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# The vulnerability of microhylid frogs, *Cophixalus* spp., to climate change in the Australian Wet Tropics

Thesis submitted by

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# STATEMENT OF CONTRIBUTION OF OTHERS

This work is co-authored with my supervisors (Prof. Steve Williams, Dr. Luke Shoo and Prof. Andrew Krockenberger). The experimental design and methods of each chapter were developed in consultation with a combination of the above-mentioned supervisors. While undertaking this research, I was responsible for partially obtaining research funding and collection and ethics permits, collecting field data, statistical analysis and interpretation, and synthesis and preparation of manuscripts for submission to a peer reviewed journals. I led the writing of all chapters, with contributions from Dr. Luke Shoo, Prof. Steve Williams and Prof. Andrew Krockenberger. For Chapter 2, previous to 2007 collection of microenvironmental data was a responsibility of Research Assistant of the Centre for Tropical Biodiversity and Climate Change at JCU, Collin Storlie, he was included as a co-author for this chapter. In Chapter 3, collaboration was established with Dr. Conrad Hoskin, who provided all the phylogenetic information for the frogs' study group. His collaboration was recognized as a co-authorship of that chapter. Charlotte Jennings and Craig Moritz performed DNA analysis in order to identify individuals from sympatric species occurring in Mt. Lewis.

I obtained financial support from Project 2.5ii4 “Impacts of climate change on rainforest biodiversity” led by my supervisor, Prof. Steve Williams, within the JCU Centre for Tropical Biodiversity and Climate Change and funded by the Marine and Tropical Sciences Research Facility (Department of the Environment, Water, Heritage and the Arts). Additional funding came from James Cook University, College of Science and Engineering, the Skyrail Rainforest Institute (2008 and 2009), and the Earthwatch Institute (2007 – 2010).

# DEDICATION

I dedicate this thesis to my family. They have been the foundation of the career I chose and the person I became. Without their support, this work would not be possible. To all the biology people I met in the way to this date; all have contributed to make this educational path richer and enjoyable. To my beautiful and little nieces and newborn nephew: Dana, Camila, and Josué, hoping they find a better world to enjoy their lives.

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# PUBLICATIONS ASSOCIATED WITH THIS THESIS

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## Primary manuscripts (in preparation)

- **Merino-Viteri, A**., L. P. Shoo, C. Storlie, and S. E. Williams. Microhabitat use and behaviour as factors for potentially buffering thermal effects on Australian climate change threatened Microhylids
- **Merino-Viteri, A**., C. J. Hoskin, L. P. Shoo, A. Krockenberger and S. E. Williams. Thermal physiology of geographically restricted mountain frogs (Microhylidae: *Cophixalus*) is more closely coupled to current environment than phylogenetic history
- **Merino-Viteri, A**., L. P. Shoo and S. E. Williams. Intrinsic and extrinsic factors limiting the distribution ranges of *Cophixalus* frogs on the Australian Wet Tropics Bioregion.

## Supplementary manuscripts (published)

- Shoo, L. P., D. H. Olson, S. K. McMenamin, K. A. Murray, M. Van Sluys, M. A. Donnelly, D. Stratford, J. Terhivuo, **A. Merino-Viteri**, S. M. Herbert, P. J. Bishop, P. S. Corn, L. Dovey, R. A. Griffiths, K. Lowe, M. Mahony, H. McCallum, J. D. Shuker, C. Simpkins, L. F. Skerratt, S. E. Williams, &J.-M. Hero. 2011. Engineering a future for amphibians under climate change. *Journal of Applied Ecology* 48:487-492
- Storlie, C., **A. Merino-Viteri**, B. Phillips, J. VanDerWal, J. Welbergen & S. E. Williams. 2014. Stepping inside the niche: microclimate data are critical for accurate assessment of species' vulnerability to climate change. *Biology Letters* 10, 20140576.

# THESIS ABSTRACT

Climate change is a global phenomenon attributable to a change in concentration of greenhouse gases in the atmosphere as a consequence of human activities since the beginning of industrialisation. The change in climatic patterns direct and indirectly affects biodiversity by, for example, promoting geographic distribution changes (elevational or latitudinal) or affecting the biological interactions of species. These effects have been proposed to more severely affect ectotherms, especially in tropical regions. Tropical regions, due to annual climatic stability, may have promoted the evolution of species with reduced thermal tolerances.

The endemic Microhylid frogs, genus *Cophixalus*, of the Australian Wet Tropics Bioregion (AWT) are considered one of the most threatened groups of vertebrates of the region by climate change. This vulnerability mainly comes from a reduced geographic distribution, restricted to a one or few mountain tops in the coldest, wettest and aseasonal parts of the AWT. These nocturnal frogs are forest floor dwellers and reproduce via direct development (i.e. eggs are deposited terrestrially and development occurs within the egg with froglets hatching from the egg capsule). During day time they seek shelter from ambient environmental conditions in refuges (mainly under logs, rocks or fallen epiphytes). Correlative models of distribution of this group of frogs predict a reduction of suitable climatic area in the future, however the mechanisms behind the potential changes are poorly understood. The ecological characteristics of *Cophixalus* and their high regional species richness (thirteen species) make this group ideal to test hypotheses about factors that influence their geographical distribution limits and potentially will influence their response to future climates.

A more accurate assessment of the vulnerability of these *Cophixalus* frogs to climate change depends on the understanding of the conditions the species actually are exposed to and their sensitivity to environmental change. Exposure requires accurate microenvironmental data and an understanding of seasonal or daily behavioural activity, while sensitivity can be assessed by directly quantifying the physiological thermal tolerances of the animals.

*Cophixalus* frogs show a consistent daily and seasonal activity pattern showing adaptations to exploit available refuges with potentially better buffering capacity, which may be important to face warmer conditions in the future.

Based on behavioural observations, a sequence of actual exposure conditions for *Cophixalus* frogs was constructed. Exposure conditions were found to be behaviourally buffered from ambient air conditions (by nocturnality and microhabitat use). This magnitude of the buffering varies with season, being higher during the warmest months.

Sensitivity of *Cophixalus* frogs was directly measured using several techniques including a dynamic method to determine critical thermal maximum and minimum. The experimental device increased or decreased temperature at a rate of approximate 1˚C/minute, until organisms lost righting capacity. The analysis of the sensitivity of organisms to climate change must be based on empirical evidence, to

improve the assessment of future climate impacts. Observed thermal tolerances and limits of the Australian Wet Tropics *Cophixalus* frogs was not predicted by phylogenetic history, but instead was correlated with contemporary environmental conditions associated with the geographical distribution of individual species. *Cophixalus* frogs have narrow thermal ranges that may be explained by their narrow geographical restriction to rainforest habitats. Historical stability of environmental conditions within this ecosystem, has been proposed as a major driver of species distribution patterns and subsequent geographic species richness in this region.

The maximum thermal tolerance ($CT_{max}$) of the eleven species of *Cophixalus* examined, ranged from 28.1 °C to 35.9 °C, and the minimum ($CT_{min}$) ranged from 8.6 °C to 14.3 °C. Thermal tolerance range was extremely narrow compared with other tropical anurans, even for the species with the widest tolerance, *Cophixalus exiguus* (25.5 °C). Mean and mode of the preferred temperature showed similar values ranging from 20 °C to 29 °C across the 13 species.

The combination of measured thermal tolerances, microenvironmental exposure and ecology of species will be fundamental for the development of more accurate assessment of vulnerability to climate change. For restricted species, the analysis of these factors may also allow the identification of parameters that may be limiting their distributions. In this study, available evidence suggests that thermal physiology parameters of individual *Cophixalus* frogs, such as $CT_{max}$ is correlated to different distribution characteristics (e.g. maximum and mid-altitud of the species distribution).

These data also allow us to determine whether species are actually filling their fundamental thermal niche. It is suggested that some species are not filling their niche. This finding may open the possibility to explore conservation strategies that may include translocation of individuals to regions where they are not currently present but environmental conditions would not act as a limitation.

When predictions of temperature change are included in the analysis, the results may be used to guide decisions about the regions and time frames when conservation strategies are required. Climate conditions under present, and two future frames (2035 and 2065) were evaluated. Our results suggest that some species may be experiencing supressed fitness due to environmental warming even under current environmental conditions. This decrease is related to exposure to conditions warmer than their preferred temperature. The exposure to these stressful conditions are expected to ocurr first in the lower elevational areas of the distribution and increase in magnitude into the future.

One of the most important findings of this research is that our data suggests that no population of any *Cophixalus* species occurring in the AWT will be exposed to temperatures that directly cause fatalities within the timeframes evaluated. This is important because it suggests that drastic interventions to avoid local extinctions will not be necessary by 2065. However, the potential for serious indirect, or synergistic, impacts remains.

It is possible too that physiological responses to environmental parameters other than temeperature may limit distributions of *Cophixalus* species. Another contribution of this study was to develop an experimental apparatus capable of quantifying tolerance to dessication in hard field conditions. We provide a description of the experimental apparatus and detailed methodology as a input for future studied focused on this environmental paramenter and also include some preliminary data on desiccation rates as an appendix. *Cophixalus* frogs show an increase of water loss with temperature, implying an increase in vulnerability to drying in the future. *Cophixalus neglectus* in particular seems to be highly sensitive to desiccation. *Cophixalus saxatilis* presents low levels of desiccation that can be explained by larger size of the species and relative low relation between surface-area and volume of individuals. *Cophixalus monticola* shows low water loss rates compared to close relatives of similar size. This pattern may be related to specialisation on *Limnospadix* palms as a preferred microhabitat which may offer less protection against water loss than other microhabitats.

The combined information assembled in this research addresses some of the important knowledge gaps identified by national authorities, needed to improving the understanding of mechanisms behind climate change impacts. This new knowledge provides a basis for the development of more robust and accurate conservation strategies that will increase the success of adaptive management focused on the conservation of species threatened with extinction, such as the Australian endemic *Cophixalus* frogs.

# TABLE OF CONTENTS

The vulnerability of microhylid frogs, *Cophixalus* spp., to climate change in the Australian Wet Tropics

# LIST OF TABLES

# LIST OF FIGURES

# Chapter 1. General Introduction

## 1.1 Climate change

The Intergovernmental Panel on Climate Change (IPCC) has established with a high degree of confidence that human activities are altering regional and global climates (IPCC, 2013). The magnitude and direction of change in climatic perturbation varies geographically but includes an increase in the temperature of land and ocean surfaces, decrease in the extent of ice sheets, increase of sea level, and altered carbon and other biochemical cycles (IPCC, 2013). Global mean surface temperatures have increased by 0.65 to 1.06 °C between 1880 and 2012 (IPCC, 2013) .

Global data reveal that 2016 was the hottest year on record (Mann et al., 2017; National Oceanic and Atmospheric Administration, 2017; Tollefson, 2016) with 2017 likely to exceed 2016. Regional estimates for Australia indicate an increase of mean surface temperature of 1.1 °C from 1910 to 2013 (McInnes et al., 2015) with more extreme changes in both daytime maximum and minimum temperatures. It is also likely that the frequency of heat waves has increased in several regions including Australia (IPCC, 2013). A further mean temperature increase of 0.3 - 1.1 °C is projected by 2030 and of 1–3.9 °C by 2090 over the Australian Wet Tropics Bioregion (AWT) depending on the emission' scenario (McInnes et al., 2015). These climatic changes are expected to have profound impacts on natural ecosystems and biodiversity (IPCC, 2013).

There is now evidence that contemporary climate change is already affecting biodiversity and is now one of the most threatening processes to biodiversity (Parmesan & Yohe, 2003; Root et al., 2003; Watson, 2016). Modelled increases in temperature may be affecting physical and biological systems across the planet, and changing geographic distribution, phenology, and physiology of individual species (Root et al., 2003; Rosenzweig et al., 2008; Walther et al., 2005, 2002). Additionally, this increase in temperature has extended favourable conditions for exotic species invasion, and has altered the natural ecological interactions and community dynamics (Hughes, 2000). For example, there is a risk of catastrophic levels of extinction within the rainforest vertebrates of the AWT of Australia as climate changes (Williams et al., 2003). Williams et al., (2008) suggested the effects of climate change on biodiversity would depend on the degree of environmental change and the relative vulnerability of the species and processes within the ecosystems. Successful environmental management and the protection of biodiversity into the future will depend on management and policy decisions informed by comprehensive assessments of vulnerability (Williams et al., 2017).

Globally, amphibians are more threatened than either birds or mammals and are declining more rapidly (Stuart et al., 2004). Climate change is a key factor implicated in the total or local extinction of some amphibian species in Latin America, Europe, and Australia (Alford et al., 2007; Bosch et al., 2007; Pounds et al., 1999, 2006). Amphibians are thought to be especially sensitive to any change in their environments for several reasons: their permeable skin, their different reproductive modes linked to microhabitat specificity, and their complex life cycles (Lips, 1998). Any environmental change can affect one or more of these features; for example contaminants in air or water will affect the internal organs, or exposure to higher temperatures will increase body water loss and force the amphibians to migrate to more congenial climates (Duellman & Trueb, 1994). Amphibians are particularly vulnerable to desiccation, and most will die if 40–50% of body mass is lost through water evaporation (Peters, 1983).

Climate change is not expected to impact all species equally (Buckley et al., 2013; Deutsch et al., 2008; Foden et al., 2013; Huey et al., 2012). Species more prone to extinction have one or several characteristics in common: limited climatic ranges, restricted habitat requirements, reduced mobility, or isolated or small populations (Foden et al., 2013; Huey et al., 2012; Millenium Ecosystem Assessment, 2005; Williams et al., 2008). Species physiologically constrained, as has been proposed for tropical ectotherms, are particularly vulnerable to climate change because of their limited adaptation capability to new environmental conditions (Bernardo & Spotila, 2006; Tewksbury et al., 2008).

## 1.2 Australian Wet Tropics Bioregion

The Wet Tropics Bioregion, located in north eastern Australia, has been a UNESCO World Heritage Area since 1988 because of its high levels of biodiversity and unique fauna and flora (UNESCO, 2016).

The region is characterized by mountain ranges with an old geological history that started 420 million years ago. Movement of crust plates, mountain raising, erosion and volcanic history are part of the processes involved on shaping the actual mountain profile of the region (Bain & Draper, 1997; Hugall et al., 2002). The AWT has around 10 000 km$^2$ of rainforest over three major landscapes spanning an elevational from sea-level to 1622 meters above sea level at Mt. Bartle Frere: uplands and tablelands of the Great Dividing Range, Intermediate eastern escarpment and the lowland coastal plain.

The AWT comprises rain forest habitats with an unique composition of endemic flora and fauna (Goosem & Tucker, 1995; Williams & Pearson, 1997) encompassed within complex environmental space (Nix, 1991; Turton & Sexton, 1996). The annual mean rainfall is an important abiotic parameter providing favourable conditions for rainforest vegetation ranging from 1200 to 8000 mm. Rainfall patterns within the AWT are highly seasonal with summer months (Dec – Mar) accounting for ~60% of the yearly total (Goosem & Tucker, 1995).

The AWT comprises less than 0.1% of the Australian land area, however it contains a disproportionately high abundance of biological diversity, particularly regionally endemic flora and fauna, evolved during its 415 million years of history (Goosem & Tucker, 1995). For example, 29 – 60% of the Australian frogs, reptiles, birds, and mammals can be found within the AWT (UNESCO, 2016; Williams et al., 2016). A recent report on the State of the Wet Tropics Vertebrate Fauna identifies 155 species considered to be of high conservation significance based on being regionally endemic (66 species), phylogenetically ancient and unique (16 spp.), rare (80 spp.) and/or threatened (66 species) (Williams et al., 2016). It has been suggested that historical climate stability may explain the high species diversity observed among the present-day AWT community (VanDerWal et al., 2009; Williams & Pearson, 1997).

Despite ~80% of the original forest cover still remaining in the region, anthropogenic activities have altered the integrity of the habitats, especially in the lowlands (DSITIA, 2014; UNESCO, 2016). Habitat destruction and fragmentation are major threats to biodiversity, especially before protection was declared. Extensive research suggests that climate change has the potential to cause mass extinction events within the AWT endemic fauna (Hilbert & Williams, 2003; Williams et al., 2016; Williams et al., 2003). These impacts in the region are likely to be driven by changes in frequency of extreme weather events, rainfall patterns, lifting cloud cover, fire regimes and, changes on population distribution ranges (Hilbert et al., 2014).

## 1.3 Australian Microhylid frogs

The impacts on biodiversity described above will potentially have severe impacts on microhylid frogs. The frog family Microhylidae is distributed in the Americas, Africa (including Madagascar), India, Southeast Asia, Papua New Guinea and northern Australia (Van der Meijden et al., 2007). Within the Microhylid family there exists a diversity of life-history strategies, morphologies, and reproductive systems; however, they are described mainly as snout-mouthed fossorial frogs eating ants or termites. The species occurring in Papua New Guinea and Australia present direct development of terrestrial eggs, this means they lay their eggs in moist areas on land, the embryo is confined to the egg, and are directly born as froglets (Haddad & Prado, 2005; Van der Meijden et al., 2007).

Within Australia, frogs in the family Microhylidae are restricted to northern Queensland and the northern margins of the Northern Territory (Cogger, 1992; Hoskin & Hero, 2008; Vanderduys, 2012; Zweifel, 1985). In Australia, the family includes two genera: *Cophixalus* (18 species) and *Austrochaperina* (five species) (Frost, 2016). 18 species are endemic to the AWT, 15 *Cophixalus* and three *Austrochaperina* (Hoskin, 2004, 2012). Recent research has dramatically increased knowledge of this group of frogs including genetics-based phylogenetics, taxonomic revisions, and ecology (calls, distributions and breeding biology of almost all the species). Other important works on this family include topics such as elevational distribution and abundance (Hoskin & Higgie, 2005; Shoo & Williams, 2004), biogeography (Graham et al., 2006), ecological differences between rare and common species (Williams et al., 2006; Williams, 2007), vocal activity (Brooke et al., 2000; Hauselberger & Alford, 2005), and conservation planning (Hero et al., 2015; Moritz et al., 2001). Geographic extent of environmental suitability has been characterised using correlative distribution models (Appendix 1.1), which model their distribution as a function of climate (Williams, 2006; Williams et al., 2010b).

The microhylid frogs of the genus *Cophixalus* can be described as small (14–35 mm), terrestrial breeders, morphologically conservative with cryptic lifestyles in the leaf-litter of rainforests (Hoskin, 2004; Shoo & Williams, 2004; Williams, 2007). Most of the species in the AWT occur over 800m, and are restricted to a few mountaintops (Williams, 2007). Like other endemic vertebrates of the AWT, these species occur in cool, wet and relatively aseasonal environments (Williams et al., 2003). Graham et al. (2006) suggested that the present pattern of species diversity in this group has been affected by vicariance among historical refugia as well as by the persistence of the species within the refugia through time.

## 1.4 Potential climate change impacts on *Cophixalus* frogs

Globally, 30% of frogs of the genus *Cophixalus* are threatened with extinction (Stuart et al., 2008). Several of the *Cophixalus* frogs are recognised in the IUCN red list mainly as a consequence of their extremely restricted geographical distributions (Hero et al., 2006). This characteristic is expected to makes these species particularly vulnerable to any biotic or abiotic change in their environments (e.g. changes on prey abundance, or changes in precipitation patterns).

Historical processes, during the last 20 million years (estimated age of the genus, Van der Meijden et al., 2007) have included dramatic environmental fluctuations that have likely shaped the present distribution pattern of species (VanDerWal et al., 2009; Williams et al., 2010a). The restriction of these species to cool, wet mountain-tops may also imply adaptations to specific environmental conditions (Williams & Hero, 1998), but also a topographic limitation since there is no more high elevation space to colonize while warming continues.  The projected vulnerability of the species will depend on how exposed the individuals will be to the change in the environment, and the resilience of the species given by physiological tolerances (Williams et al., 2008).

Direct impacts of climate change on endemic vertebrates, such as microhylid *Cophixalus,* could be driven also by changes of water cycles in these environments (McJannet et al., 2007b, c). These frogs are direct developers which means that their eggs and metamorphs are likely to be particularly exposed to drying conditions on the forest floor. Williams et al. (2003) proposed that a shift in cloud cover due to an increase in temperature would impact leaflitter dwelling organisms such as microhylid frogs.

This effect of a reduction in the frequency of cloud is expected to be more important during winter months when the moisture from cloud dripping is a major component of total water inputs in this ecosystem (McJannet et al., 2007a). Warming will increase the rate of evaporation and reduce humidity at ground level, worsening this problem.

Bioclimatic modelling of the current and future potential distribution of AWT vertebrates have considered this group of mountain-top restricted *Cophixalus* frogs as the most threatened organisms in the region (Williams et al., 2003). Habitat with suitable climate will rapidly decrease as temperature increases. Similar pattern of decline have also been predicted for mountain-top plants in the region (Costion et al., 2015). Under a “business as usual” future climate scenario (RCP 8.5), six of the top 15 most threatened species in the AWT are microhylid frogs (Williams et al., 2016).

## 1.5 Project justification

Current climate change impacts on biodiversity have been commonly based on correlations between species occurrence and current and future environmental conditions (Pacifici et al., 2015). From a species perspective, these potential impacts could be inaccurate since other aspects of ecology and physiology are not directly taken into account (e.g., physiological limits) (Kearney & Porter, 2004; Kearney et al., 2010a, 2010b; Pacifici et al., 2015). One of the main difficulties of accurately predicting climate change is that there is little information about the physiological thresholds of species (Bernardo et al., 2007). Additionally, most of the current predictions about climate change impacts have not considered micro-variation in environmental conditions within the habitats, and the specific space and range of environmental conditions needed or preferred by the species (Kearney et al., 2010b, 2013; Kennedy, 1997). This is important because a combination of behaviour and microhabitat buffering can potentially reduce the estimated vulnerability of a species (Williams et al., 2008)

Microhylid frogs are considered to be one of the most threatened vertebrate groups by climate change within the AWT (Williams et al., 2016; Williams et al., 2003; Williams & Hilbert, 2003). This evaluation has been possible because of the available baseline data on taxonomic, behavioural, and ecological information for this group. This provides foundational information to develop new and innovative methodologies to study the impact of climate change, and test assumptions of correlative models by directly examining physiological preferences and tolerances and the extent of microenvironmental buffering of exposure.

This project aims to improve the assessment of the potential climate change impacts on the Australian frogs of the genus *Cophixalus* (Fig. 1.1), by:

1) empirically evaluating the actual microenvironmental conditions that frogs are experiencing in the wild;
2) determining the thermal preferences and tolerances of microhylid species;
3) interrogating intrinsic and extrinsic factors that may be limiting the geographic distribution of the frogs; and
4) using improved mechanistic information to better predict how further changes in environmental conditions might affect the geographic extent of species in the future.

These data will enable a more realistic assessment of which species are most threatened by climate change under likely future scenarios. Ultimately, these data will help inform the design of management interventions and target management resources towards those species and places that are most likely to be vulnerable to future increased temperatures, seasonality and extreme climatic events.

## 1.6 Thesis structure

This research aims to develop an improved approach to forecasting biological impacts of climate change by interrogating processes that operate at the scale of an organism to limit the geographic range of species. The approach is applied to eleven species of endemic frogs of the genus *Cophixalus* in the Wet Tropics of Northeastern Australia. The study is comprised of several components:

Chapter 2 considers the microenvironmental conditions that *Cophixalus* frogs experience within their habitat. These actual exposure conditions have been analysed considering the use of specific microenvironments, but also considering the behaviour of organisms in this ecosystem (diurnal or nocturnal). Based on actual behavioural observations gathered for several species of *Cophixalus* frogs at different periods of the year, the quatification of the exposure conditions at different study locations are compared.

Chapter 3 quantifies the thermal physiology parameters for eleven species of *Cophixalus*. Assessed parameters include Critical Thermal Maxima ($CT_{max}$), Critical Thermal Minimum ($CT_{min}$) and Preferred Temperature ($T_{pref}$). A phylogenetic test is performed to determine whether physiological tolerances are more closely related to current environmental conditions or instead are a product of the shared evolutionary history of closely related species.

Chapter 4 specifically explores whether the distribution of *Cophixalus* frogs is primarily constrained by limits in physiological tolerances (fundamental niche), physical barriers to dispersal (potential niche), or both. Both thermal limits (minima and maxima) are considered independently. The empirical data allow us to combine abiotic conditions and species traits in order to understand mechanisms limiting the distribution of *Cophixalus* frogs in the AWT.

Finally, Chapter 5 provides a comprehensive analysis of the *Cophixalus* frogs regarding changes in the thermal environment. This mechanistic approach combines empirical measurements of the actual thermal exposure of the frogs across the elevational gradient of the AWT and the physiological tolerances of the frogs. The analysis enables a more detailed assessment of the true vulnerability of each species across three time frames (current, 2035, 2065). This knowledge is then used to identify areas for potential conservation interventions that might be implemented by management authorities to mitigate impacts.

It is important to acknowledge that the original design of this research included the evaluation of desiccation rates of *Cophixalus* frogs in the AWT. Williams et al. (2010) highlight the importance of moisture-related bioclimatic variables on limiting the potential distribution of *Cophixalus* frogs in correlative models. These results suggest that moisture may be an important factor limiting the distribution of these frogs in the AWT and that a future change in precipitation or inputs of moisture from the orographic cloud bank might affect their populations. The development of ecophysiological experiments in difficult field conditions necessitated the construction of experimental apparatus not commercially available. As appendix 1, we provide a description of the experimental apparatus, detailed methodology, and preliminary results as a input for future studies focused on this environmental parameter. Experiments were performed with the aim to determine relative differences in cutaneous water loss resistance as a proxy of desiccation risk for this group of frogs.

Experiments included the evaluation of different relative humidity (dry air, 50%, 70%, and 90%) and temperature (15 °C, 20 °C, and 25 °C) treatments of individual frogs during an experimental period of 30 minutes at an air flow rate of 15ml/min. A total of 362 experiments were performed on *Cophixalus* frogs and 77 on 3% agar models. The experimental individuals were the same used to measure thermal physiology parameters described in chapter 3 (Table 3.3).

Unfortunately, during analysing data, it was impossible to find a coherent correspondence between the observed weight loss and the calculated loss from the data gathered by the experimental apparatus, suggesting that an undetected malfunction had occurred during experimentation, making the absolute values of the moisture data not usable. Significant time and effort (many months) were spent on attempting to recalibrate the measurements using a variety of approaches. Unfortunately, it was not possible to reliably calibrate the fault. A subsequent decision was therefore made to focus the dissertation primarily on temperature. Despite this setback, we decided it would be useful to present preliminary data of relative patterns of the effect of temperature on desiccation rates as part of the warming effects discussed in chapter 5 and a patterns discussion and reccomendations for future studies on this topic in Appendix 1.

*Cophixalus aenigma* (COPAENI) *C. australis* (COPAUST) *C. bombiens* (COPBOMB)

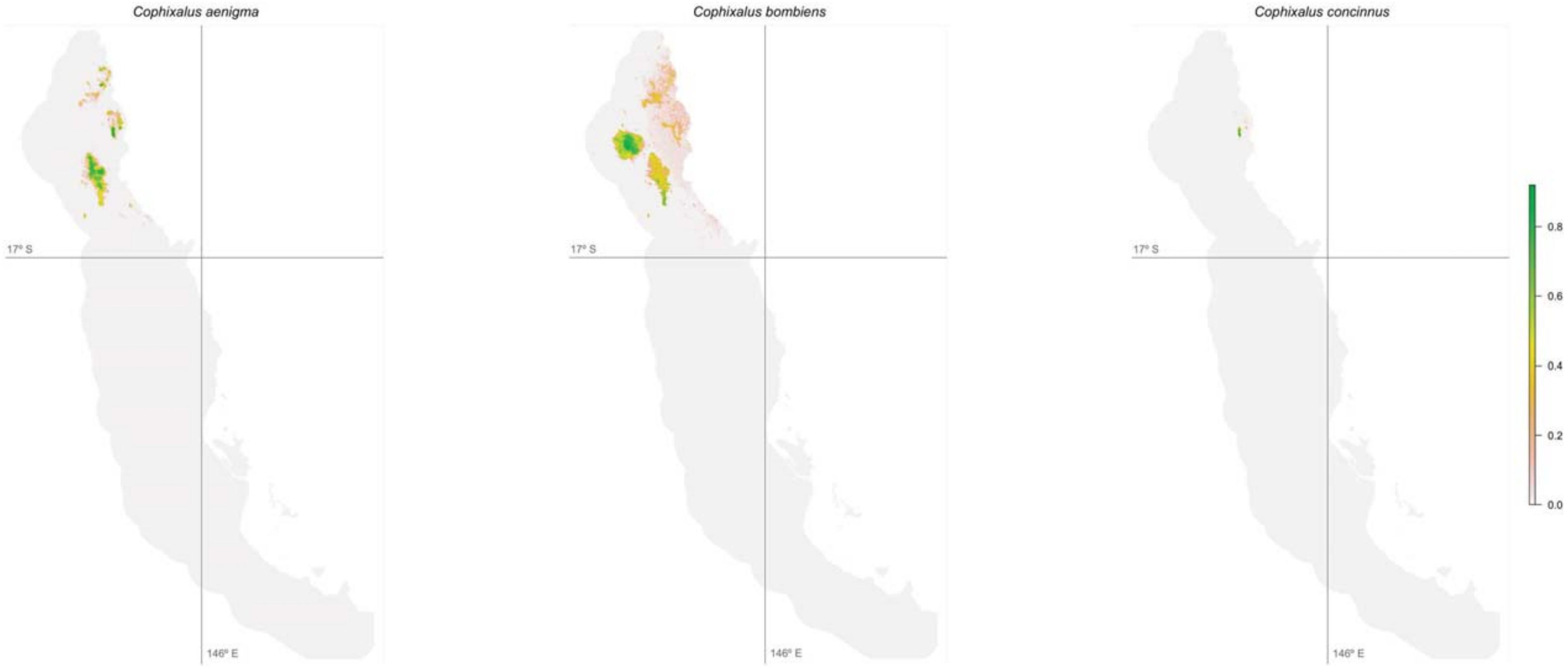
*C. concinnus* (COPCONC) Photo: Luke Shoo *C. exiguus* (COPEXIG) *C. hosmeri* (COPHOSM)

*C. infacetus* (COPINFA) *C. mcdonaldi* (COPMCDO) *C. monticola* (COPMONT)

*C. neglectus* (COPNEGL) *C. saxatilis* (COPSAXA)

**Figure 1.1.** Endemic frogs, genus *Cophixalus,* of the Australian Wet Tropics studied in this research, including species codes.

**Appendix 1.1.** Correlative Distribution Models available for Australian *Cophixalus* frogs (Williams et al., 2010b). Scale (0-1) shows climatic habitat suitability.



