in southeast Queensland (Springbrook and Lamington National Parks), and four sites in northeast New South Wales (Nightcap and Border Ranges National Parks) (Figure 5.1 and Table 5.1). These sites were selected as they are highly occupied by the target species (see Chapter 3). The sampling area is characterised by gullies in headwaters and seepage zones in subtropical rainforest of the Great Dividing Range (McDonald 2010; Anstis 2013). Field sampling took place during three separate *P. loveridgei* breeding seasons (Australian spring and summer months) from October 2011 to January 2014.

Frogs from the genus *Philoria* are a group of highly cryptic terrestrial amphibians that construct burrows under forest leaf litter or rocks. Diurnal calling surveys, with a minimum target of ten frogs / site, were conducted because calling activity for these species peaks during daylight (see Chapter 2). Visual surveys consisted of microhabitat surveys and intensive burrow searches performed by carefully excavating under leaf litter or rocks at sites where calling males were heard. *Philoria loveridgei* frogs, particularly males, were found sitting inside small flask shape burrows (≈ average dimensions depth 4.4 cm and wide 3.9 cm). Call mimicry was sometimes used to incite males to call, thereby aiding the discovery of their location.

Immediately after frogs were captured, we used a non-invasive skin swabbing technique to test for the presence and infection intensity of the amphibian fungal pathogen *Bd* (Brem et al. 2007; Hyatt et al. 2007; Kriger and Hero 2007b). This technique consisted of swabbing across the frog’s skin, using sterile dry swabs (MW113; Lakewood Biochemical CO., USA) following the methods described by Kriger et al. (2006) and Hyatt et al. (2007) for a total of 35 strokes per frog (Vredenburg et al. 2010). Swabs were immediately placed in individually labelled microtubes and stored in a dry, cool place until transported back to the laboratory and stored at 4°C. To ensure consistency in swabbing technique all frogs were swabbed by MFL (Kriger et al. 2006; Simpkins et al. 2014).
The morphological characteristics of all individuals, including body mass and size (snout-vent length, SVL), were recorded after swab samples were taken. Every frog was captured and handled at all times using individual, clean unused freezer plastic bags. All equipment used was washed with a solution of commercial bleach (1:9, bleach:water) and thoroughly cleaned between collection sites to prevent potential spread of the fungus (Phillott et al. 2010). Frog sampling was biased towards male frogs as they remain in their burrows when calling to attract females.
5.3.2 Laboratory methods and data analyses

Frog skin swabs and real-time quantitative PCR (qPCR) analyses were used to test for \textit{Bd} infection, prevalence and intensity. Swabs were analysed at San Francisco State University (California, USA), using the protocol described by Boyle et al. (2004) with the following changes: swab extracts were analysed singly instead of in triplicate (Kriger and Hero 2007a; Vredenburg et al. 2010) and BSA was added to the qPCR master mix (1\u00b0L BSA per reaction) (Garland et al. 2010).

Prevalence of \textit{Bd} was calculated as the number of frogs that were \textit{Bd} positive (when zoospore equivalents were $\geq 1$) relative to the total number of frogs sampled. Infection intensity was defined as the number of zoospore equivalents per swab. Zoospore equivalents were estimated by multiplying the qPCR genomic value by 80, as DNA extracts from swabs were diluted 80-fold during extraction and qPCR (Briggs et al. 2010; Vredenburg et al. 2010).

Pearson correlations were used to assess the relationship between altitude, body size, weight, and the intensity of \textit{Bd} infection. Analyses were conducted using R statistical software (R Development Core Team 2015).

5.4 Results

A total of 79 \textit{P. loveridgei} frogs were tested (71 males, 8 females), with \textit{Bd} detected in only seven. Overall prevalence was 8.86\% ($n = 7$, 6 males and 1 female), and the pathogen was detected in two of the eleven sites surveyed. The majority of infected frogs were from one site located in Border Ranges National Park (Bar Mt area). This site had a \textit{Bd} infection prevalence of 35.3\% ($n = 6$, 5 males and 1 female) (Table 5.1). The only other \textit{Bd} infected frog we detected was from Nightcap National Park. The park had an overall prevalence of 1.27\% and the site had a prevalence of 12.5\% ($n = 1$ male) (Table 5.1). Both sites where the pathogen was detected are located in the southern limits of \textit{P. loveridgei}'s distribution range in New South Wales, Australia (Figure 5.1). These sites have a difference in altitude of 300 m; the Nightcap site was the lowest area surveyed in this study. Altitude however, was not significantly correlated with \textit{Bd} infection ($r = -0.174$, $P = 0.124$, $n = 79$).