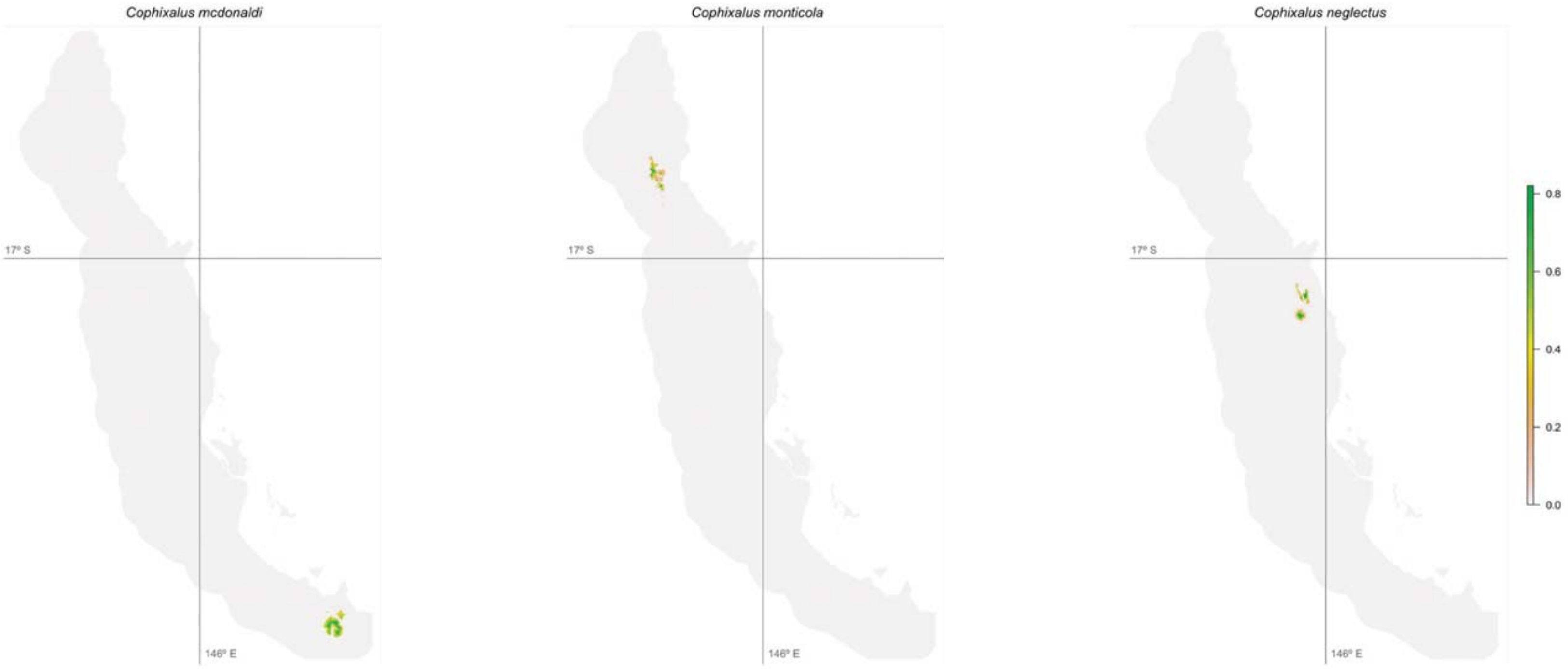
Cophixalus aenigma

Cophixalus bombiens

Cophixalus concinnus

**Appendix 1.1** Correlative Distribution Models available for Australian *Cophixalus* frogs (Williams et al., 2010b). Scale (0-1) shows climatic habitat suitability (Continued).

**Appendix 1.1.** Correlative Distribution Models available for Australian *Cophixalus* frogs (Williams et al., 2010b). Scale (0-1) shows climatic habitat suitability (Continued).



# Chapter 2. Microhabitat use and behaviour protect species from climate conditions: Implications for a warming world

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## 2.1 Summary

Quantifying exposure to changes in the environment is required to accurately assess the potential effects of climate change on animal species. The conditions in specific microhabitats (e.g. under a rock or log) within a forested area are different to open air environments. The use of these microenvironments may radically modify the conditions a species is locally exposed to. In addition, activity patterns (e.g. daily or seasonal) will also affect the exposure conditions of individuals. However, quantification of the differences between the actual conditions an individual is exposed to, compared to ambient, or open air, conditions is rarely evaluated. Here, we focus on understanding the exposure conditions of a diverse group of endemic microhylid frogs in the Australian tropical rainforests. Specifically we quantify the buffering effect of microhabitats and refuges on annual mean temperature, monthly ranges of temperature, maximum and minimum temperatures on different microhabitats. We also construct a model of the exposure conditions of theoretical diurnal and nocturnal organisms and test the prediction using empirical data on microhylids, genus *Cophixalus*, considered by the IUCN red list as threatened by climate change. Our comparisons between macro environment and micro conditions under canopy, under logs, in soil and refuges show little differences in the annual mean temperature but important differences during the warmest and coldest periods. Because of their nocturnal activity period, Microhylid frogs are exposed to a narrower set of conditions than diurnal organisms, suggesting lower exposure for this group to future warmer environments and during extreme heat events. Furthermore, behavioural observations suggest there is also potential for Microhylid frogs to utilise available refuges with greater buffering capacity if necessary. This research demostrates the importance of including buffering effects and species-specific behaviour to improve the assessment of species vulnerability to future climate change.

## 2.2 Introduction

Tropical regions of the planet are experiencing the warmest conditions in the last two million years (Bush, 2002; Mann et al., 2017; National Oceanic and Atmospheric Administration, 2017) with a further increase of 4.8 °C projected by the end of the century depending on different potential emission scenarios (IPCC, 2013). Uncertainty about how species will respond to this rapid change is a major obstacle for prioritizing conservation resources to respond to this emerging challenge (Williams, et al., 2008). Tropical species might be particularly vulnerable to warming because they often have narrow thermal tolerances and are already living close to their upper thermal limits (Deutsch et al. 2008; Huey et al. 2009; Tewksbury et al. 2008). Dillon et al. (2010) have demonstrated that tropical species will be forced to adapt their physiology more rapidly than their temperate counterparts. Contemporary warming has already been implicated in geographical range shifts of tropical species (Chen et al., 2009; Pounds et al., 2007; Williams et al., 2016). Colwell et al. (2008) predict widespread elevational shifts in plants and insects in Central America, but movement to more favourable microenvironments at the same elevation has also been proposed (Bush, 2002). The vulnerability of species with very low dispersion abilities will depend on their physiological tolerances and interactions between behaviour and microhabitat use to survive any environmental change (Williams et al., 2008). The trigger of the responses to any change in the environment will be the actual exposure to those new conditions, which may be mediated by natural history aspects such as circadian cycles and the degree of climatic buffering provided by specific microhabitats (Williams et al., 2008).

Studies that link knowledge of the physiological sensitivity of organisms to exposure conditions in the environment will be the most instructive in predicting vulnerability to future climate change (Deutsch et al., 2008; Williams et al., 2008). However, estimating the realized exposure of organisms can only be partially inferred from widely available data on macroclimate and requires detailed understanding of the buffering influence of microhabitat (Kennedy, 1997) and potential for behavioural thermoregulation (Kearney et al., 2009a). Better knowledge of the potential for both of these factors to decouple exposure of organisms to regional climate trends is therefore critical to determining how closely species are actually living to their upper physiological tolerances (Scheffers et al., 2014a, b; Williams et al., 2008).

Furthermore, the future survival of organisms with low dispersal capacities will depend on the chances of finding tolerable conditions within the future warmer environments. The identification of these environments and their management has the potential to improve biodiversity persistence (Shoo et al., 2011b). Empirical information on the capacity of microhabitats to function as refuges from thermal stress is expected to be useful for informing management actions under possible future climate change scenarios. For example, localised deployment of shade cloth (Mitchell et al., 2008) or more widespread reinstatement of vegetation (Hansen et al., 2010) have already been proposed to lower nest temperatures of selected reptile species threatened by climate change.

Vertebrate ectotherms generally use environmental sources of heat to control their body temperature (Brattstrom, 1979) and habitat selection and activity periods affect conditions they experience in the wild (Navas, 1996). The primary thermal challenge for most ectothermic terrestrial animals in tropical environments is to stay cool (Kearney et al., 2009b). Any change in the environment will directly affect the exposure conditions of organisms that lack a capacity for physiological thermoregulation (Angilletta Jr., 2009).

Structurally complex ecosystems provide a mosaic of microenvironmental conditions that buffer species from regional affecting climate (Scheffers et al., 2013, 2017; Shoo et al., 2010). Consequently, species behaviours and selection of microhabitats have the potential to buffer them from the potential negative effects of changing climate

(Kearney & Porter, 2009; Kearney et al., 2009b; Scheffers et al., 2014a). This phenomenon will potentially be a critical factor affecting species vulnerability via the provision of buffering from extreme climate events (Scheffers et al., 2014b). Extreme climate events are predicted to increase into the future (Meehl & Tebaldi, 2004) and are now widely considered to be a fundamental factor in understanding future vulnerability (Williams et al., 2016). However, Storlie et al. (2013) found significant differences between available climatic layers describing macroclimate of the region and actual daily maximum and minimum temperatures at ground level. This finer-scale thermal data should provide a more accurate reflection of the actual exposure experienced by ground-dwelling species and thereby a more robust estimate of their vulnerability (Storlie et al., 2014).

Here, we characterize the potential for thermal buffering in a tropical rainforest system, the Australian Wet Tropics Bioregion (AWT), and illustrate this effect on an example taxa, the endemic Microhylid frogs, genus *Cophixalus*. These data will be linked to physiological sensitivity of the frogs (Chapter 3) in order to assess the actual vulnerability of these organisms to climate change (Chapter 5). The Australian *Cophixalus* frogs are small, direct developing, nocturnal amphibians that utilise refuges during the day. Refuges primarily consist of logs, rocks, and epiphytes fallen from trees (Hoskin & Hero, 2008). However frogs can also be found underground or within fallen logs, cracks in rocks, and under bark on trees (Williams & Hero, 1998). Some very specialized species seek refuge in leaf litter accumulated between the leaves of *Linospadix apetiolatus* palms or in cavities in boulder fields (Hoskin & Hero, 2008; Shoo & Williams, 2004). Most species are restricted to one or few mountaintops where the environmental conditions are wet, cool, and aseasonal (Shoo & Williams, 2004). The genus contains representative taxa that are thought to be among the vertebrates most imminently threatened by climate change in the region (J. M. Hero et al., 2006; L. P. Shoo, Olson, et al., 2011; S. E. Williams et al., 2003, 2016) (Hero et al., 2006; Williams et al., 2016; Williams et al., 2003). Specifically, we quantify buffering associated with canopy cover and shelter within microhabitats on the forest floor (soil and under logs). We discuss implications of thermal buffering as a mechanism that can enable species to regulate exposure to stressful environmental conditions and show how this is conditional on activity patterns of organisms.

## 2.3 Methods

### 2.3.1 Study location

Field characterization of microclimate was carried out at 25 rainforest sites in the Wet Tropics Bioregion, located in north eastern Australia. Sites spanned available elevational (from 100 to 1600 m) and latitudinal (16°15'S to 19°00'S) variation in Australian Wet Tropics Bioregion (Figure 2.1) and included the distribution range of the 11 species of *Cophixalus* frogs tested in this study.

### 2.3.2 Daily activity patterns of Microhylid frogs

Two locations (Mount Bellenden Ker and Carbine Tablelands) where chosen to perform the behavioural observations on Microhylid frogs. These locations compile the logistics, representative species and access to Microhylid frogs refuges needed to accomplish the intensive sampling method (see below).

Three *Cophixalus* species (*Cophixalus hosmeri*, *C. aenigma* and *C. monticola*) were monitored at Mount Lewis in Carbine Uplands (Table 2.1). Mount Lewis was surveyed six times in different months between March 2009 and April 2010. The surveys covered summer and winter seasons. Mount Bellenden Ker was monitored twice, one time in summer and a second time in winter. The species monitored at this location was *Cophixalus neglectus*.

In all cases, individual frogs were located in diurnal refuges and dusted with fluorescent powder (Graeter & Rothermel, 2007). The refuges were then monitored hourly between 15:00 and 24:00, and between 3:00 and 7:00, on occasions where no rain was falling. An ultraviolet torch was then used to follow the trail left behind by the dusted frogs. Departure and return times from and to the refuges were recorded. By combining behavioural observations with environmental measurements, we created a sequence of the actual exposure conditions for these frogs.

### 2.3.3 Microclimate gathering data

We set HOBO Micro Weather Stations and DS1923 temperature/humidity logger iButton® protected from direct solar radiation and precipitation to record ambient air temperature (1.5m above ground) and soil temperature (five cm under the ground surface) at each of the 25 sites under the tropical rainforest canopy. DS1921G Thermochron® loggers were also installed under standard sized logs (diameter: 16.7 ± 6.8 cm; length 155.7 ± 74.4 cm) at the same locations. The size of these logs allowed us to obtain microenvironment data for medium-term periods (i.e. multiple months and years) without conditions being confounded by substantial decay of logs. Air temperature, soil temperature and under log temperature information were summarized for one-hour intervals for the period from November 2006 to March 2010.

In addition, DS1923 temperature/relative humidity logger iButton® were set in 26 confirmed Microhylid frog' refuges spread across locations in the region between 100 and 1600 m. Refuges comprised four epiphytes fallen from trees, ten rocks and 12 logs (Table 2.2). Refuges were monitored between March 2009 and December 2010.

To characterize thermal conditions within microhabitats, we derived four bioclimatic parameters, for every collection site: annual mean temperature, mean monthly range, warmest monthly mean maximum temperature and coldest monthly mean minimum temperature. For complete details how these variables were derived see Houlder et al. (2003). Field locations with more than one month of missing data were excluded from analysis. Data from two permanent monitoring sites and two microhylid refuges were excluded.

### 2.3.4 Aggregating the environmental data

We compared field estimates of microclimate with a reference set of broad-scale environmental conditions generated using Anuclim 5.1 software (Houlder et al., 2003) and an 80 meter resolution Digital Elevation Model (created from GEODATA 9 Second DEM Version 2; Geoscience Australia, http://www.ga.gov.au/). These are simplified climate grids derived from elevation-sensitive interpolations of open air weather station data. ANCOVA analysis, using Air Temperature (open air conditions) as a covariate, was used to test differences between microhabitats and behaviour (hypothetical diurnal or nocturnal organisms - see below).

We simulated potential realized exposure temperatures of model organisms accounting for both microclimate conditions and behavioural shuttling between nocturnal ambient exposure and diurnal retreat sites (i.e. under log shelters). Diurnal species were assumed to emerge from retreat sites at 06:00 and return at 18:00 hours and an inverse activity period was assumed for nocturnal species. Activity times for Microhylid frogs were based on behavioural observations in the field (see below).

## 2.4 Results

### 2.4.1 Microhabitat's buffering effects

Thermal conditions of the studied microhabitats covaried with macroclimate but were also buffered by microhabitat (under canopy, soil, underlog, and *Cophixalus* refuges) (Fig. 2.2, see also Table 2.3 and Appendix 2.1 for summary of statistical tests). The degree of buffering for mean annual temperature varied according to the refuge type (F= 3.530; $p < 0.01$), with estimates of buffering (intercepts comparison) from ambient air conditions, ranging from 1.4 to 3.2 °C (Table 2.3) cooler than macroenvironmental conditions (Fig. 2.2a). Our results did not show significant interaction between macroclimate and microhabitat type on microenvironmental conditons, suggesting that environmental temperature is the major thermal change source. This implies that changes in macroenvironment will directly influence mean thermal conditions in microhabitats. The same general pattern was repeated for the warmest monthly mean temperatures (Table 2.3; Fig. 2.2c).

The influence of macroenvironmental temperature and refuge type on microhabitats thermal regimes was also evident for the warmest (F= 622.733; $p < 0.01$ and F= 33.457; $p < 0.01$, respectively) and the coldest month (F= 881.577; $p < 0.01$ and F= 134.045; $p < 0.01$, respectively) of the year. However, the degree of microhabitat buffering was different between seasons. The buffering effect during summer was much higher than for annual mean temperature, ranging from 9.6 to 15.6 °C cooler. The buffering effect during the coldest month of the year maintained microhabitats at temperatures that were warmer than ambient air conditions by 1.3 to 2.2 °C (Table 2.3; Fig. 2.2d).

Microhabitats also reduced the variability of thermal conditions compared with ambient air temperature. Mean monthly thermal ranges showed less variation in soil, underlog and microhylid refuges habitats (in that order) than was the case for ambient air conditions (Fig. 2.2b, Table 2.2). A wider variation in temperature was found under canopy temperature, however it too showed less variation than ambient air conditions (Fig. 2.2b, Table 2.2).

### 2.4.2 Microhylid's behavioural observations

Behavioural activity was found to be highly consistent across individuals both daily and seasonally. Sixty-eight of 75 individuals emerged from refuges between 18:00 and 19:00 and all the individuals that were still being monitored at 05:00 (27 individuals) returned to their refuges between 05:00 and 06:00 (Table 2.1). These activity times were assumed to be a characteristic for all the species of *Cophixalus* in the AWT and was subsequently used as a basis to estimate potential exposure conditions across the day.

Across all the observed individuals, 22.7% did not return to the refuge where they were observed the previous day, and only 2 individuals never left the refuge.

### 2.4.3 Behavioural buffering and actual *Cophixalus* frogs exposure conditions

The potential buffering effect of behaviour was first explored considering a putative diurnal and a nocturnal terrestrial organism. This approach revealed less variation in mean hourly temperatures experienced by a nocturnal organism than a diurnal organism (Fig. 2.3) which experienced extreme minimum and maximum temperatures at 6am and noon, respectively (Fig. 2.3 c and d).

The combination of being nocturnal and the buffering provided by daytime refugia dramatically reduces the exposure of microhylids to temperature, particularly maximum temperatures. A more complex sequence of microenvironmental conditions was derived after accounting for the observed Microhylid frogs behaviour. Ambient air conditions influence behavioural exposure conditions during the warmest (F= 285.634, $p<0.01$) and the coldest month (F= 146.748, $p< 0.01$). It is evident that the exposure conditions of *Cophixalus* frogs are very similar to a nocturnal organism receiving a cooling buffering of almost 9 °C during the warmest month (Fig.2.4a; Table 2.3). During winter frogs receive a buffering from cold of around 1 degree (Fig.2.4b; Table 2.3). The complete summary of results is in Appendix 2.1.

## 2.5 Discussion

A reliance on coarse macro-scale estimates of exposure conditions limit efforts to infer climate change vulnerability based on an assumption that exposure is equal for any organism living in a specific environment. Determining actual exposure conditions based on microenvironmental measurements and natural history of organisms promise to improve the accuracy of any vulnerability assessment. We show that thermal regimes in rainforest microhabitats are on average slightly cooler than in open air situations but diverge dramatically in the warmest and coolest months when conditions are expected to be most stressful to organisms. In the case of the Australia Wet Tropics, our data suggest a buffering of 1.4 (soil) to 3.2 °C (microhylid refuge) from annual mean temperature depending on the microenvironment. These estimates of buffering are consistent with empirical evaluations from other localities and microenvironments (Bohlman et al., 1995; Fetcher et al., 1985; Ibanez et al., 2013; Scheffers et al., 2014a, b).

The strength of this decoupling effect depends on the spatial and temporal scale, but also the type of microhabitat used or behaviour a species exhibits. Differentiating and separately analysing data from the warmest and coldest periods reveals that buffering provided by microenvironments can vary at time scale of days, months, and years. Soil, underlog or the frog refuges showed large differences when compared with open air conditions. These are not trivial differences. For example, maximum temperature offsets of up to 10 degrees between open air conditions and microhabitats during the warmest months are even higher in magnitude than regional temperature changes predicted for this century. Lower maximums in warm months and higher minimums in cool months also translate into more moderate and less variable daily and seasonal fluctuations in temperature in those microhabitats. Our under canopy results (15 °C of buffering) may be overestimated because it seems warmer environments show less buffering than cooler ones, where the buffering effect was estimated using regression lines intercepts. Organisms using well insulated microhabitats potentially benefit from environmental conditions that are isolated from thermal extremes but also less variable. Species may prefer using microenvironments with a high frequency of temperatures close to their thermal physiological optimum (Huey et al., 2009; Huey & Stevenson, 1979). In general, nocturnal organisms face their warmest exposure temperature while resting in their diurnal refuges. These conditions may help some species with their physiological processes, such as increasing digestion rates (Navas, 1996).

Behaviour also plays an important role in decoupling conditions experienced by organisms from ambient conditions in their environment. This may be especially important in tropical ectotherms, which are more likely to possess restricted physiological tolerances than their temperate counterparts (Deutsch et al., 2008; Tewksbury et al., 2008) and may be experiencing environments closer to their thermal physiological limits (i.e. low thermal safety margin). We show that nocturnal and diurnal species shuttling between the same microhabitats (but at different times of day) experience very different thermal regimes. Diurnally exposed ectotherms experience more moderate minimum temperatures but much higher maximums than nocturnal organisms (Fig. 2.3). This suggests that, if thermal tolerances are similar between all the organisms from that environment, diurnally exposed organisms are likely to be living closer to their upper thermal limit, and would present smaller thermal safety margin, than nocturnal organisms. Aspects of actual thermal exposure in organisms are limited in literature, so in this case, we provide evidence that the Australian frogs of genus *Cophixalus* experience a similar exposure to that of a theoretically predicted nocturnal animal. Better integration of this kind of empirical data is still needed in vulnerability assessments.

Exposure that is decoupled from ambient conditions via behavioural and habitat buffering becomes even more important when assessing the likely impacts of extreme climatic events such as heat waves. The magnitude, frequency and duration of heat waves are all expected to increase under modelled future climates (IPCC, 2013). It has been proposed that microhabitat buffering has to reduce the negative effects of extreme climatic events (Scheffers et al., 2013, 2014a). Shoo et al. (2010) demonstrated that mountaintop boulders buffer macro environmental conditions for *Cophixalus concinnus* in the Australian rainforest. They found boulders buffer temperature by up to 10 °C from open air conditions.

Though not examined here, thermal buffering can also be linked to other microclimatic variables important to physiological tolerances, especially desiccation in amphibians. For amphibians, desiccation risk is an important factor influencing survival. Wygoda (1984) reported the influence of the habitat in the control of temperature in anurans through differential evaporative water loss, which is also an important aspect on Microhylid frogs.

For nocturnal and diurnal organisms, refuges are very important to keep warm during very cold extreme events even when some ectotherms can be active at very low temperatures (Navas, 1996; Navas et al., 2008). However, for microhylid frogs no restrictions in activity periods were recorded during the behavioural surveys at different times of the year (Table 2.1). Regular activity was observed in most of the cases where the individual moved several meters away from refuges during the night activities and then moved back to the refuges towards dawn (personal observations).

The observations of some *Cophixalus* individuals not returning to the same refuge each day is relevant in a climate change context. Although this behaviour was potentially due to disturbance caused by researcher intrusion, it nevertheless shows that these frogs can move short distances looking for other suitable refuges. This demonstration of plasticity could enable individuals to locate better refuges if conditions deteriorate beyond the capacity of the original refuge to adequately buffer the frog against increasing temperature or desiccation.

In many studies assessing species vulnerability to future climate change, correlative species distribution models are used to predict potential impacts of climate change. However, these models assume the absence of buffering from macro environmental conditions or differential species exposure (Kearney et al., 2009b; Kennedy, 1997). The inclusion of buffering and behaviour in these methodologies will show a more realistic and accurate assessment of the potential effects of climate change in this and other groups of Australian ectotherms.

## 2.6 Acknowledgements

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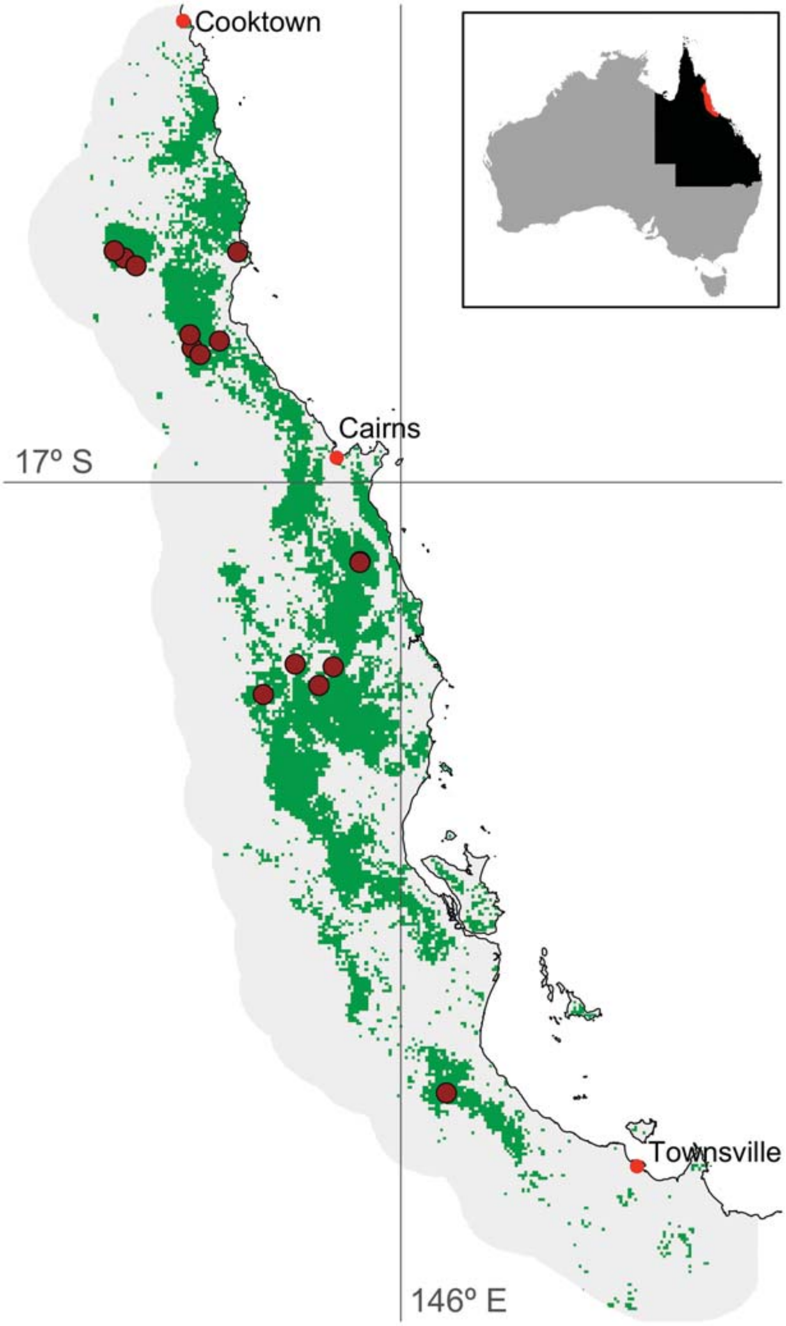

**Figure 2.1**. Locations of microenvironmental data collection sites within the Wet Tropics Region (brown circles). Current extent of rainforest in the AWT is shown in green.

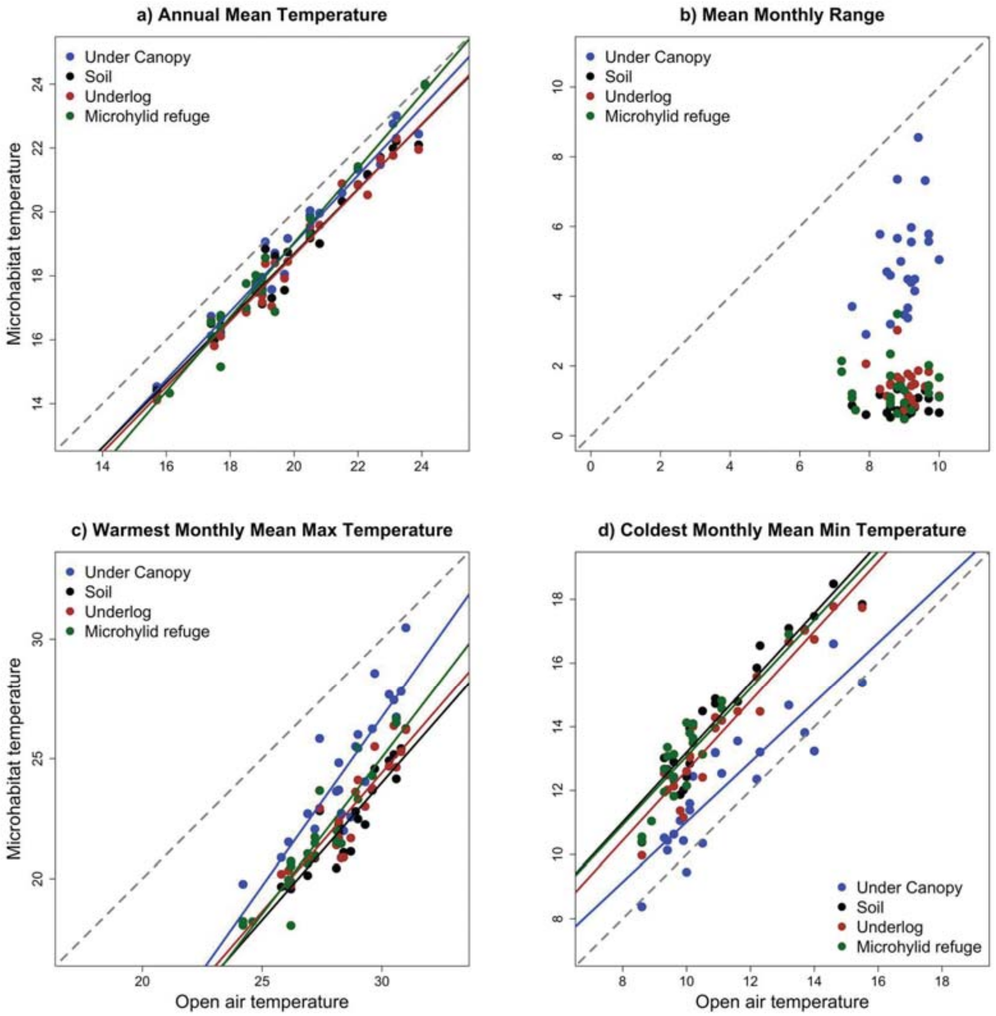

**Figure 2.2**. Comparison between different microhabitat environmental variables with the same variables under open air conditions (grey dashed line) for 23 analysed locations throughout the AWT.

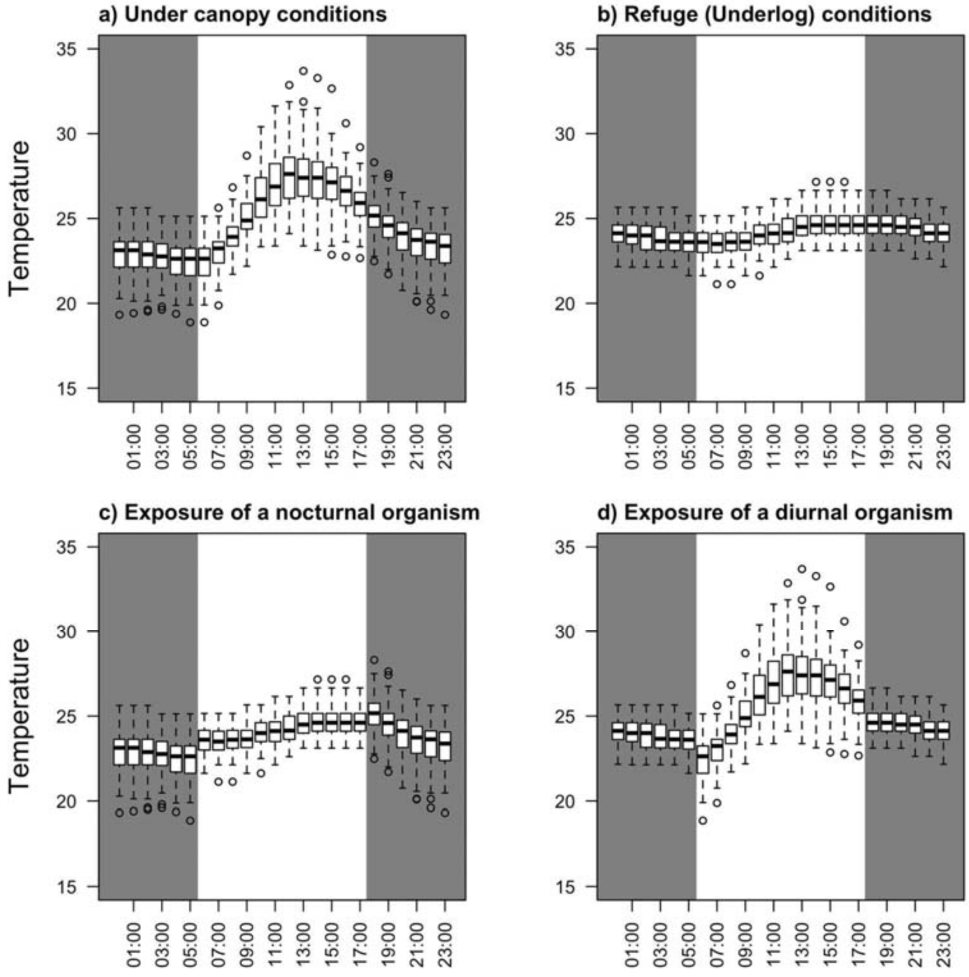

**Figure 2.3.** Averaged hourly temperatures for under rain forest canopy (a) and under log (b) in a lowland location (Carbine region, 100 m) in December. Lower panels show how under canopy and under log temperatures can be integrated to emulate the potential exposure of a nocturnal (c) and a diurnal (d) organism. Grey background represents nighttime.