The overall infection intensity was relatively low with an average of $34.2 \pm 47.7$ zoospore equivalents (mean $\pm$ standard deviation). Infection intensity ranged from as low as 0.79 to 124.8 zoospore equivalents. The frog presenting the highest infection intensity (124.8 zoospore equivalents) was from the Nightcap site (Table 5.1).
Male frogs weighed an average 2.2 ± 0.7 g and had a mean SVL of 25.7 ± 0.3 mm. Female frogs were bigger than male frogs weighing an average 2.75 ± 0.6 g and had a mean SVL of 28.8 ± 0.2 mm. No association was found between sex of the frog and infection status. Size and weight of frogs were not significantly correlated with Bd intensity (size; $r = 0.051$, $P = 0.658$, $n = 79$; weight; $r = -0.136$, $P = 0.233$, $n = 79$). No clinical signs of chytridiomycosis were observed in any of the frogs surveyed (Berger et al. 1998; Berger et al. 2005c).

5.5 Discussion

This study confirms that *P. loveridgei*, a terrestrial mountain-top frog with direct-development, is susceptible to infection by the amphibian pathogen *Bd*. Our results are the first to document the presence of *Bd* in *P. loveridgei*. We found *Bd* in two wild populations at low (683 m) and mid-high (977 m) altitude subtropical rainforest sites in mid-eastern Australia. This suggests that *P. loveridgei* populations are likely to be exposed to *Bd* at all altitudes. These results indicate low intensity of infection and low disease prevalence in *P. loveridgei* wild populations.

Infection intensity on hosts is a key variable in predicting disease dynamics in *Bd* amphibian systems (Vredenburg et al. 2010; Kinney et al. 2011). Several studies have suggested a threshold of 10,000 zoospore equivalents as benchmark where highly infected individuals usually do not recover from *Bd* infections (Vredenburg et al. 2010; Kinney et al. 2011; Reeder et al. 2012). In the present study, we found very low infection intensity, several orders of magnitude below the threshold. Our findings are in agreement with those observed in the only two other Australian direct-developing species *C. ornatus* and *A. darlingtoni* where *Bd* infection intensity was also relatively low (31 and 189 zoospores equivalents, respectively) (Kriger and Hero 2006a, 2007a). The low *Bd* infection intensities we found may be a result of the time of the year sampling was undertaken (e.g. in mid-eastern Australia summers are warm and wet) (Berger et al. 2004; Woodhams and Alford 2005; Kriger and Hero 2007b; Longo et al. 2010). A study on wild populations of two terrestrial direct-
Table 5.1 Location, altitude, geographic position, *Bd* prevalence and intensity of sites surveyed in this study.

<table>
<thead>
<tr>
<th>Site</th>
<th>National Park</th>
<th>Latitude (°)</th>
<th>Longitude (°)</th>
<th>Altitude (m)</th>
<th># frogs (# infected)</th>
<th><em>Bd</em> site prevalence</th>
<th><em>Bd</em> zoospore equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bar Mt picnic area</td>
<td>Border Ranges</td>
<td>-28.45743</td>
<td>153.13272</td>
<td>1083.1</td>
<td>12 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bar Mt</td>
<td>Border Ranges</td>
<td>-28.45725</td>
<td>153.12484</td>
<td>977.1</td>
<td>17 (6)</td>
<td>35.3 %</td>
<td>19.10 ± 28.6</td>
</tr>
<tr>
<td>Brindle Creek</td>
<td>Border Ranges</td>
<td>-28.37883</td>
<td>153.06997</td>
<td>773</td>
<td>1 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Binna Burra 1</td>
<td>Lamington</td>
<td>-28.21606</td>
<td>153.19702</td>
<td>892.8</td>
<td>5 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Binna Burra 2</td>
<td>Lamington</td>
<td>-28.25444</td>
<td>153.20199</td>
<td>1020.6</td>
<td>4 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Binna Burra 3</td>
<td>Lamington</td>
<td>-28.22087</td>
<td>153.19271</td>
<td>859.9</td>
<td>4 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mt Nardi</td>
<td>Nightcap</td>
<td>-28.53798</td>
<td>153.29052</td>
<td>683.4</td>
<td>8 (1)</td>
<td>12.5 %</td>
<td>124.8</td>
</tr>
<tr>
<td>Best of all lookout</td>
<td>Springbrook</td>
<td>-28.24178</td>
<td>153.26462</td>
<td>986.3</td>
<td>16 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bilborough new</td>
<td>Springbrook</td>
<td>-28.23111</td>
<td>153.28775</td>
<td>867.4</td>
<td>1 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bilborough 2</td>
<td>Springbrook</td>
<td>-28.23011</td>
<td>153.28720</td>
<td>848.5</td>
<td>6 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bilborough bridge</td>
<td>Springbrook</td>
<td>-28.22776</td>
<td>153.28551</td>
<td>815.2</td>
<td>5 (0)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
developing frogs *Eleutherodactylus coqui* and *Eleutherodactylus portoricensis*, in Puerto Rico, found a synergistic interaction between *Bd* prevalence and infection intensity and seasons (Longo et al. 2010). In this study, authors propose that lower infection intensity (mean number of zoospores) in the wet and warm season and higher infection intensity in the dry and cool season is a result of differential behavioural patterns. Aggregation behaviour in the host was observed during the dry season, while dispersion of host, via territoriality, occurs during the wet season. Additionally, Berger et al. (2004) found that infected Australian frogs from Queensland and New South Wales frequently died during winter, when temperatures are low and in the optimal growth range of *Bd*, in contrast with summer months when mean temperatures are high and can restrict *Bd*. This could explain the relatively high prevalence of *Bd* infection observed in the study site Border Ranges National Park, where sampling took place over the Australian warm and wet summer months. Heavily infected terrestrial frogs remain inactive and hidden at their retreats in dry and cool conditions, hindering their identification. In contrast, during warm and wet seasons frogs have lower infection levels and are more active in the forest, increasing the probability of sampling an infected frog and yielding higher prevalence estimates (Longo et al. 2010). In wild *P. loveridgei* populations, higher *Bd* infection intensity may be found in seasons of the year we did not sample (e.g. cool and dry winter). Gathering data for this cryptic frog species in non-breeding months however is an extremely difficult task because individuals remain in their underground burrows. Because high *Bd* infection intensity implies susceptibility to chytridiomycosis (Vredenburg et al. 2010), the low level we discovered in this frog species could suggest low predisposition to the infection, due to a more effective immune system, antimicrobial skin peptides or the presence of skin microbiota (Harris et al. 2006; Harris et al. 2009; Rollins-Smith 2009). A *Bd* susceptibility laboratory experiment on *C. ornatus*, a terrestrial breeding species, indicated no mortality when exposed to high concentrations of *Bd* zoospores and even clearing of infection (Hauselberger 2011). Taken together these results suggest that terrestrial amphibian species are less vulnerable to *Bd* infection and that this could be related to innate immune systems, antimicrobial peptides or skin microbiota (Hauselberger and Alford 2012).