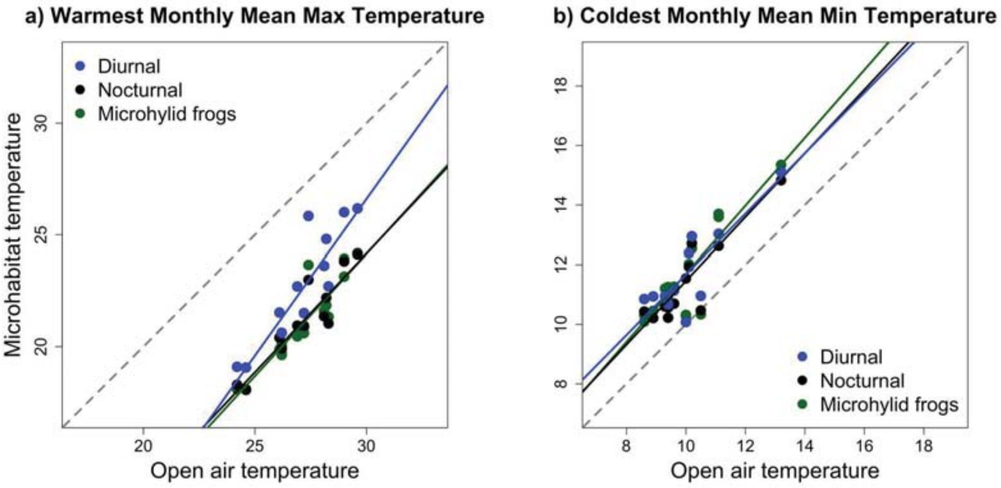

**Figure 2.4.** Comparative exposure conditions for a putative diurnal and a nocturnal organism and Microhylid frogs exposure relative to open air conditions (grey dashed lines).

**Table 2.1.** Summary of behavioural observations on Microhylid frogs at two localities in the Wet Tropics Bioregion.

| | | | | Percentage (and number) of individuals | | | |
|---|---|---|---|---|---|---|---|
| **Region** | **Elevation** | **Date** | **Frogs found** | **Leaving refuges 18:00 – 19:00** | **Returning between 5:00 – 6:00*** | **Never left refuge** | **Not coming back to same Refuge % (N)** |
| **Carbine Uplands** | 1200 | Mar 31, 2009 | 14 | 100 | 100 (9) | - | 35.7 (5) |
| | | Jun 26, 2009 | 22 | 90.9 | 100 (10) | - | 54.5 (12) |
| | | Aug 5, 2009 | 9 | 77.8 | - | 11.1 (1) | - |
| | | Oct 20, 2009 | 11 | 81.8 | 100 (2) | - | - |
| | | Nov 9, 2009 | 4 | 100 | - | - | - |
| | | Apr 13, 2010 | 8 | 100 | 100 (5) | - | - |
| **Bellenden Ker Mt.** | 1600 | Jul 16, 2009 | 2 | 100 | - | - | - |
| | | Feb 23, 2010 | 5 | 80 | 100 (1) | 20 (1) | - |
| **TOTAL** | | | 75 | | 100 (27) | 2.6 (2) | 22.7 (17) |

*Percentage of frogs and number of individuals still being monitored at that time

**Table 2.2.** Locations and type of microhylid refuge studied along the Wet Tropics Region.

| Wet Tropics Subregion | Refuge ID | Elevation | Type |
|---|---|---|---|
| Windsor Uplands | WU001 | 1100 | Epiphyte |
| | WU002 | 1300 | Log |
| | WU009 | 1100 | Log |
| | WU010 | 900 | Rock |
| | WU012 | 1300 | Log |
| Carbine Uplands | CU002 | 1000 | Rock |
| | CU003 | 1200 | Epiphyte |
| | CU1 | 1200 | Rock |
| | CU6 | 1200 | Rock |
| | CU8 | 1200 | Log |
| | CU021 | 1000 | Rock |
| | CU026 | 600 | Rock |
| | CU027 | 400 | Log |
| | CU22 | 1200 | Rock |
| Mount Bellenden Ker | BK001 | 1600 | Log |
| | BK002 | 1600 | Log |
| | BK003 | 1400 | Log |
| Atherton Uplands | AU001 | 800 | Log |
| | AU004 | 600 | Log |
| | AU006 | 600 | Rock |
| | AU008 | 1000 | Log |
| | AU009 | 400 | Epiphyte |
| | AU014 | 1000 | Log |
| Spec Uplands | SU001 | 1000 | Epiphyte |
| Thornton Lowlands | TL001 | 100 | Rock |
| | TL002 | 100 | Rock |

**Table 2.3.** Linear regression equations ($y=ax+b$) of microhabitat temperatures as a function of the open air conditions (weather station temperature data).

| Variable | Microhabitat | No. sites | a | b | Multiple R2 | F | *p* |
|---|---|---|---|---|---|---|---|
| Annual mean temperature | Under canopy | 23 | 1.07588 | -2.52089 | 0.9652 | 859 | <0.01 |
| | Soil | 23 | 1.00352 | -1.37338 | 0.9672 | 913.2 | <0.01 |
| | Underlog | 23 | 1.03062 | -1.95273 | 0.9681 | 941.3 | <0.01 |
| | MH refuge | 24 | 1.10643 | -3.18511 | 0.9108 | 194.1 | <0.01 |
| Warmest monthly mean maximum temperature | Under canopy | 23 | 1.4095 | -15.5951 | 0.8202 | 141.4 | <0.01 |
| | Soil | 23 | 1.11634 | -9.59157 | 0.8814 | 230.3 | <0.01 |
| | Underlog | 23 | 1.1552 | -10.2619 | 0.8773 | 221.6 | <0.01 |
| | MH refuge | 24 | 1.1871 | -10.9833 | 0.7662 | 122.9 | <0.01 |
| | Diurnal | 23 | 1.401 | -15.422 | 0.8196 | 90.85 | <0.01 |
| | Nocturnal | 23 | 1.063 | -7.727 | 0.8722 | 136.5 | <0.01 |
| | MH exposure | 24 | 1.09395 | -8.66956 | 0.8577 | 120.5 | <0.01 |
| Coldest monthly mean minimum temperature | Under canopy | 23 | 0.9678 | 1.3118 | 0.7767 | 107.8 | <0.01 |
| | Soil | 23 | 1.10401 | 2.16873 | 0.8926 | 257.7 | <0.01 |
| | Underlog | 23 | 1.09056 | 1.79894 | 0.8887 | 247.5 | <0.01 |
| | MH refuge | 24 | 1.315868 | 0.001241 | 0.816 | 84.24 | <0.01 |
| | Diurnal | 23 | 1.0168 | 1.5051 | 0.642 | 35.87 | <0.01 |
| | Nocturnal | 23 | 1.07 | 0.7559 | 0.762 | 64.04 | <0.01 |
| | MH exposure | 24 | 1.1421 | 0.2714 | 0.7282 | 53.57 | <0.01 |

**Appendix 2.1**. Statistical results from ANCOVA analyses performed to test the differences between microenvironments considering Open Air conditions as a covariable of the observed temperature. The results are separated for each model applied to consider differences on slopes and intercepts of the regression lines, and this table summarizes the results for the effects on microenvironments (under canopy, soil, underlog and microhylid frogs' refuges) and behavioural exposure conditions (diurnal, nocturnal, Microhylid frogs exposure).

| | **Slopes** | | | | | | **Intercepts** | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Formula: Microhabitat ~ Open air * kind** | | | | | | **Formula: Microhabitat ~ Open air + kind** | | | |
| | **F** | ***p*** | **F** | ***p*** | **F** | ***p*** | **F** | ***p*** | **F** | ***p*** |
| **Microenvironments** | | | | | | | | | | |
| Annual mean temperature | 2807.51 | <0.01 | 3.53 | 0.0172 | 1.019 | 0.3872 | 2806.126 | <0.01 | 3.529 | 0.0172 |
| Warmest month | 622.733 | <0.01 | 33.457 | <0.01 | 2.002 | 0.118 | 606.88 | <0.01 | 32.61 | <0.01 |
| Coldest month | 881.577 | <0.01 | 134.045 | <0.01 | 1.582 | 0.209 | 875.3 | <0.01 | 133.1 | <0.01 |
| **Behaviour** | | | | | | | | | | |
| Warmest month | 285.634 | < 0.01 | 20.691 | < 0.01 | 1.772 | 0.179 | 278.69 | < 0.01 | 20.19 | < 0.01 |
| Coldest month | 146.748 | < 0.01 | 0.761 | 0.472 | 0.167 | 0.847 | 150.801 | < 0.01 | 0.782 | 0.462 |

# Chapter 3. Thermal physiology of geographically restricted mountain frogs (Microhylidae: *Cophixalus*): current environment vs. phylogenetic history

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## 3.1 Summary

The realized geographical distribution of an organism is the result of a complex set of biotic and abiotic factors governing the potential distribution in space and time. An improved understanding of the interaction of all these factors is required to better predict how species will actually respond to global climate change. *Cophixalus* frogs of north-eastern Australia occur in the wettest and least seasonal areas of the Wet Tropics, with most species restricted to one or few mountaintops. *Cophixalus* species have been previously identified as a taxonomic group that is highly vulnerable to projected future climate change. These species present a unique opportunity to investigate the factors limiting distributions and how they may be affected by climate change. Here, we use experimental thermal physiology to quantify key intrinsic species parameters ($CT_{max}$, $CT_{min}$, thermal tolerance range, and preferred temperature) for 11 species of *Cophixalus* frogs. We then test correlations with both a historical phylogeny (Blomberg's K, Pagel's $\lambda$ and Phylogenetic Signal Representation curves), and contemporary geographical distribution (maximum, minimum and mid-elevation distribution and latitude) on thermal physiology. Tests were performed using Phylogenetical Generalized Least Squares (PGLS) analyses under a Brownian motion model of evolution. $CT_{max}$ ranged from 28.1 °C to 35.9 °C, and $CT_{min}$ ranged from 8.6 °C to 14.3 °C. We find that thermal tolerance range was extremely narrow compared with other tropical anurans, even for the species with the widest tolerance, *Cophixalus exiguus* (25.5 °C). Mean and mode of the preferred temperature were broadly similar across species ranging from 20 °C to 29 °C. Variation in these thermal characteristics were not explained by phylogenetic history but, $CT_{max}$, thermal tolerance range and preferred temperature were correlated with one or more geographical distribution characteristics. $CT_{min}$ was not correlated with any geographic parameters. Our findings support the idea that the relatively specialised thermal physiology of each species has acted to constrain the distribution of this group within the region, and that modest variation in thermal physiology among species has been shaped more by prevailing climates than by shared evolutionary history. Under this premise, it is suggested that *Cophixalus* frogs may have limited physiological capacity to respond to rapid environmental change.

## 3.2 Introduction

An important question for predicting biological responses to climate change is whether the observed realized climatic niches of species (i.e. climatic conditions where a species occurs) reflect fundamental constraints to the distribution of a species (Pearson & Dawson, 2003). Conceptually, distribution might be limited by hard physiological tolerances (i.e. set of climatic conditions that a species can potentially tolerate) or it may reflect other limiting factors such as substrate or ecological interactions (Blomberg & Garland Jr., 2002; Cunningham et al., 2016; Williams & Pearson, 1997), or a combination of physiological limits and other factors. The answer to this question depends in part on the degree to which the fundamental niches of species can evolve under different environmental conditions or are largely constrained by phylogenetic history (Quintero & Wiens, 2013a, b). A pervasive pattern in nature used to explain overlapping of geographic distribution between species is phylogenetic conservatism, however the mechanisms underlying this process are poorly resolved (Peterson et al., 1999).

One way to disentangle the relative influence of environment and phylogenetic history on the fundamental niche is to examine variation in physiological tolerances among species with respect to these attributes. An emerging set of studies provide compelling evidence that climate conditions, past and recent, can mold important physiological and morphological traits even among closely related species or disjunct populations of the same species (Moritz et al., 2012; Sumner et al., 1999). On the other hand, some genetically related species appear to show high similarity in traits or retain ancestral characters as a consequence of their common origin (Hoskin et al., 2011; Moritz et al., 2009). This phenomenon, known as phylogenetic inertia, for example, has also been proposed to be responsible for the apparent retention of some maladaptive ecological traits observed in species (Sih et al., 2000; Van Buskirk, 2009). However, it is unclear whether rapid evolution of the climatic niche or phylogenetic conservatism predominate among many taxonomic groups as relevant traits have not been quantified for the vast majority of species. Further investigations are therefore needed to determine the pervasiveness of different mechanisms governing the realized niches of species, particularly among geographically restricted species imminently threatened by climate change.

The *Cophixalus* frogs of the wet tropics rainforests of north-eastern Australia provide an ideal group to interrogate competing hypotheses. The geographic distributions of the 13 species endemic to the Wet Tropics are well known (Hoskin & Hero, 2008; Hoskin, 2004, 2012; Williams et al., 2010b). These are generally restricted to the coldest, wettest and aseasonal parts of the region, and over half the species have highly localized distributions to one or few mountain tops (Hoskin & Hero, 2008; Shoo & Williams, 2004). For example, *Cophixalus concinnus* is restricted to the uplands ( >1080 m elevation) of Thornton Peak, which has a summit of 1374 m (Hoskin & Higgie, 2005). A well resolved phylogeny (Hoskin, 2004; Zweifel, 1985) enables assessment of the effect of evolutionary history on ecological traits, and specifically on thermal physiological traits.

It has been proposed that vegetation history of the Wet Tropics Bioregion includes contractions of cool environments to the uplands, overlain by more recent contractions and expansions of rainforest associated with the Pleistocene glacial cycles. This has shaped species richness patterns and current distribution across the Wet Tropics (Harrington & Sanderson, 1994; VanDerWal et al., 2009; Williams & Pearson, 1997). As for other rainforest biota of the Wet Tropics, these broad-scale climatic changes would have shaped the distribution of microhylid frogs, and possibly ecological traits such as physiological tolerances (Moritz et al., 2012). The *Cophixalus* frogs are the most elevationally restricted vertebrate group in the Wet Tropics, and are predicted to be at high risk of extinction from human-induced climate change (Stuart et al., 2008). The basis of this elevational restriction, and the basis of climate modelling studies, is assumed to be physiological tolerances. Intolerance to warmer temperatures has anecdotal support in punctual observations of mountain-top *Cophixalus* frogs losing their righting response when held in hand (Zweifel, 1985; C. Hoskin, pers. obs.).

Although *Cophixalus* are restricted to relatively cool, moist environments, there is nonetheless significant variation in geographic range size and elevational restriction across the 13 Wet Tropic species. While *C. concinnus* is restricted to the summit of Thornton Peak and *C. neglectus* to the highest peaks of the Bellender Ker Range, other species like *C. australis* and *C. infacetus* have relatively large distributions that include lowland areas. Here, we quantify the thermal physiology of 11 species of *Cophixalus* frogs and test whether variation in temperature-related physiological traits across the studied species is better explained by relatedness or by current environment. Finally, we discuss the implications of our findings with respect to perceived vulnerability of these species to projections of future climate change.

## 3.3 Methods

### 3.3.1 Study sites and geographical distribution data

Empirical estimates of physiological tolerance were derived for eleven of the thirteen species of *Cophixalus* frogs that occur in the Wet Tropics of northeastern Australia. *Cophixalus ornatus* and *Cophixalus hinchinbrookensis* were not included in the study because taxonomic uncertainty at the time of study meant that it was not possible to appropriately sample these species. One population per species was studied, except for *C. australis* that has a larger geographical distribution than the other species , and for this reason two populations were sampled. The twelve locations sampled, encompass representative species from a diverse range of rainforest environments from Mt. Finnigan Lowlands in the north to Mt. Elliot Uplands in the south, spanning an elevational range of 150 to 1600 m (Table 3.1; Figure 3.1).

For each studied species, we gathered information on attributes of geographic distribution as proxies for past and recent environmental variation that may have been important in shaping physiological tolerances in amphibians. These were highest and lowest distribution elevations and mid-elevation (mid-point of the extreme elevations) derived from published references (Hoskin & Hero, 2008; IUCN, 2014; L. P. Shoo & Williams, 2004; Shoo et al., 2010; Williams et al., 2010b). The mean annual temperature, the maximum temperature of the warmest month, and the minimum temperature of coldest month were obtained for every study collection site from adjusted climate layers for the Wet Tropics Bioregion at a 250 m resolution (Storlie et al., 2013) (Table 3.1).

### 3.3.2 Thermal physiology

All physiology experiments were performed in the field in order to minimise stress and better reflect the acclimation status to local environment conditions (Pintor et al., 2016). At each study site, one to 17 adult individuals of each species were tested. All individuals were exposed to the same series of measurements and in the same order after collecting. These were: a) preferred temperature; b) desiccation experiments (mentioned in the thesis structure section and discussed in chapter 5); c) critical thermal minima; and, d) critical thermal maxima. The frogs were kept in individual plastic containers with damp paper towel and exposed to ambient temperature fluctuations while in captivity. The experiments were performed as soon as possible (between two and 72 hours after collection). Frogs were not fed during captivity.

All tested frogs were marked by toe clipping (toe I of left foot) and released at the point of capture after the experiments were completed. All experiments were performed between January 2009 and November 2010.

### 3.3.3 Preferred temperature

The preferred temperature measurements were performed within 12 hours following collection. Each measurement consisted of setting each frog into a temperature gradient (8 °C – 40 °C) formed from a 10mm thick aluminium strip (170cm long and 6cm wide). Temperature was measured every minute at twelve equally spaced, fixed thermocouples (Type T- calibrated against a certified mercury-in-glass thermometer) and recorded on a DT500 Data Logger (DataTaker Inc). Frogs were monitored for a duration of 120 minutes via time-lapse photography at one minute intervals. This enabled recording the exact position of the frog along the gradient to be determined at each time, which was then used to determine the temperature at that point. The base of the gradient was covered with a damp cloth to avoid hydric stress on animals.

Individual frogs were introduced at a standard point near the hot end of the gradient and allowed to move freely and select a preferred temperature. The first 20 minutes of each experiment were discarded, assuming this to be an adequate time for a frog to move and settle on their preferred temperature. Observed positions of frogs were linked with a time matching routine developed in R programming language (R Core Team, 2014), to a location specific temperature within the gradient. Temperatures between thermocouple positions were calculated, in the same programing routine, from the polynomial (4th order) function of temperature and position determined across all 12 points.

We derived a mean temperature occupied by each individual and then averaged across individuals within each species (Tracy & Christian, 2005). The same protocol was followed for modal temperature obtained from a frequency temperature distribution.

### 3.3.4 Critical thermal tolerances and thermal breadth

The critical thermal maximum ($CT_{max}$) and minimum ($CT_{min}$) experiments were based on the Hutchinson's dynamic method (Lutterschmidt & Hutchison, 1997). The experimental apparatus consisted of a hermetic plastic chamber immersed in a water bath that was either warmed or cooled at a rate of approximate 1˚C/minute in order to avoid acclimation. The chamber was rotated slowly throughout the trial (~ 30 minutes). Critical temperatures were established by determining the temperature at which frogs were unable to recover a normal posture after two consecutive manual rotations of the chamber. After experiments were finished, individuals were set on a water bath at ambient temperature (approx. 20˚C) to promote recovery from exposure to extreme temperatures. There was no mortality associated with these measurements.

Real-time temperatures were read directly inside the chamber using a thermocouple (Type T, calibrated against a certified mercury-in-glass thermometer) connected to a Sable System TC-1000 thermocouple meter. The tubing that allowed the thermocouple to be inserted into the chamber also helped to avoid an increase of pressure inside the chamber which might be expected to result from a change in temperature. Critical thermal measurements were all performed within 72 hours of collection.

For analytical purposes, we utilized a mean estimate of $CT_{max}$ and $CT_{min}$ derived by aggregating data from different individuals within the same species. An additional metric termed "Thermal tolerance range" was also estimated as the difference between the $CT_{max}$ and $CT_{min}$ for each species (Gutiérrez-Pesquera et al., 2016).

### 3.3.5 Phylogenetic signal

Data gathered from closely related species violates statistical independence on observations (Felsenstein, 1985). Phylogenetic relatedness among the studied species was derived from a phylogenetic tree available from previous molecular systematics works (Hoskin, 2004; Hoskin et al., 2011). The branches of the phylogeny were transformed and optimised by using a maximum likelihood lambda transformation in order to assure the phylogenetic tree is ultrametric (Paridis et al., 2004). Phylogenetic analysis was carried out using an R software routine (R Core Team, 2014) developed using functions from ape (Paridis et al., 2004), picante (Kembel et al., 2010), geiger (Harmon et al., 2008), PVR (Santos et al., 2013), phytools (Revell, 2012), and caper (Orme et al., 2013) packages.

In order to determine if observed thermal physiology traits of *Cophixalus* frogs differ from a random distribution, a Randomization Test for Phylogenetic Signal was performed for each thermal parameter ($CT_{max}$, $CT_{min}$, thermal range, and preferred temperature), using Blomberg's *K*-statistic and comparing it to 1000 simulations in a randomization test (Blomberg et al., 2003).

The Brownian motion model of trait evolution states that the cumulative change on a trait from the common ancestor until present of a group of species is proportional to the topology and branch length described by the evolution path of the species, suggesting a phylogenetic signal on data (Felsenstein, 1985). Blomberg's *K*-statistic (Blomberg et al., 2003) and Pagel's $\lambda$ (Pagel, 1999) are presented for each of the thermal physiology traits to assess for similarity of trait evolution to Brownian motion model (Münkemüller et al., 2012). Also, a statistics based method is presented for each of the physiology traits, based on a Phylogenetic Eigenvector Regression (PVR) analyses (Diniz-Filho et al., 1998). PVR allows visual determination of phylogenetic rates in thermal parameters given an evolutionary tree. The results are presented through Phylogenetic Signal Representation curves (PSR) where additive eigenvalues from model fit of each parameter is plotted against a proportion of the eigenvalues explained by the phylogeny. If a PSR curve is similar to the predicted one from the phylogeny (45° reference line), then the trait evolution implies an important phylogenetic signal evolving under a Brownian model of evolution and its position compared to the reference line shows the rate of evolution (Gouveia et al., 2014; Santos et al., 2013). If the curve bends below the reference line (slower rate of evolution), it may imply niche conservatism, while the opposite scenario may imply niche evolution (faster rate of evolution) (Santos et al., 2013; Wiens et al., 2010).

### 3.3.6 Geographical analyses

We used a PGLS analyses under Brownian motion model of evolution, in caper package for R (Orme et al., 2013), to test if thermal physiology characteristics are related to geographical distribution parameters when accounting for species relatedness (Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016). In addition to the species-level geographical characteristics described in 3.3.1 section, we also compared the thermal physiology traits to the site-specific elevation and latitude were the tested organisms were collected (Gutiérrez-Pesquera et al., 2016). Statistical significance was calculated for each of these linear regressions.

## 3.4 Results

### 3.4.1 Thermal physiology parameters

The mean and the mode preferred temperatures were highly correlated ($r^2$= 0.88, F= 93.57, $p$<0.01). This result shows no difference in pattern and allow us to use either of the two measurements (Appendix 3.1). The mean of the preferred temperature ranged between 20 °C (*Cophixalus concinnus*) to 29 °C (*Cophixalus infacetus*). A very similar rank was observed for species, considering the mode preferred temperature per species (19.1 °C to 28.4 °C) (Table 3.2).

Critical thermal minima for the eleven studied species ranged from 8.6 °C (*Cophixalus mcdonaldi*) to 14.3 °C (*Cophixalus concinnus*) (Table 3.3). The lowest critical thermal maxima was 28.1 °C (*Cophixalus aenigma*) and the highest was 35.9 °C (*Cophixalus exiguus*). Thermal tolerance range estimation revealed that the most constrained species is *Cophixalus aenigma* (14.2 °C) and the species with the widest range is *Cophixalus exiguus* (25.5 °C). There was no evident relationship between minimum and maximum thermal tolerance ($r^2$= 0.06, F= 0.624, $p$=0.45). As expected, $CT_{max}$ showed a positive relationship with mean preferred temperature ($r^2$= 0.623, F= 14.861, $p$>0.01) and thermal range ($r^2$= 0.757, F= 28.029, $p$=0). $CT_{min}$ was negatively related only to thermal range ($r^2$= 0.488, F= 8.564, $p$=0.017) (Figure 3.2). Both *Cophixalus saxatilis* and *C.*

*infacetus* showed high $CT_{max}$ and $CT_{min}$. Other groups of species showed high $CT_{max}$ and low $CT_{min}$ (*C. exiguus*) and vice versa (*Cophixalus concinnus*, *C. aenigma*).

The thermal tolerances data are summarized on Table 3.3. The results for *C. concinnus* and *C. aenigma* must be regarded as indicative only because of the low number of individuals tested especially for $CT_{min}$.

### 3.4.2 Phylogenetic signal

The observed mean of the preferred temperature was not randomly distributed across species of *Cophixalus* frogs (*K*-statistics = 1.211, $p = 0.038$). The rest of the thermal parameters data were not explained by phylogenetic tree topology. This pattern was corroborated by Pagels' $\lambda$ values. Pagels' $\lambda$ close to 0 imply data less similar to expected by evolution path for every species (Pagel, 1999), that would be the case for $CT_{max}$, $CT_{min}$ and thermal range (Table 3.4). Preferred temperature measurements showed some effect of phylogenetic history, however, estimate values varied between parameters: mean ($\lambda \approx 1$) and mode ($\lambda = 0.119$). Parameters with values similar to 1 (for both, Pagels' $\lambda$ and Blomberg's *K*-statistics) suggest an adjustment of data to Brownian motion model of evolution and, consequently, high phylogenetic signal (Garamszegi, 2014). Taken together, these results suggest that thermal tolerances have been influenced by forces other than phylogenetic inertia.

All of the thermal physiology parameters show a slower rate of change than expected by the Brownian motion model (negative values of area under the PSR curve; Table 3.4 and Figure 3.3), implying little influence of phylogenetic history on the tested parameters (Garamszegi, 2014; Gouveia et al., 2014).

### 3.4.3 Geographic analyses

Several physiological parameters were associated with geographical characteristics of the species (Table 3.4 and Figure 3.4). $CT_{max}$ was negatively related with the maximum and mid elevation of the distribution range of the species and also with the elevation of the study sites (PGLS, F= 7.057, $p$= 0.026; F= 9.644, $p$= 0.013; F= 19.037, $p$< 0.01 respectively). Thermal tolerance range was correlated with the elevation of the study sites (PGLS, F= 86.371, $p$< 0.01). The mean of the preferred temperature was negatively correlated with maximum, mid and minimum elevation of the distribution of the species (PGLS, F= 0.419, $p$= 0.031; F= 0.558, $p$< 0.01; F= 0.368, $p$ < 0.048 respectively). The mode of the preferred temperature was negatively correlated with the maximum and mid elevation of the geographical distribution range and the elevation of the collection sites. $CT_{min}$ was not associated with any independent variables. Latitude was not associated with any of the thermal physiology parameters.

## 3.5 Discussion

The realized distribution of a species is potentially shaped by a complex combination of different factors including, phylogenetic history (Wiens & Graham, 2005), past and present climate (Sunday et al., 2014; VanDerWal et al., 2009), geographical constraints (Sunday et al., 2012), and interactions with biotic factors (competing species, diseases, prey, predator) (Cunningham et al., 2016). We tested the role of geographical factors (elevational and latitudinal distribution) and relatedness (phylogenetic history) in determining the distributions of *Cophixalus* frogs across the mountains of the Wet Tropics. Overall we found thermal physiology of *Cophixalus* frogs was strongly associated with elevational distribution, with limited detectable influence of phylogenetic relatedness.

Both critical thermal limits showed no phylogenetic signal within the genus. This pattern was also found for thermal tolerance range. Preferred temperatures presented evidence of being shaped by Brownian motion model, and consequently having little phylogenetic signal on the trait (Pagel's $\lambda$ and Blomberg's *K*-statistic $\approx$ 1; Table 3.4). PSR curves showed that thermal physiology traits on *Cophixalus* frogs changed at a slower rate than expected from the topology of the phylogeny using Brownian motion model of evolution (Figure 3.2), suggesting more conservatism than niche change on these traits. These findings contrast with other anuran studies where $CT_{max}$ of adults showed high phylogenetic signal (Gouveia et al. 2014) and where $CT_{min}$ of larvae showed high phylogenetic signal and $CT_{max}$ moderate levels (Gutiérrez-Pesquera et al. 2016). However, these studies examined macroecological anuran community associations that may not apply to a taxonomically specific, highly-localised, elevationally restricted organisms like *Cophixalus* in the Australian Wet Tropics.

It is important to consider that different processes underly the evolution of species traits, and the intrinsic evolution rates of species may shape similar (or different) phylogenetic signal levels (Revell et al., 2008). The results may change if different models of evolution are tested with the same phylogeny and trait data.

The effect of latitude on thermal tolerances has been studied to compare trait adaptations between species from tropical and temperate ecosystems (Gaston et al., 2009; Ghalambor et al., 2006; Stevens, 1989). Here, we included latitude as an analysis factor as it has been found to be useful in interpreting global variation in thermal physiology. In our case we do not find an association between latitude and thermal parameters, though the absolute difference in latitude in our study area is limited (3.9°).

Previous studies have shown that thermal physiology traits (especially $CT_{min}$) are inversely correlated with elevation (Ghalambor et al., 2006; Gutiérrez-Pesquera et al., 2016). The same pattern has been also proposed for other ectotherm communities, including skinks occurring in the Wet Tropics (Moritz et al., 2012). Interestingly, our data do not show an environmental association for $CT_{min}$ (Table 3.5). In other studies changes in $CT_{min}$ across elevation are generally greater than for $CT_{max}$ (Ghalambor et al., 2006; Gutiérrez-Pesquera et al., 2016) and this has been hypothesized to reflect a key factor allowing species to colonize and perform activities in cold environments (Gutiérrez-Pesquera, 2016; Navas, 1996, 1997; Wiens et al., 2006). For *Cophixalus* frogs, $CT_{max}$, thermal tolerance range, and preferred temperature were associated with one or several of the geographic distribution variables of each species (Table 3.5; Figure 3.3), suggesting that geographical distribution may have shaped these thermal parameters through time more than phylogenetic history.

$CT_{min}$ did not show a phylogenetic signal or a correlation with any of the geographical characteristics tested, despite the fact that *Cophixalus* frogs occur in the coldest regions of the AWT. These results may suggest that additional factors not studied here may be influencing their tolerance to cold, or potentially the evolutionary history of the species shaped the parameter on environmental conditions that no longer exist in present time in the Wet Tropics (this subject will be examined further in Chapter 4). Most of the *Cophixalus* frogs in the study region are able to tolerate the actual minimum temperature for its correspondent collection sites (Tables 3.1 and 3.3). It is important to consider that behavioural and refuge buffering from macroenvironmental climate may also help these frogs to avoid extreme conditions (see previous chapter), implying that geographic characteristics of their distribution have not shaped this thermal parameter.

These results for $CT_{max}$ and the unusual results for $CT_{min}$ remained after repeating the geographical analyses using collection sites rather than species and even including potential exposure conditions these frogs may have at their collection sites (Appendixes 3.2 and 3.3, respectively). This last analysis aimed to discard the potential confounding effect of acclimation to local environmental conditions on physiological tolerances. The results support the potential importance of $CT_{max}$ being shaped by geographical parameters and having an important role on limiting the warm end of the distribution of *Cophixalus* frogs. This environmental limitation will be important when ambient temperature increases due to climate change. Small changes in temperature may produce important changes in species fitness depending on thermal physiology (Huey et al. 2012). For *Cophixalus* frogs in the AWT, this situation will be more important in the warm border of their distribution (lower regions) (See chapter 5).

$CT_{min}$ has been shown to vary in a greater magnitude than $CT_{max}$ across elevational gradients, including amphibian larvae in the Ecuadorian Andes (Gutiérrez-Pesquera, 2016), however this pattern may be biased by strong cold adaptations of organisms occurring in elevations over 2000 m (Tejedo, M pers. com.). This seems also to be the case for direct developing frogs of the genus *Pristimantis* in South America (Pintanel P., unpublished data). It is possible that the lack of evidence of correlations of $CT_{min}$ with any ecological factor, in our study, is related to the reduced elevational range in where these frogs occur (0 – 1600 m). This lack of correlation may also imply no direct potential effect on this thermal tolerance in a new warmer environment, since AWT ambient (and microhabitat) temperature is projected to increase and consequently to move away from the thermal limit.

It is expected that tropical ectotherm species present narrower physiological tolerances than temperate communities (Deutsch et al., 2008; Tewksbury et al., 2008). It is noteworthy then that, *Cophixalus* frogs from northeastern Australia have shown narrower thermal tolerance ranges compared to other amphibian species in both temperate and tropical communities: supporting the hypothesis of narrower thermal tolerances in the tropics (Janzen, 1967). Gutiérrez-Pesquera et al. (2016) showed that a tropical amphibian community in Brazil presents narrower tolerances compared to a Palearctic temperate community (34.2 °C vs. 38 °C on average, respectively). A similar study on an elevational gradient in the Ecuadorian Andes also showed a similar thermal range for tropical anurans (Gutiérrez-Pesquera, 2016). Although these studies were carried out on tadpoles, we could speculate that the pattern may hold for adult tolerances. None of the species tested by Gutiérrez-Pesquera et al. (2016) and Gutiérrez-Pesquera (2016) showed tolerances as narrow as *Cophixalus exiguus*, the species with the widest tolerance in our study (25.5 °C). Narrow thermal tolerances may be more prevelant in species living in aseasonal environments (Ghalambor et al., 2006), and is expected to increase risk of extinction under contemporary climate change

(Tewksbury et al., 2008). This empirical evidence supports modelling predictions about the gloomy future fate of the *Cophixalus* frogs in the AWT (Williams et al., 2003).

These results imply that for *Cophixalus* frogs, environmental factors have shaped their thermal physiology more than the phylogenetic history of the species. These present or past environmental factors should be limiting the contemporary realized geographical distribution of the species. Potential changes in wet vegetation in the Australian tropics have been proposed as major driver of biodiversity richness (VanDerWal et al., 2009). These changes may have promoted isolation of populations on mountain tops that maybe influenced natural history traits (Moritz et al., 2012). If this is the case, any change in environmental conditions may dramatically affect the survival of these kind of restricted species.

Plasticity on thermal tolerances in anurans has been poorly studied; however, tadpoles in a tropical elevational gradient have shown levels of Acclimation Ratio Response similar or higher than temperate species (Gutiérrez-Pesquera, 2016). A better understanding of the potential adaptive capacity of these traits will be important to assess the potential impacts of climate change on these species (Huey et al., 2012; Williams et al., 2008).

Due to the low vagility of these species, the impacts of environmental thermal change will depend on the resilience of the species to survive in their own environments by tolerating or avoiding conditions close to their physiological tolerances. It has been proposed that rates of climatic niche evolution needed to maintain resilience in the face of anthropogenic climate change should be faster than any known record (Quintero & Wiens, 2013a). Consequently, the capacity of the species survival will depend on buffering from these environmental conditions, via behaviour or microhabitat use (Williams et al., 2008). Microhabitat use has been widely studied on Australian *Cophixalus* frogs (Hoskin, 2004; Hoskin & Hero, 2008; Hoskin & Higgie, 2005; Shoo et al., 2010), and its buffering potential was analysed in the previous chapter. Storlie et al. (2014) showed that microclimate projected data better match $CT_{max}$ of some of the *Cophixalus* frogs and this is also supported by supplementary analysis here (Appendix 3.2). The exploitation of this microhabitat will be fundamental for the survival of some species to anthropogenic climate change (Williams et al., 2008), especially for species with narrow physiological tolerances, as we present here for *Cophixalus* frogs.

Our results have provided a better understanding on what may be the potential factors limiting the distributio of *Cophixalus* frogs in the AWT focusing on the effects of phylogenetic and geographical characteristics on the thermal physiology traits. This new knowledge is fundamental for better and more accurate assessments of potential climate change impacts in this group of threatened and restricted Australian vertebrate species.

## 3.6 Acknowledgements

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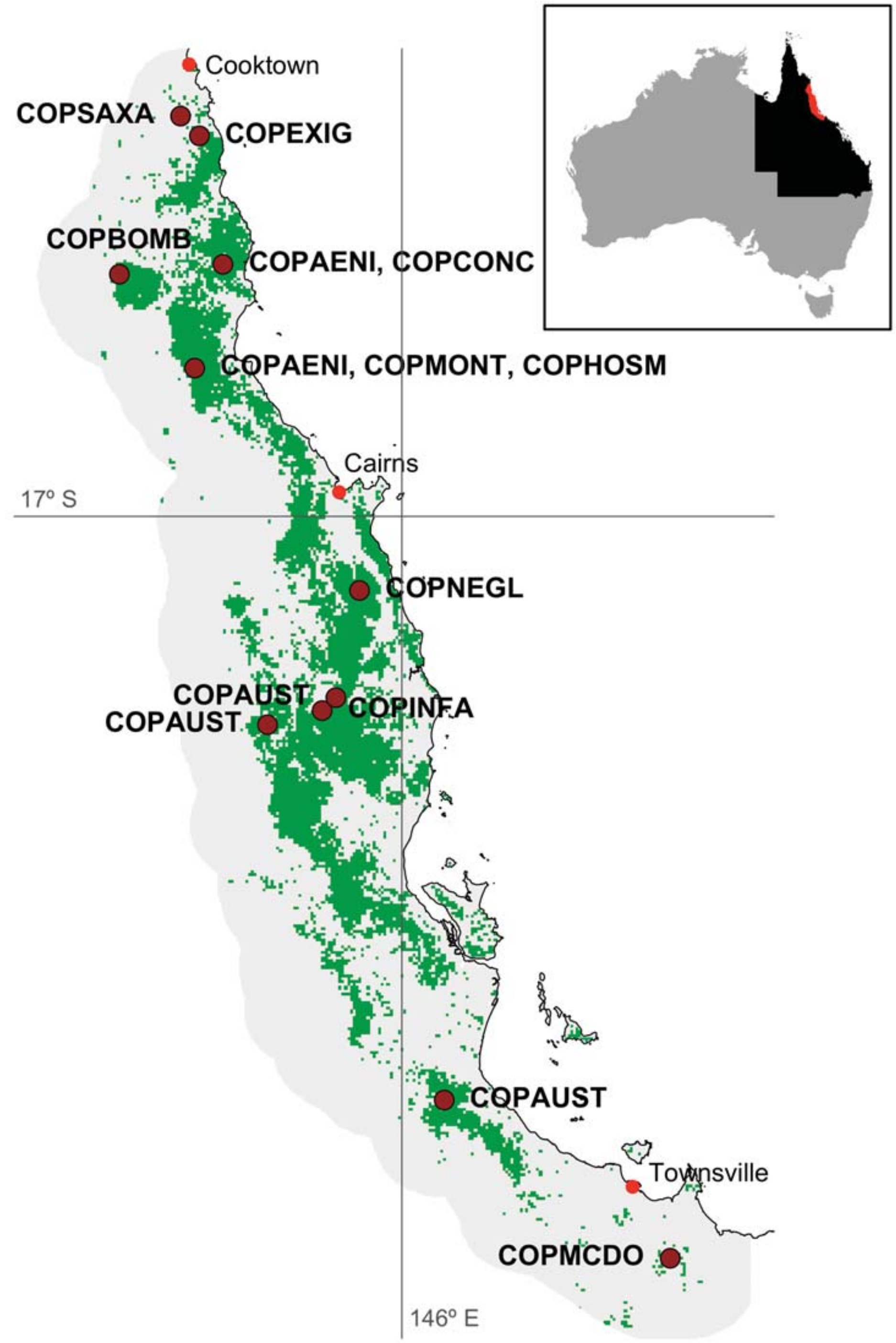


**Figure 3.1.** Location of thermal physiology data collection sites (brown circles) and species tested across the Wet Tropics Region. The current extent of the Wet Tropics rainforest is shown in green. *Cophixalus* codes represent a combination of genus and species names (Figure. 1.1).

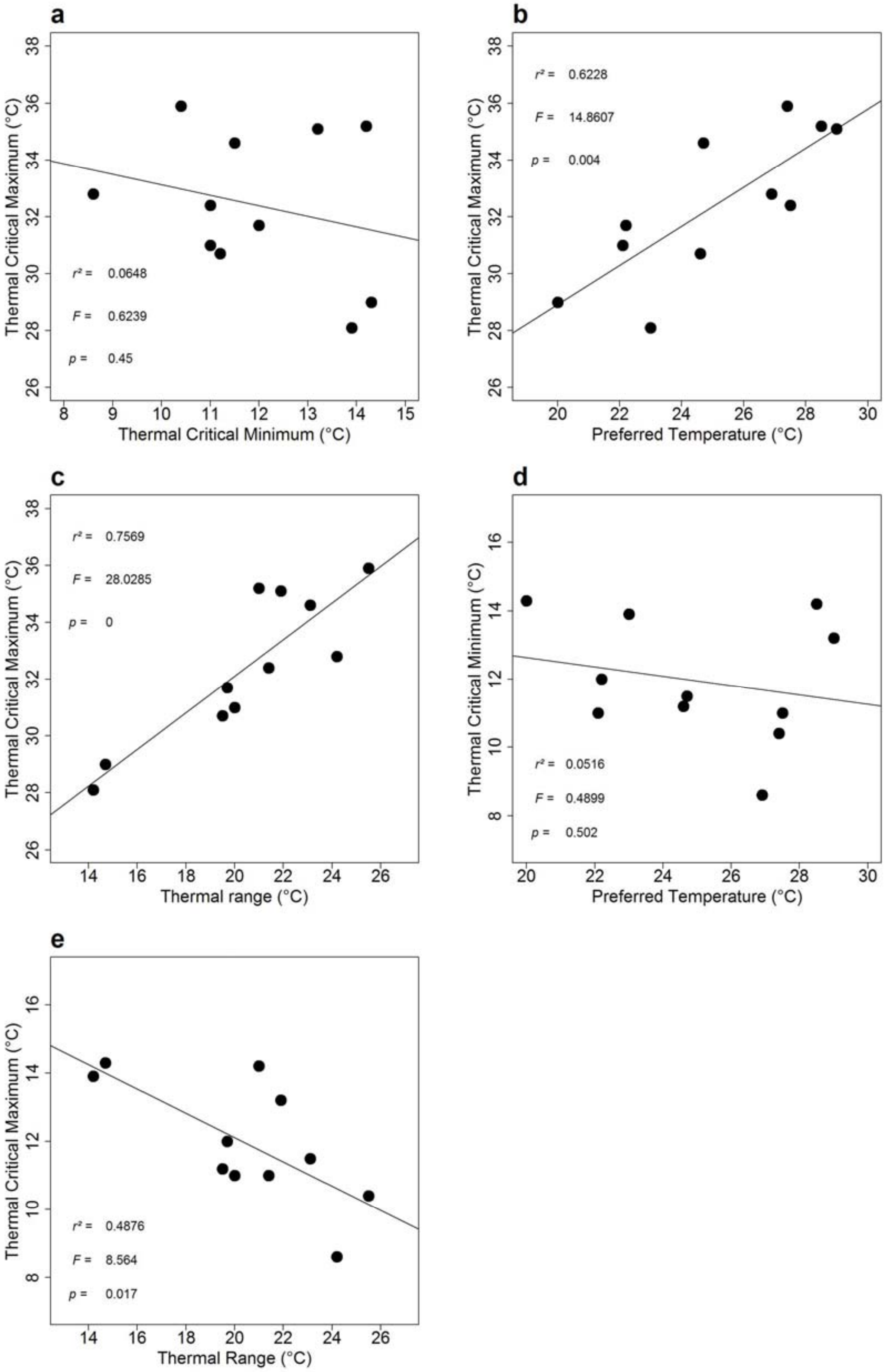
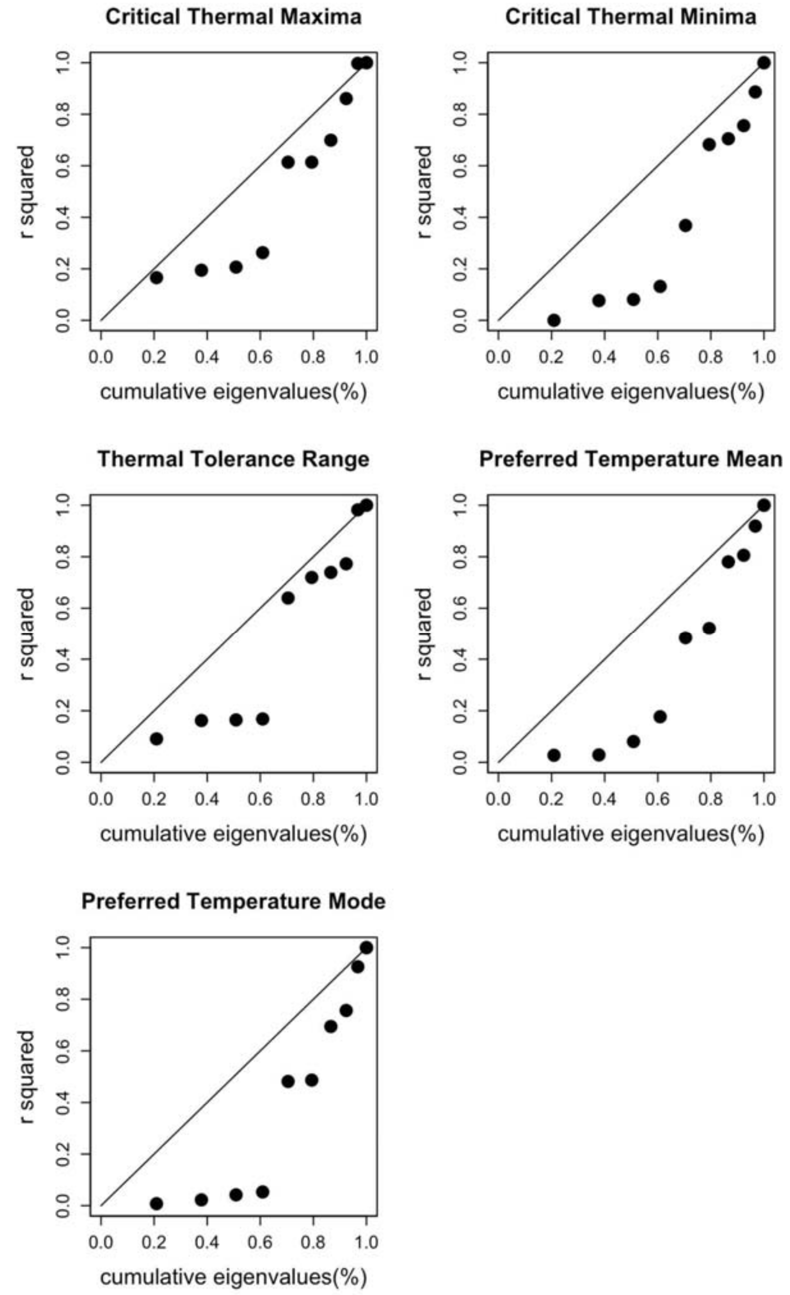


**Figure 3.2.** Linear regression of pairwise thermal physiology traits of *Cophixalus* frogs from the Wet Tropics Bioregion. Statistical results ($r^2$, F, and $p$ values) are shown within the graph. Regressions b, c, and e showed significant relationships.



Critical Thermal Maxima

Critical Thermal Minima

Thermal Tolerance Range

Preferred Temperature Mean

Preferred Temperature Mode

**Figure 3.3.** Phylogenetic signal representation (PSR) curves for thermal physiology traits of Australian *Cophixalus* frogs of the Wet Tropics Bioregion. All parameters show slower evolution rate than and do not approaches the Brownian motion evolution model represented by the 45° line.

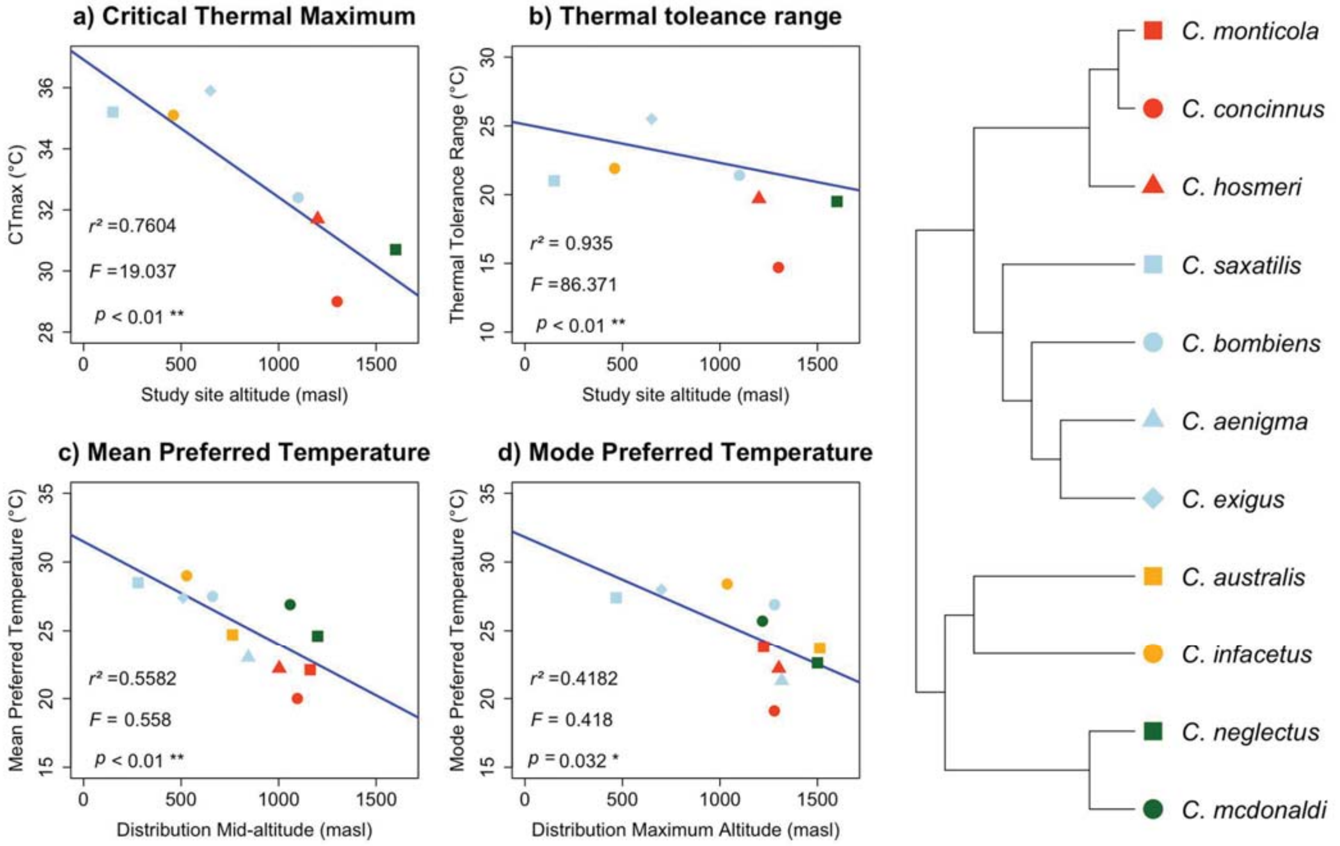


**Figure 3.4.** Statistically significant regression of thermal physiology traits of *Cophixalus* frogs from the Wet Tropics Bioregion with edited phylogeny used for this analysis. Colour points show position of the species within the phylogeny.

**Table 3.1.** Experimental sites visited along the Wet Tropics Bioregion, their elevation, geographical distribution, and thermal characteristics for eleven *Cophixalus* species. The distributional parameters are reported in the literature (Hoskin & Hero, 2008; IUCN, 2014; Shoo & Williams, 2004; Shoo et al., 2010; Williams et al., 2010b). Elevation sites and geographical distribution in m.a.s.l. Climate data generated by Storlie et al. (2013).

| **Species** | **AWT Subregion** | **Study site elevation** | **Geographical distribution** | | | **Temperature data (°C)** | | |
|---|---|---|---|---|---|---|---|---|
| | | | **Maximum elevation** | **Minimum elevation** | **Mid elevation** | **Maximum** | **Mean** | **Minimum** |
| *Cophixalus aenigma* | Carbine uplands | 1200 | 1317 | 372 | 844.5 | 20.2 | 16.1 | 11.3 |
| | Thornton Uplands | 1300 | | | | 21.5 | 17.9 | 13.6 |
| *C. australis* | Atherton Uplands | 1000 | 1512 | 15 | 763.5 | 22.9 | 17.1 | 11.1 |
| | | 400 | | | | 30.1 | 21.9 | 13.8 |
| | Spec Uplands | 1000 | | | | 24.4 | 18.4 | 11.6 |
| *C. bombiens* | Windsor Uplands | 1100 | 1280 | 42 | 661 | 20.2 | 16.6 | 11.7 |
| *C. concinnus* | Thornton Uplands | 1300 | 1279 | 913 | 1096 | 21.5 | 17.9 | 13.6 |
| *C. exiguus* | Finnegan Uplands North | 650 | 700 | 320 | 510 | 25.6 | 21.7 | 17.1 |
| *C. hosmeri* | Carbine uplands | 1200 | 1302 | 704 | 1003 | 20.2 | 16.1 | 11.3 |
| *C. infacetus* | Atherton Uplands | 460 | 1037 | 20 | 528.5 | 28.8 | 21.3 | 13.5 |
| *C. macdonaldi* | Mt. Elliot Uplands | 1100 | 1218 | 900 | 1059 | 24.5 | 19.3 | 13.4 |
| | | 900 | | | | 23.1 | 18.9 | 13.7 |
| *C. monticola* | Carbine uplands | 1200 | 1224 | 1100 | 1162 | 20.2 | 16.1 | 11.3 |
| *C. neglectus* | Mt. Bellenden Ker | 1600 | 1622 | 900 | 1261 | 19.2 | 14.6 | 9.3 |
| *C. saxatilis* | Finnegan Lowlands – Shiptons Flat | 150 | 468 | 90 | 279 | 30.0 | 23.9 | 17.8 |

**Table 3.2.** Preferred temperature for eleven species of *Cophixalus* frogs from the AWT. The mean of the parameter is presented with their standard deviation (SD) and standard error (SE). Species are ordered based on mean value of preferred temperature. Values are presented in °C.

| **Species** | **n** | **Mean** | **SD** | **SE** | **Mode** |
|---|---|---|---|---|---|
| *Cophixalus infacetus* | 5 | 29 | 2.1 | 0.2 | 28.4 |
| *C. saxatilis* | 8 | 28.5 | 3.2 | 0.3 | 27.4 |
| *C. bombiens* | 9 | 27.5 | 4.4 | 0.4 | 26.9 |
| *C. exiguus* | 6 | 27.4 | 2.7 | 0.3 | 28 |
| *C. mcdonaldi* | 10 | 26.9 | 4 | 0.4 | 25.7 |
| *C. australis* | 21 | 24.7 | 2.6 | 0.2 | 23.7 |
| *C. neglectus* | 17 | 24.6 | 3.8 | 0.4 | 22.6 |
| *C. aenigma* | 6 | 23 | 4 | 0.4 | 21.3 |
| *C. hosmeri* | 6 | 22.2 | 2.6 | 0.2 | 22.2 |
| *C. monticola* | 4 | 22.1 | 5 | 0.6 | 23.8 |
| *C. concinnus* | 7 | 20 | 1.8 | 0.2 | 19.1 |

**Table 3.3.** Thermal tolerances for eleven species of *Cophixalus* frogs from the AWT. The mean of each parameter is presented with their standard deviation. Species are ordered based on Thermal Critical Maxima ($CT_{max}$). Values are presented in °C.

| **Species** | **n** | **$CT_{min}$** | **SD** | **n** | **$CT_{max}$** | **SD** | **Thermal range** |
|---|---|---|---|---|---|---|---|
| *Cophixalus exiguus* | 7 | 10.4 | 1.4 | 6 | 35.9 | 1.0 | 25.5 |
| *C. saxatilis* | 5 | 14.2 | 1.9 | 5 | 35.2 | 0.5 | 21.0 |
| *C. infacetus* | 5 | 13.2 | 0.7 | 5 | 35.1 | 0.7 | 21.9 |
| *C. australis* | 16 | 11.5 | 3.4 | 17 | 34.6 | 1.7 | 23.1 |
| *C. mcdonaldi* | 5 | 8.6 | 0.8 | 8 | 32.8 | 0.7 | 24.2 |
| *C. bombiens* | 6 | 11.0 | 2.4 | 10 | 32.4 | 3.2 | 21.4 |
| *C. hosmeri* | 5 | 12.0 | 5.4 | 4 | 31.7 | 0.4 | 19.7 |
| *C. monticola* | 4 | 11.0 | 0.2 | 4 | 31.0 | 0.8 | 20.0 |
| *C. neglectus* | 14 | 11.2 | 2.6 | 13 | 30.7 | 2.6 | 19.5 |
| *C. concinnus* | 1 | 14.3 |  | 6 | 29.0 | 2.0 | 14.7 |
| *C. aenigma* | 2 | 13.9 | 5.6 | 3 | 28.1 | 2.3 | 14.2 |

**Table 3.4.** Pagel's λ (Pagel, 1999), *K*-statistics (Blomberg et al., 2003), and area under the Phylogenetic Signal-Representation (PSR) curves (Diniz-Filho et al., 2012) values for the five thermal traits of *Cophixalus* frogs in the Wet Tropics Bioregion.

| **Variable** | **λ** | ***K*-statistic** | ***p*** | **Area under PSR curve** |
|---|---|---|---|---|
| $CT_{min}$ | $5e^{-5}$ | 0.684 | 0.676 | -0.243 |
| $CT_{max}$ | $5e^{-5}$ | 0.880 | 0.267 | -0.14 |
| Thermal breadth | $5e^{-5}$ | 0.692 | 0.67 | -0.165 |
| Preferred temperature mean | 1.097 | 1.211 | 0.038* | -0.234 |
| Preferred temperature mode | 0.119 | 0.810 | 0.417 | -0.266 |

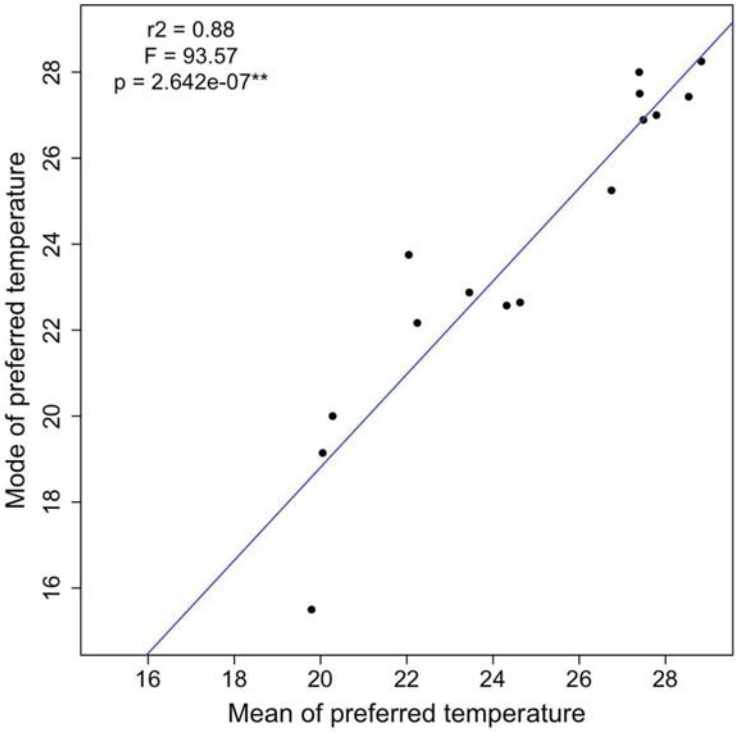
**Table 3.5.** Summary of phylogenetic generalized linear models (PGLS). The values in bold font show the significant (*) and highly significant (**) regressions. AltRange represents the elevational range of the species; ColAlt represents the elevation of experimental sites; Lat represents the latitude of the experimental sites; Max. elev, Min.elev and Mid.elev represent the parameters showing the actual elevational distribution of the frogs. Breadth represents the Thermal Tolerance Range and Mean.Tp and Mode.Tp represent the mean and the mode of the Preferred Temperature.

| PGLS | λ | AIC | Slope | | SE | Intercept | | SE | r2 | F | *p* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Breadth ~ AltRange | 0 | 61.77394 | 0.00034 | ± | 0.00 | 20.24224 | ± | 2.14 | 0.0018 | 0.016 | 0.901 | |
| **Breadth ~ ColAlt** | **0** | **9.49512** | **-0.00284** | **±** | **0.00** | **25.11254** | **±** | **0.32** | **0.93504** | **86.371** | **< 0.01** | ** |
| Breadth ~ Lat | 1 | 29.57244 | 0.76639 | ± | 0.93 | 35.38763 | ± | 15.60 | 0.10099 | 0.674 | 0.443 | |
| Breadth ~ Max.elev | 0 | 60.4766 | -0.00368 | ± | 0.00 | 24.77188 | ± | 4.15 | 0.11285 | 0.113 | 0.312 | |
| Breadth ~ Mid.elev | 0 | 60.04909 | -0.00441 | ± | 0.00 | 24.1232 | ± | 3.11 | 0.14667 | 1.547 | 0.245 | |
| Breadth ~ Min.elev | 0 | 60.76715 | -0.00248 | ± | 0.00 | 21.68708 | ± | 1.67 | 0.08911 | 0.880 | 0.373 | |
| $CT_{max}$ ~ AltRange | 0 | 54.99243 | 0.00079 | ± | 0.00 | 31.8653 | ± | 1.57 | 0.0177 | 0.018 | 0.697 | |
| **$CT_{max}$ ~ ColAlt** | **0** | **28.88943** | **-0.00448** | **±** | **0.00** | **36.91464** | **±** | **1.09** | **0.76036** | **19.037** | **< 0.01** | ** |
| $CT_{max}$ ~ Lat | 0.718 | 39.50124 | 0.59566 | ± | 1.62 | 42.85716 | ± | 27.02 | 0.02199 | 0.135 | 0.726 | |
| **$CT_{max}$ ~ Max.elev** | **1** | **49.67961** | **-0.00568** | **±** | **0.00** | **39.43531** | **±** | **2.64** | **0.43949** | **7.057** | **0.026** | * |
| **$CT_{max}$ ~ Mid.elev** | **0** | **47.17773** | **-0.00613** | **±** | **0.00** | **37.4794** | **±** | **1.73** | **0.51726** | **9.644** | **0.013** | * |
| $CT_{max}$ ~ Min.elev | 0 | 50.52046 | -0.00362 | ± | 0.00 | 34.17369 | ± | 1.05 | 0.34584 | 4.758 | 0.057 | |
| $CT_{min}$ ~ AltRange | 0 | 46.77471 | 0.00045 | ± | 0.00 | 11.62306 | ± | 1.08 | 0.01217 | 0.012 | 0.747 | |
| $CT_{min}$ ~ ColAlt | 0 | 31.2763 | -0.0012 | ± | 0.00 | 13.31045 | ± | 1.26 | 0.14428 | 1.012 | 0.353 | |
| $CT_{min}$ ~ Lat | 0 | 32.5135 | 0.07488 | ± | 0.90 | 13.39434 | ± | 14.76 | 0.00116 | 0.007 | 0.936 | |
| $CT_{min}$ ~ Max.elev | 0 | 46.43482 | -0.00115 | ± | 0.00 | 13.26624 | ± | 2.19 | 0.04223 | 0.397 | 0.544 | |
| $CT_{min}$ ~ Mid.elev | 0 | 45.91151 | -0.00172 | ± | 0.00 | 13.3562 | ± | 1.64 | 0.08673 | 0.855 | 0.379 | |
| $CT_{min}$ ~ Min.elev | 0 | 46.07936 | -0.00114 | ± | 0.00 | 12.48661 | ± | 0.86 | 0.07268 | 0.705 | 0.423 | |