*Philoria loveridgei* are found in small isolated populations forming small groups of discrete burrows in gullies at stream headwaters. The low *Bd* prevalence (8.86%) we found could be a result of their breeding behaviour and microhabitat inhabited (non-stream dwelling and a lack of constant running water). Terrestrial amphibians with direct-development spend a reduced amount of time in contact with water, hence limiting their probability of getting infected with *Bd*
(Lips 1999; Lips et al. 2006; Kriger and Hero 2007a). Alternatively, low Bd prevalence could also be explained by the isolated patchy distribution of P. loveridgei probably limiting disease transmission.

Results presented here are the first to confirm that P. loveridgei frogs are susceptible to Bd infection, supporting the only two other reports of wild individuals of C. ornatus and A. darlingtoni infected with Bd (Kriger and Hero 2006a, 2007a). There are several hypotheses that can explain the proposed enzootic state between Bd and terrestrial breeding amphibian species: 1) host microhabitat selection limits Bd transmission and growth (breeding and living habitat), 2) microorganisms in the environment limits Bd transmission and growth, 3) the thermal environment limits Bd transmission and growth, 4) host behaviour limits Bd transmission and growth, 5) host immune system limits Bd transmission and growth, 6) host skin microbiota limits Bd transmission and growth, 7) Bd strain and 8) a dilution effect causes Bd to fade out of host population. Below we discuss how each of these could apply to P. loveridgei.

Host microhabitat selection influences host-pathogen dynamics (Kriger and Hero 2007a; Rowley and Alford 2007; Brem and Lips 2008; Gründler et al. 2012). Although, it had been hypothesised that terrestrial frogs could be more resistant to Bd as they lack aquatic life stages, terrestrial breeding species are infected by the pathogen in the wild (Lips et al. 2003; Bell et al. 2004; Burrowes et al. 2004; Lips et al. 2006; Brem and Lips 2008; Longo et al. 2010; Catenazzi et al. 2011). In general, species that depend on aquatic environments have higher Bd infection levels and prevalence than terrestrial species (Lips et al. 2006; Kriger and Hero 2007a; Rowley and Alford 2007; Becker et al. 2014), and terrestrial species have lower Bd infection intensity and prevalence presumably due to the lack of use of water bodies (Lips 1999; Lips et al. 2006; Kriger and Hero 2007a). These could explain the low infection levels and prevalence found in this study. Aquatic micro-predators may directly limit Bd zoospores and lower Bd transmission (Buck et al. 2011; Hamilton et al. 2012; Schmeller et al. 2014). Thermal environments can influence disease dynamics including prevalence (Berger et al. 2004; Retallick et al. 2004; Drew et al. 2006; Kriger and Hero 2007b, a; Longo et al. 2010). Several laboratory studies have explored the thermal ecology of Bd and concluded that cooler temperatures (<23°C) increase Bd pathogenicity and increased temperatures decrease it (Longcore et al. 1999; Woodhams et al. 2003; Berger et al. 2004; Piotrowski et al. 2004). Additionally, various field studies have found evidence supporting this pattern (Woodhams and Alford 2005; Kriger and Hero 2007b; Kriger et al. 2007). For example, prevalence was related to annual environmental changes, in
*Litoria wilcoxii* (a stream breeding species), with higher prevalence levels in winter and early spring months when the weather is cool and dry (Kriger and Hero 2007b). *Philoria loveridgei* sites are located on mountain-top rainforests where thermal regimes are suitable for *Bd*. Host behaviour is also thought to strongly influence *Bd* transmission, where it can occur via direct contact between an infected frog and an uninfected frog or it can occur via a *Bd*-contaminated substrate and an uninfected frog (Piotrowski et al. 2004; Rachowicz and Vredenburg 2004; Lips et al. 2006; Rowley and Alford 2007). Both laboratory and field based studies have demonstrated *Bd* transmission through direct frog-to-frog contact (Parris and Cornelius 2004; Rowley and Alford 2007; Longo and Burrowes 2010). Frog to frog contact increases when frogs aggregate, as was shown in direct-developing *E. coqui* from Puerto Rico (Joglar et al. 2007; Longo and Burrowes 2010). Although similar clumping behaviour has not been observed for *P. loveridgei*, they are found in small groups in discrete burrows in gullies at stream headwaters. Disease could be transmitted amongst individuals within these groups, particularly between males and females when in amplexus.

*Bd* transmission can also occur from frog to frog via environmental contact. For example, previous studies have found that *Bd* zoospores can swim and persist for some time in running or still water (Johnson and Speare 2003; Parris and Cornelius 2004; Piotrowski et al. 2004; Rachowicz and Vredenburg 2004; Lips et al. 2006; Rowley and Alford 2007). *Bd* has also been found on substrates such as moist rocks, sticks and leaf litter (Richards-Zawacki 2010). Moreover, long distance movement of stream breeding amphibian species could disperse the pathogen onto terrestrial substrates encountered by terrestrial amphibians that never go to water, thus acting as vectors for disease dispersal (Retallick et al. 2004). The frequency of contact with an infected frog or contaminated environment is related to host behaviour and will facilitate *Bd* infection in direct-developing species, such as *P. loveridgei*.

Host immune systems in amphibians are also known to play a key role in *Bd*-host disease dynamics. For example, several antimicrobial peptides have been recognised as an important mechanism to reduce *Bd* infection (Rollins-Smith 2009; Woodhams et al. 2010; Walke et al. 2011). Little is known about the immune defences of *P. loveridgei*. Amphibian skin microbiota such as skin bacteria may also affect *Bd* transmission and *Bd*-host disease dynamics. Laboratory studies have demonstrated that several species of bacteria present on amphibian skin may inhibit *Bd* growth and consequently reduce infection intensity (Harris et al. 2006; Woodhams et al. 2007b; Harris et al. 2009; Lam et al. 2010; Walke et al. 2011). Another factor
that could affect susceptibility to \emph{Bd} infection is the strain of \emph{Bd} present in the host populations. Recent population genetics studies have found that different genetic strains of \emph{Bd} are associated with epizootic and enzootic sites (Rosenblum et al. 2013), and laboratory experiments revealed differences in amphibian time till death and mortality rates for individual infected with different \emph{Bd} strains (Berger et al. 2005b; Fisher et al. 2009a; Gahl et al. 2012). High host diversity may also reduce the effects of chytridiomycosis. A laboratory experiment reported lower \emph{Bd} infection values when comparing multi-host treatments with single-host treatments, particularly, when a terrestrial host was included, as this species showed decrease infection levels while aquatic neighbouring species experience increased infection (Becker et al. 2014).

The \emph{Bd} infection intensity and \emph{Bd} prevalence pattern we observed in \emph{P. loveridgei} suggest an enzootic state between the host and pathogen. Despite finding populations of \emph{P. loveridgei} infected with \emph{Bd}, we found no clinical signs of chytridiomycosis or death related to this illness, nor have they been reported elsewhere. Additionally, no population declines related to \emph{Bd} have been documented for any Australian direct-developing amphibian species (Hines et al. 1999; Hero et al. 2006; Hauselberger and Alford 2012) in contrast with reports from other parts of the world (Bell et al. 2004; Burrowes et al. 2004; Lips et al. 2006; Longo and Burrowes 2010; Longo et al. 2013). These results imply that Australian direct-developing amphibian species are surviving in an enzootic state with \emph{Bd} perhaps through an innate immune response that could be providing them with protection against \emph{Bd}, or by some other method described in the above hypotheses. Brem and Lips (2008) suggests that terrestrial amphibians may share similar skin microbial fauna that provides them with protection to \emph{Bd} and in turn make them less susceptible as a group. A study of four Australian frogs showed higher \emph{Bd} resistance in species with powerful skin peptide defences when experimentally infected with \emph{Bd} (Woodhams et al. 2007b). Other studies have shown the inhibitory role of skin bacteria in the growth of \emph{Bd} (Harris et al. 2006; Woodhams et al. 2007a; Harris et al. 2009; Walke et al. 2011). Future research should focus on investigating the bacterial skin community in Australian direct-developing amphibian species to elucidate their possible role in \emph{Bd} infection susceptibility. Additionally, research should focus on how this microbial community is acquired in these terrestrial species (Walke et al. 2011).