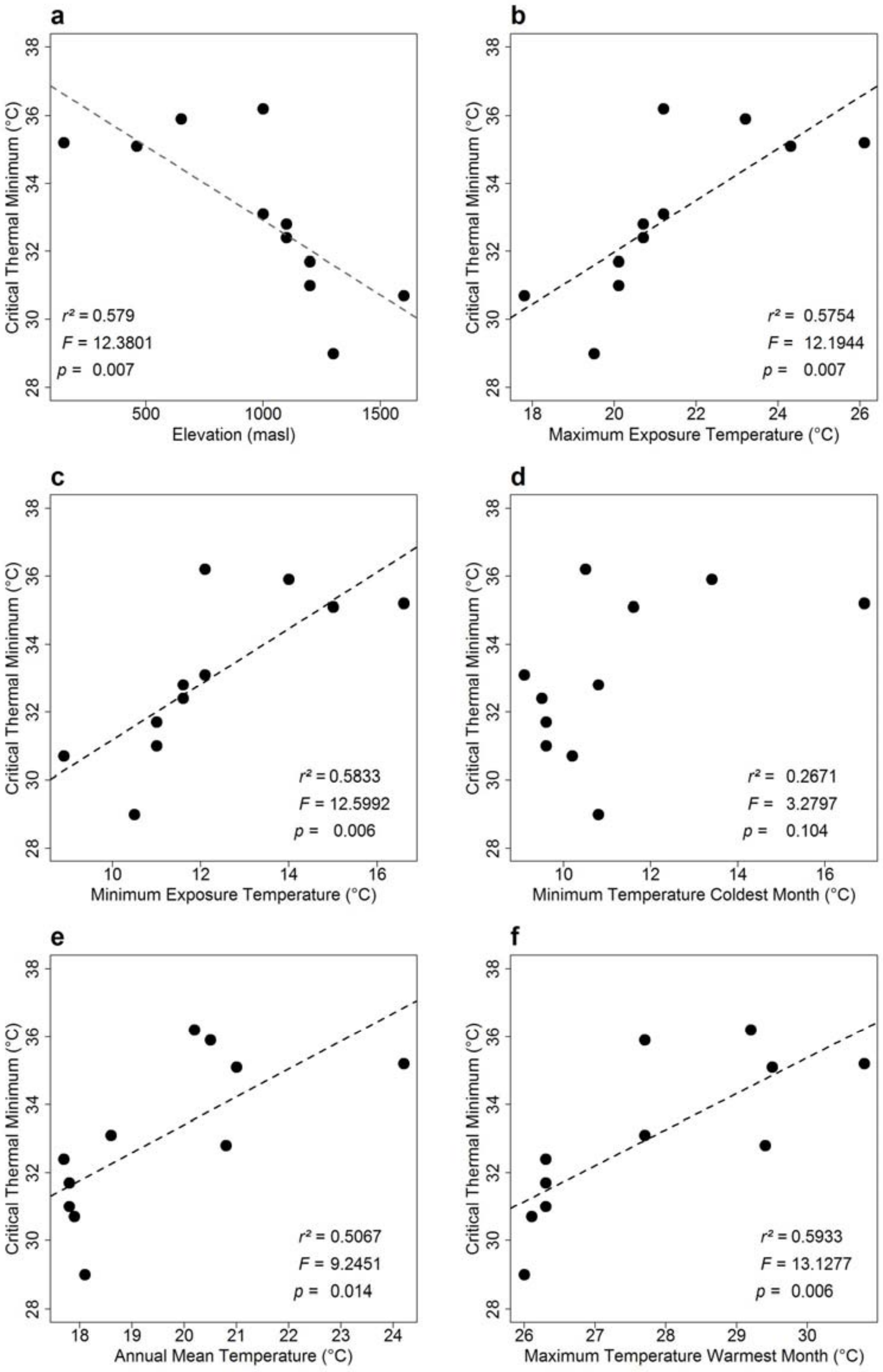
**Table 3.5.** Summary of phylogenetic generalized linear models (PGLS). The values in bold font show the significant (*) and highly significant (**) regressions (continued).

| PGLS | λ | AIC | Slope | | SE | Intercept | | SE | r2 | F | P | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean.Tp ~ AltRange | 1 | 55.58677 | -0.00047 | ± | 0.00 | 26.00903 | ± | 2.03 | 0.00522 | 0.005 | 0.833 | |
| Mean.Tp ~ ColAlt | 0.920 | 36.30448 | -0.00425 | ± | 0.00 | 29.9911 | ± | 2.04 | 0.49156 | 0.492 | 0.053 | |
| Mean.Tp ~ Lat | 1 | 41.49144 | -0.70107 | ± | 1.97 | 14.39507 | ± | 32.87 | 0.02075 | 0.127 | 0.734 | |
| **Mean.Tp ~ Max.elev** | **1** | **49.6753** | **-0.00544** | **±** | **0.00** | **31.95553** | **±** | **2.64** | **0.41879** | **0.419** | **0.031** | ***** |
| **Mean.Tp ~ Mid.elev** | **0.824** | **46.91374** | **-0.00751** | **±** | **0.00** | **31.48586** | **±** | **1.91** | **0.55818** | **0.558** | **< 0.01** | ****** |
| **Mean.Tp ~ Min.elev** | **0.481** | **51.6201** | **-0.00439** | **±** | **0.00** | **27.3524** | **±** | **1.19** | **0.3678** | **0.368** | **0.048** | ***** |
| Mode.Tp ~ AltRange | 0 | 58.54414 | 0.00064 | ± | 0.00 | 24.02842 | ± | 1.84 | 0.00844 | 0.008 | 0.788 | |
| **Mode.Tp ~ ColAlt** | **0** | **37.97171** | **-0.0053** | **±** | **0.00** | **29.8774** | **±** | **1.92** | **0.5882** | **8.570** | **0.026** | ***** |
| Mode.Tp ~ Lat | 0.585 | 44.39284 | 0.09029 | ± | 2.13 | 26.74048 | ± | 35.42 | 0.0003 | 0.002 | 0.968 | |
| **Mode.Tp ~ Max.elev** | **0.327** | **52.72224** | **-0.00615** | **±** | **0.00** | **31.81681** | **±** | **2.94** | **0.41825** | **0.418** | **0.032** | ***** |
| **Mode.Tp ~ Mid.elev** | **0** | **50.20064** | **-0.0073** | **±** | **0.00** | **30.5045** | **±** | **1.99** | **0.53558** | **0.536** | **0.010** | ***** |
| Mode.Tp ~ Min.elev | 0 | 54.20331 | -0.00415 | ± | 0.00 | 26.49098 | ± | 1.24 | 0.33175 | 0.332 | 0.064 | |

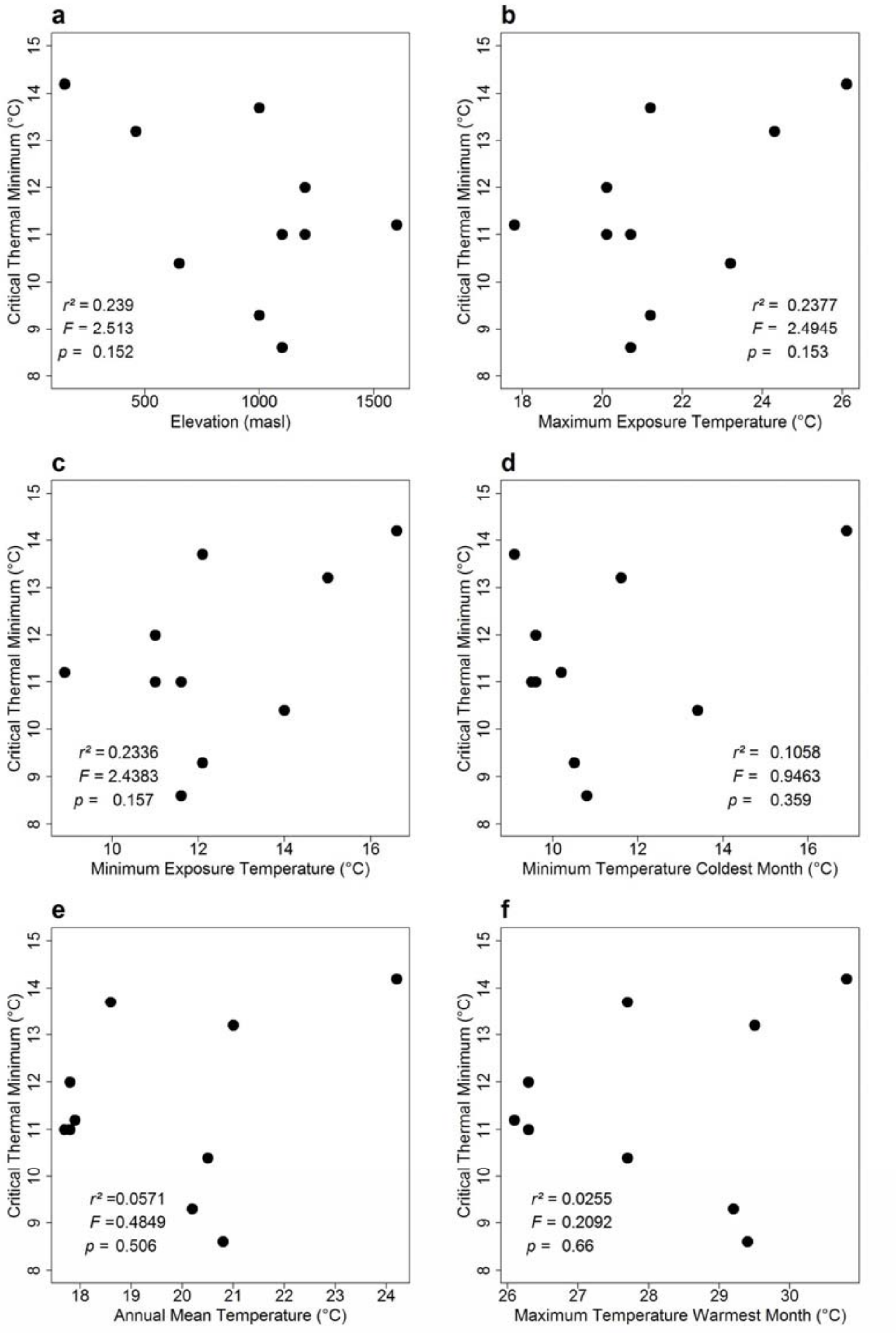
**Appendix 3.1**. Regressions between preferred temperature parameters (Mean and mode)



**Appendix 3.2**. Ordinary Least Square Regressions results for CT$_{max}$ and elevation, extreme macroclimate variables for the AWT (Storlie et al., 2013), and actual Microhylid frogs extreme exposure conditions in the AWT (see next chapter) by study locations. All the linear regressions were significant except for the minimum temperature of the coldest month. *Cophixalus aenigma* data was excluded because of the low number of observations (*n*=3).



**Appendix 3.3**. Ordinary Least Square Regressions results for $CT_{min}$ and elevation, extreme macroclimate variables for the AWT (Storlie et al., 2013), and actual Microhylid frogs extreme exposure conditions in the AWT (see next chapter) by study locations. None of the linear regression were statistically significant. *Cophixalus aenigma* and *C. concinnus* data were excluded because of the low number of observations (*n*=1 and *n*=2, respectively).



# Chapter 4. Unexpected variety of geographical distribution constraints on the endemic and restricted *Cophixalus* frogs of northeastern Australia

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## 4.1 Summary

Understanding the factors that determine the geographic distribution of species is a fundamental aspect of ecology and biogeography that is now becoming even more important with the increasingly severe impacts of anthropogenic climate change.
An element of adapting to and managing climate change impacts on natural ecosystems relies on accurate predictions of the changes that climate change will have on the limits of the geographical distribution of a species. These factors may involve direct impacts via species physiological preferences and tolerances or indirect impacts via biotic/abiotic interactions and barriers to movement. Robust predictions are more complicated if we consider that the boundary of a species distribution can change over time and be influenced by multiple factors simultaneously. Sadly, comprehensive analyses of these potential thresholds are limited in the literature by insufficient knowledge on individual species traits. To address this problem, we have gathered available information on thermal physiology, ecology and distribution for eleven species of *Cophixalus* frogs in the Wet Tropics Bioregion of Northeastern Australia. This unique group of frogs make a significant contribution to the biodiversity values of the Wet Tropics World Heritage Area as they are endemic, geographically restricted rainforest specialists. The microhylids are primarily restricted to the uplands of the mountains in the region and many species are endemic to just one or a couple of mountain tops with very limited potential to move to maintain suitable climate space under climate warming. These factors combine to make these frogs, one of the most threatened groups of vertebrates in Australia. Here, we significantly improve our understanding of microhylid distributions by considering a combination of species ecology, the realised distribution ranges of the frogs, measured physiological tolerances and species specific exposure conditions. Using this information, we modelled fundamental niches during the warmest and the coldest periods of the year across the elevational range of the species within the Wet Tropics Bioregion. We present evidence that even closely related species may have different factors limiting their realized geographical distribution. Direct physiological limitations are important for some species at the cold boundary of the distribution (*C. bombiens* and *C. monticola*). On the other hand, geography is a limiting factor for *C. bombiens, C. australis*, and *C. infacetus* at warm boundary and *C. exiguus, C. mcdonaldi*, and *C. saxatilis* at their cold boundary. *Cophixalus monticola* also showed geographical limitation in distribution at the cold border suggesting interactions between several factors. Species underfilling the

theoretical fundamental niche at the warm boundary suggests other (biotic or abiotic) factors besides geography and temperature may be driving the distribution threshold. Several species showed overfilling of the potential niche at the cold boundary suggesting that the use of microhabitats with better thermal buffering may be a key factor in their survival at high elevations. This explanation can be suggested for *C. concinnus* occurring at boulder fields at Thornton Peak, where our analysis shows a complete mismatch between potential and realized elevational distribution. None of the species was limited by $CT_{max}$, suggesting minimal direct threat from environment warming. This improved understanding of the factors limiting the distributions of *Cophixalus* species will improve the assessment of the potential vulnerability of each species to climate change impacts, thereby enabling more informed management and policy decisions and species-specific management strategies aimed at climate change adaption in the region.

## 4.2 Introduction

Geographical distribution of species are the result of a complex interaction between different aspects of physiology, ecology, behaviour and evolutionary history (Gaston et al., 2009). Physiology can shape the fundamental niche of a species, restricting the environmental space to where it can find all the conditions required to indefinitely accomplish all the metabolic functions (Hutchinson, 1957; Kearney & Porter, 2004; Peterson & Soberón, 2012). This fundamental niche will not necessarily be filled, especially in the presence of significant biotic interactions (prey/predator, diseases, etc.), or limitations on dispersal (Peterson & Soberón, 2012). These processes can influence realised distributions of species or populations; however, the environment plays an important role (Bozinovic et al., 2011; Snyder & Weathers, 1975; Stevens, 1989, 1992), not only in the present but also in the past (VanDerWal et al., 2009; Wiens et al., 2006).

Tropical ectotherms, because of their exposure to a less variable thermal environment, may have adapted their physiological traits to a narrower set of conditions (e.g. temperature) than similar species in temperate zones, which are exposed to a wider set of environmental conditions during annual seasons (Janzen, 1967; Tewksbury et al., 2008). This narrower set of conditions may influence the actual geographical range where a species occurs. Stevens (1989) proposed that these physiological constraints can explain what he called Rapoport's rule, where the species in the tropics have smaller geographical distributions than similar species in high latitudes. Amphibians have been shown to have thermal tolerances matching the range of temperatures available in their environments (Snyder & Weathers, 1975) and tropical tadpoles have narrower tolerances than temperate ones (Gutiérrez-Pesquera et al., 2016). Since elevational variation is analogous in many ways to latitudinal variation (Bozinovic et al., 2011), physiology is usually considered to be an important constraint for distribution of species along elevational gradients. Janzen (1967) proposed that these physiological constraints for each species may produce dispersal consequences. A tropical mountain could be a more effective dispersal barrier than the same mountain in the temperate zones due to species having evolved within a narrower range of environmental conditions within the tropics than in more temperate areas.

Physiology has often been ignored as part of ecogeographical analyses (Bozinovic et al., 2011), however, it is now one of the most important parameters to be considered, given the predicted consequences of ongoing climate change (Gaston et al., 2009). Projections based on future climate scenarios predict that organisms will be forced to move pole wards or to higher elevations in order to maintain themselves in suitable environments (Chen et al., 2011; Laurance et al., 2011). Indeed, these trends have already been observed in many systems across many taxa worldwide and have far reaching consequences both for biodiversity, natural ecosystems and human wellbeing (Pecl et al., 2017). However, lack of empirical data describing the thermal physiology of most species makes it difficult to determine the true parameters affecting the species distribution boundaries and consequently impede making robust predictions about their potential sensitivity to climate change. Williams et al. (2008) emphasised that to understand the vulnerability of species to climate change it is important to consider both the real exposure of the species to the environmental change and their intrinsic sensitivity to the change.

This situation is especially important for species having narrow geographical (latitudinal and elevational) distribution ranges or specialized ecology (e.g. habitat or microhabitat use). These characteristics are usually typical of restricted endemic species, which arguably need more specific future conservation attention.

*Cophixalus* frogs of northeastern Australia and the Wet Tropics Bioregion present several of these characteristics, offering an opportunity to test several of the aspects (biotic or abiotic) that may be constraining the frog's distribution and speculate about their potential responses to changes in thermal regimes in that environment.

The microhylid frogs of the genus *Cophixalus* are small, terrestrial breeders that are, morphologically conservative, with cryptic lifestyles in the leaf-litter of rainforests (Hoskin, 2004; Shoo & Williams, 2004; Williams, 2007). Most of the species in the AWT are restricted to one or few mountaintops (Williams, 2007). Like other endemic vertebrates of the AWT, these species occur only in cool, wet and relatively aseasonal environments (Williams et al., 2003). Graham et al. (2006) suggested that the present pattern of species richness in this group has been affected by vicariance among historical refugia as well as by the persistence of the species within the refugia through time. This evolutionary history may have produced adaptations in these frogs (e.g. narrow physiological tolerances) that may have direct implications under climate change scenarios.

Here, we evaluate whether the realized elevational distribution ranges of *Cophixalus* frogs match their expected elevational ranges based on the actual exposure environmental range and their physiological tolerances. We explore how the limits to the actual distribution are affected by topography within their geographical distribution. Finally, we explore how future changes on thermal regimes in the AWT might affect the populations of *Cophixalus* frogs at both the upper (cold) and lower (hot) distribution boundaries.

## 4.3 Methods

### 4.3.1 Thermal tolerances

The critical thermal maximum ($CT_{max}$) and minimum ($CT_{min}$) were obtained for eleven of the thirteen species of *Cophixalus* frogs that occur in the Wet Tropics of northeastern Australia. Only *Cophixalus ornatus* and *Cophixalus hinchinbrookensis* were not included because at the time of this study *C. australis* and *C. hinchinbrookensis* were considered as mere populations of *C. ornatus* (Hoskin, 2012). Considering that most of the species have restricted geographical distributions, only one population per species was studied, except for *C. australis* that has a larger geographical distribution, and *C. aenigma* that was found at two locations. *Cophixalus mcdonaldi* was tested from two elevations (1100 and 900 m) in the same AWT subregion (Mt. Elliot uplands). The twelve locations visited, encompass representative species from a diverse range of rainforest environments from Finnegan Lowlands in the North to Elliot Uplands in the South and were selected spanning an elevation range from 150 to 1600 m (See Table 3.1; Figure 3.1).

More details about the methods have been described in section 3.3.3.

Results for *Cophixalus aenigma* ($CT_{max}$ and $CT_{min}$) and *C. concinnus* ($CT_{min}$) must be considered with caution because the number of individuals tested is low.

### 4.3.2 Thermal exposure of *Cophixalus* frogs in the AWT microhabitats

Temperature/relative humidity loggers (iButton® DS1923) were set in 26 confirmed Microhylid frog refuges spread across locations in the region between 100 and 1600 m. Refuges comprised four epiphytes fallen from trees, ten rocks and 12 logs (Table 2.1). Refuges were monitored between March 2009 and December 2010. Loggers were also set at 1.5 m above the ground at the same places as the refuge to measure ambient air conditions. Data were gathered at 1 hour resolution.

We simulated the realized exposure based on activity times for Microhylid frogs verified with behavioural observations in the field (see Chapter 2).

Methods are described in detail in sections 2.3.1 and 2.3.4.

The warmest and coldest monthly mean temperatures were derived from exposure sequences from each site and then linked to the elevation of the site (Table 2.1). A linear regression was applied to the warmest and coldest temperatures, according to elevational gradient. Regression lines were drawn to the warm temperatures ($a= 26.973382$, $b= -0.005744$, $r^2= 0.9454$, $p<0.001$) and the cold temperatures ($a= 17.41083$, $b= -0.005315$, $r^2= 0.8061$, $p<0.001$). These regression lines were used to describe the annual extreme temperatures microhylid frogs are exposed to in the AWT (Fig. 4.1).

### 4.3.3 Geographical distribution and potential topographic constrictions for *Cophixalus* frogs

For each studied species, we collated information on their geographic distribution, including recorded elevational range. Generally, the highest elevational record corresponded to the top of the highest mountain range within the species distribution range. This information was derived from published and on-line references (Hoskin & Hero, 2008; IUCN, 2014; Royal Geographical Society of Queensland, 2016; Shoo et al., 2010; Williams et al., 2010b) (Table 4.1). For *Cophixalus saxatilis*, there is no reported top elevational limit, so we assumed its maximum elevation to be the highest point within its distribution.

### 4.3.4 Elevational estimate of the potential fundamental niche for *Cophixalus* frogs

The potential elevational range where frogs can find thermal conditions within their tolerances was defined for each species within the elevational range of the AWT. This range of elevations was limited by the intercept between the $CT_{max}$ and the maximum potential exposure, which describes the lowest elevation (warm boundary) at which that species would be able to survive, and the intercept between $CT_{min}$ and the potential minimum exposure temperature, which sets the highest elevation (cold boundary) at which that species may survive. The polygon formed between these limits represents the potential fundamental thermal niche of the species.

### 4.3.5 Niche filling condition

To assess if the potential fundamental niche of the species is filled, we compared the actual elevational limit of the species against the limit imposed by thermal tolerances. The differences between the potential and the realized distribution range determines if the species is overfilling or underfilling its potential range at both of the (warm/low and cold/high) boundaries. A visual demonstration of the model developed to quantify the thermal constraints in the available AWT microclimate envelope is shown Figure 4.1.

In cases where the species are underfilling the cold boundary of the niche we assessed the effect of topography within the actual distribution range of the species. We considered all the species might reach sea level as the lowest potential elevation if physiology tolerance allowed it.

## 4.4 Results

### 4.4.1 Thermal tolerances

The critical thermal maximum ($CT_{max}$) and minimum ($CT_{min}$) for the eleven *Cophixalus* species are summarized in Table 3.3. $CT_{max}$ ranged from 28.1 °C to 35.9 °C and $CT_{min}$ from 8.6 °C to 14.3 °C.

### 4.4.2 Thermal space for the rainforest in the AWT

Figure 4.2 shows the thermal range along the altitudinal gradient for the AWT Rainforest. As expected, it shows a decrease in temperature according to elevation for maximum and minimum limits. The rate of change of temperature is similar between the two parameters. Data show a lapse rate in temperature of -0.4 °C per 100m increase in elevation for maximum temperature and a change of -0.5 °C per 100m for minimum temperature. The intercepts show a difference between the warm and cold limit of the AWT rainforest temperature of 15.9 °C, ranging from 15.3 °C to 31.2 °C at sea level.

### 4.4.3 Thermal exposure space for *Cophixalus* frogs in the AWT

Exposure conditions for *Cophixalus* varied with elevation at a rate of 0.6 °C and 0.5 °C per 100m increase in elevation for maximum and minimum temperatures respectively. The intercepts of the regression lines show a difference of 9.6 °C at sea level, ranging from 17.4 °C to 27 °C.

### 4.4.4 Niche filling condition

The geographical characteristics of the distribution of the *Cophixalus* frogs are presented in Table 4.1 and the niche filling results are summarized in Figure 4.3. $CT_{max}$ limits the warm side of the distribution (Fig. 4.2). Species can not survive in lowland environments where the temperatures they are exposed to are higher than their physiological tolerances and conversely they can not survive at high elevations where they are exposed to temperatures below their $CT_{min}$.

When we compared the potential thermal niche of the frogs to their actual elevational range (Fig. 4.2), the data show that none of the species are currently restricted by their physiological tolerances at their warmer boundary (lower distribution elevation). This implies that every species tested may have the capacity to survive at sea level. *Cophixalus bombiens, C. australis* and *C. infacetus* have been recorded at very low elevations so it is possible to consider them as filling their potential niche. Two species (*Cophixalus bombiens* and *C. monticola*) fill the cold boundary of the distribution. Six species (*C. aenigma, C. australis, C. concinnus, C. hosmeri, C. infacetus* and *C. neglectus*) show some degree of overfilling the potential niche. *C. exiguus, C. mcdonaldi,* and *C. saxatilis* underfill their potential niche, and are also topographically constrained since all of them are restricted by the top elevation within their geographical distribution range.

## 4.5 Discussion

A better understanding of the potential limits on the distribution of *Cophixalus* frogs in the AWT is an important advance on informing potential conservation strategies facing ongoing climate change. The knowledge gathered here will allow authorities to identify species and places where vulnerability is high based on much more robust assessment of vulnerability than provided by correlative distribution models as it implicitly includes measured physiological tolerances. This enables managers and policy makers to focus conservation strategies on species and specific boundaries of the distribution where the species is most vulnerable (e.g. low elevation populations) and adaptation actions can be most effective.

The lack of comprehensive studies analysing thermal physiology, actual exposure, and realized distribution of organisms (Bozinovic & Pörtner, 2015; Sexton et al., 2009) makes it difficult to compare these results to other similar geographical climate change predictions.

The only general pattern found was that all *Cophixalus* species were shown to be physiologically capable of tolerating the thermal environments across the region, even in the hotter lowlands. This was an unexpected result given that many of the species are restricted to cool mountain tops. Further, exposure conditions for *Cophixalus* frogs are buffered from the macroclimate found within the rainforest of the AWT by the combination of microhabitat, and diel activity period (nocturnal) (Chapter 2, Fig. 4.1).

Three species occur at elevations close to sea level (*Cophixalus bombiens, C. australis*, and *C. infacetus*) suggesting that they have potentially filled their fundamental niches. Other groups of species are underfilling their thermal niche at the lower elevation warm boundary. *Cophixalus saxatilis* is an endemic species restricted to the boulder fields (Hoskin & Hero, 2008) present in Black Trevethan Range region - a characteristic that maybe is limiting the lowest distribution range of the species. Boulder fields have previously been shown to provide high levels of temperature buffering (Shoo et al., 2010). Boulder microhabitats will undoubtedly provide an environment for the microhylids that significantly buffers both the temperature and moisture conditions for this species. *Cophixalus monticola*, is another special case; it uses *Lynospadix apetiolatus* palms at around 1.5 m above ground as refuges (Hoskin, 2004; Hoskin & Hero, 2008). It is possible that this microhabitat is less buffered than rocks, fallen ferns or logs at ground level. Its maximum exposure may be higher than estimated here which may approach the limitation imposed by their thermal tolerances; however, its elevational range may also be related to the distribution of the palm at Mt. Lewis (the reported elevational range of the palm is from 400 to 1300 m, but it is also present at Mt. Spurgeon, though there are no elevational reports for this mountain range; Dowe, 2010). The other species underfilling their thermal niche at the warm boundary include *Cophixalus aenigma, C. exiguus, C. hosmeri, C. mcdonaldi*, and *C. neglectus*. These species may be limited by other environmental factors such as moisture availability or biotic factors such as presence of competing species.

When we examine the cold boundary of the distribution of these frogs, we found that *Cophixalus monticola* and *C. bombiens* fill most of their potential thermal niche based on the physiological tolerances. Interestingly, for *C. monticola* this limitation fits the maximum elevation available within its distribution range, suggesting that topography may have shaped its thermal tolerance and acts as an inescapable limit to its distribution. *Cophixalus exiguus, C. mcdonaldi,* and *C. saxatilis* underfill their potential at the cold boundary and would seem to be limited by the available elevational range rather than by an intrinsic physiological limitation. Under a climate change context these results are relevant since there would be the potential to translocate these species to other places of higher elevation if required whilst remaining within physiological limits (Shoo et al., 2011a). *Cophixalus aenigma, C. australis, C hosmeri, C. infacetus* and *C. neglectus* overfill their potential thermal fundamental niche. This situation implies that these species are already exposed in their current distribution to temperatures that are outside their tolerances, a situation that should not be possible (Sexton et al., 2009). The most plausible explanation is that these species may occupy more efficient microhabitats than the specific refugia monitored in this study enabling them to buffer their exposure more than predicted here. This has been suggested for other species that have been shown to be latitudinally overfilling their potential niche (Sunday et al., 2011). In chapter 2, we present behavioural observations that suggest frogs may change diurnal refugia implying the capacity to select different levels of buffering. This buffering could potentially protect individuals more than suggested by our analyses. This behavioural plasticity will also be important when assessing the options of species to face climate change (Williams et al., 2008). For *Cophixalus aenigma*, the large difference between actual and potential limits can be explained by an underestimated thermal tolerance due to the low number of individuals tested for $CT_{min}$. This may also be the case for *C. concinnus*, albeit this species is known to occur in boulder fields at the top of Thornton peak, where it benefits from significant thermal buffering resulting in a stable microenvironment (Shoo et al., 2010). At low temperatures the boulder fields offer a warmer environment (Shoo et al., 2009), increasing the exposure temperature and more closely matching the distribution of the species. This suggests that boulder fields are not only important for buffering maximum temperatures but also provide buffering on the cold boundary of distributions. Managing or manipulating refuge sites offers some potential for adaptive management via manipulation of exposure in microhylid frogs or other species that utilise similar diurnal refuges.

Maintaining tolerable levels of moisture / humidity can be a very important factor for the distribution of amphibians (Dood Jr., 2009; Duellman & Trueb, 1994; Heyer et al., 1994). The AWT rainforest humidity is influenced by rainfall and by cloud stripping. Cloud stripping is the process of condensation from clouds in the rainforest canopy and has been shown to be an extremely important factor in determining water input into these montane forests particularly during the dry winter (McJannet et al., 2007a). Rainfall is highly variable across the year (150mm - 600mm/month) throughout most of the region (Pearson, 1994). The assessment of other environmental parameters and the responses of the frogs to changes in those parameters will significantly improve our understanding of the relative vulnerability of the region's biodiversity.

It is also important to consider that distribution limits may be imposed by limitations on survival of other life history stages different to adults. Australian Microhylid frogs present direct development of terrestrial eggs that are laid in moist soil or leaf litter. It is highly likely that the most severe limitations of temperature and moisture are imposed on the survival of eggs and embryos and/or the small metamorphs. Their small body size would make them highly susceptible to lethal water loss under dry and/or hot conditions.

Understanding the determinants of geographical range limits is of fundamental importance for the potential adaptive management of endemic ectotherms with restricted distribution ranges. *Cophixalus* frogs have highly restricted geographic and elevational ranges that result in predictions of high vulnerability under projected future climate change (Williams et al., 2016; Williams et al., 2003). Surprisingly, our results suggest thermal tolerance is not likely to be a major constraint on the altitudinal distributions of these microhylid frogs, especially at the warm border of the distribution.

Increasing our understanding of the ecophysiological mechanisms determining their distributions is important for informing conservation managers on the specific factors that are important and helping them to make informed conservation decisions about potential management actions when they become necessary. To integrate as many factors (e.g. link thermal tolerances to environmental variables) as possible as part of the potential climate change impacts is fundamental for more accurate assessments (Bozinovic & Pörtner, 2015).