In summary, our study is the first to report \emph{Bd} infections in wild populations of \emph{P. loveridgei}. No population declines have been reported for this species suggesting populations co-exists with \emph{Bd} in the wild and are not severely affected. Although our results suggest that
populations of *P. loveridgei* have a low risk to decline due to *Bd* infection, we recommend undertaking seasonal monitoring of populations to better evaluate the conservation status and manage this threatened species. We discussed several factors that may stabilise the *Bd*-host relationship in other systems. Further research on the effectiveness of these factors in Australian terrestrial direct-developing frogs is needed to fully describe infection dynamics and pathogen-host interactions.

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5.7 References


CHAPTER 6-
General Discussion

6.1 Introduction

Evidence clearly shows that climate change has affected ecosystems and individual species, challenging species survival and increasing extinction rates (Bellard et al., 2012; Foden et al., 2013; Moritz and Agudo, 2013). Assessing the possible threats climate change poses to a species requires information about its vulnerability (Williams et al., 2008; Pacifici et al., 2015). Vulnerability assessments generally involve identifying which aspects of a species ecological and evolutionary biology determine their vulnerability (Williams et al., 2008). Vulnerability to climate warming, however also depends on species sensitivity and exposure (Williams et al., 2008), with some species more vulnerable than others (Moritz and Agudo, 2013). For example, it has been reported that thermal specialist, particularly species that require cool conditions, are highly vulnerable to a warming environment (Laurance et al., 2011). Ectotherms, and particularly amphibians, are also highly vulnerable to the effects of climate change (Carey and Alexander, 2003; Corn, 2005; Deutsch et al., 2008; Hagger et al., 2013; Li et al., 2013). Despite the identification of some vulnerable groups, uncertainty remains as to what extent and which species will be most vulnerable? (Williams et al., 2003; Thomas et al., 2004; Pimm, 2008; Blaustein et al., 2010; Penman et al., 2010; Moritz and Agudo, 2013). A general integrative framework was developed by Williams et al. (2008), proposing that a species’ vulnerability would depend on its sensitivity (biotic vulnerability), exposure (regional and local), resilience and potential to adapt (evolutionary and ecological) (Figure 6.1). Since then, several theoretical frameworks focusing on multiple factors involved in a species vulnerability to climate change have been developed. All agree that, sensitivity and exposure are critical (Kearney and Porter, 2009; Rowland et al., 2011; Huey et al., 2012; Foden et al., 2013; Moritz and Agudo, 2013) and that a species vulnerability to climate change involves the synergistic combination of intrinsic (sensitivity) and extrinsic (exposure) factors (Williams et al., 2008).
Figure 6.1 A general framework to assess species vulnerability to global climate change (Williams et al., 2008).

Species sensitivity involves physiological tolerance limits (critical thermal limits, preferred body and activity temperatures, and metabolic rates), ecological traits (behaviour, habitat or trophic specialization, life history characteristics, biotic and abiotic interactions) and genetic diversity. Exposure factors involve regional (mesoscale changes in means and extremes of temperature and precipitation) and local (microhabitat and topographic buffering) environment (Williams et al., 2008; Moritz and Agudo, 2013).

Following Williams et al. (2008) framework, my research makes original contributions to the scarce existing knowledge about the sensitivity and exposure factors involved in the vulnerability of P. loveridgei to climate change. A recent assessment of subtropical rainforest vertebrate species of eastern Australia suggests P. loveridgei could be highly vulnerable to
climate change, because it is a regionally endemic specialist restricted to montane rainforest (Hagger et al., 2013). Hagger et al. (2013) came to this conclusion using limited published data suggesting that *P. loveridgei* possesses traits consistent with those associated with declining frog populations in upland areas within Australia (restricted distribution range, low clutch size, low resilience and low recolonization potential) (Hagger et al., 2013). No specific measurements, however, were done of *P. loveridgei* sensitivity or exposure. My research project was designed to assess the likely effects of climate change on different aspects of *P. loveridgei* behavioural biology, by directly measuring different sensitivity (Chapters 2, 4 and 5) and exposure (Chapters 3 and 4) factors. More specifically, it aimed to provide knowledge on the calling phenology, distribution, thermal tolerances and disease susceptibility of *P. loveridgei* (Figure 6.2).

My research aimed to study factors influencing *Philoria loveridgei* sensitivity and exposure by:

1) Investigating the relationships between temperature, rainfall and calling phenology (time and duration) (Chapter 2).
2) Modelling current distribution, detectability and occupancy, and forecasting future distribution changes under different climate change scenarios (Chapter 3).
3) Examining critical thermal limits, preferred body temperatures and thermal environments (Chapter 4).
4) Assessing the susceptibility and infection status of *P. loveridgei* with the pathogen *Batrachochytrium dendrobatidis* (Chapter 5).