## 4.6 Acknowledgments

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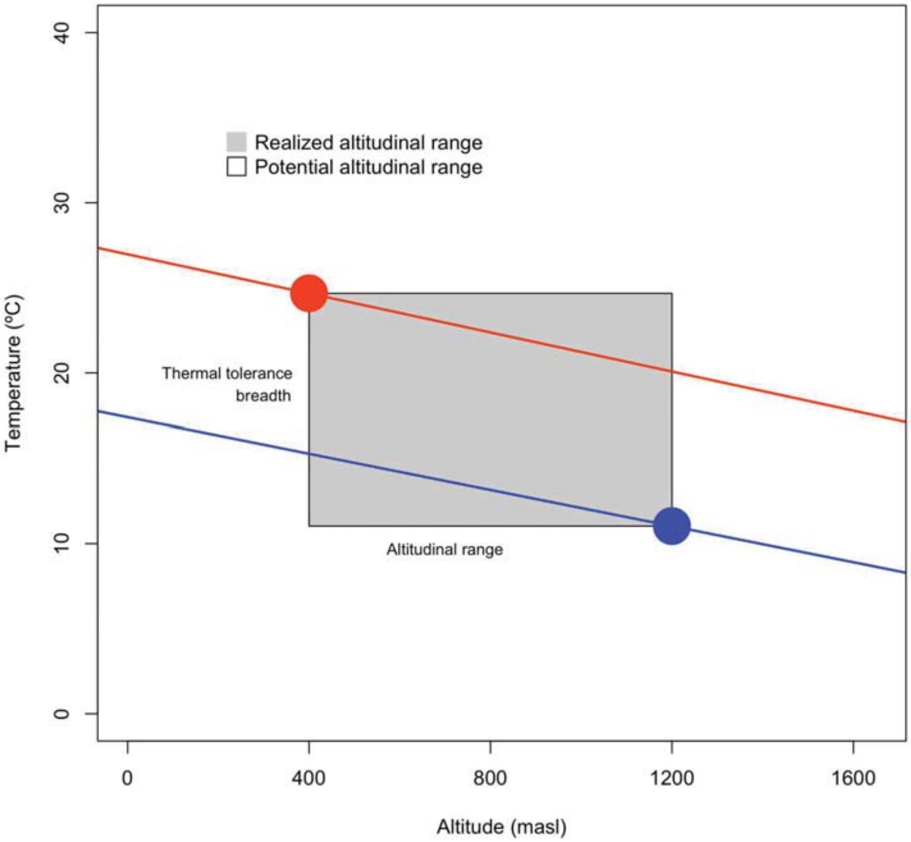


**Figure 4.1.** Theoretical approach considering the potential thermal exposure conditions for *Cophixalus* frogs along the elevation gradient of the AWT. Red line shows maximum exposure and blue line minimum exposure. Red dot represents $CT_{max}$ and blue dot represents $CT_{min}$. The intersection between maximum exposure and $CT_{max}$ limit the potential lowest (warm) boundary of the distribution of the species and the intersection between $CT_{min}$ and minimum exposure determines the potential maximum elevation (cold boundary) of the distribution. Grey polygon shows the actual elevational distribution range of the species. In this case the species is filling the potential (fundamental) niche.

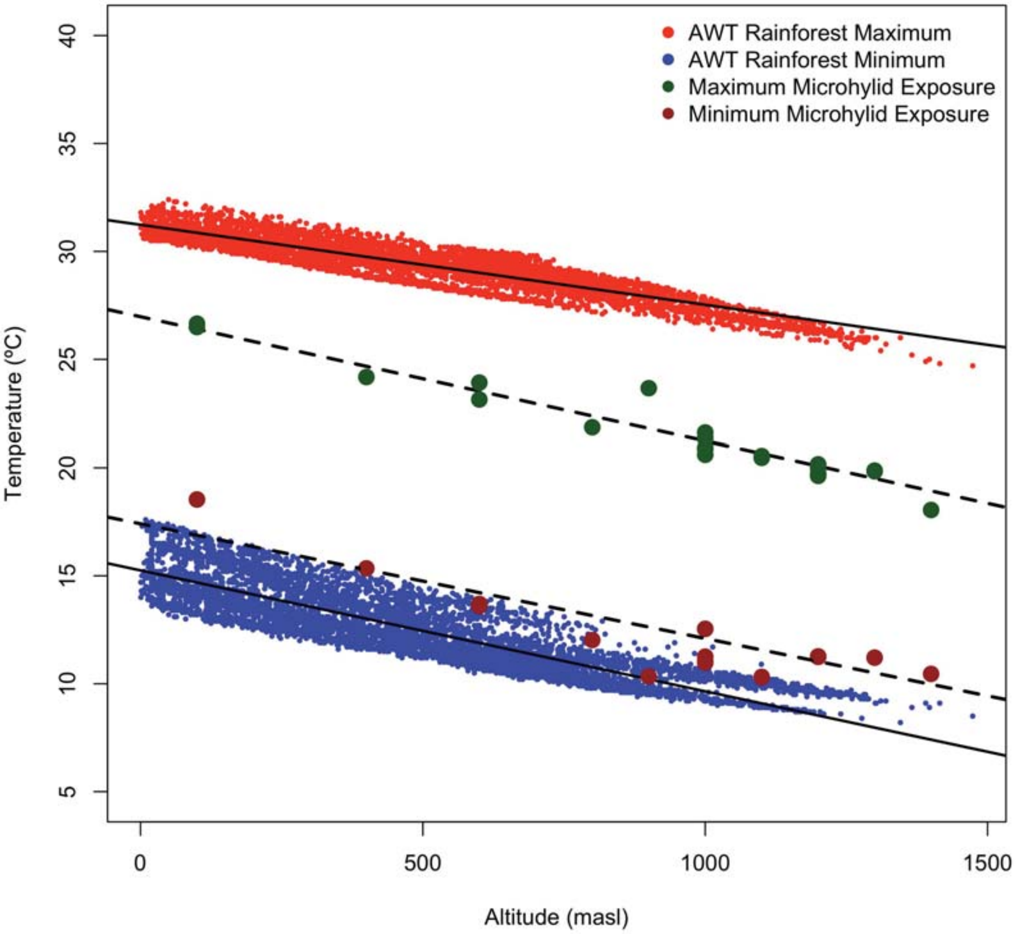
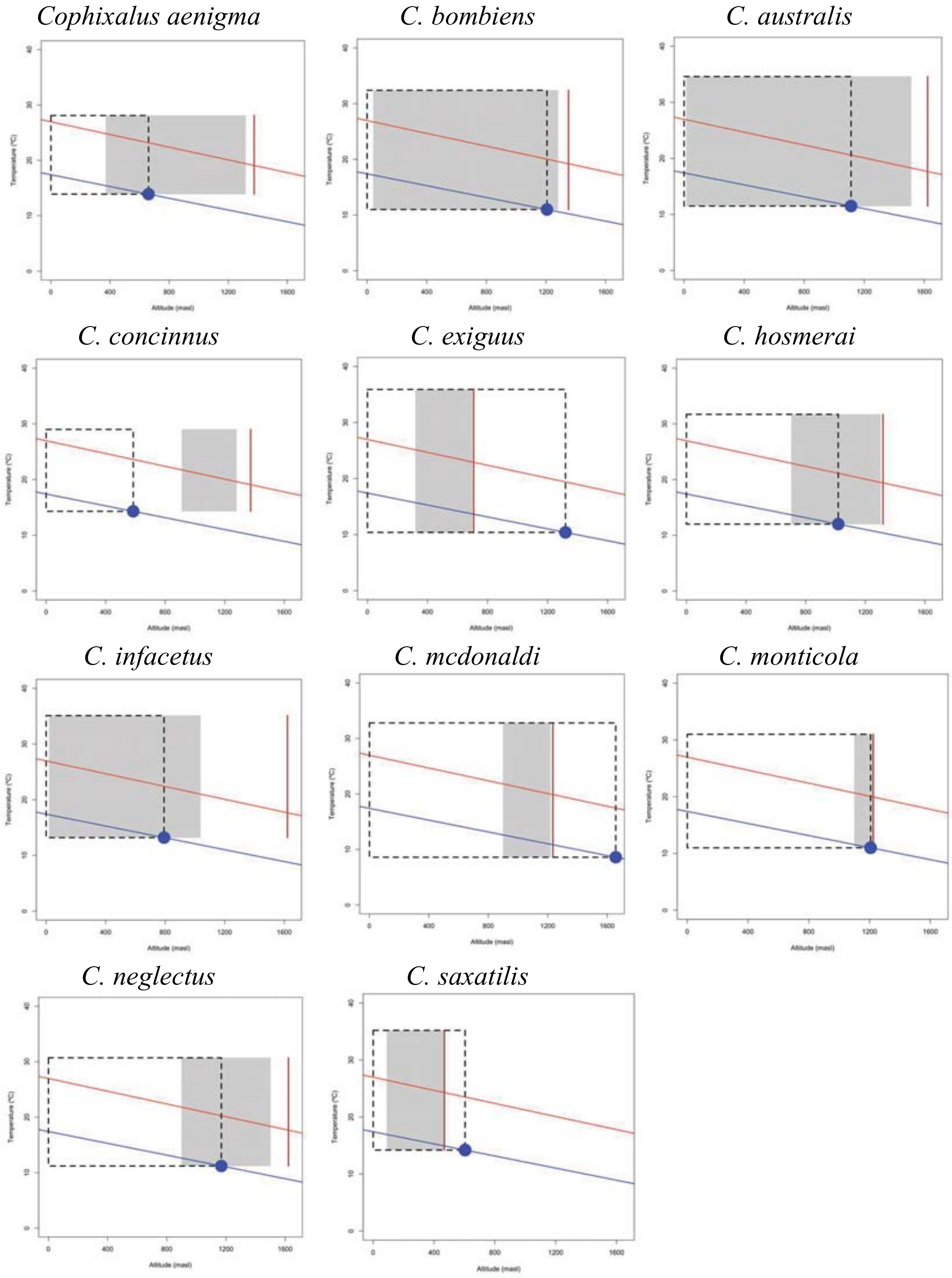


**Figure 4.2.** Warmest and coldest temperature within the Australian Wet Tropics rainforest across elevation for macroclimate (red and blue dots respectively with linear regressions represented as black solid lines) and exposure conditions for each species of Microhylid frogs (green and maroon dots respectively with linear regressions represented as black dashed lines).



*Cophixalus aenigma*

*C. bombiens*

*C. australis*

*C. concinnus*

*C. exiguus*

*C. hosmerai*

*C. infacetus*

*C. mcdonaldi*

*C. monticola*

*C. neglectus*

*C. saxatilis*

**Figure 4.3.** Microhylid exposure temperature, thermal tolerances, elevational distribution range and potential elevational fundamental niche for 11 species of *Cophixalus* in the AWT. Grey box represents the elevational range reported for every species. Vertical brown line represents the reported maximum altitude within the frogs' geographical distribution. Red and blue solid lines represent maximum and minimum microhylid frogs' exposure along the elevational gradient of the AWT. Blue dot represents the altitude where exposure conditions reaches $CT_{min}$, becoming the cold boundary of the fundamental niche of the species (black dotted line). Additional explanation of the graph is available in Figure 4.1.

**Table 4.1.** Geographical distribution of *Cophixalus* frogs of the AWT (maximum and minimum elevation) and highest point within their distribution range. Data from published and on-line references (Hoskin & Hero, 2008; IUCN, 2014; Royal Geographical Society of Queensland, 2016; Shoo et al., 2010; Williams et al., 2010b)

| **Species** | **Actual geographical distribution** | | **Potential highest elevation** | |
|---|---|---|---|---|
| | **Maximum elevation** | **Minimum elevation** | **Location** | **Elevation** |
| *Cophixalus aenigma* | 1317 | 372 | Thornton Peak | 1375 |
| *C. australis* | 1512 | 15 | Bartle Frere | 1622 |
| *C. bombiens* | 1280 | 42 | Mt. Windsor | 1351 |
| *C. concinnus* | 1279 | 913 | Thornton Peak | 1375 |
| *C. exiguus* | 700 | 320 | Big Tableland | 708 |
| *C. hosmeri* | 1302 | 704 | Mt. Spurgeon | 1318 |
| *C. infacetus* | 1037 | 20 | Bartle Frere | 1622 |
| *C. mcdonaldi* | 1218 | 900 | Mt. Elliot | 1235 |
| *C. monticola* | 1224 | 1100 | Mt. Lewis | 1224 |
| *C. neglectus* | 1622 | 900 | Bartle Frere | 1622 |
| *C. saxatilis* | 468 | 90 | Black Mountain (Black Trevethan Range) | 468 |

# Chapter 5. Are Australian *Cophixalus* frogs imminently threatened by climate warming?

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## 5.1 Summary

Climate change is an inminent threat to biodiversity worldwide. Potential impacts have commonly been forecast using species distribution models and qualitative inferences from observations of natural history. Robust predictions of potential impacts on specific geographical areas is key to developing cost-effective management actions to minimise future biodiversity losses. Here, we provide a vulnerability analysis incorporating both sensitivity and exposure, using an ecophysiological approach, comprising physiological traits of 11 species of *Cophixalus* frogs (sensitivity) and the actual conditions (exposure) these species of frogs experience in their specific microhabitats across an elevational gradient in the Australian Wet Tropics bioregion (AWT). Exposure analyses include consideration of the specific thermal microenvironmental conditions, the degree of buffering from open air conditions and activity patterns and behaviour of the species. We also include a projected increase in thermal conditions in their environments and project the potential thermal risk for these species by 2035 and 2065. Based on these data, geographical regions of the AWT where species are most likely to be affected by climate warming are identified at the three analysed time frames. Our findings suggest that four of 11 species analysed may already be experiencing thermal conditions that exceed their preferred temperature potentially resulting in decreased fitness (*C. concinnus, C. australis, C. aenigma* and *C. hosmeri*). Negative effects on fitness are expected to be most prominent at the lowest elevational range of the distribution of these species but the threshold will ascend over time. A similar but delayed scenario is expected under future projected climates for *C. bombiens* (from 2035) and *C. monticola* (from 2065). The remaining five species are expected to be thermally buffered by their thermal physiology compared to the under risk species. None of the species will face complete thermal deficit (environmental conditions over their thermal critical maximum) across their distribution by 2065, however it is important to consider that the entire distribution range for *C. concinnus* from 2035, and almost the whole distribution of *C. monticola* in 2065 are predicted to exceed preferred temperatures, making them the most threatened species in the AWT. Thornton, Carbine, Atherton, Kirrima and Spec regions were identified as regions where a decrease in fitness is mostly to be experienced by affected *Cophixalus* frogs. We discuss the implications of these findings to focus adaptive management on species and geographical regions where conservation actions are needed most.

## 5.2 Introduction

Increasing temperature is one of the most evident effects of anthropogenic climate change. Temperature directly and indirectly influences many physiological and behavioural processes in organisms and thereby is a key driver of the distribution of most species (Huey et al., 2012). Several hypotheses link the geographical distribution area of ectothermic organisms to temperature (Bozinovic et al., 2011; Janzen, 1967; Stevens, 1992). These hypotheses imply temperature may limit latitudinal or elevational boundaries of species distribution, especially through its role as key factor determining the fundamental niche of the species (Kearney & Porter, 2004).

However, the processes through which temperature affects organisms are still unknown for many species and often interpretation is based on theories with little empirical validation (Bozinovic et al., 2011).

Thermal physiology is often ignored when assessing potential impacts of climate change, which is one of the most important threats to biodiversity in the present and future (Bozinovic et al., 2011; Rosenzweig et al., 2008). Climate change vulnerability can be assessed considering a framework where the risk of being affected depends on the interaction of species sensitivity and actual exposure to those changes (Williams et al., 2008). Species' thermal tolerance is an important parameter when measuring the actual sensitivity of a species and to assess how much thermal environmental change the species may bear in its natural habitats without human intervention (Deutsch et al., 2008; Duarte et al., 2012; Sunday et al., 2014).

Thermal regimes within a habitat depend on several factors such as microhabitat use based on behaviour, activity periods, ontogenetic stage, etc. (Gouveia et al., 2014; Gutiérrez-Pesquera et al., 2016; Scheffers et al., 2013, 2014). Organisms will be exposed only to a subset of the whole thermal variation present in that ecosystem (Kennedy, 1997). This actual exposure may have shaped some metabolic processes in species (Storlie et al., 2014) and its assessment is fundamental to accurately assess the potential impacts of climate change (Williams et al., 2008).

Comparisons of present and future correlative models of species distribution predict not only polarward movements but also elevational or multidirectional moves tracking suitable conditions (Thomas et al., 2004; VanDerWal et al., 2012). These models are an important tool to address potential impacts of climate change, albeit they have been critized because of their correlative approach and some of their inherent assumptions (Kearney et al., 2010a). The climate information used in these models represent macroenvironmental conditions which can not necessarily be directly extrapolated to the actual conditions organisms are exposed to in their natural habitats (Kennedy, 1997; Storlie et al., 2014; Chapter 2 in this document).

A more direct approach based on empirically measured physiological tolerances and linked to microenvironmental conditions can be a more powerful test of the influence of temperature on ectotherms (Andrew et al., 2013; Deutsch et al., 2008; Duarte et al., 2012). This approach calculates a warming tolerance (WT) range, which represents the difference between the maximum thermal tolerance and the maximum exposure temperature. WT represents the amount of temperature that a species may tolerate before facing a detrimental physiological thermal deficit (TD - when exposure is over tolerance), resulting in intolerable exposure in that location and potentially resulting in localised extinction. Therefore, warming tolerance (WT) provides a quantitative metric, based on measured physiology responses, that can be used as a metric for relative species vulnerability to climate change (Bozinovic et al., 2011).

These thermal effects are based on the potential consequences of increasing ambient temperature in ectotherms by the modification of performance (fitness) within the environment (Huey et al., 2012). Due to the asymmetric shape of a thermal performance curve in ectotherms (Appendix 5.), warming conditions may affect species in different ways. If a species is exposed to conditions under the optimum temperature, it can still have the chance to improve its performance in that environment until reaching optimum temperature (Thermal Safety Margin - TSM). However, if the same amount of temperature change pushes the exposure conditions over the optimum temperature, the decrease in performance in that new environment will be considerably lower than the original one (Huey et al., 2012).

Increasing our understanding of the direct processes and mechanisms relating increasing temperature under global climate change and the vulnerability of species is important for helping environmental policy makers and managers to make more informed decisions. More robust information about the relative vulnerability of each species and the exact spatial and temporal dimensions of that vulnerability will increase the capacity of managers to design more effective adaptation strategies aimed at reducing impacts.

Here, we present empirical data describing the thermal safety margins and warming tolerance ranges of eleven *Cophixalus* frogs and we analyse the potential impacts of predicted increases of temperature for 2035 and 2065 in the AWT. We provide empirical information of $CT_{max}$, preferred temperature and the maximum potential exposure conditions along the available elevational gradients of each *Cophixalus* species. This approach will determine where and when particular populations of each species will become threatened by climate warming in order to focus conservation strategies on species, space and time within the region.

## 5.3 Methods

### 5.3.1 Critical Thermal Maxima ($CT_{max}$)

The critical thermal maximum ($CT_{max}$) were obtained for eleven of the thirteen species of *Cophixalus* frogs occurring in the Wet Tropics of northeastern Australia. Only *Cophixalus ornatus* and *Cophixalus hinchinbrookensis* were not included because they were still formally under the name *Cophixalus ornatus* when this study was carried out. Most of the species have restricted geographical distributions, so only one population per species was studied, except for *C. australis* that has the largest geographical distribution and *C. aenigma* that was found at two locations in two different Wet Tropics subregions. *Cophixalus mcdonaldi* was tested from two elevations (1100 and 900 m) in the same Wet Tropics subregion. The twelve locations visited encompass representative species of the genus from a diverse range of rainforest environments from Finnegan Lowlands in the North to Elliot Uplands in the South, spanning an elevation range from 150 to 1600 m (Table 3.1; Figure 3.1).

Detailed methods for measuring $CT_{max}$ are described in section 3.3.3.

Results for *Cophixalus aenigma* must be considered with caution because the number of individuals tested is low.

### 5.3.2 Preferred temperature

In this climate change effects assessment, we consider the preferred temperature ($T_{pref}$) as a proxy for optimum temperature since some evidence shows a linear relationship between those two parameters in another group of ectotherms (reptiles) (Huey et al., 2009, 2012). Evidence of a correlation between these two parameters is also mentioned for tadpoles by Fry (1947). The preferred temperature experiments were performed within the first 24 hours following frog collection. Each experiment consisted of individually setting two frogs into a two lane metal linear gradient where a range of temperatures was offered to the animals (8 °C – 40 °C). Temperature was logged and recorded every minute at twelve equally separated points along the gradient using a DT500 Data Logger (dataTaker Inc). Frogs were monitored during 120 minutes via time lapse photography at one minute intervals. This enabled recording the exact position of the frog along the gradient to be ascertained at any given time. Gradient floor was covered with a water soaked cloth to avoid hydric stress on animals.

More detailed methods for this parameter are described on section 3.3.1.

### 5.3.3 Maximum thermal exposure for *Cophixalus* frogs in the AWT

Temperature/relative humidity loggers (iButton® DS1923) were set in 26 confirmed Microhylid frogs' refuges spread across locations in the region between 100 and 1600 m. Refuges comprised four epiphytes fallen from trees, ten rocks and 12 logs (Table 2.1). Refuges were monitored between March 2009 and December 2010. Additional loggers were set at 1.5 m above ground where refuges were found. Data were gathered at 1 hour resolution.

We simulated potential realized exposure based on activity times for Microhylid frogs verified with behavioural observations in the field (described in Chapter 3).

More detailed methods are described in detail in sections 2.3.1 and 2.3.4.

Warmest monthly mean temperatures were derived from exposure sequences from each site and then linked to the elevation of that location (Table 2.1). A linear regression was applied to the warmest and coldest temperatures, according to elevation. Regression lines were drawn to the warm temperatures ($a= 26.973382$, $b= -0.005744$, $r^2= 0.9454$, $p<0.001$) and the cold temperatures ($a= 17.41083$, $b= -0.005315$, $r^2= 0.8061$, $p<0.001$). These regression lines were used to describe the annual extreme temperatures microhylid frogs are exposed to in the AWT (Fig. 4.1).

### 5.3.4 Geographical distribution for *Cophixalus* frogs

For each studied species, we gathered information of their geographic distribution, including their maximum and minimum elevations. This information was derived from published and on-line references (Hoskin & Hero, 2008; IUCN, 2014; Royal Geographical Society of Queensland, 2016; Shoo et al., 2010; Williams et al., 2010b) (Table 4.1). For *Cophixalus saxatilis*, there is no reported maximum limit, we assumed the maximum elevation within its distributional range as its geographic limit.

Distribution data for *Cophixalus* species were provided by the Centre for Tropical Biodiversity and Climate Change data base (Williams et al., 2010b) and data collected during this study. Correlative models were developed using accuCLIM climate estimates (Storlie et al., 2013) and fitted using MaxENT algorithms (Phillips et al., 2006) (Appendix 1.1). These maps of habitat suitability were transformed into binary presence/absence maps using the maximum training sensitivity plus specificity logistic threshold provided by the algorithm. A map for *C. australis* was not available because of its recent description, the map presented is a modification from the *C. ornatus* map based on regions where Hoskin (2012) suggested this species occurs. Elevation at each presence pixel in the map was extracted from a 80m resolution Wet Tropics Bioregion digital elevation model resampled at the same extent and resolution of correlative models using functions of *raster* package (Hijmans, 2016) in R software (R Core Team, 2014).

### 5.3.5 Thermal safety margin and warming tolerance for present environmental conditions at *Cophixalus* frogs' distribution

Thermal safety margin (TSM) and warming tolerance (WT) was calculated for each species following Deutsch et al. (2008). However, we endeavoured to make the analysis more realistic for *Cophixalus* frogs, by using the actual exposure conditions of microhylid frogs mediated by the combination of microhabitat temperatures and activity patterns instead of simple ambient temperature. Since exposure conditions depend on elevation at which a population is found, we calculated the metrics at the lowest and highest elevations where the species occurs for present environmental conditions. TSM is the difference between maximum exposure temperature and the preferred temperature. The difference between the maximum exposure temperature and the Critical Thermal Maxima (CTmax) represents the Warming Tolerance (WT) (positive difference). Theoretical scenarios of the potential effects of temperature change that species may face at present or future environmental conditions are graphically summarized in Figure 5.1.

### 5.3.6 Estimating current and future vulnerability to increasing temperature of *Cophixalus* populations

We estimated thermal threat for *Cophixalus* frogs by determining the maximum elevation at which each species will face a decrease in performance (exposure temperature over $T_{pref}$) or thermal deficit (exposure temperature over $CT_{max}$) at three time frames (Current, 2035 and 2065). We used future mean temperature increases predicted for summer months (December-February) in the AWT (Hilbert et al., 2014). These predictions are summarized in Table 5.1.

The estimates of vulnerability to thermal impacts are based on the combination of both physiology and location and can therefore be used to generate geographically explicit assessments of vulnerability. We mapped the areas in which each species will face a decrease in performance. Maps of the species affected at each of the three time frames (current and two future times) were derived to illustrate potential future impacts of climate change on *Cophixalus* distributions.

## 5.4 Results

### 5.4.1 Thermal physiology parameters

The preferred temperature, critical thermal maximum thermal safety margin and warming tolerance for each species is presented in Table 5.2. The mean of the preferred temperature ranged between 20 °C (*Cophixalus concinnus*) and 29 °C (*Cophixalus infacetus*) and the critical thermal maxima between 28.1 °C (*Cophixalus aenigma*) and 35.9 °C (*Cophixalus exiguus*).

### 5.4.2 Exposure conditions for *Cophixalus* frogs

Maximum exposure temperatures for each species were calculated according to the lowest and highest elevation point for each species. The actual maximum temperature that each species was exposed to varied from 20.7 °C (*C. monticola*) to 26.9 °C (*C. australis* and *C. infacetus*) at the lowest recorded elevations and from 18.3 °C (*C. australis*) to 24.3 °C (*C. saxatilis*) at the highest elevations. $CT_{max}$ was higher than the exposure temperatures of the lowest point of the distribution for all the studied species. The exposure ranges for each species are summarized in Table 5.2.

### 5.4.3 Thermal safety margin

At present environmental conditions, the TSM estimates ($T_{pref}$ - Exposure temperature) for seven species ranged from 0.8 °C (*C. bombiens*) to 5.1 °C (*C. mcdonaldi*) at the lowest elevation, while for the other four species (*Cophixalus australis, C. hosmeri, C. concinnus* and *C. aenigma*) exposure temperature exceeds their $T_{pref}$. At their highest elevation, estimates for TSM across species range from 0.4 °C (*C. concinnus*) to 8 °C (*C. infacetus*). This results suggest that at present conditions, lowland populations of some species may be facing effects of exposure to temperatures higher than their optimum and consequently reducing their performance in the environment. The TSM values for each species is summarized in Table 5.2.

### 5.4.4 Warming tolerance

The Warming Tolerance (WT) estimates ($CT_{max}$ – Exposure temperature) ranged from 3.3 °C (*C. aenigma*) to 11 °C (*C. mcdonaldi*) at their lowest elevation of distribution and from 8.7 °C (*C. aenigma*) to 16.3 °C (*C. australis*) at their highest side of the distribution. The complete WT estimates for each Microhylid species are summarized in Table 5.2.

### 5.4.5 Present and future thermal threat for *Cophixalus* frogs

Four species are currently exposed to temperatures above their preferred temperature in some parts of their distribution: *Cophixalus australis, C. hosmeri, C. concinnus* and *C. aenigma*. Assuming that $T_{pref}$ is a proxy for the optimum thermal performance (Huey et al., 2012), this situation implies that their performance may already be comprimised. Our approach predicts that lowland populations of these species may be experiencing negative impacts on their fitness below specific elevations for each species: *Cophixalus australis* ~396 m, *C. hosmeri* ~831 m, *C. concinnus* ~1214 m and *C. aenigma* ~692 m. These elevational limits will shift higher while warming continues. By 2035, *C. bombiens* will be included in this group of species facing negative impacts occurring below 100 m. By 2065, *C. monticola* will also face reduced performance at elevations less than 1197 m. The description of the increasing negative impacts on fitness across the elevational gradient for each species is detailed in Table 5.3. A graphical comparison of the thermal situation for each species is presented in Figure 5.2. For a subset of affected especies (*C. aenigma, C. hosmeri, C. bombiens*, and *C. monticola*), the geographical regions affected at the different studied time frames are presented in Figure 5.3.

## 5.5 Discussion

The bulk of studies predicting the potential impacts of climate change on species have been based on species distribution models based on contemporary climate and projected into the future using potential scenarios of future climate (Araújo et al., 2006; Austin & Van Niel, 2011; Hijmans & Graham, 2006; Pearson & Dawson, 2003; Phillips & Dudík, 2008; Townsend Peterson, 2006). Little consideration has been given to the many other context-specific variables that directly influence either the conditions the organisms are exposed to in their natural habitat and ecology or the role of complex indirect mechanisms influencing, or buffering, potential climatic impacts on the species (Williams et al., 2008). It is assumed, in the absence of physiological data, that these correlative approaches capture the combination or sum of all variables influencing the distribution of the species.

Our results show the potential effect of temperature increase on populations of eleven species of *Cophixalus* frogs in the AWT. Our approach determines vulnerability to warming based on the actual exposure conditions these frogs will have across their elevational range considering behavioural and microhabitat buffering and their actual maximum thermal tolerance ($CT_{max}$). An important finding of this study is that, our data suggest that no population of any *Cophixalus* species occurring in the AWT will be exposed to temperatures that directly cause fatality (exceeding the Thermal Deficit - TD) (Table 5.3). Although none of the species are expected to face a TD, it is important to consider that exposure conditions at the lowest distribution elevation of *C. aenigma* are predicted to be close to the thermal critical maxima by 2065. Nonetheless, our results suggest pervasive decline in ecological fitness directly attributable to temperature increase that may cause population extinctions even where species stay within their thermal tolerance range.

Huey et al. (2012) proposed different scenarios of environmental warming effects, depending on the environmental conditions a population is exposed to. In a scenario where a species is exposed to conditions below its thermal optimum, a warming climate may produce an increase in species' fitness ability; while if the species is exposed to conditions over its thermal optimum, the fitness ability will rapidly decrease. *Cophixalus* frogs show both effects. Some species currently experience conditions under their optimal temperatures and as warming occurs an increase in thermal performance may be expected. This seems to be the case of *Cophixalus exiguus, C. infacetus and C. mcdonaldi* even under future scenarios out to 2065. The opposite scenario is shown for four other species (*C. aenigma, C. australis, C. concinnus,* and *C. hosmeri*), where even at current thermal conditions, in some parts of their range they are exposed to thermal regimes over their preferred temperature suggesting the likelihood of existing reduction on performance. *Cophiixalus bombiens* will start facing a decrease in performance from 2035.

For species facing imminent thermal threat, behavioural buffering (staying in refuges at maximum exposure times) or selecting better insulated refuges will be fundamental for their survival. *Cophixalus concinnus* occurs in habitats characterized by the presence of boulder fields (Hoskin & Hero, 2008; Williams, 2007) which have been shown to provide highly buffered microenvironments from ambient air conditions (Shoo et al., 2010). This insulation is probably more effective than the refuges used to create the exposure conditions of our analysis, so it is possible that the outlook for this species is more positive than the analyses presented here would suggest (Sunday et al., 2012; Williams et al., 2008). Another special case is *C. monticola*, which uses *Linospadix* palms as a refuge. It will be important to assess the buffering effect this refuge provides to the frog to determine the actual vulnerability of the species and to consider not only the direct impacts of climate change on this microhabitat but the potential for the species to modify its behaviour and microhabitat use to use a more buffered microhabitat such as under leaf litter or logs. Several authors have proposed that understanding this kind of relationships will significatively improve models of potential impacts of climate change (Araújo & Luoto, 2007; Pearson & Dawson, 2003; Van der Putten et al., 2010; Williams et al., 2008).

Our analysis uses a thermal exposure approach for *Cophixalus* frogs in the AWT that already considers a basic behavioural and microhabitat use buffering (See chapter 2), and its interaction with thermal physiology (See chapter 3) to provide a more accurate method for assessing the direct impacts of increasing temperature. This approach may be relevant when considering a future environment with more frequent and longer lasting heat waves (Meehl & Tebaldi, 2004). Buckley & Huey (2016) suggest an increase on the importance of extreme weather as a factor involved in biodiversity loss. Understanding extreme events has been recently emphasised as a priority research topic (Williams et al. 2017) and the projections of impacts using direct ecophysiological analyses such as the ones presented here will be much more robust than predictions based on long-term climatic averages such as those used in the majority of studies using correlative species distribution models. A similar point has to be made considering other weather parameters such as projected changes in rainfall seasonality and cloud stripping, which is an important source of water in the mountain tops of the Australian tropical forests (McJannet et al., 2007b). Still et al. (1999) suggested the importance that the potential change in cloud formation elevation and evapotranspiration in montane forest will have on mountain fauna. All these variables (macro, micro and extreme climate linked to other parameters such as species physiology) show the complexity of predicting future scenarios of how climate change will affect biodiversity (Dowd et al., 2015).

In general, anurans have low vagility and remain at specific geographical regions (Becker & Zamudio, 2011; Navas, 1996). This characteristic reduces the options of organisms to cope with permanent changes in environmental parameters, as generated by climate change. *Cophixalus* frogs have shown to be exposed to buffered conditions from ambient temperatures due to behavioural adaptations and present plasticity on refugia selection (chapter 2). Our results suggest that the environmental change pressure will start at lower distribution populations and the survival of this organisms will depend on their capacity of exploiting more effective buffering refuges under future climate.

*Cophixalus concinnus* is an extreme case since its complete elevational distribution will be exposed to harmful ambient thermal conditions by 2035.

Another important factor that may affect survival of organisms under climate change scenarios is the degree on which organisms may acclimatize their thermal sensitivity (Stillman, 2003). Acclimation capacity may ameliorate the potential effects of warmer environmental conditions (Gunderson & Stillman, 2015). It has been proposed that amphibians in temperate ecosystems may have grater acclimation capacity than tropical counterparts due to the exposure to seasonality thermal variation in extreme latitudes (Gunderson & Stillman, 2015); however, Gutiérrez-Pesquera (2016) demonstrated that Acclimation Ratio Response between tropical and temperate tadpoles do not show significant differences. Acclimation capacity has also been reported for other ectothermic organisms occurring in the AWT (Pintor et al., 2016). The actual acclimation capacity for *Cophixalus* frogs has not been assessed and need to be addressed, in order to improve our approach to assess the thermal impacts of climate change in this group of species.

Survival of biodiversity will depend on population or ecosystem management in order to maximize individuals survival (Shoo et al., 2011a). The effectiveness of the conservation strategies will depend on the level of scientific data supporting the assessment of the potential impact, the timeliness of the action, and the continuity of intervention evaluation (Williams et al., 2017). Our work identifies not only the *Cophixalus* species potentially affected by climate change but what specific AWT subregions will host affected species and when the potential impacts will take place. The Australian Wet Tropics regions where species are affected by a decrease in population performance are consistent along the three studied time frames (current, 2035 and 2065); however, affected regions will increase in size while warmer conditions reach higher zones (Fig. 5.3). Thornton region will host two species of *Cophixalus* frogs facing this problem (*C. aenigma* and *C. concinnus*) and Carbine region, by 2065, will have three species threatened by climate warming (*C. aenigma*, *C. hosmeri* and *C. monticola*).

Based on our results, intervention for *Cophixalus* frogs may include localised supplementation of the number or quality of microhabitats with high buffering capacity (e.g. large logs, boulders). This approach not only will provide thermal buffering, but may also provide refuge from dessication stress which is fundamental for amphibian survival (Shoo et al., 2011a). Our preliminary observations of dessication rates on these frogs indicate that there is an increase in desiccation rates across all species when temperature increases from 15 °C to 20 °C, suggesting higher risk in future warmer environments (Appendix 4). As expected, it seems desiccation rates are explained by the amount of skin exposed, represented here by the mass of the organism (Appendix 5). The lowest desiccation rate was observed for *C. saxatilis* which may be explained by the difference in size of the species. *Cophixalus saxatilis* is around 5 times bigger than the rest of the species and the surface/volume ratio decreases with size, reducing its risk of desiccation. However, *Cophixalus monticola*, similar in size to the rest of *Cophixlus* species, also exhibited a relatively low level of desiccation when temperature increased. This result is particularly important because *C. monticola* was identified as one of the four species most imminently threatened by increased temperature (see above) and may suggest the presence of a cutaneous resistance mechanism. This characteristic may be developed in association with the use of *Limnospadix* palms as refuge, which may provide less protection against water loss than other microhabitats. Finally, results from the additional experiments indicate that dessication, rather than temperature directly, might be an important threatening factor for *C. neglectus*. *Cophixalus neglectus*, the best sampled species (n=20), seems to dramatically increase its water loss rate with temperature compared to the other species. This pattern may explain the restriction of this species to cool and moist mountaintop environments.

Future management could trial direct, localised augmentation of moisture in the form of artifitial misting during extreme drying events. Lessons from a previous effort to employ artificial misters to stabilise the decline of Kihansi spray toad and wetland plants affected by water diversion for hydroelectricity in Tanzania may be instructive here (Krajick 2006, Quinn et al. 2005). Such augmentation strategy might be especially effective during dry summers or in the winter season when water input is predicted to decrease (changes in rainfall seasonality and cloud stripping). In a extreme event of environmental warming, translocation of individuals within AWT areas where favourable conditions are maintained in the future may also be a plausible option. Our results inform environmental authorities and will improve the development of strategies focused on minimising the loss of species in the AWT.

## 5.6 Acknowledgements

The authors thank the financial support of James Cook University and the Marine and Tropical Sciences Research Facility to fund the fieldwork and the Ph. D. scholarship of AMV. The Skyrail Rainforest Foundation provided funding to AMV in order to research micro environmental conditions of microhylid frogs between 2007 and 2010. Earthwatch Institute funded part of the fieldwork and organized volunteers to help during the research period. This research was authorized under the Queensland Parks & Wildlife permit WITK05468508. Additional comments and help were provided by Dr. April Reside.

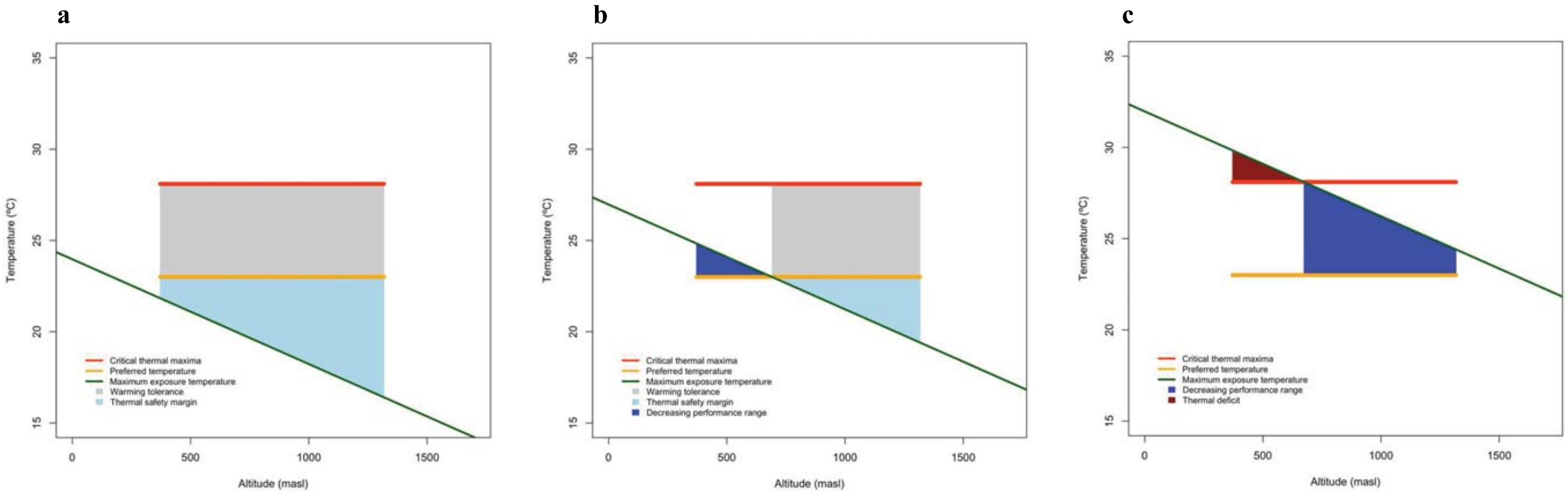
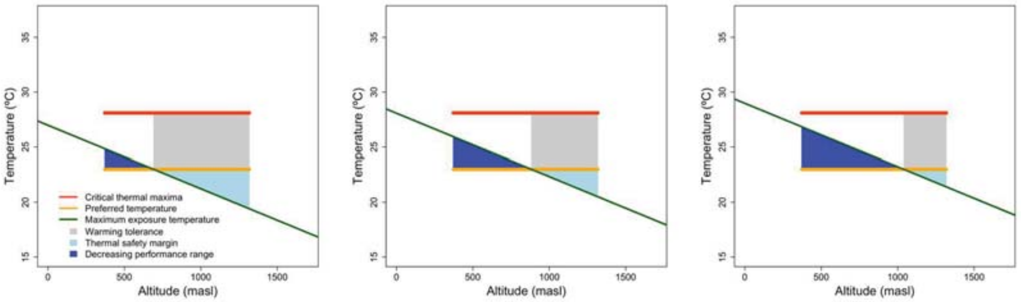


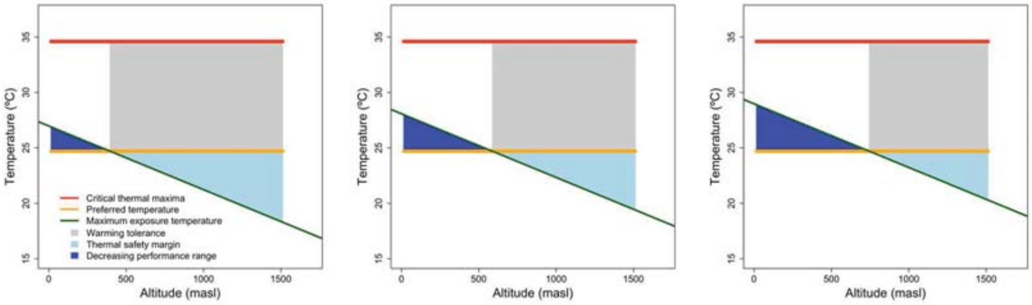
**Figure 5.1.** Theoretical potential effects of environmental conditions along the elevational distribution range of a species, based on thermal physiology parameters, actual exposure and the current elevational distribution of the species. At present conditions (a), a species is exposed to a certain maximum thermal regime (green line). If exposure conditions are under the preferred temperature ($T_{pref}$ )(orange line), the species has a Thermal Safety Margin (light blue range), where an increase of temperature can even be favourable because species may increase performance while getting exposed close to a more preferred temperature (a proxy for optimum temperature). All the populations have a range of temperatures known as Warming Tolerance Range (grey range). In a warming scenario (b), exposure conditions increase and populations exposed to higher temperatures than $T_{pref}$ will experience a decrease in performance (blue range). Upper populations will still keep a thermal safety margin and their complete warming tolerance. In an extreme warming scenario (c) the lowland populations will be exposed to conditions over its maximum thermal tolerance ($CT_{max}$) (red line), facing a thermal deficit that will imply a force to migrate upwards or species unable to migrate will face local extinctions. Higher elevation populations will be experiencing different degrees of reduced performance.

**Present** **2035** **2065**

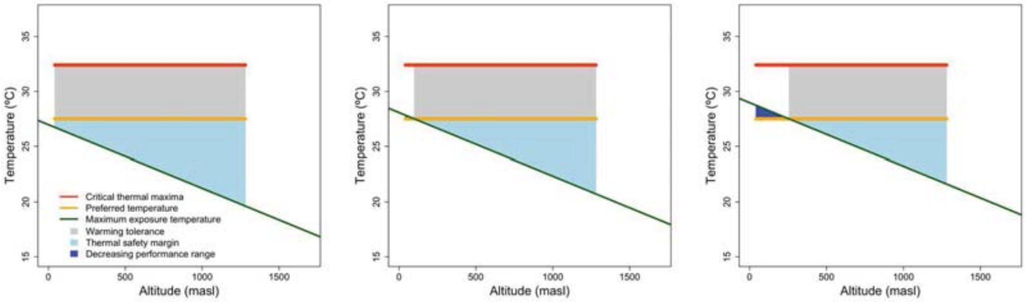
*Cophixalus aenigma*



*C. australis*



*C. bombiens*



*C. concinnus*

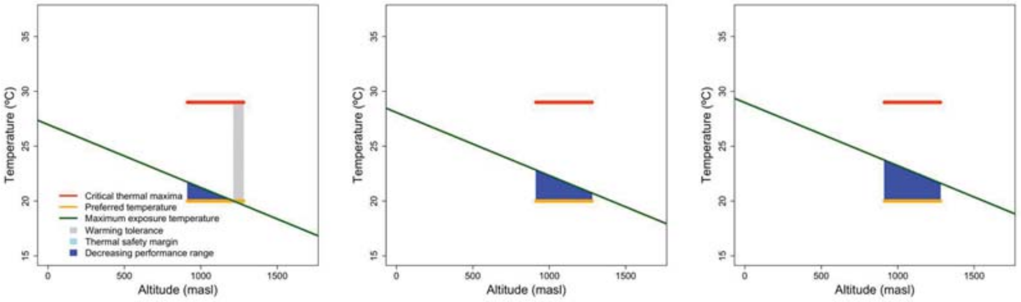
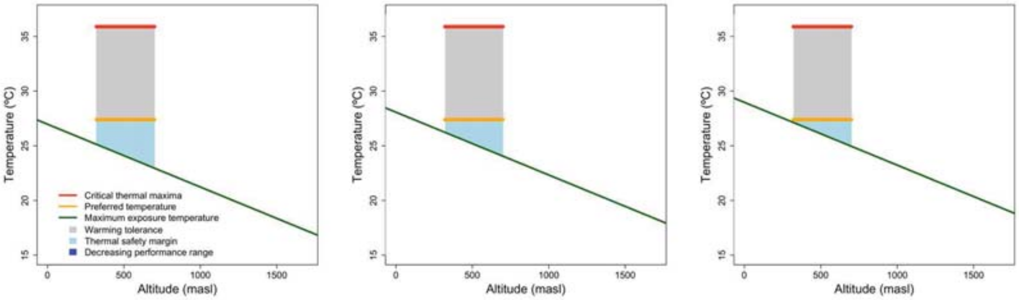


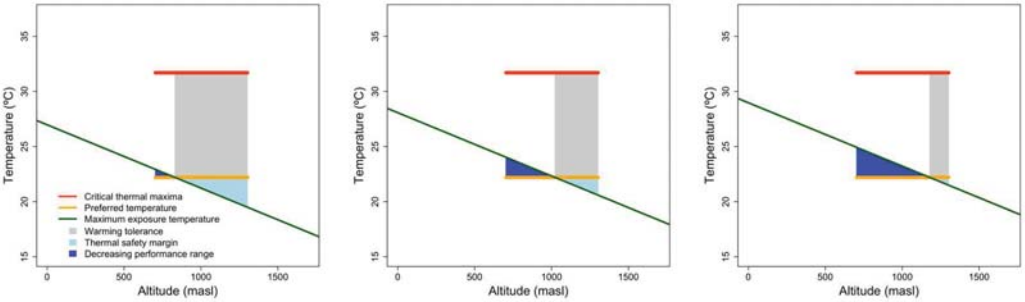
**Figure 5.2.** Thermal safety margin and warming tolerance range for *Cophixalus* species occurring in the AWT. These metrics are presented along the elevational gradient of each species and for two future time frames (2035 and 2065).

**Present** **2035** **2065**

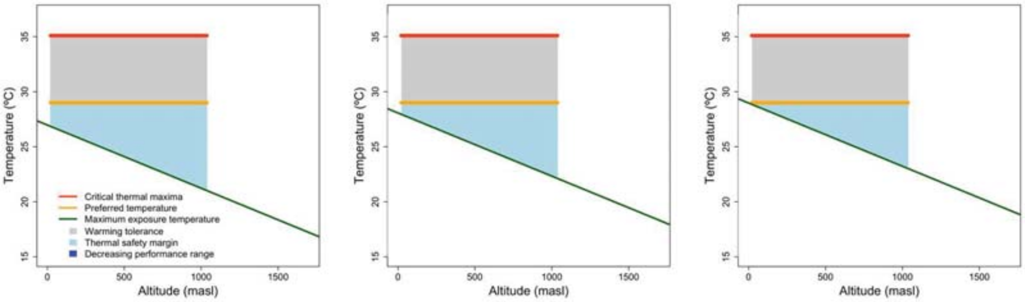
*Cophixalus exiguus*



*C. hosmerai*



*C. infacetus*



*C. mcdonaldi*

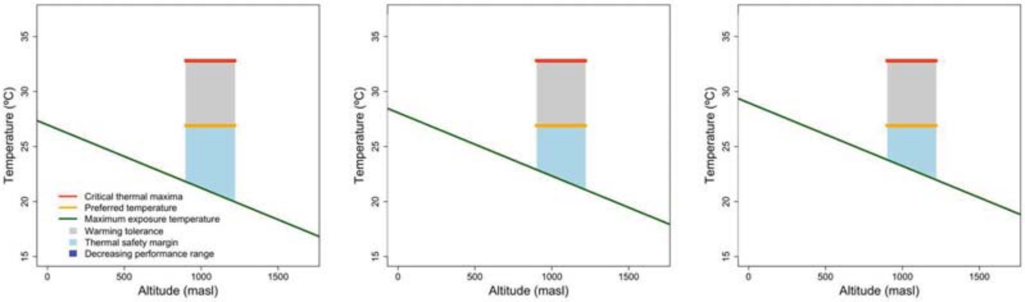


**Figure 5.2. Continued**. Thermal safety margin and warming tolerance range for *Cophixalus* species occurring in the AWT. These metrics are presented along the elevational gradient of each species and for two future time frames (2035 and 2065).

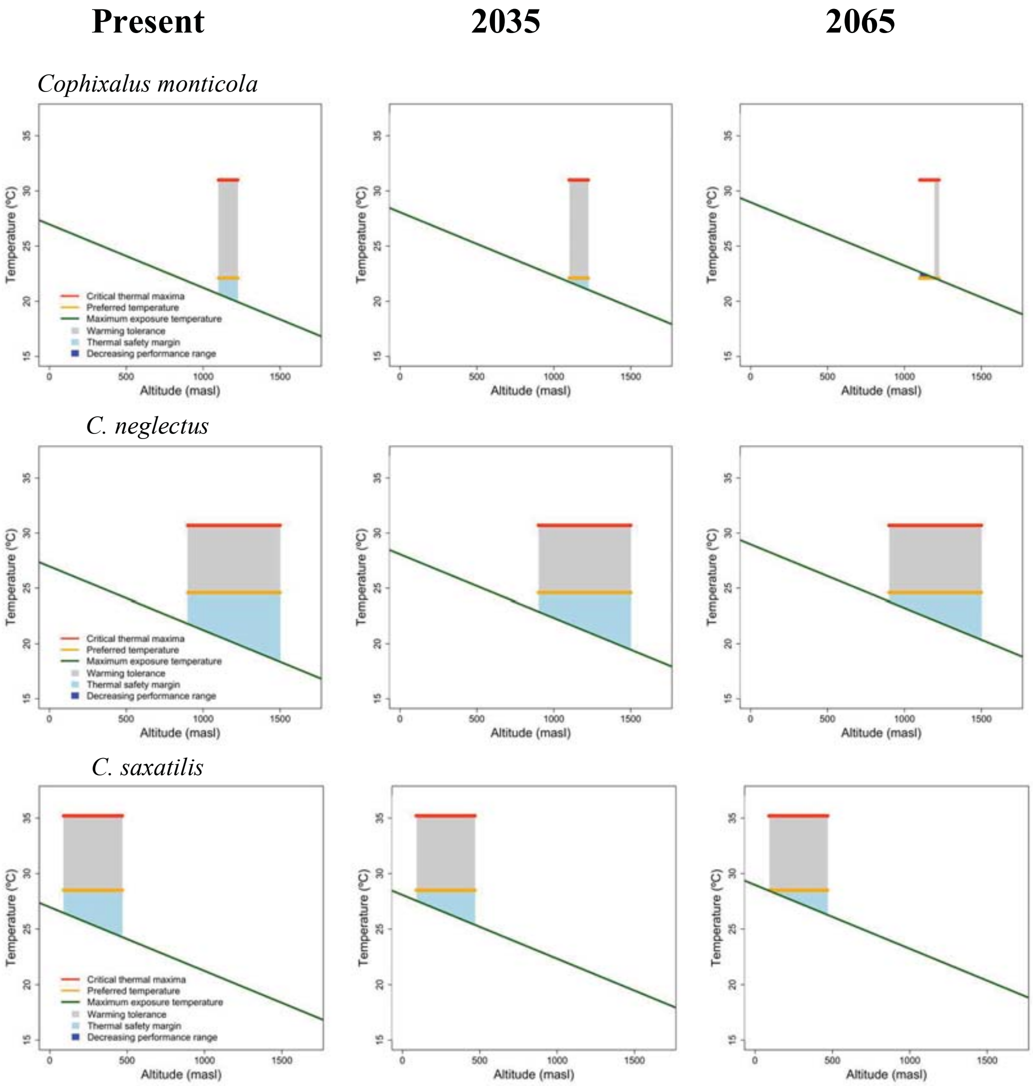
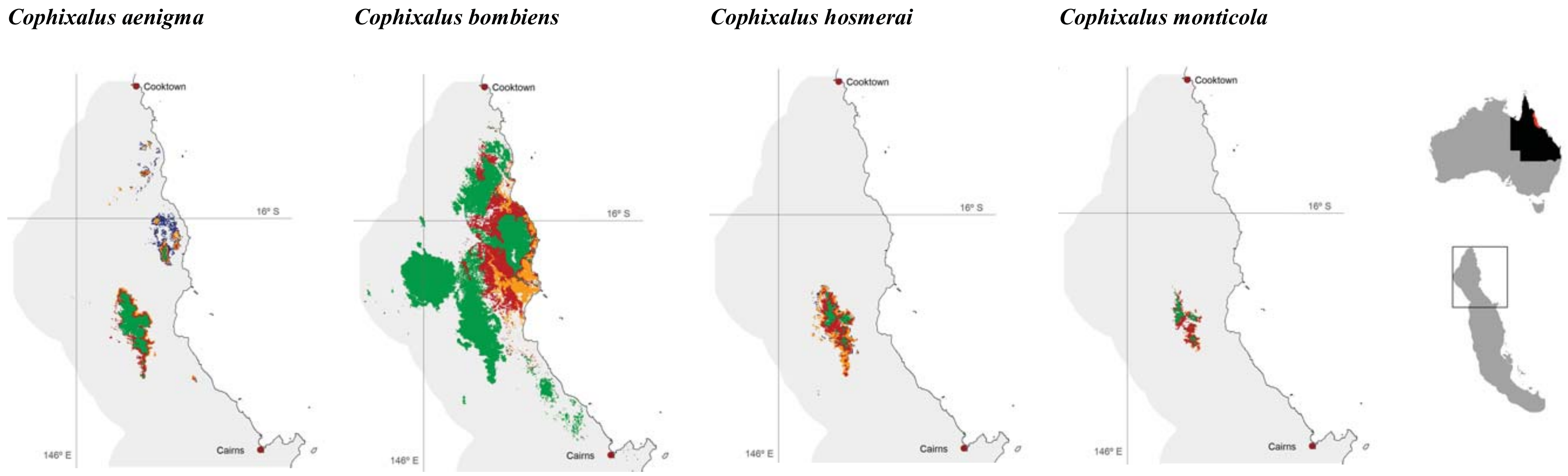


**Figure 5.2. Continued**. Thermal safety margin and warming tolerance range for *Cophixalus* species occurring in the AWT. These metrics are presented along the elevational gradient of each species and for two future time frames (2035 and 2065).


Cophixalus aenigma

Cophixalus bombiens

Cophixalus hosmerai

Cophixalus monticola

**Figure 5.3.** Geographic distribution of a subset of *Cophixalus* frogs showing regions where a decrease in fitness due to thermal conditions is expected for current climate (blue regions), by 2035 (orange regions) and by 2065 (red regions). Green regions show areas where, until 2065, climate will present favourable conditions in the future (exposure conditions under $T_{pref}$).

**Table 5.1.** Predicted changes in temperature for the summer months in the Wet Tropics Bioregion for years 2035 and 2065. This prediction is based on 42 global climate models in a medium emission scenario (RCP 4.5) with a baseline for 1986-2005 period (Hilbert et al., 2014).

| **Season** | **Year** | **Temperature range** | **Mean increase** |
|---|---|---|---|
| Summer period (Dec-Feb) | 2035 | 0.2 - 1.9 | 1.1 |
| | 2065 | 0.6 - 3.4 | 2 |

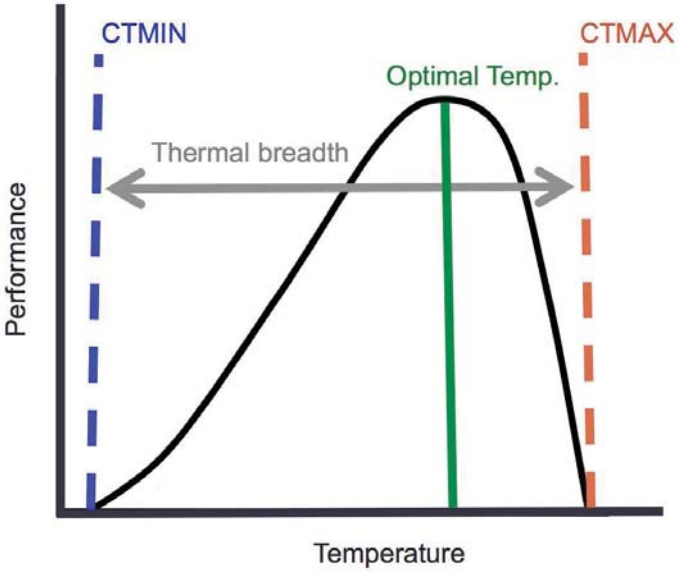
**Table 5.2**. Thermal Safety Margin and Warming Tolerance range for eleven species of *Cophixalus* frogs at the lowest and highest elevation of their distribution. Species are ordered by decreasing the Warming Tolerance Range at the highest elevation population.

| | **Thermal Physiology** | | **Exposure Conditions** | | **Thermal Safety Margin** | | **Warming Tolerance** | |
|---|---|---|---|---|---|---|---|---|
| **Species** | **CT$_{max}$** | **T$_{pref}$** | **Low** | **High** | **Low** | **High** | **Low** | **High** |
| *Cophixalus australis* | 34.6 | 24.7 | 26.9 | 18.3 | - | 6.4 | 7.7 | 16.3 |
| *C. infacetus* | 35.1 | 29 | 26.9 | 21 | 2.1 | 8 | 8.2 | 14.1 |
| *C. exiguus* | 35.9 | 27.4 | 25.1 | 23 | 2.3 | 4.4 | 10.8 | 12.9 |
| *C. bombiens* | 32.4 | 27.5 | 26.7 | 19.6 | 0.8 | 7.9 | 5.7 | 12.8 |
| *C. mcdonaldi* | 32.8 | 26.9 | 21.8 | 20 | 5.1 | 6.9 | 11 | 12.8 |
| *C. neglectus* | 30.7 | 24.6 | 21.8 | 18.4 | 2.8 | 6.2 | 8.9 | 12.3 |
| *C. hosmeri* | 31.7 | 22.2 | 22.9 | 19.5 | - | 2.7 | 8.8 | 12.2 |
| *C. monticola* | 31 | 22.1 | 20.7 | 19.9 | 1.4 | 2.2 | 10.3 | 11.1 |
| *C. saxatilis* | 35.2 | 28.5 | 26.5 | 24.3 | 2 | 4.2 | 8.7 | 10.9 |
| *C. concinnus* | 29 | 20 | 21.7 | 19.6 | - | 0.4 | 7.3 | 9.4 |
| *C. aenigma* | 28.1 | 23 | 24.8 | 19.4 | - | 3.6 | 3.3 | 8.7 |

**Table 5.3.** Elevational ranges for *Cophixalus* frogs of the AWT and maximum elevation at which populations may be decreasing performance and facing thermal deficit due to change in thermal regimes at three time frames (Current, 2035 and 2065).

| | **Elevational distribution** | | **Reduced performance** | | | **Thermal deficit** | | |
|---|---|---|---|---|---|---|---|---|
| **Species** | **Minimum** | **Maximum** | **Current** | **2035** | **2065** | **Current** | **2035** | **2065** |
| *Cophixalus concinnus* | 913 | 1279 | 1214 | 1279 | 1279 | - | - | - |
| *C. hosmeri* | 704 | 1302 | 831 | 1023 | 1179 | - | - | - |
| *C. aenigma* | 372 | 1317 | 692 | 883 | 1040 | - | - | - |
| *C. australis* | 15 | 1512 | 396 | 587 | 744 | - | - | - |
| *C. bombiens* | 42 | 1280 | - | 100 | 257 | - | - | - |
| *C. monticola* | 1100 | 1224 | - | - | 1197 | - | - | - |
| *C. exiguus* | 320 | 700 | - | - | - | - | - | - |
| *C. infacetus* | 20 | 1037 | - | - | - | - | - | - |
| *C. mcdonaldi* | 900 | 1218 | - | - | - | - | - | - |
| *C. neglectus* | 900 | 1500 | - | - | - | - | - | - |
| *C. saxatilis* | 90 | 468 | - | - | - | - | - | - |

**Appendix 5.1**. Thermal performance curve with terms studied in this thesis. Modified from Gutiérrez-Pesquera (2016).



# Chapter 6. General conclusions

Multiple lines of evidence suggest that many species in the Australian Wet Tropics face drastic impacts due to global warming. Just a few degrees increase will produce reductions on the habitat availability mainly of endemic species including frogs of the genus *Cophixalus*. Most of the species of this genus are considered as threatened in the UICN Red List due to their limited distribution range and the high chances of population disturbances by stochastic events. Other experts suggest climate change as an additional potential cause of extinction for this group of frogs. This research has explored key factors that may be limiting the distribution of this group of endemic vertebrates. By understanding these factors, the prediction of the potential changes of environmental change is expected to enable the development of more cost-effective conservation strategies to respond to this emerging challenge.

Previous predictions based on correlative models of distribution of these frogs, presented reductions of species distribution area, with suitable climate in the region due to an increase in temperature; however, the mechanisms behind this potential effects remained poorly understood.

Geographical and ecological characteristics of these frogs determine their vulnerability to changes in environmental conditions. Geographic restriction to one or few mountain tops and to the coldest, wettest and aseasonal regions of the AWT, their little size, and their reproductive mode, are examples of aspects that increase their risk of being affected by climate change.

Under these considerations, the measurement of behavioural patterns and thermal conditions in frogs' microenvironments enables a quantitative description of the actual conditions these animals are exposed to and the collection of thermal physiology parameters allow to determine the sensitivity of these frogs to potential changes in thermal conditions. These two basic aspects of the ecology of the species are fundamental for a robust assessment of climate change impacts.

The data gathered here will inform environmental authorities to develop more effective conservation strategies, focused on the relative vulnerability of each species across space (specific areas of the AWT) and time (specific time frames). More informed assessment of vulnerability provides managers and policy makers with the knowledge and capacity to make more informed and effective decisions about allocation of resources for conservation and adaptation aimed at minimising extinction threat to this important group of Australian endemic vertebrates, the Microhylid frogs.

## 6.1 Summary of major findings

Chapter 2 focused on determining the role of behaviour and refuge use in order to understand the actual exposure conditions these frogs experience. Our results revealed that *Cophixalus* frogs have consistent patterns of activity depending on species, regions, and seasons. Individuals generally leave diurnal refuges between 17:00 h and 18:00 h and return to their retreat sites between 05:00 h. and 06:00 h.