The results chapters of this thesis are structured as a series of manuscripts, with their own abstract, introduction, methods, discussion and reference list. This final chapter focuses on synthesising the key findings of the data chapters to evaluate *P. loveridgei* vulnerability to climate change and to recommend management and conservation actions. A conceptual diagram summarising the results used to evaluate *P. loveridgei* vulnerability to climate change is shown in Figure 6.2. Specific detailed discussions of methods and results used to evaluate the vulnerability are provided in each of the results chapters.
6.2 Sensitivity

6.2.1 Philoria loveridgei calling phenology

Daily temporal variation in anuran calling activity is species-specific with each species displaying distinct activity peaks (Bridges and Dorcas, 2000; Oseen and Wassersug, 2002; Todd et al., 2003; Steelman and Dorcas, 2010; Willacy et al., 2015). The calling activity of P. loveridgei was detected across all hours of the day however, a diurnal calling pattern was identified, with higher calling frequency during early morning hours (5:00 – 10:00). Results of this study refine the P. loveridgei breeding season by identifying the core calling period (months with the highest frequency of calling), in November and December.

In general, the most common abiotic factors influencing amphibian breeding behaviour are temperature and rainfall (Bridges and Dorcas, 2000; Oseen and Wassersug, 2002; Todd et al., 2003; Kirlin et al., 2006; Saenz et al., 2006; Steelman and Dorcas, 2010; Willacy et al., 2015). The calling phenology of P. loveridgei was primarily influenced by rainfall, varying...
between seasons (2011-2014). This activity was further influenced by temperature. I hypothesise that spring and early summer rainfall events increase soil moisture, and hence increase *P. loveridgei* nest moisture (i.e. small burrows in soft and moist soil) and subsequently form a suitable environment for egg and larval survival. These early summer rainfall events would therefore cue the onset of the calling, associated with breeding. Although the onset of calling follows the start of the wet season, it finishes before the end of the wet season. This further suggests that early rainfall events are associated with the terrestrial breeding behaviour of *P. loveridgei*. The eggs of *P. loveridgei* are deposited in a thick jelly with no free water, inside burrows constructed in moist soil. Larval development also takes place in the jelly (Seymour et al., 1995). Heavier rainfall events, at the end of the season could be detrimental for development due to potential nest flooding.

Amphibian breeding phenology is strongly associated with environmental cues, making this taxonomic group vulnerable to phenological changes due to climate change (Corn, 2005; Parmesan, 2007; Todd et al., 2011). Warming temperatures may have a stronger effect on early breeder species (Todd et al., 2011). Breeding timing could affect amphibian populations by altering the synchrony in life-history events on interacting species (i.e. predatory-prey interactions and competitive dynamics) (Blaustein et al., 2010; Yang and Rudolf, 2010; Todd et al., 2011). Reduced rainfall may affect reproductive phenology because breeding cues will be less frequent (e.g. rainfall events), and available breeding sites will become scarce. Species with direct-development may be more vulnerable and experience increased mortality due to drier climate conditions, with direct impacts on all life stages (Donnelly and Crump, 1998; Bickford et al., 2010; Lemckert and Penman, 2012). For example, eggs and tadpoles of these terrestrial species may become more vulnerable to mortality from drying (Donnelly and Crump, 1998; Lemckert and Penman, 2012). Drier environments may also force adult amphibians to congregate in water sources or near moist microhabitats, increasing competition and facilitating disease transmission (Bickford, 2005; Joglar et al., 2007; Bickford et al., 2010; Longo and Burrowes, 2010). *Philoria loveridgei* breeding phenology could be vulnerable to changes in temperature and precipitation patterns that could lead to alterations in population structure and dynamics (e.g. larval survivorship), which could result in population declines.

### 6.2.2 *Philoria loveridgei* thermal tolerance

Thermal sensitivity tests, critical thermal limits of *P. loveridge*, described a narrow thermal tolerance range (*CT*$_{\text{min}}$ = 6.8°C and *CT*$_{\text{max}}$ = 30.4 °C), mainly limited by *CT*$_{\text{max}}$. Preferred body temperatures exhibited a narrow range (7.3 - 25°C) reflecting the narrow
thermal range experienced in subtropical rainforests. It has been posited that frogs inhabiting tropical and subtropical mountains and experiencing small temperature variations, may have limited acclimation ability in rapidly changing thermal environments thereby increasing their vulnerability to global warming (Wake and Vredenburg, 2008; Seebacher et al., 2015). Overall, these thermal physiology results suggest that currently the thermal biology of \( P. \) loveridgei is well within climate change predictions. Climate change however, will increase maximum air temperatures to match \( P. \) loveridgei \( C_{T,\text{max}} \), suggesting possible physiological stress.

**6.2.3 Philoria loveridgei susceptibility to chytridiomycosis**

Susceptibility to the amphibian fungal pathogen disease, chytridiomycosis, was confirmed for the first time in wild \( P. \) loveridgei populations. The fungal pathogen \( Bd \) was detected in two \( P. \) loveridgei populations in low (683 m) and mid-high (977 m) altitude subtropical rainforest sites. Results indicate very low infection intensity (34.2 ± 47.7 zoospore equivalents) and low disease prevalence (8.86%). No clinical signs of chytridiomycosis or population declines were documented in this study or have been reported for \( P. \) loveridgei. These results suggest wild populations of \( P. \) loveridgei co-exist with \( Bd \) in an enzootic state between the host and pathogen, however host-pathogen dynamics could be altered with climate change (Rohr et al., 2008; Li et al., 2013). A warming climate will alter the thermal environment of \( P. \) loveridgei becoming thermally suitable for \( Bd \).