Behavioural observations provided evidence of frogs changing their diurnal refuge. This evidence of plasticity suggests a capacity for these species to search for refuges with greater buffering, for example under larger logs, if conditions deteriorate under climate change. However, this will be limited by the absolute maximum refuge potential, that is the "best-available" refuge in their habitats.

The daily behaviour and microhabitat use provides access to a buffered set of environmental conditions that are partially decoupled from ambient air temperature. The buffering effect of behaviour and microhabitat use is seasonally variable. Microhabitats keep the animals cooler in summer and warmer during winter than ambient air conditions.

Chapter 3 focused on the thermal physiology ($CT_{max}$, $CT_{min}$, thermal range, and preferred temperature) of the *Cophixalus* frogs of the AWT and its relationship with their evolutionary history and characteristics of their elevational distribution.

Only preferred temperature was shown to be correlated with the evolutionary history of this group of frogs. This parameter ranged between 20 °C to 29 °C. The result possibly reflects the adaptation of these frogs to this long-term stable ecosystem.

$CT_{max}$ ranged from 28.1 °C to 35.9 °C. This parameter was correlated with several geographic characteristics of the distribution of these frogs. This correlation may suggest upper temperature tolerance may have had an important role in shaping the distribution of this group of frogs.

Since these frogs are restricted and adapted to the coldest thermal conditions in the AWT, tolerance to cold has arguably not been important for this animals. That may explain why $CT_{min}$ did not show correlation with either the phylogeny or elevational geographical parameters. This parameter ranged from 8.6 °C to 14.3 °C.

*Cophixalus* frogs showed extremely narrow thermal ranges (14.2 – 25.5 °C). Although narrow ranges might be expected for organisms adapted to a long-term stable ecosystem such as the Australian tropical rainforest, tolerance ranges of *Cophixalus* frogs were still less than those reported for other tropical amphibian communities in other continents.

The thermal fundamental niche of an organism can be determined by the combination of its physiological thermal tolerances and its actual exposure conditions in its natural habitat. These parameters describe the geographical space where the organism may occur; however, different kind of biotic (e.g. prey) or abiotic (e.g. topography) interactions may impede the fully occupancy of that area. Chapter 4 tested the thermal fundamental niche filling for this group of frogs. An important contribution of our work is to perform this analysis with empirically measured data, which is usually lacking for most species.

Our findings show that none of the *Cophixalus* species in the AWT are restricted by physiology at the warm (lower elevation) border of their distribution. At the cold border (top elevation) some species match their distribution with topographic limits (*C.*

*saxatilis, C. mcdonaldi, C. hosmerai, C. exiguus*) or with a combination with physiology (*C. monticola*).

Some species seem to be overfilling their thermal niche at the cold border. This observation may be related to the species finding refugia with better buffering capacity such as boulder fields at the top of Thornton Peak for *C. concinnus*.

Species with topographic limitations, (e.g. *C. mcdonaldi*) but with thermal tolerances that would allow them to survive in other regions where climate is favourable, may be suitable candidates for translocation in an extreme case of increase in temperature.

Finally, chapter 5 focused on a vulnerability assessment of thermal increase in the AWT, for *Cophixalus* frogs. This chapter shows that even at present thermal regimes, there are regions (lowland populations) where species may be facing a decrease in fitness due to an exposition to thermal conditions higher than $T_{pref}$. This area will continue to grow as climate warming continues in the region.

The most important result shows that none of the studied *Cophixalus* frogs are expected to face thermal deficit (exposure over $CT_{max}$) by 2065. This implies that none of the species are predicted to face local extinction in the region within this time frame. However, *C. aenigma* shows for this period its exposure close to its thermal tolerance.

All these data and analyses are fundamental for improving the assessment of the vulnerability to environmental changes. In addition, our findings are capable to guide environmental authorities focus conservation strategies in specific areas and time frames where they are needed. These strategies will improve the effectiveness of conservation in this global important biodiversity area.

## 6.2 Future research directions

This research has focused and deeply analysed the potential effects of changes in temperature regimes in the region on *Cophixalus* frogs populations. An important factor that must be addressed is the potential plasticity of the thermal parameters studied here. This plasticity may allow the frogs to modify their tolerances according to the change in conditions and is likely to be a useful area of future inquiry.

Global anthropogenic climate change implies changes in several environmental parameters other than temperature, including changes in moisture patterns. A priority for future research is analysing moisture availability in the region and desiccation tolerance in the *Cophixalus* frogs. The unavoidable limitation of deeper analysis of desiccation data gathered during this research has prevented robust conclusions about the effect of moisture on limiting the distribution of the *Cophixalus* frogs in the AWT. The preliminary general patterns mentioned in this research provide insights that may guide future specific research on *Cophixalus* species.

*Cophixalus neglectus* seems to be a very moisture-sensitive species. Research focussed on determining the actual tolerance to desiccation on natural conditions would be important for its potential conservation management. The assessment of different elevation populations could provide information about where conservation actions may be needed first.

The potential cutaneous resistance of *C. monticola* could be interesting to explore with reference to "lower than expected" desiccation rates of other similar sympatric species (*C. hosmeri* and *C.  aenigma)*. The use of different microhabitats within the same rainforest by these species would also be instructive to evaluate the level of protection to desiccation provided by the refuge and whether this is related to the degree of cutaneous resistance to desiccation.

It is important to state that no assessment of climate change vulnerability will be complete until the physiological tolerances and exposure conditions of each ontogenic life stage is considered. This will allow us to understand if the geographic range of the species is limited by any of these stages. It could be expected that the eggs and hatchlings of microhylid frogs could be particularly vulnerable, especially to desiccation.

The lack of information about biological interspecific interactions is a pervasive problem in vulnerability assessment. Understanding the interactions and synergies between abiotic and biotic interactions for each species remains fundamental to improving our knowledge about comprehensive understanding of climate change impacts.

Finally, a major challenge will be to design and evaluate the cost-effectiveness of practical management actions that may form part of the conservation toolkit to maximise persistence of vulnerable species under climate change.

# Reference List

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# Appendix1. Notes on desiccation rates in *Cophixalus* frogs of the Australian Wet Tropics

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## Summary

Climate change is modifying environmental conditions in natural ecosystems worldwide. One of the parameters that will be changed is water availability in direct and indirect forms: rain patterns, cloud formation, increase of sea level and temperature, etc. Water availability is an important factor that may limit geographic distribution of species and changing water inputs will affect natural population dynamics. Amphibians are expected to be particularly exposed to changes in water availability due to their permeable skin. Here, we present a basic assessment of water loss rates in 11 species of *Cophixalus* frogs restricted to the AWT exposed to different environmental conditions. The experiments required development of an appropriated device to test the effect of different thermal and relative humidity regimes. Unfortunately, subsequent analysis of data suggested that an undetected malfunction had occurred during experimentation, making the absolute values of the moisture data not usable. Nevertheless, we decided it would be useful to present preliminary data on relative patterns of the effect of temperature on desiccation rates. Results show an increase of water loss rates in direct response to increasing temperature, and body size seems to be an important factor moderating observed patterns of water loss. However, departures from this general pattern were also found for a subset of species (*Cophixalus neglectus*, *C. saxatilis* and *Cophixalus monticola*) which we suggest might be attributable to intrinsic species characteristics such as lack of cutaneous resistant mechanisms, distinctive size or use of specific microhabitats, respectively. Due to a failure in data gathering deeper analysis were impossible. Desiccation risk is important for amphibians and its assessment is fundamental for reducing the effects of climate change.

## Introduction

Amphibians are vertebrates with a high sensitivity to environmental changes. This characteristic is mainly related to their permeable skin (Duellman & Trueb, 1994; Pessier, 2002). The amphibian skin allows vital functions such as: gas exchange, water and nutrients absorption, secretion of chemicals for pathogen and predator defense, within others (García-París, Montori, & Herrero, 2004; Pessier, 2002). The optimal development of amphibians will depend of the selection of microhabitats with favorable levels of temperatures, humidity and food availability (Carey et al., 2001; Wells, 2007). Changes in environmental factors may have important impacts on wild anuran populations (Blaustein & Wake, 1990; Kiesecker, Blaustein, & Belden, 2001; Pounds et al., 2006;

Wake, 1991). Availability of moisture has been proposed to be an important factor limiting the geographical distribution of amphibians (Duellman & Trueb, 1994).

Adaptations of the skin to reduce evaporative water loss has been fundamental for the survival of amphibians and reptiles in terrestrial ecosystems (Gans, Krakauer, & Paganelli, 1968; Krakauer, Gans, & Paganelli, 1968; Young, Christian, Donnellan, Tracy, & Parry, 2005). The physiological process for which amphibians skin constantly lose water is known as evaporative water loss (Tracy, 1972; Lillywhite, 1975; Spotila & Berman, 1976; Wells, 2007). The rate of water loss depends on habitat, microhabitat use, activity period, and posture (Pough et al., 1983; Tracy, 1976; Tracy et al., 2008; Young et al., 2006). The challenge of maintaining a positive water balance has promoted the development of morphological, physiological and behavioral adaptations in anurans (Barbeau & Lillywhite, 2005; Toledo & Jared, 1993).

Thicker skin, lipid and serum secretion, skin colour changes, reduction of exposed skin through posture are all adaptations developed to reduce water loss (Colbert, 1969; Pough, et al., 1983; Shoemaker et al., 1987). Evaporative water loss of most ‘typical’ amphibians resembles the evaporation rate of a free water surface, implying no mechanisms of skin resistance against desiccation (Spotila & Berman, 1976; Young et al., 2005). Some species have developed mechanisms to avoid desiccation, reducing the rate of cutaneous water loss. These anurans, known as ‘atypical’, seem to present low rates of water loss that coincide with low levels water available in their habitats (Buttemer, 1990; Loveridge & Withers, 1981; Shoemaker et al., 1972; Wygoda, 1984; Young et al., 2005). Ecological habits seem to be correlated to anuran water loss. This process has been reported even for closely related species, due to differences in microhabitat environmental conditions (Wygoda, 1988, 1984; Young et al., 2005, 2006).

Studies to quantify adaptations of the skin to avoid evaporative water loss have typically used models with the same characteristics of real frogs and having the maximum rate of evaporation. One of the most used methods is the assessment of water loss in 3% agar frog models (Buttemer, 1990; Buttemer & Thomas, 2003; Spotila & Berman, 1976; Wygoda, 1984; Young et al., 2005, 2006). If a real frog water loss is similar to the models, it means that the frog has no cutaneous mechanism; lower water loss will mean the presence of a mechanism to avoid desiccation (Navas & Araujo, 2000; Tracy et al., 2007; Young et al., 2005, 2006).

The *Cophixalus* frogs of the wet tropics rainforests of north-eastern Australia provide an ideal group to understand how water availability may limit the distribution of organisms. The geographic distributions of the 13 species endemic to the Wet Tropics are well known (Hoskin & Hero, 2008; Hoskin, 2004, 2012; Williams et al., 2010b). These are generally restricted to the coldest, wettest and aseasonal parts of the region, and over half the species have highly localized distributions to one or few mountain tops (Hoskin & Hero, 2008; Shoo & Williams, 2004). It has been proposed that the distribution of these frogs may be highly influenced by water availability (Hilbert & Williams, 2003; Williams et al., 2010; Williams, Bolitho, & Fox, 2003).

Future modifications of environmental conditions and the understanding of the physiology limitations of the species may help to more accurately predict population changes in amphibians (Riddell et al., 2017; Riddell & Sears, 2015). Climate change will modify several environmental parameters that may also indirectly affect the rates of water loss, such as temperature (Withers et al., 1984; Rothermel & Semlitsch, 2002; Wells, 2007; Riddell et al., 2016). Here, we evaluated the rates of water loss of adult individuals (and their corresponding agar models) of 11 species of *Cophixalus* frogs of the AWT under different conditions of relative humidity and temperature. This comparative analysis will allow us to determine the most resistant and the most sensitive species of *Cophixalus* frogs to water loss. The understanding of this process will contribute to improving conservation strategies to save these species from harmful effects of climate change.

## Methods

Experiments were performed with the aim to determine relative differences in cutaneous water loss resistance as a proxy of desiccation risk for this group of frogs. An experimental apparatus was designed and assembled specifically for this study (see Figure 7.1). The main component was a portable FoxBox Respirometry System (Sable Systems International). This device contains a pump, a flow control, a flow-meter, and $O_2$ and a $CO_2$ analyzer. The FoxBox pumped air at a constant rate of 15ml/min into a DG-4 Dewpoint Generator (Sable Systems International) to control the level of moisture in air flow. An experimental glass chamber (length: 3.8 cm and diameter: 2 cm) was set into a PTC-1 temperature cabinet (Sable Systems International) connected to a PELT-5 temperature controller (Sable System intenational). Into the cabinet a RH-300 Water Vapor Analyzer read the moisture released by the frogs' skin. Afterwards, air flow returned to FoxBox for flow control. Moisture, air flow, flow temperature, $O_2$ and $CO_2$ levels were collected in the FoxBox internal memory every 5 seconds. Moisture was removed from air flow by a Dierite® scrubber (CA S number 7778-18-9, Sigma-Aldrich) before air returns to FoxBox. $CO_2$ was removed from air flow before $O_2$ measurement by an Ascarite® scrubber (CA S number 1310-73-2, Sigma-Aldrich). Cabinet temperature and relative humidity in air flow were controlled by thermistor probes connected to the corresponding devices (thermocouples in scheme). In case of experiments with dry air, a cylinder with Drierite® (length: 30 cm and diameter: 4 cm) was connected to the beginning of the air intake tubing, to completely remove moisture from incoming environment air. FoxBox collected data was analysed using LabAnalist X software (Chappell, 2010). Water loss rates were adjusted by original weight of the individuals and presented as percentages of weight loss.

Desiccation rates were measured for eleven of the thirteen species of *Cophixalus* frogs occurring in the AWT. Measurements were taken in the field at eleven locations that covered the distribution ranges of these species and an elevational range of 150 to 1600 m (Table 3.1; Figure 3.1). Frogs were kept at ambient temperature in individual plastic containers with damped paper towel to allow the animals to keep fully hydrated.

For each individual, the experimental conditions were set at a constant air flow of 15 ml/min. Temperature and relative humidity combinations were tested as follows:
20 °C 0% RH; 20 °C 50% RH; 20 °C 70%; 20 °C 90%; 15 °C 0%RH and 25 °C 0%RH. For *C. neglectus* and *C. australis*, due to greater availability of specimens, all temperature (15 °C, 20 °C, 25 °C) and relative humidity (0%, 50%, 70%, 90%) combinations were tested.

Experiments consisted of an initial 10 minute run as base line without the frog in the experimental chamber, then a 30 minute experiment with the frog in the experimental chamber and a 10 minute run as a second base line to confirm that measurement levels returned back to regular values without the frog in the chamber. The skin of each frog was dried and urine removed by gently pressing the animal with a paper towel prior the experiment. Frogs were weighed at a 0.001 resolution at the beginning and at the end of the 30 minutes experiment (A&D Weighing FX-300i milligram balance). Depending on the number of individuals collected during each fieldtrip, time between experiments ranged between 4 and 8 hours, allowing rehydration of the organisms. This procedure was repeated, as many times as real frog tested for that species, with 3% agar models with similar characteristics of the *Cophixalus* frogs.

FoxBox collected data were analysed using LabAnalist X software (Chappell, 2010). Water loss rates were adjusted by original weight of the individuals and presented as percentages of weight loss.

## Results

A total of 362 experiments were performed on *Cophixalus* frogs and 77 on 3% agar models. The experimental individuals were the same used to measure thermal physiology parameters described in chapter 3 (Table 3.3). Percentage of weight loss was calculated for each individual using the frogs weight prior to the experiment as a baseline. The percentage of weight loss for *Cophixalus* frogs for experiments with dry air and three temperatures (15 °C, 20 °C and 25 °C) is presented in Figure 7.2.

Unfortunately, during subsequent data analysis (post experiments), it was impossible to find a coherent correspondence between the observed weight loss and the calculated weight loss from the data gathered by the experimental apparatus. This suggested that an undetected malfunction had occurred during experimentation, making the absolute values of the moisture data not usable. Significant time and effort (many months and several people) were spent on attempting to recalibrate the measurements using a variety of different approaches. Unfortunately, we were unable to retrospectively decipher and calibrate the fault with sufficient reliability. A subsequent decision was therefore made to focus the dissertation primarily on the impact of temperature on *Cophixalus* frogs. Despite this setback, we decided it would be useful to present data on relative patterns of the effect of temperature on desiccation rates.

Desiccation rates generally increased with temperature for all the species (Figure 7.2). However, a disproportionately high increase in desiccation rate was apparent for *Cophixalus neglectus* at the highest temperature (25 °C) and desiccation rates of *C. saxatilis* remained low at all the temperature regimes. Water loss was negatively correlated with body size in *Cophixalus* frogs (Figure 7.3). *Cophixalus monticola* showed lower water loss rates compared to close relatives of similar body size.

# Discussion

Williams et al. (2010) highlight the importance of moisture-related bioclimatic variables on limiting the potential distribution of *Cophixalus* frogs in correlative models. Our results suggest that moisture may be an important factor limiting the distribution of these frogs in the AWT and that a future change in precipitation or inputs of moisture from the orographic cloud bank might affect their populations.

We specifically designed a sophisticated experimental apparatus to evaluate impact of temperature and humidity on water balance in *Cophixalus* frogs (see Figure 7.1). Although we believe that the design is well suited to the purpose and generalizable, we were unable to determine the source of experimental error in this application (i.e. difference between observed and predicted water loss). Possible explanations include a variable gas leak in any of the joints between tubing and devices, a gas leak in a little piece of plexiglass allowing a thermocouple to measure the temperature of the air flow, voltage variation from batteries used in the field, a variable data gathering fault in FoxBox. We recommend for future experimentation to check tubing and connections between all the devices, to run checks and calibrations in parallel with experiments in laboratory previous to field work, the use of 3% agar models is recommended (to avoid additional complications such as animal moving). If discrepancies are not solved, it would be important to replace devices.

Our preliminary observations of desiccation rates on these frogs indicate that there is an increase in desiccation rates across all species when temperature increases from 15 °C to 20 °C, suggesting higher risk in future warmer environments (Figure 7.2). As expected, it seems desiccation rates are explained by the amount of skin exposed, represented here by the mass of the organism (Figure 7.3). The lowest desiccation rate was observed for *C. saxatilis* which may be explained by the difference in size of the species. *Cophixalus saxatilis* is around 5 times bigger than the rest of the species and the surface/volume ratio decreases with size, reducing its risk of desiccation. However, *Cophixalus monticola*, similar in size to the rest of *Cophixlus* species, also exhibited a relatively low level of desiccation when temperature increased. This result is particularly important because *C. monticola* was identified as one of the four species most imminently threatened by increased temperature (see Chapter 5) and may suggest the presence of a cutaneous resistance mechanism. This characteristic may be developed in association with the use of *Limnospadix* palms as refuge, which may provide less protection against water loss than other microhabitats. Finally, results from the additional experiments indicate that dessication, rather than temperature directly, might be an important threatening factor for *C. neglectus*. *Cophixalus neglectus*, the best sampled species (n=20), seems to dramatically increase its water loss rate with temperature compared to the other species. This pattern may explain the restriction of this species to cool and moist mountaintop environments.

Research focussed on determining the actual tolerance to desiccation on natural conditions would be important for its potential conservation management. The assessment of different elevation populations could provide information about where conservation actions may be needed first. Intervention for *Cophixalus* frogs may include localised supplementation of the number or quality of microhabitats with high buffering capacity (e.g. large logs, boulders). This approach not only will provide thermal buffering, but may also provide refuge from desiccation stress which is fundamental for amphibian survival (Shoo et al., 2011a).

The potential cutaneous resistance of *C. monticola* could be interesting to explore with reference to "lower than expected" desiccation rates of other similar sympatric species (*C. hosmeri* and *C. aenigma)*. The use of different microhabitats within the same rainforest by these species would also be instructive to evaluate the level of protection to desiccation provided by the refuge and whether this is related to the degree of cutaneous resistance to desiccation.

Global anthropogenic climate change implies changes in several environmental parameters other than temperature, including changes in moisture patterns. A priority for future research is analysing moisture availability in the region and desiccation tolerance in the *Cophixalus* frogs. The unavoidable limitation of deeper analysis of desiccation data gathered during this research has prevented robust conclusions about the effect of moisture on limiting the distribution of the *Cophixalus* frogs in the AWT. The preliminary general patterns mentioned in this research provide insights that may guide future specific research on *Cophixalus* species.

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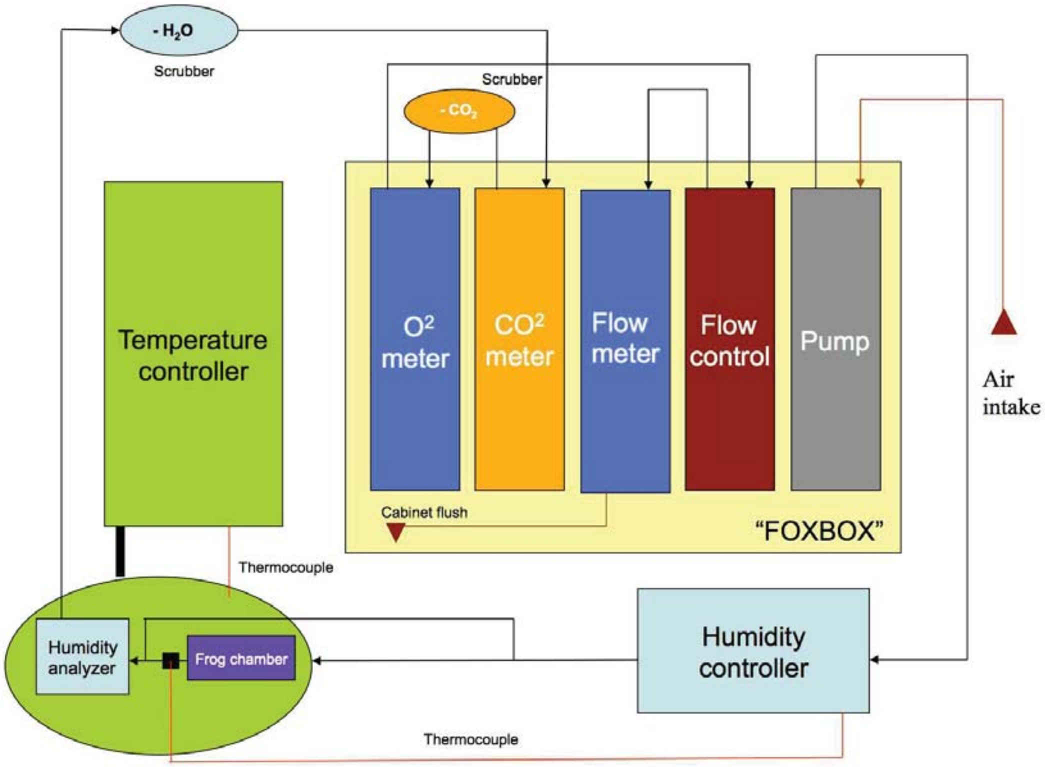

**Figure 7.1.** Schematic of the experimental apparatus developed to measure desiccation rates of *Cophixalus* frogs in the field.

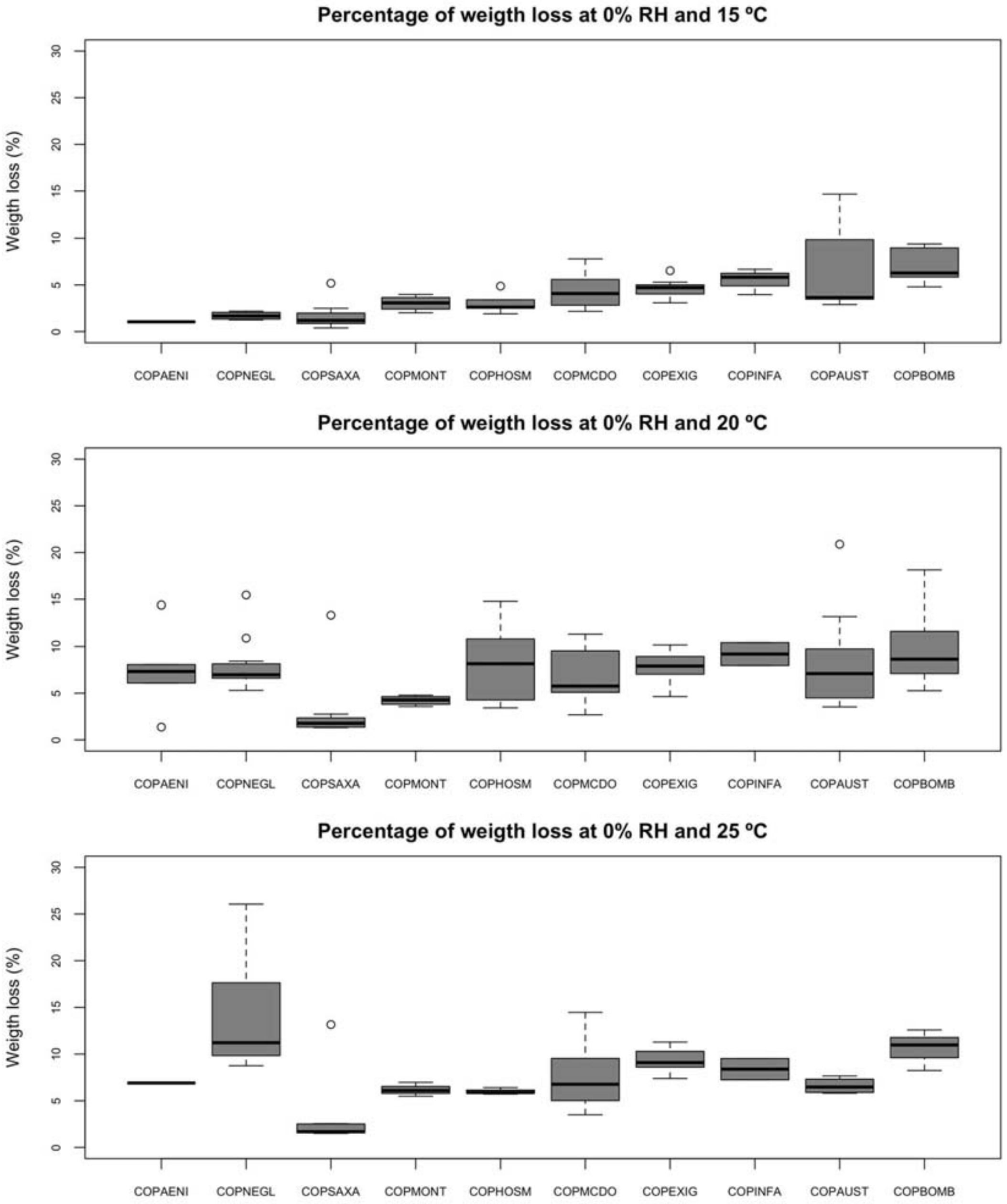

**Figure 7.2.** Comparative percentages of weight loss on desiccation experiments (30 minutes) for *Cophixalus* species of the AWT at three different temperatures. Dry air flow was set at 15ml/min. Species are ascending ordered according to the species mean desiccation rate at 15 °C. *Cophixalus* codes represent a combination of genus and species names (Figure. 1.1).

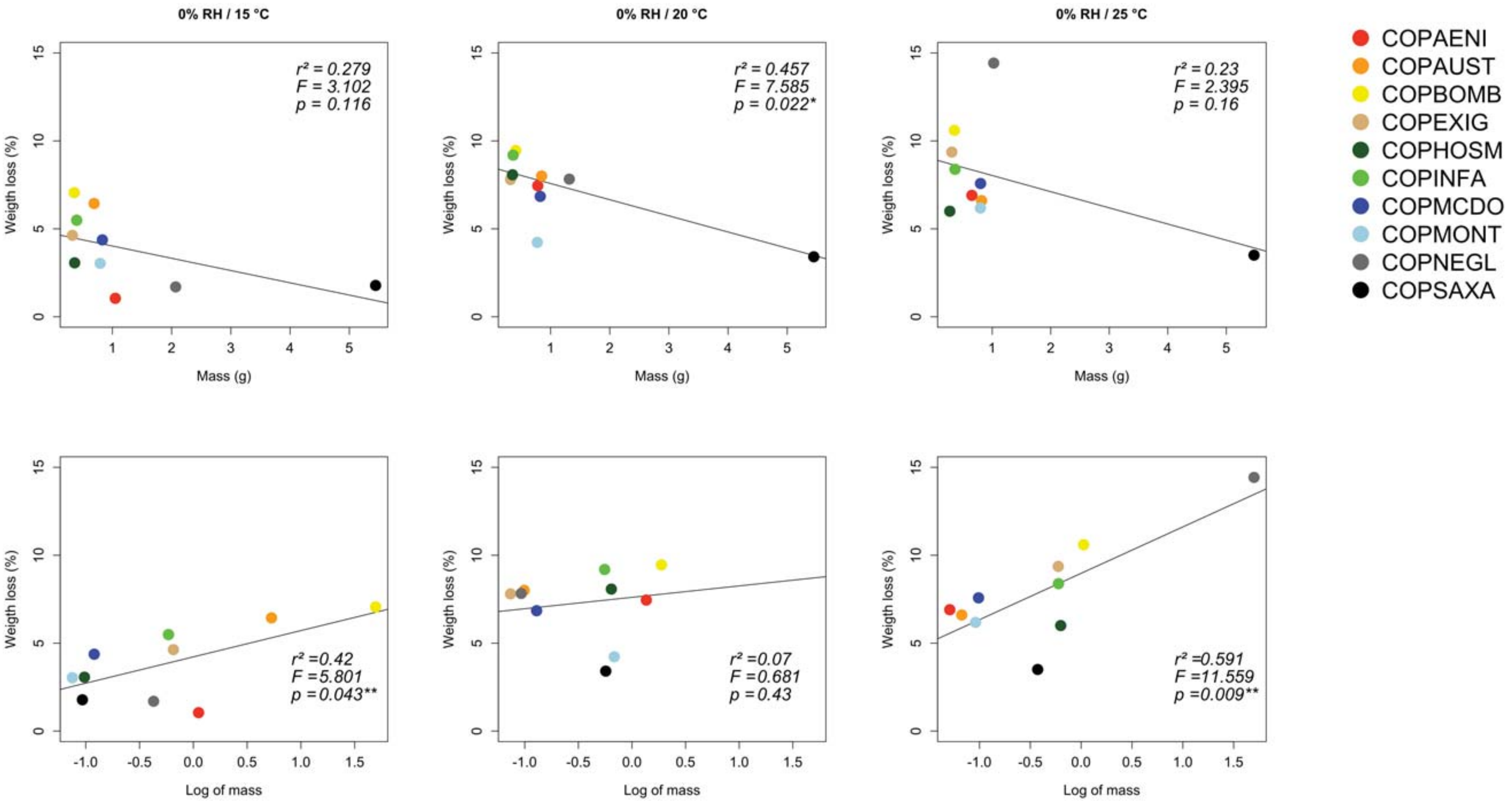

**Figure 7.3.** Linear regressions between percentage of weight loss (top row) and log transformed mass (bottom row) of individual frog species under three experimental conditions. Experiments were performed with a dry air flow of 15ml/min. *Cophixalus* codes represent a combination of genus and species names (Figure. 1.1).

# Abbreviations

AWT: Australian Wet Tropics Bioregion

COPAENI: *Cophixalus aenigma*

COPAUST: *Cophixalus australis*

COPBOMB: *Cophixalus bombiens*

COPCONC: *Cophixalus concinnus*

COPEXIG: *Cophixalus exiguous*

COPHOSM: *Cophixalus hosmerai*

COPINFA: *Cophixalus infacetus*

COPMCDO: *Cophixalus mcdonaldi*

COPMONT: *Cophixalus monticola*

COPNEGL: *Cophixalus neglectus*

COPSAXA: *Cophixalus saxatilis*

$CT_{max}$: Critical Thermal Maximum

$CT_{min}$: Critical Thermal Minimum

PGLS: Phylogenetic generalized least squares regressions

PVR: Phylogenetic eigenvector Regression

$T_{pref}$: Preferred Temperature

TSM: Thermal Safety Margin

WT: Warming Tolerance