**6.3 Exposure**

**6.3.1 Philoria loveridgei distribution, detectability and occupancy modelling**

The current distribution of \( P. \) loveridgei was modelled throughout a restricted geographical range, mainly within national parks in the mountains of mid-eastern Australia. The two variables associated to \( P. \) loveridgei distribution were precipitation in the wettest period and maximum temperature of the warmest period. Occupancy was high (0.773) throughout the current \( P. \) loveridgei distribution range with different environmental variables influencing detectability (temperature and rainfall) and occupancy (canopy cover). Future predictive models forecast a substantial loss, to varying degrees, of suitable habitat areas for \( P. \) loveridgei under both moderate and extreme climate change scenarios. These results describe an increasingly narrow and fragmented geographical distribution for \( P. \) loveridgei suggesting a high vulnerability to climate change.
6.3.2 *Philoria loveridgei* thermal environment

Thermal environment and sensitivity results described that *P. loveridgei*, a subtropical mountain-top ectotherm, is a thermoconformer and thigmotherm. Thermal environment results demonstrated that *P. loveridgei* microhabitats (i.e. leaf litter burrows) are a relatively stable environment that effectively buffer extreme temperatures and could provide a refuge from temperatures extremes. Closed canopy rainforest is also a thermally stable environment providing a thermal buffer. Overall, these results suggest that the vulnerability of *P. loveridgei* to climate change could be reduced.

6.4 *Philoria loveridgei* vulnerability to climate change

Examining different factors affecting *P. loveridgei* sensitivity and exposure to climate change allowed an assessment of this species vulnerability. As expected, *P. loveridgei* biology is influenced by rainfall and temperature patterns making this ectotherm potentially vulnerable to climate change. Collectively, the results of this research suggest that *P. loveridgei* (a subtropical mountain-top ectotherm) is vulnerable to climate change, although it may have some potential to respond and survive.

*Philoria loveridgei* could be thought highly vulnerable to climate change if we only analysed the results of future prediction models, which forecasted an extended loss of suitable habitat under different climate change scenarios (Chapter 3). This result is corroborated by predictions of subtropical rainforest range contractions in the area inhabited by *P. loveridgei* (Mellick et al., 2013). *Philoria loveridgei* distribution is dependent on canopy cover hence this species is dependent on rainforest. Predicted rainforest contraction will further limit the availability of suitable habitat for *P. loveridgei*, increasing its vulnerability to climate change. These assessments, however, are based on large scale climate models that measure a species exposure under global climate change scenarios. Regional and local fine scale exposure measures are needed to refine these predictions and to more accurately assess risk under climate change.

One way for *P. loveridgei* to respond to the predicted climate change range contractions would be to shift its distribution. It has been proposed that the capacity of a species to survive will be related to their ability to move to new areas (Thomas et al., 2006). The general expected trend is for species to move to higher altitudes in response to a warming environment (Parmesan and Yohe, 2003; Lawler et al., 2009; Forero-Medina et al., 2011). Mountain-top amphibian species, such as *P. loveridgei*, are constrained as they lack an altitudinal range area that will allow them to move up hill, jeopardising their survival.
Therefore, *P. loveridgei* survival capacity will be associated with its capacity to acclimate, shifts in microhabitat choice and the evolutionary response of physiological tolerances (Williams *et al.*, 2008; Kearney *et al.*, 2009; Seebacher *et al.*, 2015).

Although an important trait to evaluate climate change vulnerability, thermal sensitivity information for most species is scarce (Williams *et al.*, 2008; Huey *et al.*, 2012; Moritz *et al.*, 2012). *Philoria loveridgei* exhibited a narrow thermal tolerance range mainly limited by $CT_{\text{max}}$, which could lead to a high vulnerability to climate warming (Chapter 4). Rising environmental temperatures could exceed $CT_{\text{max}}$ of cool-adapted ectotherms physiological limits exposing them to physiological stress which could lead to extinction (Sinervo *et al.*, 2010; Laurance *et al.*, 2011). Warming tolerance results described maximum air temperatures were higher than *P. loveridgei* estimated $CT_{\text{max}}$, suggesting this species could face physiological stress in the near future (Chapter 4).

The extent of the effect of a warming environment however, could be eased by thermal habitat buffering (e.g microhabitat buffering) and behavioural adaptation (Moritz and Agudo, 2013; Scheffers *et al.*, 2013). Microhabitat warming tolerance results described a buffer of air temperatures indicating the possibility of this species to mitigate the effects of rising environmental temperatures to an extent (Chapter 4). *Philoria loveridgei* could tolerate environmental warming by maintaining its burrowing behaviour, and extreme events could be effectively buffered under low and moderate global warming scenarios. Thermally buffered environments (i.e. rainforest and burrows) are expected to play a major role in reducing *P. loveridgei* vulnerability to climate change. Future climate change models however, predict rainforest contractions that will immediately restrict *P. loveridgei* distribution (Mellick *et al.*, 2013). Landscape features (e.g aspect, slope, vegetation cover and soil moisture) will be important characteristics to consider when attempting to ameliorate the effects of climate change (Moritz and Agudo, 2013).

If extreme predicted global warming scenarios eventuate (IPCC, 2014), microhabitat buffering will be less effective and fail to provide adequate refuge for *P. loveridgei*. In this case refugia may not be enough to dampen the expected increase in the frequency of extreme climate events such as heat waves and cyclones. Extreme events, such as heat waves, have already been shown to significantly affect some species causing severe impacts in Australia (e.g. flying foxes and white lemuroid possum) (Williams *et al.*, 2003; Welbergen *et al.*, 2008).
Although *P. loveridgei* susceptibility results suggest populations co-exist with *Bd* and do not to suffer any clinical or population effects, host-pathogen dynamics could be altered with climate change (Rohr et al., 2008; Li et al., 2013). A study on Australian anuran declines suggested a climate-pathogen interaction hypothesis where consecutively warmer years were likely to predispose upland anuran populations to pathogens (e.g. *Bd*) leading to declines (Laurance, 2008). Under current climate conditions chytridiomycosis does not appear to be a threat to populations of *P. loveridgei*, however this disease may be constrained by the cool rainforest environment and could increase with climate change.

Climate change will not only increase air temperatures, but will also affect rainfall and cloud patterns, which would lead to changes in environmental moisture content (e.g. soil moisture) (Pounds et al., 2006; IPCC, 2014). The effects of altered rainfall patterns on *P. loveridgei* biology will be important in relation to its vulnerability to climate change. Changes in precipitation patterns could lead to alterations in *P. loveridgei* phenology, because this behaviour is influenced by rainfall (Chapter 2). I hypothesise that changes in rainfall and cloud patterns will play a large role in *P. loveridgei* vulnerability to climate change. Spring and early summer rainfall events increase soil moisture, which increases *P. loveridgei* nest moisture and subsequently, increases egg and larval survival. This strong reliance on highly moist microhabitats could increase *P. loveridgei* vulnerability. Reduced rainfall could reduce environmental moisture, particularly reducing soil moisture, causing hydrological stress and consequently egg and larvae mortality (Seymour et al., 1995).

### 6.5 Management and conservation strategies

Research focused on understanding how a species is likely to respond to climate change is key in assessing its vulnerability and can guide management and conservation efforts (Moritz and Agudo, 2013). A fundamental management and conservation strategy is to design reserve networks that protect suitable habitat that will protect biodiversity *in situ* (Bellard et al., 2012). Mitigating the effects of climate change for *P. loveridgei* could be done by securing large areas with suitable habitat. Maintaining mountain-top areas of eastern Australia as national parks would be a crucial management strategy to ensure this species survival, as they are dependent on subtropical rainforests.

Another management strategy would be to reduce human induced changes to canopy cover. Dense rainforest act as thermal buffers against predicted extreme temperatures, playing a major role in buffering *P. loveridgei* microhabitats. Additionally, expanding forested
areas in already existent national parks could be a good approach to manage this species and others facing similar impacts.

Management efforts for potentially vulnerable species, such as P. loveridgei, should also focus on minimizing other stressors on population viability and continuously monitor of population dynamics (Moritz and Agudo, 2013). For example, monitoring host-pathogen dynamics could detect disease outbreaks in known populations. Future work should focus on continuing to monitor P. loveridgei disease dynamics to elucidate trends which could anticipate disease outbreaks.

6.6 Future directions for research

Accurately predicting a species vulnerability to climate change is a complex process involving correct identification of the relationships between its environmental, life history and community interactions (Williams et al., 2008; Bellard et al., 2012; Moritz et al., 2012). The aim of this study was to measure several exposure and sensitivity factors to comprehensively understand P. loveridgei vulnerability to climate change. Due to time and budget restrictions however, not all factors could be evaluated.

Although I investigated several impacts of rising temperatures on adult P. loveridgei, this study did not account for potential impacts of temperature on other life stages (eggs, larvae, metamorphs and juveniles). Any decrease in egg and larval survival could reduce juvenile recruitment rates and affect population size which would lead to adult population declines. Therefore, investigating the potential impacts of temperature on other life stages of this species is critical for evaluating further vulnerability.

A second limitation of this thesis was to accurately account for changes in environmental moisture. Changes in rainfall patterns are far more difficult to estimate and different climate change scenarios predict wildly different outcomes, making environmental moisture even harder to predict. Alterations in rainfall patterns and soil moisture are likely to reduce egg and larval survivorship, hence urgent attention is required to understand this relationship. Future research should focus on investigating changes in evaporative water loss, soil moisture and their impacts on larval survivorship. Future models should also look at incorporating these aspects to improve predictions of species vulnerability to climate change.
6.7 References


